

INFORMATION ON FRONTIERS PUBLICATION

Issue: Scientific articles published on Frontiers Marine Science based on ASI collected data

Background

The ACCOBAMS Survey Initiative (ASI), coordinated by the ACCOBAMS Secretariat in 2018-2019, was the first ever large-scale survey of cetaceans in the Mediterranean and Black Seas, providing a unique opportunity to collect data on cetaceans combining visual monitoring methods – from aircrafts and boats – with acoustic detection methods. All the riparian countries of the ACCOBAMS area jointly participated in this exceptional effort along with more than 40 national and international organizations.

The success of ASI extends beyond the significant data and unprecedented scientific results on cetacean populations in the macro region. Its impact is also evident in regional cooperation, capacity building, communication, awareness, and, most importantly, conservation outcomes.

These scientific results have been published in a dedicated Research Topic in the peer-reviewed journal *Frontiers in Marine Sciences*: "[The ACCOBAMS Survey Initiative \(ASI\): Implementing Large Scale Surveys for Marine Megafauna in the Mediterranean and Black Seas](#)".

Since their publication, the impact of the articles reached a total of 28,792 views, with 27,272 article views, 6,456 article downloads, and 1,520 topic views.

**ARTICLES PUBLISHED IN THE SPECIAL FRONTIERS MARINE SCIENCE PUBLICATION
DEDICATED TO THE ACCOBAMS SURVEY INITIATIVE ON**

“IMPLEMENTING LARGE SCALE SURVEYS FOR MARINE MEGAFUNA IN THE MEDITERRANEAN AND BLACK SEAS”

Panigada, S., Ridoux, V., Amaha Ozturk, A., Bejaoui, N., Boisseau, O., eds. (2024). *The ACCOBAMS Survey Initiative (ASI): implementing large scale surveys for marine megafauna in the Mediterranean and Black Seas*. Lausanne: Frontiers Media SA. doi: 10.3389/978-2-8325-5698-6

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The ACCOBAMS Survey Initiative (ASI): implementing large scale surveys for marine megafauna in the Mediterranean and Black Seas

Edited by

Simone Panigada, Vincent Ridoux, Ayaka Amaha Ozturk, Nejla Bejaoui and Oliver Boisseau

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The ACCOBAMS Survey Initiative (ASI): implementing large scale surveys for marine megafauna in the Mediterranean and Black Seas

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Editorial: The ACCOBAMS Survey Initiative (ASI): implementing large scale surveys for marine megafauna in the Mediterranean and Black Seas

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Editorial on the Research Topic

[The ACCOBAMS Survey Initiative \(ASI\): implementing large scale surveys for marine megafauna in the Mediterranean and Black Seas](#)

In recognizing the need for robust data on the conservation status of cetacean populations in the Mediterranean ecosystem, the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) proposed a synoptic survey of the entire region, the ACCOBAMS Survey Initiative (hereafter 'ASI'). The ASI was planned as a multi-species survey, collecting data on cetaceans and other large marine vertebrates, such as sea turtles, sharks and rays and marine birds. The survey consisted of an aerial component covering most of the Agreement area, whereas a vessel-based component was implemented in areas expected to be important for deep diving cetaceans. The core aim of the ASI was to estimate the abundance and determine the distribution of cetaceans and other marine megafauna in the Mediterranean and Black seas. [Panigada et al.](#) present a summary of the visual line-transect distance sampling aerial surveys, which covered 77% of the Mediterranean Sea to monitor all the relevant Mediterranean habitats and the species therein. The aerial component of the ASI occurred between June and August 2018. Overall, eight planes monitored more than 55,000 km along predetermined transects, from the Gulf of Cadiz to the west to the Israeli coast in the east, over an area of almost 2 million km². [Cañadas et al.](#) present a thorough description of summer distributions and densities of cetaceans, underlining a strong longitudinal gradient, from low densities in the east to high densities in the west, a pattern shared by most cetaceans. [Paiu et al.](#) present the results of the aerial surveys conducted in the Black Sea in 2019, completed under the umbrella of the ASI, within the framework of the CeNoBS project "Support MSFD implementation in the Black Sea

through establishing a regional monitoring system of cetaceans (D1) and noise monitoring (D11) for achieving GES". These aerial surveys yielded the first insights into overall abundance, density and distribution, providing regional baseline values and density maps for all three species of cetacean (*Delphinus delphis ponticus*, *Tursiops truncatus ponticus*, *Phocoena phocoena relicta*) in the Black Sea during the summer months, to be used for the elaboration of effective conservation measures and to address national and international requirements (e.g., achieving and maintaining GES (Good Environmental Status), and national conservation plans for cetaceans). Boisseau et al. summarize the results of ship-based visual and acoustic distance sampling surveys, focusing on those areas known or expected to be important for deep-diving species (sperm whales *Physeter macrocephalus* and Cuvier's beaked whales *Ziphius cavirostris*), as well as to survey those areas for which it was not possible to carry out aerial surveys. Lerebourg et al. used acoustic data to model habitats of sperm whales to fill knowledge gaps on distribution of the species in the Mediterranean Sea. These models highlighted a higher concentration of sperm whales in the western basin and the Ionian Sea along the Hellenic Trench, than in the eastern basin in summer. DiMatteo et al. analyzed data on marine turtles collected during the ASI effort and combined them with data collected between 2003 and 2018 to estimate distribution and abundance throughout the Mediterranean Sea.

The ASI was also an opportunity to develop or refine tools and methodologies. Nivière et al. developed an *ad-hoc* open-source plugin (PelaSIG) for QGIS 3 to facilitate and standardise the different steps before and after distance sampling surveys. The plug-in comprises tools for survey preparation, automatic data checking, visualisation and presentation of survey effort and sightings and is designed to process aerial datasets collected with the dedicated SAMMOA software, during marine megafauna surveys. The paper analyzed the use of the plug-in with the dataset from the aerial component of the ASI, using a multi-target protocol. Ollier et al. matched visual and acoustic events recorded in a double platform setting during the vessel-based component of the ASI to estimate visual and acoustic detection probabilities for small cetaceans in the Mediterranean Sea. Their results illustrate how passive acoustic monitoring can be used as an independent platform in Mark-Recapture Distance Sampling (MRDS) to estimate the detection probability, highlighting the importance of using dual-platform vessel surveys to estimate detection probability, and improve robustness of abundance estimates. Sol et al. analyzed temporal patterns in acoustic detections of small delphinids in the western Mediterranean. They highlighted a strong diel rhythm with as much as five times more detection during the night than during the day. The authors discussed putative ecological mechanisms underlining these patterns and recommended that these patterns should be considered when estimating dolphin abundance from acoustic detections collected during both day and night.

Finally, the ASI allowed the assessment of several anthropogenic stressors that are of major conservation concern for cetaceans and other marine megafauna. Popov et al. overlapped bycatch rates in the Black Sea with robust abundance estimates made during the CeNoBS project, confirming that bycatch poses the most serious threat to harbour porpoises in the Black Sea, and stressing the

urgent need for implementation of mitigation measures to reduce bycatch immediately in order to allow the population to survive in the basin. The CeNoBS project also allowed Frassà et al. to investigate the habitat preferences of the three subspecies of cetacean in the Black Sea, with the aim of developing habitat models to estimate the probability of their presence and using the habitat models in support of environmental status assessments in relation to human stressors, such as shipping noise. The ASI effort also allowed the collection of data on marine debris, which can have harmful effects on marine mammals through both entanglement and ingestion (Deudero and Alomar, 2015). Lambert et al. (2020) provide the first abundance estimate of floating mega-debris in the Mediterranean and mapped distributions at the basin-wide scale. Perna et al. analysed presence and abundance data of cetaceans in relation to marine plastic litter – collected through the Plastic Busters MPAs (PB MPAs) project – to assess the risk of exposure for cetaceans to this growing threat.

Initially developed to improve knowledge of cetaceans in the ACCOBAMS area, the ASI has been also crucial to fulfil European Union (EU) Regulations and Directives, specifically the Habitats Directive and the Marine Strategy Framework Directive (Authier et al., 2017), as well as other relevant instruments, including, but not limited to, the "Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean" of the Barcelona Convention, the "Convention on the Protection of the Black Sea Against Pollution" known as the Bucharest Convention, the "Convention on the Conservation of Migratory Species and Wild Animals" (also known as CMS or Bonn Convention), and the "Berne Convention on the Conservation of European Wildlife and Natural Habitats". The ASI survey is the first step towards establishing a long-term monitoring program across the entire ACCOBAMS area, and, as such, it creates the environment for further basin-wide monitoring efforts using systematic, shared, coordinated and comparable methods in the future. The information gathered will further enhance knowledge of cetacean status, facilitating the development of informed measures for conservation and mitigation, as well as supporting the implementation of the above-mentioned international obligations. Furthermore, the outcomes of this survey will support both place- and threat-based conservation efforts in the ACCOBAMS area, through the identification of Important Marine Mammal Areas (IMMAs) and Cetacean Co-occurrence with Human activities (CCH).

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Basin-wide estimates of loggerhead turtle abundance in the Mediterranean Sea derived from line transect surveys

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Loggerhead turtles are a globally vulnerable species of marine turtle. Broad-scale patterns of distribution and abundance can provide regional managers a tool to effectively conserve and manage this species at basin and sub-basin scales. In this study, combined aerial and shipboard line transect survey data collected between 2003 and 2018 were used to estimate distribution and abundance throughout the Mediterranean Sea. Approximately 230,000 linear kilometers of survey effort, from seven different surveying organizations were incorporated into a generalized additive model to relate loggerhead density on survey segments to environmental conditions. Two spatial density models estimating loggerhead density, abundance, and distribution were generated – one a long-term annual average covering 2003–2018 and another covering the summer of 2018, when a basin-wide aerial survey, the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area Survey Initiative, was performed. Both models were adjusted for availability bias using dive data from loggerhead turtles tagged with time depth recorders. Mean abundance for the long-term average model was estimated as 1,201,845 (CV=0.22). The summer 2018 abundance estimate was 789,244 turtles and

covered a smaller area than the long-term average. These estimates represent the first basin-wide estimates of abundance for loggerhead turtles in the Mediterranean not based on demographic models. Both models predicted similar distributions, with higher abundance predicted in the northern Adriatic Sea, central Mediterranean basin, Tyrrhenian Sea, and south of the Balearic Islands. Lower densities were predicted in the eastern Mediterranean Sea and the Aegean Sea. The highest density areas generally did not coincide with previously established adult loggerhead turtle foraging areas, which are typically neritic, indicating the models are predominantly predicting oceanic distributions, where most of the survey effort occurred. Juvenile loggerhead turtles are predominantly oceanic and comprise most of the population, but care must be taken when using these models as they may not accurately predict distribution of neritic foraging areas, where subadult and adult loggerheads can often be found. Despite this limitation, these models represent a major step forward for conservation planning and understanding basin-wide distribution and abundance patterns of this species.

KEYWORDS

density estimation, marine turtle, abundance, Mediterranean, line transect, availability bias

1 Introduction

Estimates of abundance and distribution of a population are prerequisites for effective conservation and management at appropriate spatial and temporal scales. Deriving such estimates can be challenging for large animals at sea such as mammals and sea turtles, where collecting observation data is often logistically and financially challenging.

Spatial density models have proven to be an effective technique for estimating population abundance and distribution for cryptic marine taxa (Forney et al 1995; Roberts et al., 2016; Hammond et al., 2021) such as marine mammals. These techniques are readily applied to marine turtles (Gómez de Segura et al., 2006; Benson et al., 2007; Lauriano et al., 2011; Eguchi et al., 2018; Fortuna et al., 2018; Welch et al., 2019), though spatial density models of marine turtles at sea are less common than for marine mammals, potentially due to their availability to be observed when mature females come ashore to nest, offering researchers an avenue of observation and sampling not available for taxa that spend their entire lives at sea. Managing sea turtles requires studies from multiple types of data and lines of evidence, including at sea observation, in order to holistically manage populations.

Loggerhead turtles (*Caretta caretta*) are distributed globally in all temperate ocean basins (Wallace et al., 2010), are globally vulnerable (Casale and Tucker, 2017), and in the Mediterranean

Sea are the most common sea turtle species. Nesting beaches for Mediterranean loggerhead turtles are found primarily in the Eastern Mediterranean (Casale et al., 2018). In the Western Mediterranean, limited nesting occurs (Casale et al., 2018), but has been increasing in the last decade, with females coming from both Mediterranean and Atlantic populations, in what may be potential colonization (Maffucci et al., 2016; Carreras et al., 2018).

After hatching, Mediterranean loggerhead turtles make their way into the sea where they entrain as oceanic juveniles throughout the offshore areas of the Mediterranean basin, occasionally venturing as far as the Atlantic coast of Portugal. As they mature, juveniles generally transition to neritic foraging areas once they reach approximately 60 cm curved carapace length (Carreras et al., 2006; Casale and Mariani, 2014; Clusa et al., 2014; Snape et al., 2016; Cardona and Hays, 2018; Casale et al., 2018), though some loggerheads remain in oceanic foraging areas.

In the Western Mediterranean, juvenile loggerhead turtles from the Northwest and Northeast Atlantic subpopulations mix with resident Mediterranean loggerhead turtles, determined by genetic stock assignment of animals captured in Mediterranean waters (Laurent et al., 1998; Wallace et al., 2010; Carreras et al., 2011; Tolve et al., 2018; Loisier et al., 2021). Juveniles from Atlantic subpopulations can stay in Mediterranean waters for as many as ten years (Eckert et al., 2008; Revelles et al., 2008; Clusa et al., 2014), though the number of animals that remain this long

is unclear. The proportion of juvenile loggerhead turtles of Atlantic origin can be higher than 30 percent in some areas (Carreras et al., 2006; Carreras et al., 2011). Individuals of Mediterranean origin have not been detected in the western or southern Atlantic basins.

Estimates of adult female nesting populations in the Mediterranean Sea show a modest increasing trend and are approximately 15,000 for loggerhead turtles (Casale and Heppell, 2016), although this is likely an underestimate given the lack of comprehensive surveys of all potential nesting habitats. The best demographic estimates for the total loggerhead turtle population originating in the Mediterranean Sea solely uses the number of nesting females as a starting point, and ranges from 0.8–3.4 million (Casale and Heppell, 2016) but does not include juveniles of Atlantic origin. This demographic model can provide an independent comparison to abundance estimates derived from visual surveys, with caution, given the lack of inclusion of turtles of Atlantic origin.

Loggerhead turtles, regardless of origin, experience significant threats in the Mediterranean Sea including bycatch in fisheries, climate change, coastal development, and marine pollution (Casale et al., 2018; Lucchetti et al., 2021). These threats affect multiple life stages of loggerhead turtles across the entire basin, necessitating management of this species at basin-wide scales to be effective.

Spatially explicit estimates of basin-wide distribution and abundance can help prioritize in-water areas or regions for conservation measures. Spatially explicit estimates of abundance and distribution for loggerhead turtles based on line transect surveys exist in limited areas of the Mediterranean such as the Adriatic Sea, off the coast of Spain, and the Pelagos sanctuary (Gómez de Segura et al., 2003; Gómez de Segura et al., 2006; Lauriano et al., 2011; Fortuna et al., 2018). However, these surveys have not been combined to provide a comprehensive estimate of distribution and abundance basin-wide, and none cover the eastern basin.

Recent efforts for marine mammals in the Mediterranean Sea (Cañadas et al., 2018; Mannocci et al., 2018) and the United States (Roberts et al., 2016) have shown that it is possible to combine multiple line-transect surveys from across a region into a distance sampling framework to produce spatially explicit estimates of distribution and abundance. Distance sampling (Buckland et al., 2001) provides a method to relate observed perpendicular distances of animals on a survey trackline to animal abundance. These abundances can be related to environmental covariates, allowing abundance to be predicted based on the underlying environment. The resulting predictions are often referred to as spatial density models and are usually predicted at the resolution of the underlying environmental covariates. This also allows for extrapolation into areas and times where surveys did not occur, but where similar environmental conditions can be found. Here we follow the general method to generate spatial density models laid out by Miller et al. (2013).

In the last 20 years, many line transect surveys in the Mediterranean Sea sighted loggerhead turtles, and have been conducted to follow distance sampling protocols. In 2018, a basin-wide aerial survey was conducted by the Agreement for the Conservation of Cetaceans of the Black Sea, Mediterranean Sea, and Contiguous Atlantic Area (ACCOBAMS) Survey Initiative that included observations of loggerhead turtles using a strip transect methodology. Strip transects differ from distance sampling in that all animals within a certain distance from the sampling platform are assumed to be detected. In distance sampling, there is an assumption that the proportion of animals detected decreases with increasing distance from the trackline and that this relationship can be modeled. Line transect and strip transect survey methodologies are both compatible for inclusion into a spatial density model as both can be broken into segments with an estimated abundance that can be linked to environmental covariates.

Another important consideration for predicting abundance from line transect data is accounting for the probability of detecting an animal on the trackline (i.e., at a perpendicular distance of 0), or across the entire strip for strip sampling. This is affected by two factors: 1) availability bias, which is failing to detect animals because they are unavailable to be seen (e.g., hidden or submerged while diving) and 2) perception bias, where observers fail to detect animals present at or near the surface (Pollock et al., 2006). The combined probability of detection on the trackline, or across the strip, is referred to as $g(0)$. Distance sampling assumes that $g(0)=1$, but this is rarely the case in practice and unless correction factors are applied to lower the probability of detection, abundance will be underestimated.

Further complicating availability bias estimation for turtles is the mean sea surface temperature in the Mediterranean Sea varies from 15 to 26°C over the course of the year (Pastor et al., 2020). As ectotherms, turtles respond to changes in temperature by changing dive behavior (Mrosovsky, 1980; Bentivegna et al., 2003; Broderick et al., 2007; Hochscheid et al., 2007), with longer dive times during cool months than warm months. Turtles may also alter their dive behaviors depending on the habitat they occupy, changing how long they are available at the surface to be seen.

Here we present spatial density models of both a long-term average of loggerhead turtle distribution and abundance, as well as estimates based solely on the summer 2018 aerial survey, corrected for availability bias. The models provide robust estimates of abundance and distribution of loggerhead turtles across the whole Mediterranean Sea on which to base conservation and management decisions at basin-wide and subbasin scales, regardless of population of origin.

The long-term and summer 2018 loggerhead turtle models found in this paper were first described in the grey literature technical reports Sparks and DiMatteo (2020) and ACCOBAMS (2021), respectively. This article provides updated discussion

and methods, a summer 2018 abundance estimate adjusted for availability bias, and input from regional data providers not found in either technical report.

2 Methods

2.1 Study area and data

The study area includes eight regions of the Mediterranean Sea (Figure 1), previously defined in Mannocci et al., 2018 for ease of referring readers to subregions. Line transect survey data from seven different organizations were used, covering 229,598 linear km of effort, split between 56,171 km of shipboard surveys and 173,427 km of aerial surveys (Table 1). Details on the aircraft used and survey speeds and heights can be found in Sparks and DiMatteo (2020) and ACCOBAMS (2021). Included data from these surveys spanned 2003–2018 and covered all months of the year, though there are differences in survey coverage between months. Surveys covered all major regions within the Mediterranean Sea, but coverage was sparse or absent in some regions of the eastern and southern Mediterranean. Figure 1 shows the geographic coverage of incorporated surveys and associated sightings.

All surveys but the ACCOBAMS Survey Initiative (ASI) survey were usable in a distance sampling framework, and we ensured that they contained all the requisite components to perform distance sampling – time, location, the number of

animals detected (e.g. group size), and perpendicular distance to the animal(s) from the trackline (Buckland et al., 2001). The ASI survey used a strip transect sampling protocol.

Line transects were split into 5-km segments, the same resolution as the finest scale candidate environmental covariates for the spatial density models. Not all transects could be split into perfectly even 5-km segments, so an algorithm was used to split the segments as close to 5-km as possible (Mannocci et al., 2018).

All loggerhead turtle sightings, as well as unidentified hardshell turtle sightings, were used in the model (Table 1). In aerial surveys it can be difficult to discriminate loggerhead from green turtles (*Chelonia mydas*); hence, in some surveys, sightings were assigned to the species most likely to occur in the region (loggerhead turtles) or all recorded as unidentified. Unidentified sightings could reasonably be assumed to be mostly loggerhead turtles based on their much larger population than green turtles (Casale and Heppell, 2016). With few exceptions green turtles are limited to the Eastern Mediterranean. In the western Mediterranean, 98% of stranded turtles are loggerhead turtles (Tomás et al., 2008).

2.2 Detection function fitting

Detection functions, monotonically decreasing functions used to describe the relationship between probability of detection and

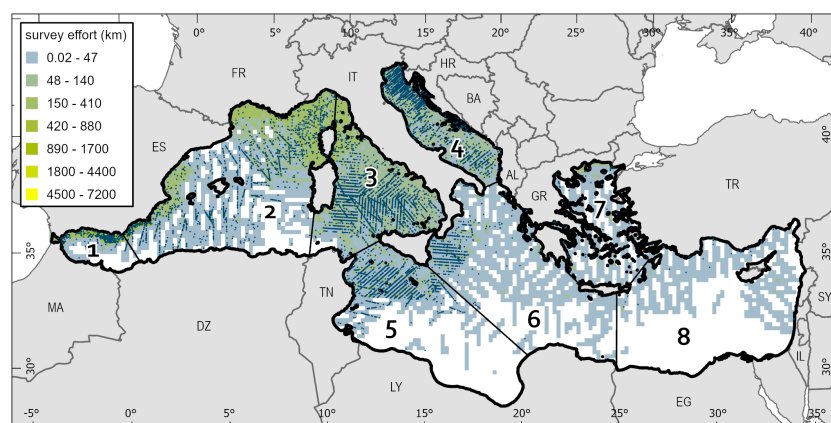


FIGURE 1

The study area within the Mediterranean Sea (black outline) over which abundance predictions are made. Subregions are separated by thinner black lines and are defined as 1) Alborán Sea/Strait of Gibraltar, 2) Algero-Provençal Basin, 3) Tyrrhenian Sea/eastern Ligurian Sea, 4) Adriatic Sea, 5) Strait of Sicily/Tunisian Plateau/Gulf of Sirte, 6) Ionian Sea/Central Mediterranean, 7) Aegean Sea, and 8) Levantine Sea. Survey effort (linear km of effort per 400 km² grid cell) is provided as a surface with turtle observations shown as small blue dots. 2-digit country codes for select countries are provided for reference (clockwise from top): ES, Spain; FR, France; IT, Italy; HR, Croatia; BA, Bosnia and Herzegovina; AL, Albania; GR, Greece; TR, Turkey; SY, Syria; IL, Israel; EG, Egypt; LY, Libya; TN, Tunisia; DZ, Algeria; MA, Morocco.

TABLE 1 Summary of survey effort and sightings used in the loggerhead turtle models.

Survey	Platform	Region	Years	Effort (linear km)	Useable Sightings	Group size >1	Notes
ASI	aerial	All subregions	2018	55,498	3745	5%	all given as hardshell
BWI, ISPRA	aerial	Adriatic Sea	2010, 2013	16,595	2010	2%	all given as hardshell
PELAGIS	aerial	Algero-Provençal basin, Tyrrhenian Sea/eastern Ligurian Sea	2011, 2012	32,240	371	2%	all given as hardshell
TETHYS, ISPRA	aerial	Algero-Provençal basin, Tyrrhenian Sea/eastern Ligurian Sea, Ionian Sea	2009–2011, 2013, 2014, 2016	61,996	5792	6%	all given as hardshell
University of Valencia	aerial	Algero-Provençal Basin	2010, 2011, 2013	7,098	81	2%	all loggerhead
Alnitak/Alnilam	shipboard	Alborán Sea/Strait of Gibraltar	1999–2011	42,094	441	7%	all loggerhead
Song of the Whale (IFAW/MCR)	shipboard	All subregions but the Adriatic Sea	2003, 2004, 2005, 2007, 2013	7,013	64	5%	62 hardshell, 2 confirmed loggerhead
Song of the Whale (ASI)	shipboard	Alborán Sea/Strait of Gibraltar, Algero-Provençal Basin, Tyrrhenian Sea/eastern Ligurian Sea, Strait of Sicily/Tunisian Plateau/Gulf of Sirte, and Ionian Sea/Central Mediterranean	2018	7,064	98	6%	31 hardshell, 67 confirmed loggerhead
TOTALS				229,598	12,602		

ASI, ACCOBAMS Survey Initiative; BWI, Blue World Institute and Italian Institute for Environmental Protection and Research; ISPRA, Italian National Institute for Environmental Protection and Research; PELAGIS, Systèmes d'Observation pour la Conservation des Mammifères et Oiseaux Marins; TETHYS, Tethys Institute; IFAW, International Fund for Animal Welfare; MCR, Marine Conservation Research.

distance to an observation (Buckland et al., 2001), were fit for the six surveys that used distance sampling protocols and were subsequently used to predict segment abundance.

Histograms of perpendicular distances were generated for each of the six surveys to explore the need for truncation. Buckland et al. (2001) recommends that distant sightings are truncated ('right truncation') to maintain a minimum probability of detection of 0.15. Left truncation, e.g. removing sightings near the trackline, is generally only used in special circumstances, such as when the trackline is not visible. This was performed for only the University of Valencia aerial surveys which used aircraft with flat windows, limiting the view of the trackline.

The ability to sight animals generally varies by survey platform and protocol. As such, it is desirable to fit separate detection functions by platform and survey if there are enough sightings to meet the recommended 60 sighting threshold for fitting robust detection functions (Buckland et al., 2001).

All surveys had more than the recommended 60 sightings, so no pooling was required between surveys or platforms. We pooled multiple years of the same survey program to be able to fit more complex detection functions, unless there was good

reason not to pool, such as different altitudes between years. Most surveys had associated survey condition and sightings covariates that allowed multi-covariate distance sampling (Marques and Buckland, 2004), meaning the probability of detection varies as a function of the covariates.

All combinations of survey condition and sighting covariates were attempted for both half normal and hazard rate detection functions, the two most common forms for the detection function (Buckland et al., 2001), both with and without cosine transformations. Additional covariates tested in combination with the others included year (for surveys with multiple years), month, and observer position. Group size was not included as a covariate, as is common for marine mammals, because turtles are not gregarious creatures and rarely aggregate except for the purposes of mating. Table 1 summarizes available sightings per survey and percent of group sizes larger than one.

Detection function model selection was based on Akaike Information Criteria (AIC), which is used to assess the trade-off between goodness of fit and model simplicity. We selected the model with the lowest AIC. If more than one 'best' model had similar AIC (within 2) we chose between them based on

goodness of fit statistics (Cramer-von Mises and Kolmogorov-Smirnoff), parsimony (degrees of freedom), and qualitative assessments of quantile-quantile (Q-Q) plots and detection plots.

The ASI aerial survey did not record perpendicular distances to sea turtles, instead opting for a strip transect approach. The survey data providers assumed all sea turtles within a 200-meter strip on either side of the plane were detected (ACCOBAMS, 2021). With a strip transect methodology, no detection function is fit and abundance per segment is calculated by dividing the number of individuals sighted by the area covered and adjusted for availability and perception bias if possible.

2.3 Correction for availability bias

No surveys obtained for this study had the requisite information to assess perception bias *in-situ*. We opted not to use published estimates of perception bias as they are very survey platform and condition specific.

Three datasets of loggerhead turtles deployed with satellite-linked, time depth recorders were used for calculating dive and surface intervals appropriate for making availability bias adjustments (Broderick et al., 2007; Hochscheid et al., 2007; Hochscheid et al., 2010; Hochscheid et al., 2013; Chimienti et al., 2020; Haywood et al., 2020; Hochscheid, 2020; Oceanographic Turtles Project, 2020; Society for the Protection of Turtles, 2020). These tags recorded dive and surface intervals, as well as georeferenced animal locations, allowing dives to be linked to specific locations. Availability bias is calculated as average surface interval divided by average surface interval plus average dive length.

Tagged animals from the three datasets were deployed in waters off Cyprus, Italy, and Spain respectively. Animals ranged across all regions of the Mediterranean Sea included in this study except for the Adriatic Sea and Aegean Sea. Table 2 summarizes the available data.

The Italy and Cyprus dive datasets had similar formats with dive duration and surface intervals given in seconds along with dates and times for the start and end of the dives. Georeferenced

locations, a combination of global positioning system (GPS) and Argos satellite system locations, were provided separately along with dates and times. Argos location classes 3, 2, 1, and 0 were considered valid as were all GPS locations. Dives were matched to the closest location within six hours on the assumption that remotely sensed environmental covariates would not change appreciably over the distance a turtle could swim in 6 hours. Dives that could not be georeferenced to a location within six hours were removed from the analysis.

For both datasets, tags were configured to consider a dive to have started when the turtle dove below 4 m. In good viewing conditions for aerial surveys (low turbidity, low Beaufort sea state, etc.) turtles can be seen as deep as 3 m, though the chances of detecting turtles decreases as depth increases (Fuentes et al., 2015; Barco et al., 2018). The viewable depth for turtles is likely shallower for shipboard surveys as the viewing angles preclude seeing down into the water column. Because of this decreased detection chance, we are likely overestimating the amount of time animals are available to be seen because some portion of the animals will be deeper than can be seen or will be harder to detect by observers. This would have the effect of underestimating abundance.

Tags for the Spain dataset were formatted to record depths over five-minute intervals and reported the average depth of the five-minute period. Each five-minute average depth was georeferenced to a location generated from a state-space switching model track of animal locations. These five-minute averages were then used to generate dive profiles. Surface and dive intervals were created from these dive profiles. Based on tag configuration, dives were defined to start when the turtles went below 3 m.

Short surfacing events of a few minutes or less may have been missed in the Spain dataset, as average depths were reported over five-minute intervals. This is likely not an issue for aerial surveys, which can generally be treated as instantaneous snapshots of the surface, but underestimation of availability could occur for shipboard surveys (overestimating abundance).

Surface intervals longer than four hours were removed from all three datasets. Surface times of this length could be indicative of a nesting event, basking, mating, or a failure of the saltwater

TABLE 2 Summary of available dive data for availability bias estimates.

Dataset	Number of tags	Number of dives	Years deployed	Life stages	Sex	Source	Primary regions
Cyprus	7	3,110	2002-2008	adult	female	post-nesting	Alborán Sea/Strait of Gibraltar, Algero-Provençal Basin
Italy	31	11,985	2005-2017	mix of adults, juveniles, and sub-adults (length, not age class, reported)	9 females, 4 males, 18 unknown	2 post-nesting, 16 rehabilitated, 13 wild-caught	Tyrrhenian Sea/eastern Ligurian Sea, Strait of Sicily/Tunisian Plateau/Gulf of Sirte, Ionian Sea/Central Mediterranean
Spain	17	36,279	2017-2020	1 adult, 15 juveniles, 1 unknown	2 female, 2 male, 13 unknown	14 wild-caught, 3 rehabilitated	Ionian Sea/Central Mediterranean, Levantine Sea

switch that indicated when the turtle was below the surface. Removing these events depresses availability estimates, increasing abundance. These long surface intervals were less than one percent of all dive records.

The 51,373 remaining georeferenced dives were stratified spatially and temporally to determine if significant differences in dive behavior, and hence availability, existed. Turtles have diurnal changes in dive behavior (Hochscheid, 2014), so only daytime dives were included in this analysis, which is also when surveys would have occurred.

The temporal split was into two broad seasons—warm and cool—based on major climatic shifts in temperature and increases in neritic turtle activity. The warm season was defined as May–October and cool as November–April. Spatial stratification was into neritic versus oceanic regions, split at the 200 m isobath, where animals would presumably be feeding on different prey and exhibiting different dive behavior (Hochscheid, 2014). This also partially addresses differences in behavior between presumed larger, neritic adults and smaller, oceanic individuals.

For the long-term model, abundance for each segment was calculated using a Horvitz-Thompson-like estimator (Borchers et al., 1998). The availability bias estimate for each segment was adjusted based on platform-specific speed, height, and viewing angles, which can be found in Mannocci et al. (2018). Bubble window aerial surveys were adjusted after Carretta et al. (2000). Flat window surveys and shipboard surveys were adjusted using equations seven and four respectively from Laake et al. (1997). For the summer 2018 model, availability bias adjustments were applied after modeling, as the unadjusted model was used in the ACCOBAMS Survey Initiative report (ACCOBAMS, 2021) and was created using methods consistent with models for other taxa presented in that report.

2.4 Spatial modeling

2.4.1 Environmental covariates

Potential environmental covariates that could be associated with marine turtle habitat were compiled for the study area. For the long-term model, six static covariates and ten dynamic covariates (three physical and seven biological) were selected that were available at a monthly resolution and spanned the temporal range of the study.

For the summer 2018 model, covariates consistent with the other ACCOBAMS models (ACCOBAMS, 2021) were used as candidates and included 18 static covariates and 10 dynamic covariates at monthly and seasonal resolution. Latitude and longitude were included as potential covariates for the summer 2018 model but not the long-term model.

Including spatial smooths is a common practice in spatial density modeling to account for variability not captured by the available environmental covariates. It was not used in the long-term model because a spatial smooth cannot easily be

extrapolated, which would necessitate using separate models for any areas of geographic extrapolation. Additionally, a spatial smooth is most influential where sightings occur, which can cause issues when survey coverage is uneven, as is the case with the long-term model.

Different sets of covariates were used for the long-term and summer 2018 models because the summer 2018 model needed to be consistent with other ACCOBAMS models and was originally produced as part of that project. Not all the covariates used in the summer 2018 model were available for the time period of the long-term model. Only contemporaneous dynamic covariates were included in both models, rather than climatological covariates, on the assumption that turtle distribution is more closely related to ephemeral conditions than long-term averages of conditions (Howell et al., 2015). Details on the covariates considered can be found in Table 3.

All covariates were processed to 5 x 5 km grid cells for the long-term model and 10 x 10 km grid cells for the summer 2018 model using a nearest neighbor resampling method. The center points of the processed grid cells were sampled and used for model prediction. Because some extreme values of covariates were poorly sampled by surveys, the extent of environmental extrapolation was assessed for the long term model using the R package *dsmextra* (Bouchet et al., 2019). The methods and results of the extrapolation analysis are presented in the Supplemental Material.

2.4.2 Density modeling

A generalized additive model (GAM) framework was used for both the long-term and summer models. The response variable, predicted abundance, was modeled with a Tweedie distribution (Foster and Bravington, 2013), which handles zero-inflated distributions well. This is useful because most segments had zero sightings and, therefore, an abundance of zero. All models were fit with the R package *mgcv* (Wood, 2011).

Candidate models for the long-term model were fit to all segments from survey data between 2003 and 2018. Only segments from the ACCOBAMS aerial survey were used for the summer 2018 model.

Predictions from the long-term model were made monthly, the finest temporal scale of the dynamic covariates. Cells from each monthly prediction were averaged into a single prediction covering the time span of the study period, creating a ‘densitology’ prediction of long-term abundance over the 16 years. This assumes that loggerhead abundance was relatively stable over this period, which may not be true given the apparent increasing trend in nesting females (Casale et al., 2018). Cells were treated as independent units for predictions.

A single model prediction was made for summer 2018, as well as a conventional abundance estimate using a 400-meter strip transect. For the conventional estimate, the density of the animals was estimated for each strip (searched area), dividing the number of animals detected by the searched area. The individual strip

TABLE 3 Candidate environmental covariates for inclusion in spatial density models.

Candidate Environmental Covariates	Source	Used in Model
Biological		
Chlorophyll	Monthly mean chlorophyll concentration at the ocean surface derived from the Mediterranean Sea Biogeochemical Reanalysis ocean model (Teruzzi et al., 2019)	Long-term, summer 2018
Net Primary Productivity	Monthly mean primary production at the ocean surface derived from the Mediterranean Sea Biogeochemical Reanalysis ocean model (Teruzzi et al., 2019)	Long-term
Phytoplankton Carbon Biomass	Monthly mean phytoplankton carbon biomass at the ocean surface derived from the Mediterranean Sea Biogeochemical Reanalysis ocean model (Teruzzi et al., 2019)	Long-term
Vertically Integrated Chlorophyll	Monthly mean of depthwise integration of chlorophyll concentration through the photic zone (Teruzzi et al., 2019)	Long-term
Vertically Integrated Net Primary Productivity	Monthly mean of depthwise integration of net primary productivity across the water column (or photic zone) (Teruzzi et al., 2019)	Long-term
Vertically Integrated Phytoplankton Carbon Biomass	Monthly mean of depthwise integration of phytoplankton carbon biomass across the water column (or photic zone) (Teruzzi et al., 2019)	Long-term
Vertically Generalized Production Model (VGPM)	Monthly net primary productivity across the water column by the VGPM model (Behrenfeld and Falkowski, 1997)	Long-term
Physical		
Sea Surface Temperature	Monthly mean sea surface temperature at the ocean surface derived from the Mediterranean Forecasting System ocean models (Nucleus for European Modeling of the Ocean [NEMO]) (Simoncelli et al., 2019)	Long-term, summer 2018
Sea Surface Temperature Standard Deviation	Monthly mean sea surface temperature standard deviation at the ocean surface derived from the Mediterranean Forecasting System ocean models (Nucleus for European Modeling of the Ocean [NEMO]) (Simoncelli et al., 2019)	Summer 2018
Bottom Temperature	Monthly mean temperature at the ocean bottom derived from the Mediterranean Forecasting System ocean models (NEMO) (Simoncelli et al., 2019)	Long-term
Salinity	Monthly mean salinity at the ocean surface derived from the Mediterranean Forecasting System ocean models (NEMO) (Simoncelli et al., 2019)	Long-term
Sea Surface Height	Monthly mean sea surface height derived from the Mediterranean Forecasting System ocean models (NEMO) (Simoncelli et al., 2019)	Summer 2018
Mixed Layer Thickness	Monthly mean mixed layer thickness derived from the Mediterranean Forecasting System ocean models (NEMO) (Simoncelli et al., 2019)	Summer 2018
Static		
Latitude	Latitude derived from segment center points	Summer 2018
Longitude	Longitude derived from segment center points	Summer 2018
Depth	Depth of seafloor derived from Shuttle Radar Topography Mission (SRTM)15 and SRTM30 bathymetry (Becker et al., 2009 and Olson et al., 2016)	Long-term, summer 2018
Slope, Contour, and Aspect	Slope of seafloor derived from SRTM15 and SRTM30 bathymetry (Becker et al., 2009 and Olson et al., 2016)	Long-term, summer 2018
Distance to Canyon	Distance to closest submarine canyon derived from the International Hydrographic Organization-International Oceanographic Commission's General Bathymetric Chart of the Oceans Gazetteer (IHO-IOC Commission, 2018).	Long-term, summer 2018
Distance to Seamount	Distance to closest seamount derived from Wurtz and Rovere (2015)	Long-term, summer 2018
Distance to Shore	Distance to shore derived from National Oceanic and Atmospheric Administration (National Oceanographic and Atmospheric Administration, 2016)	Long-term, summer 2018
Distance to Contour	Distance to the 100m, 500m, 1000m, and 2000m contours. Derived from SRTM bathymetry (Becker et al., 2009 and Olson et al., 2016)	Summer 2018
Seabed Habitat Map	Seabed habitat derived from Europe SeaMap (Populus et al., 2017)	Long-term

densities were averaged then extrapolated to the whole study area by multiplying the average density by the total area.

For both long-term and summer 2018 spatial density models, candidate models were fit with all possible combinations of available covariates attempted. The exception

was covariates that were highly correlated. Correlation was examined using Spearman's correlation coefficient, and if covariates had a score of 0.5 or higher, only one was retained. A review of scatter plots of covariate interactions did not indicate non-linear relationships were present.

The maximum degrees of freedom, or wiggleness, for the relationship between covariates and the response variable was allowed. Thin-plate regression splines with shrinkage were used to allow the effect of non-significant variables to ‘shrink’ away to zero (Wood, 2003). Given the large number of segments with sightings, no attempt was made to limit the number of covariates included in the model if they were significant. Model selection was accomplished by choosing the model with the lowest Restricted Maximum Likelihood (REML) score, which may be less prone to local minima than other selection criteria (Wood, 2011). We checked model goodness of fit by examining residuals, utilized degrees of freedom, and by qualitatively assessing models for unrealistic artifacts or predictions.

2.4.3 Uncertainty estimation

For both models, a parametric bootstrap approach was conducted to account for two sources of uncertainty: model parameter uncertainty and environmental variability as in Becker et al. (2014). After model fitting and selection, resampled parameter estimates from the selected model based on their associated uncertainty were drawn and new predictions were made based on the resampled parameters, also called posterior simulation (Bravington et al., 2021).

For the long-term model, 200 draws were made for each month, creating a set of 200 abundance predictions for each month that varied based on the uncertainty of the parameter space of the selected model relative to the underlying environment. Only one set of predictions was generated for the summer 2018 model as it was treated as a single time period. Coefficient of variation (CV) was calculated based on these abundance predictions, which combined the model parameter uncertainty and environmental variability.

An additional source of uncertainty was included in the overall CV calculation for the summer 2018 model. The delta method was employed to combine the CV from the parametric

bootstrap and the variation among the samples used in the model (individual strip transects).

3 Results

3.1 Segments and detection functions

Some sightings were removed because they were missing detection function covariates or perpendicular distances. Any survey segments with removed sightings were eliminated from all subsequent analyses. The percentage of on effort sightings eliminated from surveys ranged from 4.7% (University of Valencia) to 0% (PELAGIS) with 0.7% of sightings removed overall.

Table 4 summarizes the selected detection function, covariates, and truncation distances for all surveys where distance sampling protocols were followed. Review of the detection function goodness of fit statistics and plots indicated good fit for most detection functions.

For the TETHYS and ISPRA aerial surveys, there was some potential heaping on the trackline (e.g. more sightings on the trackline than would be expected). There was no clear way to address this heaping. However, detection functions are generally robust to this issue (Buckland et al., 2001), and the normal model selection process was applied.

The University of Valencia surveys were flown at different heights between survey years, so separate detection functions were initially attempted for the different years. There were only 34 sightings in the 2010/2011 surveys. The Q-Q plots for models fitted to those surveys indicated poor fit and could not include any covariates with so few sightings, so instead the years were combined, and survey year was included as a potential covariate. The selected detection function was the half normal, left truncated at 104 m with month as the only covariate. The Q-Q plot was not as good as for other aerial surveys, but this was unsurprising given the relatively lower number of sightings, the need for left truncation, and the different altitude between years.

TABLE 4 Summary of selected detection function models for surveys with distance sampling protocols.

Survey ¹	Platform	Selected function	Covariates	Truncation distance
BWI ISPRA	aerial	hazard rate	glare, Beaufort sea state, month	right truncation at 343 m
PELAGIS	aerial	half normal	Beaufort sea state, month	right truncation at 200 m
TETHYS ISPRA	aerial	half normal with cosine adjustment	none	right truncation at 300 m
University of Valencia	aerial	half normal	month	right truncation at 450 m, left truncation at 104 m
Alnitak/Alnilam	shipboard	hazard rate	month, observer position	right truncation at 400 m
Song of the Whale (IFAW/MCR)	shipboard	hazard rate	Beaufort sea state	right truncation at 100 m
Song of the Whale (ASI)	shipboard	hazard rate	month	right truncation at 100 m

¹Full names of survey providers were provided in Table 1.

3.2 Correction for availability bias

Significant differences in dive duration and surface interval were identified by all three stratifications: day/night, depth, and warm/cool season (t-test, all p values < 0.05). Based on this finding, availability bias estimates were generated using daylight dives only, and then stratified by season and depth. This approach yielded four different availability bias adjustments based on the mean dive and surface intervals. There was high variability among the dives, and standard deviations of the intervals often exceeded the mean (Table 5). This variability is not accounted for in the CV of the final models, as at the time of analysis there was not a computationally feasible method for propagating this uncertainty. Instantaneous $g(0)$ availability bias adjustments ranged from 0.48 for oceanic areas in the summer to 0.27 in neritic areas in the winter (Table 5).

3.3 Spatial models

The best long-term model retained all candidate covariates. It had the best deviance explained (34%) and lowest REML score of all candidate models. Figure 2 shows the functional relationships to covariates fit by the long-term model. Extreme values of chlorophyll *a*, depth, slope, and salinity were poorly sampled (see rug plots in Figure 2) and had higher associated uncertainty. For dynamic covariates, apparent preferences were for warmer temperatures, higher productivity, and lower salinity. The relationships with static covariates led to higher predicted abundance in offshore areas.

The total predicted abundance was 1,201,845 (CV=0.22, 95% CI [838,864-1,548,280]) for the long-term model and included adjustments for availability bias. Abundance values by grid cell can be seen in Figure 3A. The overall CV estimate includes both GAM parameter uncertainty and environmental variability. The abundance in the areas of geographic extrapolation was 192,826 (Figure 3A). The extent of geographic extrapolation and the percent of the total predicted abundance that was in the areas of geographic extrapolation was approximately the same (16%).

The highest predicted abundances were in the southeastern Mediterranean off the coast of Egypt, the northern Adriatic Sea, the southern Algero-Provençal Basin to the Balearic Islands, and

the Tunisian Plateau. Predicted abundance was low in most coastal areas, the Aegean Sea, and the eastern Mediterranean Sea (except for the hotspot off Egypt). An assessment of inter- and intra-annual variability of monthly abundance predictions for the long-term model is presented in the Supplemental Materials.

There were high values of CV (greater than 1) located around areas of low salinity, far distances to canyons, and extremely deep areas that were poorly sampled by surveys. For example, the northern Adriatic Sea, the Libyan coast, and off the coast of Crete all had high values of CV (Figure 3B). CV was generally lower in well sampled areas.

The selected covariates for the summer 2018 model were latitude, longitude, distance to shore, distance from canyons, mean mixed layer thickness, and standard deviation of sea surface temperature. Like the long-term model, higher densities were predicted further from shore and canyons. Turtles appeared to avoid areas at extreme ranges of mean mixed layer depth and areas with high variability in temperature (Figure 4).

The summer 2018 (uncorrected for availability bias) abundance estimate was 343,321 turtles (CV=0.03) in the whole Mediterranean except the unsurveyed southeastern region, a similar area to what was extrapolated for the long-term model. Abundance and CV values by grid cell can be seen in Figure 5. The total abundance of the spatial model was similar to the results obtained from the strip transect analysis: 329,529 individuals (CV=0.05). These results include 2,252 turtles estimated in contiguous Atlantic waters of South Portugal, to the west of the Strait of Gibraltar, where predictions were not made for the long-term model. The highest abundances were predicted in the Central and Western Mediterranean and the Adriatic Sea. The lowest abundance was predicted in the Eastern Mediterranean, approximately 20 times lower than the higher abundances predicted elsewhere.

Adjusting the summer 2018 model for availability bias using the same warm month and depth availability bias estimates as the long-term model, the corrected abundance estimate is 789,244 turtles. This estimate does not include any predictions for the southeastern Mediterranean. The long-term model predicts 1,009,550 (95% CI [787,449-1,231,651]) turtles in the same area (areas covered the ASI survey). Considering the CIs of the two estimates, there is some overlap between the predictions.

TABLE 5 Stratified availability bias, $g(0)$, estimates for the Mediterranean Sea based on *in-situ* dive data.

Season	Depth	Mean surface interval (minutes)	Mean dive duration (minutes)	Surface interval standard deviation (minutes)	Dive duration standard deviation (minutes)	# of dives	$g(0)$ instantaneous
warm	oceanic	20.3	21.8	37.2	13.9	17102	0.48
cool	oceanic	22.8	29.7	40.1	23.5	5707	0.43
warm	neritic	9.2	11.9	34.2	14.0	6549	0.43
cool	neritic	11.4	31.1	26.4	56.9	1903	0.27

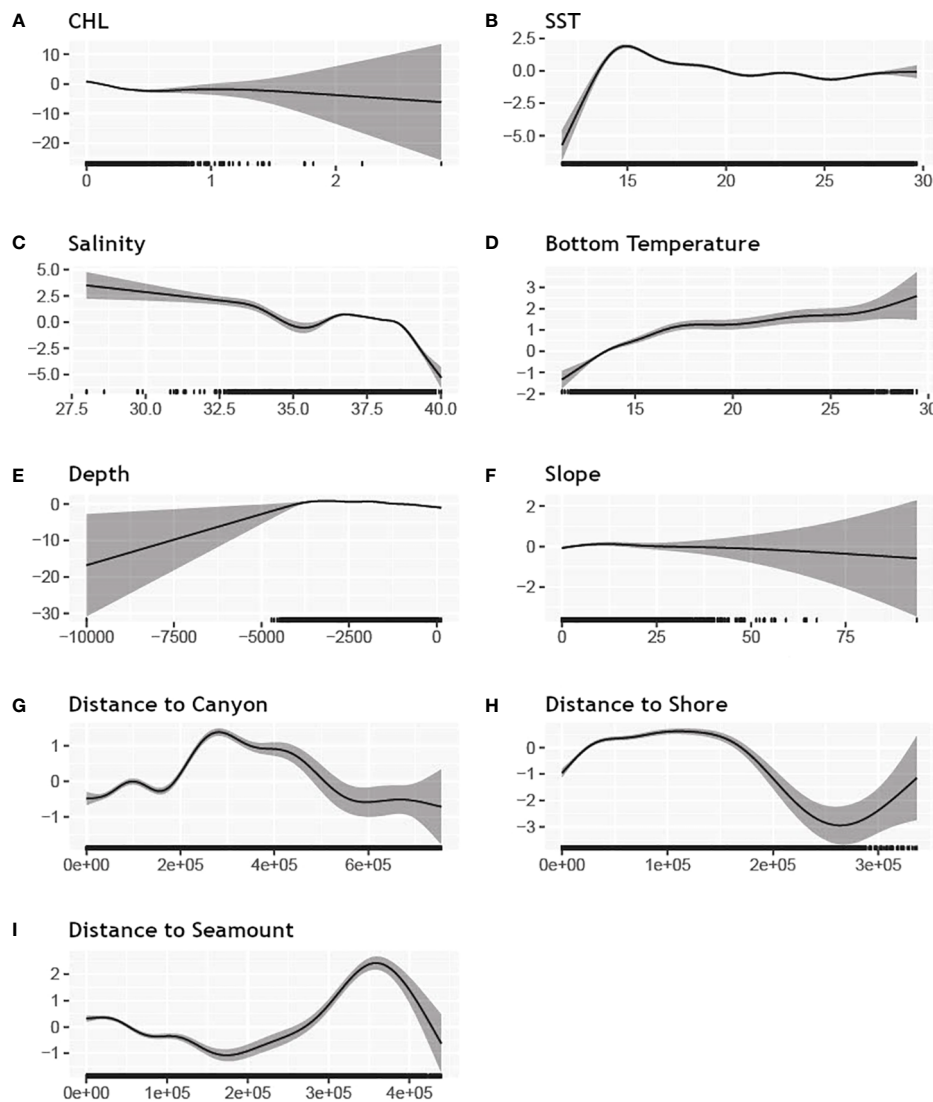


FIGURE 2

Functional plots of the relationships between environmental covariates and abundance for the long-term model. All plots are single covariate smooths with unlimited degrees of freedom. Uncertainty is shown by the gray shaded areas. Rug plots demarking sampled values are represented by ticks on the x-axis. Panels and abbreviations: (A) CHL – mean chlorophyll concentration at the ocean surface (mg/L), (B) SST – mean sea surface temperature (degrees Celsius), (C) Salinity – mean salinity (ppm), (D) BT – mean bottom temperature (degrees Celsius), (E) Depth – bottom depth (m), (F) Slope – bottom slope (degrees), (G) Distance to canyon (m), (H) Distance to shore (m), (I) Distance to sea mount (m).

4 Discussion

This study presents the first estimates of loggerhead turtle distribution and abundance across the Mediterranean Sea which are not derived from demographic modeling of nesting females, and which include loggerhead turtles of Atlantic origin. Both a long-term average estimate from 2003-2018 and a summer 2018 estimate were derived from line transect survey data to inform management at basin and regional scales. Despite sources of uncertainty in the modeling process, these models represent the best estimates of the

numbers and distribution of loggerhead sea turtles at basin-wide scales in the Mediterranean Sea.

The similarity in abundance estimates and spatial pattern between the long-term model and summer 2018 estimates may indicate that summer distribution of loggerhead turtles may be driving the patterns predicted by the long-term model. This is unsurprising given that most of the survey effort used in the long-term model occurred in warm months.

For the long-term model, seasonal models were initially attempted, as there is evidence that animals migrate seasonally from the eastern to western basins and from the eastern basin to

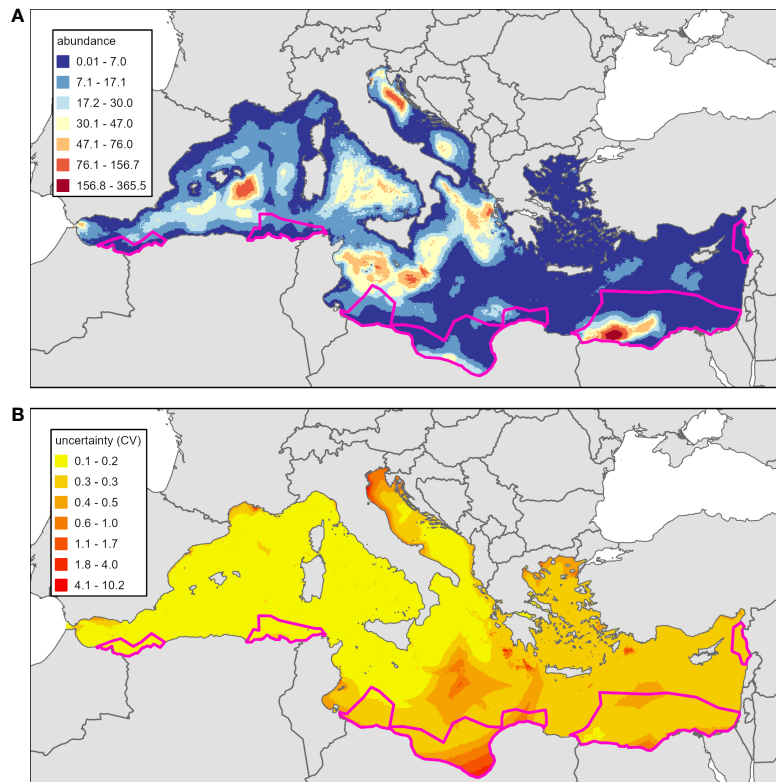


FIGURE 3

Long-term loggerhead turtle abundance predictions adjusted for availability bias (panel A) for the Mediterranean Sea and associated uncertainty, measured as coefficient of variation (panel B). The spatial scale of predictions for this figure is the number of animals in each 25km² grid cell. Areas highlighted in pink are areas of geographic extrapolation.

the Adriatic Sea (summarized in Mazor et al., 2016; Casale et al., 2018). Cool season models performed poorly and did not seem realistic, so seasonal models were not presented. This was likely driven by lower survey coverage in the cool season and less uniform spatial coverage.

Low abundance in the eastern Mediterranean was predicted by both the long term and summer models. Despite the eastern Mediterranean being the largest concentration of nesting on the region, nesting females account for a very small percentage of the total population and juveniles range widely in their oceanic stage.

While most of the spatial abundance predictions appear reasonable based on the sightings data, the hotspot off Egypt in the long-term model (area of geographic extrapolation, Figure 5) should be treated with caution given that there is no survey coverage in that area. The hotspot has the highest predicted abundance values in the model.

Rabia and Attum (2015) found evidence of loggerhead turtles stranding on the Egyptian coast, and Rabia and Attum (2020) observed sea turtles foraging in Lake Bardawil in Egypt. Additionally, the continental shelf off Egypt's Nile Delta hosts foraging sites for loggerhead turtles nesting in Cyprus and the

southern coast of the eastern Mediterranean is a migratory corridor for turtles traveling from Cyprus to the east coast of Tunisia (Snape et al., 2016; Haywood et al., 2020). Despite this evidence that turtles are present in the waters off Egypt and migrate through the area, this hotspot should be treated with caution until line transect data covering this area can be included in models.

With a few exceptions, the models predicted lower abundance in coastal and neritic areas, where large juveniles and adult turtles are known to forage, particularly in the eastern Mediterranean. These neritic foraging areas have been confirmed by multiple lines of investigation (Casale et al., 2018). Exceptions to low predictions in coastal areas were the Tunisian Plateau, the northern Adriatic Sea, and the hotspot off Egypt. It may be that not all neritic areas host the appropriate prey base for neritic turtles. More research into prey distribution would be needed to confirm this thesis.

The models can best be considered models of oceanic loggerhead turtles, which represent most loggerheads in the Mediterranean Sea (Casale and Heppell, 2016). Some adult loggerhead turtles may be found in oceanic habitats, either while migrating, or if they have never recruited to neritic areas

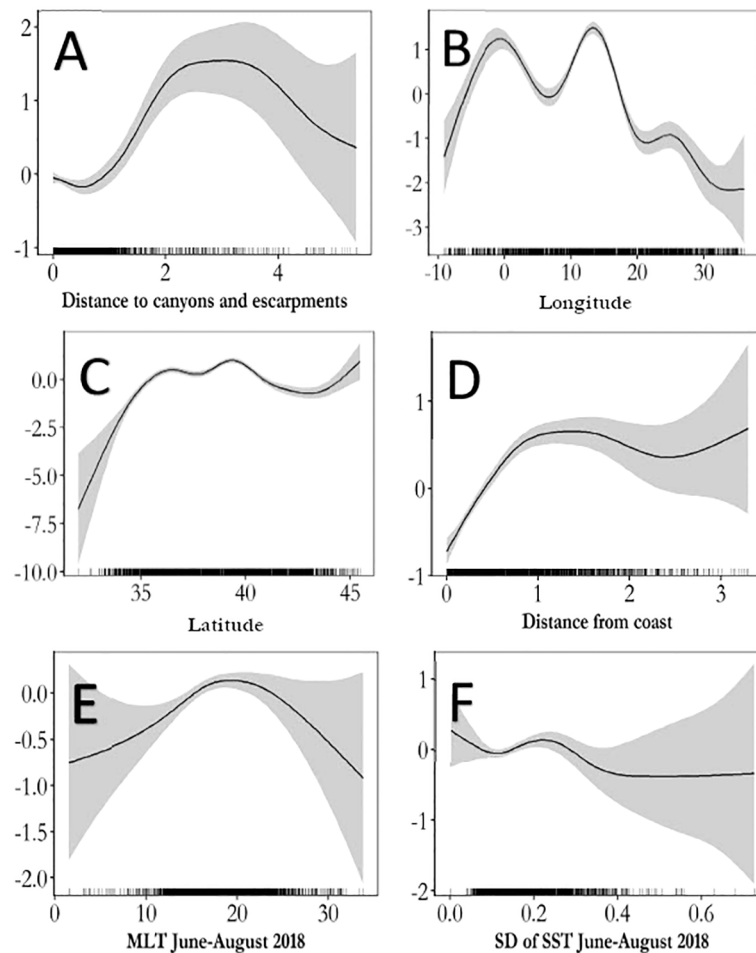


FIGURE 4

Functional plots of the relationships between environmental covariates and abundance for the summer 2018 model. All plots are single covariate smooths with unlimited degrees of freedom. Uncertainty is shown by the gray shaded areas. Rug plots demarking sampled values are represented by ticks on the x-axis. Panels and abbreviations: (A) Distance to canyons and escarpments (m), (B) smooth of longitude, (C) smooth of latitude, (D) distance from shore (m), (E) MLT – mean mixed-layer depth (m); (F) SD of SST – standard deviation of sea surface temperature (degrees Celsius).

(Luschi et al., 2018). Mediterranean loggerhead nesting females are the smallest in the world (Tiwari and Bjørndal, 2000) and small adults in other regions have been shown to sometimes remain oceanic foragers (Hawkes et al., 2006).

When considering these models for management purposes, some important neritic foraging areas for older turtles may be overlooked, specifically, large individuals of high reproductive value, which may be particularly subject to high bycatch mortality in coastal fisheries (Casale, 2011; Lucchetti et al., 2021). Coupling these models with other data sources such as satellite telemetry and stable isotope analyses will be critical for holistic management of the loggerhead turtle population in the Mediterranean Sea.

Environmental covariates were poorly sampled close to shore, and there were grid cells where predictions were not

made within 5–10 km of shore. A decision was made to not extrapolate the models into these unsampled grid cells as they were also poorly sampled by surveys. This is a source of underestimation of abundance in the model. The missing cells represent 0.05 percent of the area of the Mediterranean Sea. If estimates are needed close to shore for management purposes, extrapolating values from nearby cells is a possible solution. However, these are broad scale models intended for basin-wide and regional abundance estimation and conservation action. We caution against relying on estimates from individual cells for fine scale management.

Satellite telemetry data featured in the State of the World's Sea Turtle Report (SWOT Team, 2019) showed loggerhead turtles to be distributed almost throughout the entire Mediterranean Sea, though fewer locations were recorded in

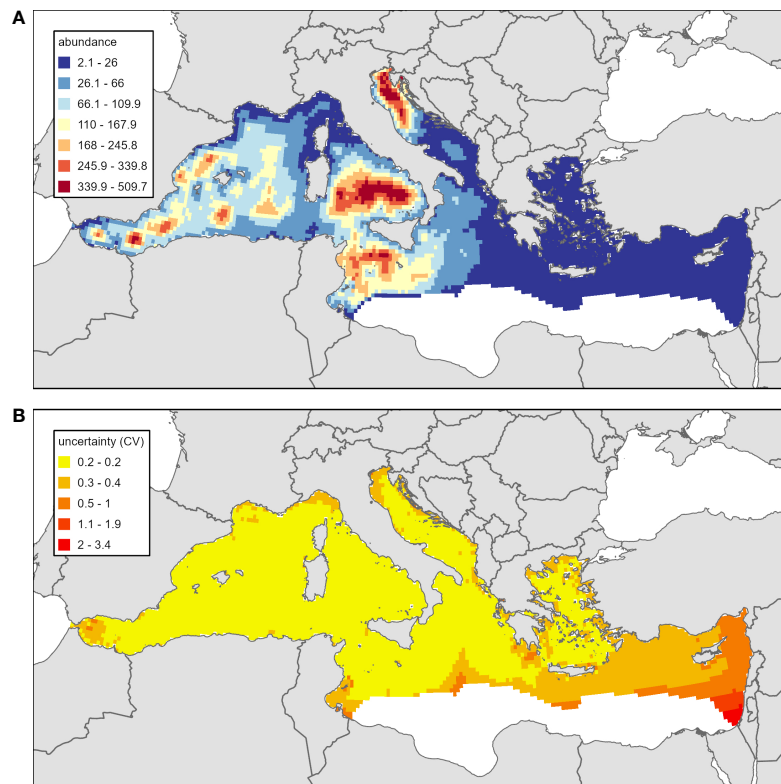


FIGURE 5

Summer 2018 loggerhead turtle abundance predictions unadjusted for availability bias (panel A) for the Mediterranean Sea and associated uncertainty, measured as coefficient of variation (panel B). The spatial scale of predictions for this figure is the number of animals in each 100km² grid cell. This differs from Figure 4 where the long-term model was predicted with 25km² grid cells. For the summer 2018 model, geographic extrapolation into unsurveyed areas of the southeastern Mediterranean Sea was not pursued.

the north-central Mediterranean and eastern Mediterranean. Higher densities of satellite telemetry locations were found in the Alboran Sea, Tunisian Plateau, Adriatic Sea, and Tyrrhenian Sea. These areas of relatively higher and lower density of satellite telemetry locations correlate well with the models' predicted distribution, though caution must be taken when comparing spatial density models to density of satellite telemetry locations. There are significant biases associated with satellite telemetry, such as deployment bias, individual behavior, and the age classes tagged. Despite these biases, the [SWOT Team \(2019\)](#) report featured hundreds of tagged animals, and the general concurrence of our model and the tag data is good.

The models presented in this study also concur well with demographic population estimates. [Casale and Heppell \(2016\)](#) presented a demographic model of the Mediterranean loggerhead turtle population based on the number of adult females, reproductive output, and assumptions about age of sexual maturity. That study represented a completely independent population estimate from the spatial density models. [Casale and Heppell \(2016\)](#) made three estimates of loggerhead turtle population, assuming age of sexual maturity at

21, 25, and 34 years old. The estimates were 1,197,087 (CI 805,658-1,732,765), 1,521,107 (CI 1,034,839-2,178,790), and 2,364,843 (CI 1,611,085-3,376,104) respectively. The population estimate from the long-term model of 1,201,845 (95% CI [838,864-1,548,280]) was statistically similar (e.g. estimates overlapped when considering uncertainty) to the 21- and 25-year scenarios but not the 34-year scenario. The summer 2018 model was only statistically similar to the 21-year scenario. This may provide support to the lower age of maturity scenarios which aligns with the small size of Mediterranean adult loggerhead females. However, it is likely the spatial density models are underestimating both abundance and uncertainty (see discussion below). The concurrence of these independence estimates lends credence to both estimates as being valid, though caution is merited in comparing the two as the demographic estimates do not include turtles of Atlantic origin and may also be underestimates of total loggerhead population.

It is important to acknowledge that both models in this study and the demographic population estimates are single point estimates of population. More research is needed to determine if changes in population abundance are occurring, such as

repeated, stratified abundance estimates covering the same area. This study, which combines multiple surveys over different time periods, geographic areas, and abundance varying with environmental covariates, is not the best study design for detecting trends. There is some evidence of colonization of the eastern basin by individuals from other nesting stocks and nesting within the Mediterranean may be increasing (Casale and Tucker, 2017; Carreras et al., 2018). Abundance estimates based on repeated stratified estimates could provide an additional line of evidence for population growth in the region.

As in most spatial density models, there are potential sources of bias in our estimates that merit discussion, even though the estimate is statistically similar to independent demographic population estimates. Possible sources of underestimation include the following: not accounting for perception bias, overestimating the amount of time animals are at the surface based on the depth at which dives were assumed to be started (3–4 m for dive data), missing cells close to shore due to missing environmental covariates, and not detecting small animals.

Aerial surveyors have indicated an ability to detect animals as small as 40 cm (Barco et al., 2018); however, animals of that size may be several years old already. Given the age structure of sea turtle populations, those missed animals represent a large fraction of the total population (Mazaris et al., 2005; Casale and Heppell, 2016).

Possible sources of overestimation of population include the following: including sightings reported as hardshell turtles that may actually be green turtles and including the dive data from Spain that may be missing some surfacing events. There were too few confirmed sightings of green turtles (less than 10) to attempt to use machine learning or other discrimination techniques to assign unidentified turtles to be either green or loggerhead turtles.

Green turtles may comprise as much as 50% of sea turtles in the Eastern Mediterranean if maximum population estimates for green turtles and minimum population estimates for loggerhead turtles are assumed (Casale and Heppell, 2016), though this extreme case seems unlikely. Mitigating this, Mediterranean green turtles recruit to near-shore neritic habitats at a curved carapace length of 27–40 cm (Türkozan et al., 2013; Bektaş, 2018) which is the close to the minimum CCL at which sea turtles are detectable by aerial survey methods (Barco et al., 2018). Since the surveys used to develop the spatial density models only partially cover near-shore habitats where larger green turtles recruit, and smaller oceanic green turtles are not likely to be detected, we expect the false positive identification of green turtles as loggerhead turtles to have low or negligible, though unavoidable, net contribution to estimates of distribution and abundance of loggerhead turtles. Green turtles can be found in the Levantine Basin, the Strait of Sicily/Tunisian Plateau/Gulf of Sirte, the Ionian Sea/Central Mediterranean, and the Aegean Sea subregions.

Overall, we posit that we are generally underestimating abundance because missing small turtles is likely the largest effect, given the size of those age classes relative to the total population. Additionally, there are more sources of possible underestimation than overestimation.

Some sources of uncertainty were not accounted for in either model, including measurement error in distances to sightings, variability in dive behavior, and uncertainty and daily variation in covariate values. The long-term model did not account for the variability from detection functions. This was not applicable to the strip transect approach used in the summer 2018 model. Because we are underestimating variability in our predictions, the reported CVs only account for a portion of the variability in the model. Actual variability may be much higher, and this should be taken into consideration when making management decisions based on these data.

These spatial density models can be used by regional managers to estimate impacts to loggerhead turtles from military training and testing exercises, offshore renewable energy projects, and fisheries interactions at broad spatial scales, with appropriate understanding of the limitations of these models. They can also serve as an important input into regional marine spatial planning efforts that require spatially explicit estimates of distribution and abundance. The results also support efforts to understand the distribution of the combined populations of Mediterranean and Atlantic loggerhead turtles in the Mediterranean Sea.

4.1 Future directions

While the models presented here represent an excellent first attempt at a basin-wide spatial density model for the Mediterranean Sea, there are improvements that can be made.

There are additional survey data that could be added to the survey dataset, mostly from the University of Valencia as well as from new winter shipboard surveys in the eastern Mediterranean associated with the ASI that was not available when this study was undertaken. The eastern Mediterranean is data poor already, and cool season surveys are a critical data gap. Inclusion of the new ASI survey and any future cool season surveys may allow for seasonal models to be fit. Drone surveys, though often at smaller spatial scales than the broad scales surveys used here, may also prove useful in surveying neritic foraging areas and can be used in a spatial density modeling framework the same way other strip survey transects can.

To address the issue of missing neritic foraging areas for larger turtles, two possible solutions are: 1) fit separate models to neritic and oceanic areas, or 2) fit a single model to all the data but use a hierarchical GAM framework with habitat included as a factor (Pedersen et al., 2019). Additional research into adult turtle behavior and distribution, particularly in the western basin, could help refine this future work. The same

hierarchical approach could be applied to the various sub regions of the Mediterranean to see if environmental relationships are similar across the regions.

While the stratified availability bias estimates used here better reflect changing dive behavior over time and space than a single estimate, a more complex treatment may be possible given the amount of available data. Modeling availability spatially in response to environmental covariates would allow for smooth relationships over time and space, unlike the current stratified estimates that have distinct boundaries spatially and temporally. Lastly, including more sources of uncertainty in the overall estimate of CV (such as dive variability) would give users of the models a better understanding of the limitations of the models. Recent work has shown this is possible but was outside the scope of this project (Bravington et al., 2021).

Data availability statement

Some of the data used in this study are currently unpublished and are not available publicly. Persons interested in acquiring the data should contact the survey organizations directly. The long-term abundance model and associated uncertainty are available at <https://seamap.env.duke.edu/models/NUWC/Med/>.

Ethics statement

This study used observations of animals previously conducted under other research efforts.

Author contributions

AD and AC were responsible for the concept of the paper; AD, AC, JR, and LS were responsible for data processing and analysis; AD, AC, and LS, were responsible for writing; SP, AM, CF, DH, GL, HP, JAR, OB, JT, and VR provided the line transect survey data used; AB, BG, DM, JH, RSa, RSn, and SH provided the dive data used for the availability bias

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analysis as well as information on sea turtle ecology in the Mediterranean.

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Conflict of interest

Author AD is employed by McLaughlin Research Corporation and CheloniData LLC.

The remaining 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.2022.930412/full#supplementary-material>

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Assessment of the bycatch level for the Black Sea harbour porpoise in the light of new data on population abundance

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Incidental catch in fishing gear (often known as bycatch) is a major mortality factor for the Black Sea harbour porpoise (*Phocoena phocoena relicta*), an endemic subspecies listed as Endangered in the IUCN Red List. The primary gear, responsible for porpoise bycatch in the Black Sea are bottom gillnets and trammel nets targeting turbot (*Scophthalmus* spp.), the most valuable commercial fish species in the Black Sea. From 2019 to 2021, a study was conducted in Bulgaria, Romania, Türkiye and Ukraine, to estimate the bycatch level in light of new information on porpoise distribution and abundance obtained from aerial surveys (CeNoBS) undertaken in 2019 as part of ACCOBAMS Survey Initiative (ASI). Bycatch data were collected by independent observers onboard turbot fishing boats (Bulgaria and Romania), complemented by questionnaire surveys and examination of stranded carcasses (in all countries). Some 48 monitoring trips took place (63 hauls by 11 different vessels). Cetaceans were caught on just over half of the trips (55%): 182 harbour porpoises, 4 bottlenose dolphins and 3 common dolphins. The median number of porpoises bycaught per trip was 1 (maximum 41) and the number of porpoises per km of net varied between 0 and 3.66 (median 0.1). Bycatch rates showed seasonal variation with marked increase in summer, compared to spring. The total annual bycatch of harbour porpoises in the Black Sea was roughly estimated as between 11 826 and 16 200 individuals. These numbers were the product of median values for effort (days/trips and vessels) and bycatch rate. Given the new estimates of porpoise abundance based on the CeNoBS survey of 2019 and reconciling abundance and bycatch estimates, harbour porpoise bycatch in the Black Sea represents between 4.6% - 17.2% of the estimated total population, depending on assumptions used. Even the most conservative estimate is among the highest worldwide and far exceeds the probable sustainable levels of around

1.0-1.7%. This study confirms that bycatch poses the most serious threat to the Black Sea harbour porpoises and that all riparian countries engaged in turbot fisheries must implement urgent measures to reduce it immediately, if the population is to survive in the long-term.

KEYWORDS

Black Sea, harbour porpoise, bycatch, gillnets, on-board observation, *Phocoena phocoena*

1 Introduction

Three species of cetaceans are found in the Black Sea that are designated as endemic subspecies: the Black Sea harbour porpoise (*Phocoena phocoena relicta* Abel, 1905); the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus* Barabash-Nikiforov, 1940); and the Black Sea common dolphin (*Delphinus delphis ponticus* Barabash, 1935) (Figures 1–3). All of them are considered as vulnerable or endangered due to several historical and current adverse factors affecting their populations (Birkun et al., 2014). Among these factors, the primary one was the commercial hunting of cetaceans in the Black Sea which was highly intensive between 1929 and 1966 when a ban was adopted by the USSR, Bulgaria and Romania. It continued in Turkish waters until 1983 (Kleinenberg, 1956; Tonay and Öztürk, 2012). Additionally, a genetic study indicated a strong reduction in the population size of Black Sea harbour porpoise (approximately 90%) in the second half of the 20th century, possibly due to massive dolphin fisheries and bycatch (Fontaine et al., 2012).

Nowadays, Black Sea cetaceans are protected in the riparian countries at both national and international levels. They are listed in the IUCN Red List, national Red Data Books, and (in EU waters) annexes II and IV of EU Habitats Directive 92/43/EEC and in Descriptor 1 (D1, Biodiversity) of EU Marine Strategy Framework Directive (MSFD) 2008/56/EC.

Incidental catch in fishing gear (henceforth referred to as bycatch) is a major threat for populations of small cetaceans in

European seas, and the greatest source of non-natural mortality for many (Dolman et al., 2016; Amaha Öztürk, 2021). In Europe, cetacean bycatch is considered under the above-mentioned EU Directives, the EU Common Fisheries Policy (CFP) and the two regional CMS (Convention for Migratory Species) agreements on the conservation of cetaceans: ASCOBANS (Agreement on the Conservation of Small Cetaceans of the Baltic, North East Atlantic, Irish and North Seas) and ACCOBAMS (Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area) - the latter one covering the Black Sea. Bycatch mortality is specifically identified as criterion D1C1 assessed in the current cycle of EU MSFD implementation for 'good environmental status' (GES) for cetaceans.

A quantitative understanding of the effect of bycatch on affected populations is key to being able to undertake (and later evaluate) effective bycatch mitigation measures (e.g. Moore et al., 2021; Wade et al., 2021). Determining an 'acceptable' removal rate (taking into account inevitable uncertainty) for a cetacean population is not easy and several suggestions have been put forward. For example, ASCOBANS agreed to a 'limit' of 1.7% of the harbour porpoise abundance as appropriate for the GES (Moffat et al., 2011; ASCOBANS, 2015). Other approaches are used elsewhere such as the estimated potential biological removal (PBR) used in the USA – this has been parameterised to be equal to 1% of the minimum



FIGURE 1
Bycaught Black Sea harbour porpoise.



FIGURE 2
Bycaught Black Sea bottlenose dolphin.



FIGURE 3
Bycaught Black Sea common dolphin.

abundance estimate for cetaceans (Wade, 1998). More recently, Manlik et al. (2022) proposed an approach they called sustainable anthropogenic mortality in stochastic environments (SAMSE): this method gives an estimate of sustainable bycatch level for bottlenose dolphins to be not more than 0.5% of abundance estimate.

Historically, several kinds of fisheries have been identified as primary sources of cetacean bycatch in the Black Sea including:

- turbot (*Scophthalmus* spp.) gillnet/trammel fishery (Vasiliu and Dima, 1990; Pavlov et al., 1996; BLASDOL, 1999; Anton et al., 2010; Radu and Anton, 2014; Tonay, 2016; Bilgin et al., 2018);
- sturgeon (Acipenseridae) trammel net fishery (Pavlov et al., 1996; Vishnyakova and Go'din, 2015a);
- dogfish (*Squalus acanthias*) gillnet fishery (Birkun et al., 2009);
- pound net fishery (Vasiliu and Dima, 1990);
- purse seining (Birkun et al., 2014); and
- pelagic trawling (Özdemir and Erdem, 2011; Birkun et al., 2014).

By far the largest bycatch (90 to 98% by number) reported by the above studies was that of the Black Sea harbour porpoise. Most cetacean bycatch was due to illegal, unreported and unregulated (IUU) fishing operations - this makes estimation of deaths difficult to obtain from the preferred method - direct observations. A previous estimation of cetacean bycatch numbers in the Black Sea (Birkun et al., 2014), based on historical data and extrapolation, suggested an annual catch of at least 20 000 animals (of which over 11 000 were in gillnets for turbot) - probably greatly exceeding any sustainable level. Tonay (2016), based on onboard observations of part of the fleet, estimated the annual bycatch of harbour porpoises to be around 2 000 animals (CV=0.37) in the Turkish western Black Sea which is the most precise sub-regional bycatch estimate available. Vishnyakova (2017) undertook a demographic study of the harbour porpoise population in the Azov Sea. It showed that bycatch was the main mortality factor for the Azov population, which declined by 60% over 13 years (2000-13). Clearly, this could be applicable to some other parts of the Black Sea.

The present study focused on developing and applying a standard approach to bycatch monitoring in the Black Sea with a view to filling the existing gaps on distribution, levels and effects of bycatch pressure on cetaceans in the region. The research was undertaken across several Black Sea riparian countries and the objective was to identify and evaluate patterns of cetacean interactions with fisheries. The methodological framework was developed for collecting field data and bycatch assessment, and the field surveys were conducted in Bulgaria, Romania, Türkiye and Ukraine between 2019 and 2021. The estimated bycatch level of the Black Sea harbour porpoise as the most affected species was compared to the most up-to-date abundance estimate derived from the summer 2019 aerial survey of the basin (Paiu et al., 2021a), which is the most comprehensive until now.

2 Material and methods

The study included information collected by questionnaire surveys and data from onboard observers, supplemented by data from cetacean stranding records and fishing effort (fleet size, annex 1). All these data were used to estimate total bycatch that was compared to total abundance estimated by the 2019 aerial survey (Paiu et al., 2021a) This approach using several sources of information is broadly following the recommendations of Wade et al. (2021). Each step is described below.

2.1 Questionnaire development and application

The questionnaire developed was based upon a review of similar exercises throughout the world and the experiences of the authors (Zappes et al., 2018; Filgueira dos Santos et al., 2021). Special attention was devoted to the aspects of fishing operations which are often concealed and missing in reports. Since cetaceans are legally protected in the Black Sea countries, fishermen have a tendency to deny or under-report bycatch, even if it occurs during legal fisheries operations. Therefore, the questionnaire was designed with indirect indicators to understand the bycatch potential of certain fishing practices, net types and operations. The final questionnaire was largely based upon that developed for the coast of the northwestern Spain (Goetz et al., 2014) supplemented with questions used in published studies from the Black, Mediterranean, Caspian Seas and the Persian Gulf (Dmitrieva et al., 2013; Jabado et al., 2015; FAO, 2019). Taking into account the completeness and comprehensiveness of the questionnaire developed by Goetz et al. (2014), its structure and principles of the interview were used in this survey. These included close-ended questions prevalence together with open-ended, 'don't know' answer options, understandability and anonymity. The list of variables used here followed Goetz et al. (2014) and Table 1 therein. Included questions were on fleet segment (vessel and gear types), number of vessels, frequency and duration of operations, net types, target and main discard species, cetacean behaviour near the fishing operations, personal attitude of fishermen to cetaceans, depredation

by cetaceans, all kinds of bycatch (including fish and birds), survival of bycaught animals, intensity and dynamics of interactions with fisheries. Voluntary interviews covering the broadest scale of the fisheries types, company sizes and port sizes across the area were conducted using local languages, in an informal environment. No personal data were collected during the interviews. Analysis of interviews followed qualitative approach such as that used by Carruthers and Neiss (2011) and Mustika et al. (2021). Mann-Whitney U tests were used for detecting statistically significant differences between samples and sub-samples when necessary.

2.2 Onboard data collection on cetacean bycatch

Onboard observers monitored catch and bycatch during regular fishing operations of gillnet fisheries targeting turbot species (*Scophthalmus maeoticus*, *S. maximus*, *S. rhombus*), the most valuable commercial fish in the Black Sea. In Bulgaria approximately 3% of turbot fishing fleet was monitored on basis of willingness of shipmasters to accept observers, and in Romania 2.4% of the active vessels able to fish with stationary nets or bottom trawling were covered by the study. Standard protocols provided by the General Fisheries Commission for the Mediterranean (GFCM) were adopted for collecting standardized information on fishing operations and bycatch of cetaceans by onboard observers (FAO, 2019). These included general data on the vessel, data on fishing operations for each vessel; general information on fishing trip (number of hauls, location, duration, catch data) and general information on bycatch of vulnerable species and existence of marine litter for each onboard observation; biological data on bycaught marine mammals. For several small-size boats that could not accommodate independent observers, data were collected by fishermen.

During the onboard data collection, 27 (43%) of the observed 63 hauls included strings fitted with acoustic deterrent devices (ADDs or pingers) as a mitigation measure: therefore, use of pingers hypothetically might have caused reduction of bycatch level. Pinger trials included two types of configurations. In 2019 mixed sets of nets were used combining active and control parts. In 2020 and 2021 trials were using pair of sets - active and control - that were situated at minimum distance of 500 m.

2.3 Cetacean stranding surveys

Strandings can be used as supplementary source of data on the cause of death, as well as demographic data, for subsequent population and health analyses (Vishnyakova and Gol'din, 2015b). During the current study, cetacean stranding data were collected as supplementary evidence for the occurrence of bycaught cetaceans ashore during the seasons of observations. Surveys were conducted by some of the authors and information from existing databases was used (Bulgaria: Popov and Meshkova, 2022; Ukraine:

Vishnyakova et al., 2021; Romania: Paiu et al., 2022; Türkiye: Paiu et al., 2021b and İÜ-TUDAV, unpubl. data).

2.3.1 Bulgaria and Ukraine

Overall data on strandings were collected by field surveys and opportunistically through citizens' reports verified by photographs. In addition, in Ukraine, specific monitoring routes, 4 km each, located near the fisheries facilities (Kurortne, Sasyk, Shagany, Lebedivka-Burnas, Chornomorsk, Odesa, Tendra, Zalizny Port, Dzharylhach), were checked for presence of cetaceans and signs of bycatch.

2.3.2 Romania

The area between Corbu and Vama Veche was under surveillance both in 2019 and 2020. The surveys were done mainly on foot and when possible, by all-terrain vehicle. Surveys were conducted by Mare Nostrum NGO-coordinated National Monitoring Network that includes volunteers and partner institutions (Dobrogea Littoral Water Basin Administration, National Agency for Fishery and Aquaculture, Dobrogea Inspectorate for Emergency Situations, Police and Coast Guard).

2.3.3 Türkiye

Data was collected through local stranding surveys, citizens' and media reports by İÜ-TUDAV Cetacean Stranding Network.

2.4 Fishing effort assessment

The most robust measure used for fisheries assessment in the Black Sea is the fleet size (the number of vessels licensed for turbot fishery) where fishing effort is quantified as the number of trips (Gómez-Munöz, 1990; McCluskey and Lewison, 2008). These data are the most consistent and the best quantified across a region with diverse practices and regulations and frequent IUU fishing (Gómez-Munöz, 1990). Each trip is equal to a fishing day and may involve one or more hauls depending on length of strings of nets that were set. It is specific for the Black Sea turbot fishery that usual soaking time of nets is longer – from 7 to 20 days. That is taken into account by the unit of effort described in part 3.2. The fishing fleet structure was analysed using GFCM reports (FAO, 2020; STECF, 2020). Additionally, the national assessments of the fleet in Bulgaria, Romania, Türkiye and Ukraine were obtained from the competent authorities upon requests or from open access sources. In view of the comparability of the estimates obtained, the GFCM reports were used as the main data source for the fleet structure assessment for Bulgaria, Romania and Ukraine. For Türkiye, the national assessment was used as the best primary source due to its more complete and detailed analysis of fleet (TUIK, 2019). Also, in Ukraine, the numbers of vessels involved in turbot fishery was assessed on site during the questionnaire survey, since the data on the number of currently operating small vessels were not included into official statistics.

2.5 Total bycatch assessment

Total bycatch for Bulgaria, Romania, Türkiye and Ukraine, which comprised the major part of observable fishing operations in the Black Sea was estimated only for the harbour porpoise. The two other cetacean species were not assessed due to the low numbers of observed bycatch which precluded the development of robust estimates. Given the inevitable uncertainty of data on fishing effort and bycatch reporting, the assessment was based on the assumptions listed below.

1. Bycatch occurs exclusively in gillnets: although different type of fishing gear, including static nets set or pelagic trawls can cause bycatch of harbour porpoises, their impact seems to be minor in comparison with the gillnets (Radu and Anton, 2014). In the past, the use of three-walled trammel nets in turbot fishery was reported (Radu et al., 2003; Samsun and Kalaycı, 2004; Tonay, 2016), but since the use of these nets is prohibited in turbot fishery, they are considered as limited to some IUU operations (Gol'din, personal data) and were not considered here.
2. All the gillnets of the mesh size 160 to 200 mm have equal potential for bycatch: historically it was suggested that nets of 120-140 mm mesh size were especially dangerous for cetaceans (Birkun et al., 2009). However, at the time of this study they are not used in the sea.
3. Most bycatch are not reported by fishermen, regardless of the fishing being legal or IUU, due to the protected status of cetaceans under national legislation; uncertainty in legal definitions of incidental catch; the absence of a code of conduct for incidental catch situations; and fear of prosecution.
4. No other vessels than members of the fishing fleet are involved in IUU operations: the well-developed legal and regulatory framework in all the countries of this study leaves little room for unregistered fishing vessels. However, the effort and scope of IUU operations, especially turbot fishing during the annual closed season, is large but difficult to estimate (Shlyakhov, 2013). Even if only a few vessels are really involved in IUU fishing, their IUU effort during the closed season of prohibition is believed to be high enough that makes it comparable to that of the legal operations.
5. Bycatch is independent of local differences in effort: large scale IUU fishing is practiced in all the countries of study, and it includes considerable effort in shelf waters of exclusive economic zones. An inevitable consequence of this practice is extensive hauling and the loss of many 'ghost' nets at sea. It is estimated that over 1 500 gillnets and entangling nets are lost annually in Turkish Black Sea alone (Dagtekin et al., 2019), the loss of turbot nets in the Istanbul region was estimated to be around 70 km in 2008-2009 (Yıldız and Karakulak, 2016). Clearly, ghost nets in remote open sea areas continue to catch fish and cetaceans.
6. There are no seasonal differences in effort as proscribed closed seasons are fully utilised by IUU operations.

Although there are legal closed seasons (between 30 and 60 days, depending on the country) for turbot in all the riparian Black Sea countries during spawning, this season is the most commercially profitable (turbot form the largest aggregations during this period) and the IUU effort is at least as intensive as the legal effort during other seasons.

7. There is a season of porpoise bycatch largely limited to four months, from April to July. This assumption is based on considerable published evidence of bycatch seasonality (Vishnyakova and Gol'din, 2015a; Paiu et al., 2017; and references therein), which was additionally confirmed by the results of this study. Importantly, this season coincides with the reproductive season of the Black Sea harbour porpoise peaking from May to July and, consequently, can be explained by aspects of its life history (Vishnyakova and Gol'din, 2015a).
8. Bycatch is linearly proportional to seasonal porpoise abundance (density): this assumption is based on assumptions 2 and 5-7. If the gillnet fishing effort is high during all the season of high bycatch rate, and the bycatch coincides with biologically important season for the Black Sea harbour porpoise (summer), the bycatch rate can be presented as a function of porpoise density.
9. Observed bycatch could be possibly lower than usual due to tested pingers as mitigation measure on 27 of 63 hauled strings of nets. Despite significant reduction of bycatch levels was observed only for 6 hauls (10% of all) that involved PAL pingers there is underlying possibility that use of pingers generally may have introduced negative bias in normal bycatch levels.

Mean values and variance were evaluated as part of the general statistics considered (Northridge and Fortuna, 2008). However, median values were considered preferable to arithmetic means as they better correspond to the non-parametric nature of bycatch events and uncertainty of the underlying distribution. Median values show less bias due to outliers – cases of extremely high bycatch events shifting the mean values. Resultant estimates of bycatch based upon the use of median values might thus be considered 'conservative'. Interquartile ranges were used for estimation of confidence intervals.

Given the data limitations and uncertainties that preclude a more sophisticated analysis, bycatch numbers are estimated solely as a function of number of vessels, bycatch per trip and number of trips during the bycatch season:

$$N_{byc} = f(\text{number of vessels; bycatch per trip; number of trips})$$

$$N_{byc} = N_v B_t N_t$$

where N_{byc} is total bycatch level per year; N_v is number of vessels; B_t is bycatch per trip and N_t is annual number of trips per vessel.

The basis for the calculations came from the onboard bycatch study in Bulgaria and Romania conducted from 2019 to 2021, supported by questionnaire surveys in four riparian countries.

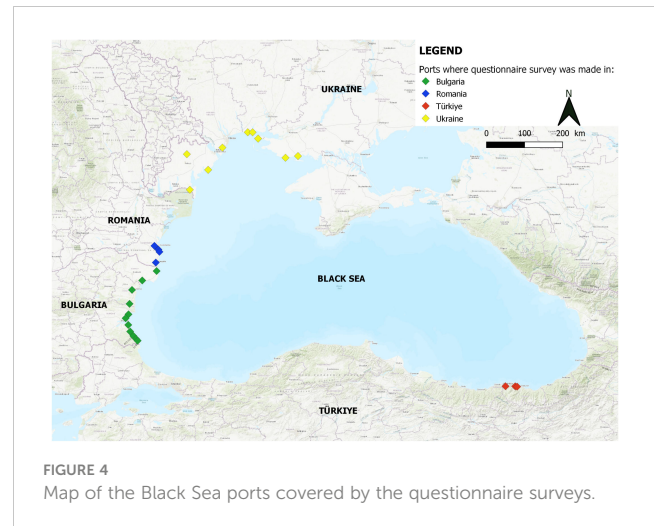
Limitation to this time and sub-region concurred with the aerial survey in summer 2019 (Paiu et al., 2021a).

3 Results

3.1 Questionnaire survey

In total, 63 interviews were conducted, 23 in Bulgaria, 15 in Romania, 8 in Türkiye and 17 in Ukraine, covering the main segments of the fleet (Table 1 and Figure 4). The individual respondents reported data from 1 to 20 boats each. No significant differences were found between countries in the statistical characteristics of samples and numerical results of the survey (Mann-Whitney U test, $p < 0.05$). The interviewees were aged between 28 and 74 years (median 47 years) and had 2-60 years of experience (median 25 years) indicating a high level of expertise and good institutional memory. Besides, 37% originated from families traditionally involved in fishing. Most of the interviewed fishermen (81%) were flexibly involved in multi-target fisheries (target species distribution is shown at the Figure 5), and 17% switched between fisheries practices within a year due to catch seasonality. Almost half (48% of all the responses) of the fishing gears reported as being in use were gillnets (Figure 6); these included nets used among multiple gears. The soaking time for gillnets involved in turbot fisheries varied from 1 to 91 days (median 12 days). In addition, the number of small vessels currently used in the north-western Black Sea sector of Ukraine for turbot fishery was specially assessed on site and estimated as 180 vessels.

In 50 of 63 interviews (79%) bycatch was reported, and 30 (48%) of respondents reported cases of cetacean bycatch: 25 of them (40% of the total sample) mentioned the harbour porpoise as the bycaught species; eight respondents (13%) mentioned the cases of bottlenose dolphins and four (6%) reported common dolphins; seven respondents mentioned bycatch of more than one cetacean species. Cetacean bycatch was reported for gillnets (24 respondents, 80% of those who reported cetacean bycatch), other stationary nets (3 respondents), purse seine nets (2 respondents) and mid-depth trawls (1 respondent). In Türkiye, it was reported that cetacean survival rate in trammel nets was higher than in the other types of stationary nets or gillnets as animals could be released alive.



Several respondents who did not report cetacean bycatch in turbot or bluefish fisheries mentioned the bycatch of species that are usually bycaught together with cetaceans, indirectly indicating possible cetacean bycatch: the great cormorant (*Phalacrocorax carbo*), whiting (*Merlangius merlangus*), sturgeons (Acipenseridae), dogfish (*Squalus acanthias*) and rays (*Raja clavata*, *Dasyatis pastinaca*). Therefore, it can be assumed that a considerable part of respondents concealed cetacean bycatch cases. In particular, in Ukraine none of the respondents reported cetacean bycatch during the current fishing season, while in Romania no cetacean bycatch was reported at all. This situation was mirrored in official records by fisheries authorities where cetacean bycatch records were missing. However, many of the respondents reported bycatch as 'historical' (at least, one or two years before the interview). Overall attitude of interviewed fishermen towards cetaceans was mostly positive or neutral. None of the respondents reported cases of intentional killing of cetaceans.

3.2 Onboard observations

Bycatch monitoring aboard fishing vessels licensed for turbot fishing was undertaken in Bulgaria from 2019 to 2021 and in Romania in 2020 (Figure 7). In total, 48 monitoring missions were made that covered 63 hauls by 11 different vessels (eight for Bulgaria

TABLE 1 Fishermen interviews by country and fishing port.

Country	Ports	Vessel type/fishery	No. interviews
Bulgaria	Balchik, Varna, Byala, Nessebar, Pomorie, Sozopol, Primorsko, Tsarevo, Ahtopol, Krapets, Sinemorets	fishing vessels 6-20 m	23
Romania	Constanta, Mangalia, Agigea, Eforie	beam trawler and small boats (4.5-10 m long)	15
Türkiye	Çarşıbaşı, Akçaabat, Faroz (Trabzon Prefecture)	purse seiners and small boats (5-10 m long)	8
Ukraine	Vylkove, Lebedivka, Bilhorod-Dnistrovskiyi, Chornomorsk, Rybakivka, Ochakiv, Pokrovka, Lazurne, Skadovsk	mid-depth trawlers and small boats (4-10 m long)	17

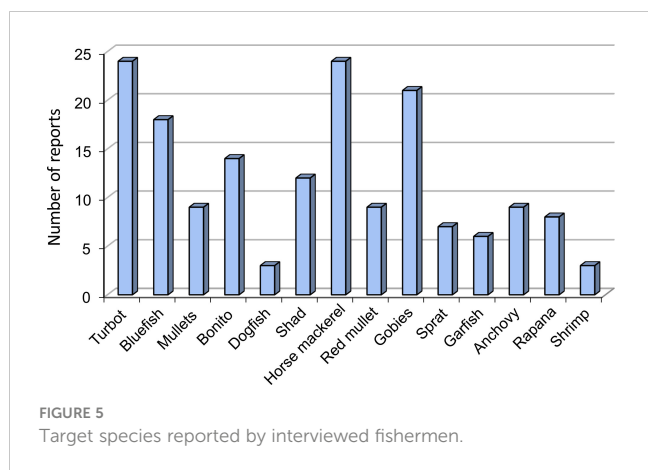


FIGURE 5 Target species reported by interviewed fishermen.

and three for Romania) during two main turbot fishing campaigns: spring and summer (Table 2). For seven of the missions (12 hauls), data were reported by fishermen who agreed to report (i.e., no observers were present). Bycatch was reported for some of these missions without observers, suggesting no bycatch was hidden by fishermen that agreed to report. Consequently, potential bias is minimal and relates more to species identification and biological data rather to bycatch level. Observations in Bulgaria were concentrated between April and July with just few hauls in October and November in line with turbot fishing effort. In Romania observations spanned between March and July. No significant differences were found between the countries and years of study (Mann-Whitney U test, $p > 0.05$). The gillnet strings observed during the hauls were between 840 and 11 760 m long (median 4 300 m) with soaking times from 7-31 days (median 16 days). In two exceptional cases, soaking time was extremely long (up to 91 days) due to bad weather.

In 36 (57.1%) of 63 hauls there were records of bycaught cetaceans. Harbour porpoises (in total, 182 individuals) were recorded in 32 hauls. In addition, bottlenose dolphins (total four individuals) were recorded in four hauls, and common dolphins (total three individuals) in three hauls. The median number of porpoises bycaught per haul was 1, the mean number was 2.89, and the maximum number was 41. The number of bycaught porpoises per km of net varied between 0 and 3.66 (median 0.1).

Standardized bycatch per unit of effort (BPUE) was calculated using the following formula:

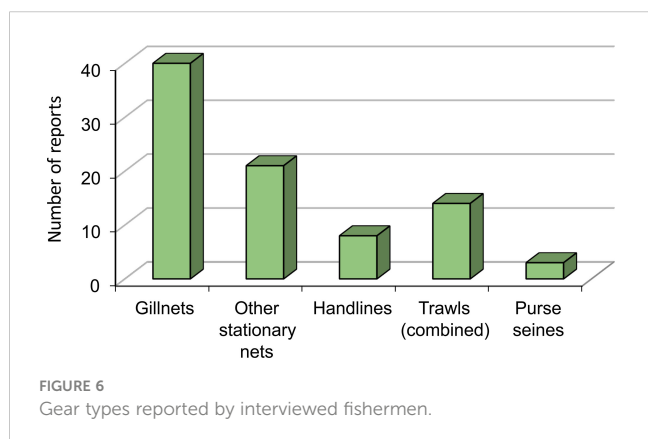


FIGURE 6 Gear types reported by interviewed fishermen.

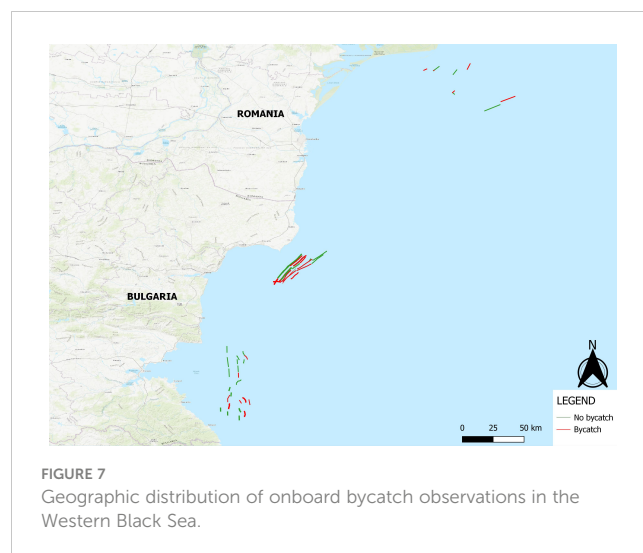


FIGURE 7 Geographic distribution of onboard bycatch observations in the Western Black Sea.

$$BPUE = \frac{\text{individuals}}{\text{day} \cdot \text{km}^2}$$

Soaking time is measured in days (1 day = 24 hours) while surface of nets was calculated in km^2 as product of length and height. In this way different height of used nets (that varied between 2 and 4 m) was taken into account.

Besides, an average bycatch rate as individuals per km of nets (ind./km) was 0.37 ind./km (SD = 0.67)

Bycatch showed strong seasonality with the high risk during the four months April to July. Most of the cases (70%) exceeding the median number of bycaught porpoises per haul were recorded in summer, between June 27 and July 29, whereas the proportion of hauls exceeding median number per km and BPUE were respectively 45% in spring and 55% in summer. BPUE was significantly higher in summer compared to spring (Mann-Whitney U test: $U=316$, $p < 0.05$). No significant difference in BPUE between years was found (Kruskal-Wallis test: $H=1541$, $p > 0.05$). The total annual bycatch per vessel varied between 0 and 95 porpoises.

Importantly, the general statistical characteristics of the cetacean bycatch, such as high variance (54.4), skewness (4.1) and kurtosis (17.7) were comparable to that of an average target fish species rather than of marine mammal bycatch usually observed in fisheries (Curtis and Carretta, 2020).

Another feature was that cetacean bycatch did not correlate to fish catch. However, both fish catch (CPUE) and cetacean bycatch (BPUE) positively correlated to net length at statistically significant levels (r was respectively 0.50 ($p < 0.05$) and 0.32 ($p < 0.001$)) and reached a maximum at 10 000 – 11 500 m length of the strings.

3.3 Stranding surveys

3.3.1 Bulgaria

Collected data on stranded cetaceans in Bulgaria for 2019 revealed 58 stranded cetaceans while at the same time onboard

TABLE 2 Results from bycatch onboard monitoring.

Boat	Country	Date	Bycatch (ind.)	Bycatch (ind./km)	BPUE	Soak time, days	Length, m	Depth, m	Type of sampling	Species		
										Dd	Pp	Tt
1	Bulgaria	8.4.2019	0	0.00	0.00	23	3 640	71	observer		0	
1	Bulgaria	12.4.2019	1	0.13	1.70	26	7 560	71	observer		1	
1	Bulgaria	10.4.2019	2	0.17	2.27	25	11 760	71	observer		2	
1	Bulgaria	11.4.2019	0	0.00	0.00	25	10 920	65	observer		0	
2	Bulgaria	10.4.2019	1	0.22	4.75	18	4 500	70	observer		1	
3	Bulgaria	12.4.2019	1	0.24	4.28	19	4 100	88	self-report		1	
3	Bulgaria	13.4.2019	1	0.23	0.00	20	4 300	88	observer		0	1
4	Bulgaria	13.4.2019	0	0.00	0.00	7	3 500	80	observer		0	
1	Bulgaria	27.6.2019	1	1.19	56.69	7	840	65	observer		1	
1	Bulgaria	1.7.2019	14	1.31	43.57	10	10 710	65	observer		14	
1	Bulgaria	2.7.2019	36	3.21	97.40	11	11 200	65	observer		36	
1	Bulgaria	6.7.2019	41	3.66	76.26	16	11 200	73	observer		41	
2	Bulgaria	6.7.2019	2	0.44	10.68	16	4 500	67	observer		2	
3	Bulgaria	6.7.2019	5	0.96	16.03	20	5 200	65	observer		5	
5	Bulgaria	8.7.2019	0	0.00	0.00	19	2 000	75	self-report		0	
6	Bulgaria	21.10.2019	0	0.00	0.00	77	2 000	60	self-report		0	
6	Bulgaria	4.11.2019	0	0.00	0.00	91	4 000	80	self-report		0	
9	Romania	5.3.2020	0	0.00	2.83	22	3 000	50	observer		0	
10	Romania	20.3.2020	1	0.25	0.00	14	4 000	70	observer		1	
10	Romania	20.3.2020	0	0.00	2.64	14	4 000	71	observer		0	
11	Romania	4.4.2020	0	0.00	2.42	21	1 500	45	observer		0	
11	Romania	10.4.2020	1	0.17	0.00	21	6 000	35	observer		1	
11	Romania	10.4.2020	1	1.25	0.00	29	800	45	observer		1	
1	Bulgaria	10.4.2020	2	0.36	0.00	21	5 600	74	observer	1	1	
1	Bulgaria	10.4.2020	0	0.00	0.00	21	5 600	74	observer		0	
1	Bulgaria	12.4.2020	2	0.17	0.00	22	11 480	65	observer		2	
1	Bulgaria	13.4.2020	2	0.17	0.00	24	11 480	76	observer		2	
2	Bulgaria	12.4.2020	0	0.00	0.00	14	8 800	65	observer		0	
2	Bulgaria	12.4.2020	1	0.13	0.00	14	8 000	83	observer	1	0	
7	Bulgaria	13.4.2020	0	0.00	4.68	17	4 200	65	self-report		0	
7	Bulgaria	13.4.2020	0	0.00	0.00	17	6 100	65	self-report		0	
7	Bulgaria	13.4.2020	0	0.00	14.88	17	6 000	65	self-report		0	
7	Bulgaria	13.4.2020	0	0.00	0.00	17	3 000	75	self-report		0	
7	Bulgaria	13.4.2020	0	0.00	32.18	17	4 000	75	self-report		0	
7	Bulgaria	13.4.2020	1	0.33	9.92	17	3 000	75	self-report		0	1
3	Bulgaria	10.4.2020	1	0.43	27.55	31	2 300	80	self-report		1	
3	Bulgaria	12.4.2020	0	0.00	6.87	15	3 200	82	observer		0	
1	Bulgaria	28.6.2020	6	0.54	0.00	12	11 200	80	observer		6	

(Continued)

TABLE 2 Continued

Boat	Country	Date	Bycatch (ind.)	Bycatch (ind./km)	BPUE	Soak time, days	Length, m	Depth, m	Type of sampling	Species		
										Dd	Pp	Tt
2	Bulgaria	28.6.2020	0	0.00	0.00	12	3 100	81	observer		0	
1	Bulgaria	4.7.2020	14	1.35	7.09	14	10 360	68	observer		14	
1	Bulgaria	16.7.2020	4	0.36	4.36	12	11 200	77	observer		4	
11	Romania	21.7.2020	1	0.20	5.13	13	5 000	55	observer		1	
11	Romania	22.7.2020	0	0.00	0.00	14	1 100	55	observer		0	
11	Romania	22.7.2020	2	0.67	15.87	14	3 000	55	observer		2	
1	Bulgaria	23.7.2020	10	0.91	2.20	11	11 000	76	observer		10	
1	Bulgaria	29.7.2020	3	0.27	0.00	13	11 200	75	observer		3	
1	Bulgaria	2.8.2020	1	0.09	0.00	10	10 640	70	observer	1	0	
2	Bulgaria	14.10.2020	0	0.00	26.67	7	3 100	45	observer		0	
1	Bulgaria	10.4.2021	4	0.40	17.81	14	10 080	80	observer		3	1
1	Bulgaria	13.4.2021	3	0.26	7.04	13	11 760	80	observer		2	1
1	Bulgaria	11.4.2021	1	0.10	20.25	15	10 080	78	observer		1	
2	Bulgaria	11.4.2021	0	0.00	0.00	15	2 500	82	observer		0	
8	Bulgaria	12.4.2021	0	0.00	0.00	16	2 700	86	observer		0	
5	Bulgaria	13.4.2021	2	1.00	6.06	15	2 000	70	self-report		2	
1	Bulgaria	2.7.2021	7	0.64	0.00	12	10 920	80	observer		7	
1	Bulgaria	3.7.2021	3	0.27	0.00	13	10 920	80	observer		3	
1	Bulgaria	4.7.2021	10	0.85	0.00	14	11 760	80	observer		10	
2	Bulgaria	4.7.2021	0	0.00	0.00	14	2 600	80	observer		0	
8	Bulgaria	15.7.2021	0	0.00	5.95	25	3 000	81	observer		0	
8	Bulgaria	15.7.2021	1	0.30	0.00	25	3 300	87	observer		1	
8	Bulgaria	15.7.2021	0	0.00	0.00	25	3 000	77	observer		0	
8	Bulgaria	15.7.2021	0	0.00	2.65	26	2 200	83	observer		0	
8	Bulgaria	15.7.2021	0	0.00	14.37	26	2 500	81	observer		0	
									TOTAL	3	182	4

bycatch data reported 104 bycaught cetaceans from only six vessels (3% of all licensed vessels for turbot fishing). This suggests that the portion of bycaught animals reaching the coast is small. At least two freshly dead stranded cetaceans were observed with clear evidence of bycatch (missing tail flukes) during the closed turbot fishing season in Bulgaria providing evidence of IUU fishing.

3.3.2 Romania

Between 2019 and 2020, a total of 154 cetaceans were recorded stranded on Romanian beaches. In 2019 the peak was reached in June and in 2020 in August. Of the 53 recorded cases in 2019, 16 of them indicated possible cetacean-fisheries interaction, 28 unidentifiable causes. While of the 101 recorded cases in 2020, only 8 could be assigned to bycatch. The large number of unidentified causes of death recorded in 2020 (84 cases) was

because the state of decomposition was too advanced to establish cause of death realistically.

3.3.3 Ukraine

No bycatch evidence was discovered during dedicated cetacean stranding surveys conducted in Ukraine near the fisheries sites. That concurred with the data from interviews, as well as with the low density of cetaceans at sea during the season of the survey. From 137 cetacean stranding cases recorded in overall in 2019-20 along the Ukrainian Black Sea coast (including all the data coming from opportunistic sources), only 20 (18 harbour porpoises and two bottlenose dolphins) indicated possible cetacean-fisheries interaction. Most cases of stranded animals with bycatch signs were recorded between May and July but they also occurred from March to November.

3.3.4 Türkiye

Between 2019-2020, a total of 73 cetaceans were recorded stranded on Türkiye coast by İÜ-TUDAV Cetacean Stranding Network, local surveys and media. Strandings of harbour porpoises were observed at high rate during spring and summer (especially in June and July) and half of them were neonates. This may be related with turbot fishery's indirect effect, which was reported before in the Black Sea. Because of the death of lactating and nursing mothers in turbot nets, neonates may have starved to death and stranded ashore (Tonay et al., 2017). The number of strandings of common dolphins was high in winter and early spring. Bycatch signs were found in six common dolphins, one bottlenose dolphin and one harbour porpoise.

The harbour porpoise represented 96% of recorded bycatch in onboard survey and 65% in stranding records and was present in all the months when bycaught cetaceans were recorded (Figure 8).

3.4 Total bycatch estimates

Total Black Sea bycatch numbers were estimated by multiplying the following parameters:

- Median bycatch of porpoises per trip (based on onboard observations): 1 (interquartile range 0-2.5)
- Median number of trips per bycatch season: based on onboard observations 7.3 (interquartile range 5.65-9.42); based on questionnaires 10 (interquartile range 6-16)
- Number of turbot fishing vessels in the Black Sea (except Georgia) (data sources: FAO, 2020; this study): 1 620

Using this simple approach that is generally conservative for the reasons given above, estimates for annual bycatch of harbour porpoises by the Black Sea fleet involved in turbot catch is either 11 826 (interquartile range 0 – 38 200) assuming trip information from onboard observers or 16 200 (interquartile range 0 – 64 800) assuming trip information from questionnaires (Figure 9).

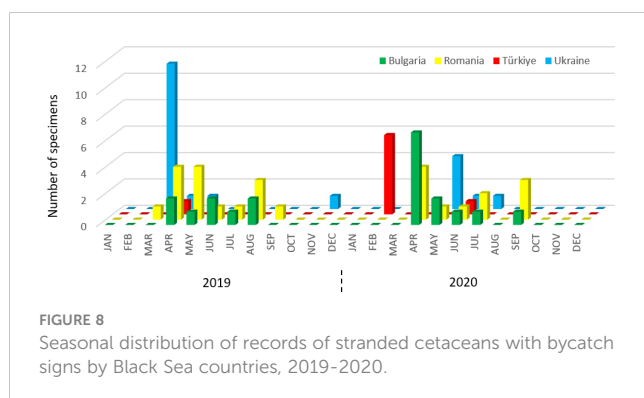


FIGURE 8 Seasonal distribution of records of stranded cetaceans with bycatch signs by Black Sea countries, 2019-2020.

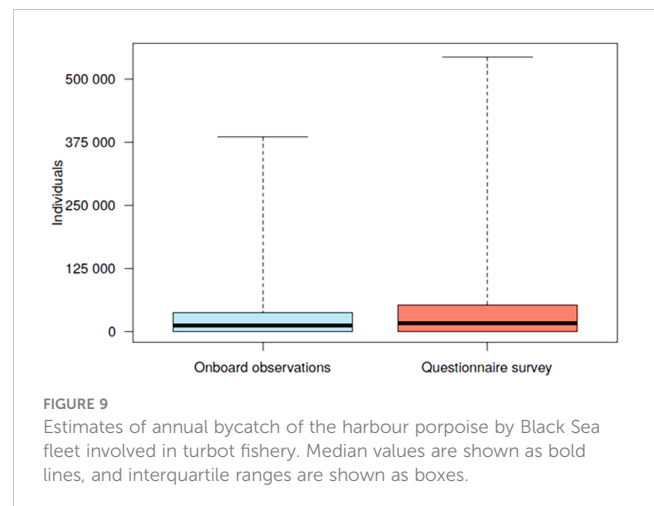


FIGURE 9 Estimates of annual bycatch of the harbour porpoise by Black Sea fleet involved in turbot fishery. Median values are shown as bold lines, and interquartile ranges are shown as boxes.

4 Discussion

Overall basin wide bycatch rate of harbour porpoises in turbot gillnet fisheries found in this study in spite of the conservative assumptions used is apparently very high. Most previous studies of harbour porpoise bycatch in the Black Sea reported an average bycatch rate as individuals per km of nets (ind./km). The comparable statistic from the present study was 0.37 ind./km – similar to a previous 2014-2018 study in Bulgarian waters when 0.31 ind./km was reported (Zaharieva et al., 2022). Older studies conducted in different regions of the Black Sea reported a wide range of bycatch rates – these are summarised here for information without any comparison of methods and assumptions (e.g. how net height and soaking time were taken into account): 0.09 ind./km in Ukraine (Pavlov et al., 1996), 1.53 ind./km in Ukraine (Birkun et al., 2009), 0.22 ind./km in Bulgaria (Mihaylov, 2011), 0.33 and 0.19 ind./km in western Türkiye (Tonay, 2016), 4.14 ind./km in central Türkiye (Gönener and Bilgin, 2009) and 0.43 ind./km in eastern Türkiye (Bilgin et al., 2018).

Strandings can be a supplementary source of monitoring bycatch. In the current study, there were some carcasses indicating the evidence of bycatch in Bulgaria, Romania and Ukraine. Besides, between January 2019-May 2020, 50 stranded cetaceans (common dolphins 58%, harbour porpoises 36%, bottlenose dolphins 4%, and unidentified delphinids 2%) were reported in Turkish Black Sea coast, of which one harbour porpoise and six common dolphins indicated the evidence of bycatch (Paiu M. et al., 2021b). Strandings of common dolphins were in winter-early spring and not related to turbot fisheries but possibly to purse seines or midwater trawls. This implies that the interaction with fishing gears other than turbot gill nets needs further investigation. Besides, the result of Bulgaria indicated that it was not possible to robustly estimate the annual bycatch from the stranding record due to the low number of strandings compared to the data obtained through onboard observations. The advanced stage of decomposition also makes it impossible to determine the cause of death. As a result, it was understood that strandings themselves can indicate the occurrence of bycatch but hardly

provide quantitative data without demographic modelling (Moore and Read, 2008).

During the current study pingers have been tested as mitigation measure in 27 of the hauls in Bulgaria. Three models of pingers have been tested: Future Oceans 10 kHz, Future Oceans 70 kHz and Porpoise Alerting Devices (PAL), frequency 10 kHz. Observed differences in bycatch levels in strings fitted with Future Oceans pingers and those without pingers were not significant in 2019 (Popov et al., 2020). The only model which showed significant decrease in bycatch (86%, $p < 0.05$) was the PAL pinger deployed on six strings. These accounted for 10% of all observed hauls with resultant overall negative bias of 8% in recorded bycatch rate. Larger scale trials in terms of coverage and duration are needed to confirm these results and test potential habituation effect.

The bycatch estimates provided here concur with the overall bycatch in turbot gillnet and trammel net fisheries (including IUU fishing) calculated for the western Turkish Black Sea coast of 2 011 and 2 294 porpoises per year in 2007 and 2008, respectively, based on bycatch per haul and the total net amount in the area (Tonay, 2016). Using an earlier value of the number of active vessels in that area as 185 (Tonay and Öztürk, 2003), the average annual bycatch of harbour porpoises per boat varied between 10.9 and 12. These mean values are a little higher than those from this study (7.3–10 depending on data source). The differences probably reflect normal interannual and interregional variation, and the rough combined estimate of annual bycatch as 10 harbour porpoises per vessel can be assumed to be a robust estimate for further monitoring, given the consistency in values despite the different approaches. Widening sub-regional coverage, i.e. further research in the eastern Black Sea is important for enhancing the accuracy of scaling or further stratified modelling (Authier et al., 2021), since most of the data for both studies come from the western Black Sea. However, the estimates from the western subregion are particularly important due to the summer concentration of the major part of the Black Sea harbour porpoise population in this area (Paiu et al., 2021a).

The data obtained during this study from several independent lines of evidence corroborate earlier suggestions on bycatch seasonality and the potentially strong impact of IUU as well as legal fisheries. They also provide similar rough estimates of overall bycatch per vessel. However, the data presented here are somewhat limited due to the relatively small sample size of observed trips/hauls. Based on the calculator of Curtis and Carretta (2020), it can be suggested that the coverage by onboard observers needed to obtain abundance estimates with a CV value of about 0.3, is at least 220 trips for the whole Black Sea. The relatively small sample size (48 trips, 63 hauls) might have affected precision of the obtained result. However, unlike many situations elsewhere when bycatch sampling effort is considered low due to the rarity of bycatch events (Authier et al., 2021), bycatch frequency in the Black Sea is high (57.1% observed occurrence rate, 95% probability of observing bycatch is achieved at 0.2% observation effort: Curtis and Carretta, 2020), and here the high variance of cetacean bycatches is observed, which is due to outliers – extremely high bycatch events. Moreover, the highest bycatch incidents could be missed and thus increasing the observation effort would only increase the variance. Also, most part of the eastern Black Sea was not covered

by this survey, data from that area are limited and probably more vessels involving bycatch are falling out of the GFCM statistics. Therefore, given the whole bulk of available evidence, despite the high figure obtained from this study, our estimates can be even underrated (rather than overrated).

Despite the small sample of observation effort, these results concurred with two previous independent studies (Birkun et al., 2014; Tonay, 2016). Birkun et al. (2014) provided a total estimated annual bycatch of porpoises in the Black Sea of 20 000 for all fishing gear types or 11 000 for gillnets, mostly based on surveys conducted in 2006–08 and 2012–13. Applying our method to the data of Birkun et al. (2014) increases the total for all gear to 25 000 porpoises. Alternatively, if we use the annual bycatch rate per vessel in 2007–08 calculated for western Turkish Black Sea fleet (after Tonay, 2016) for the whole basin' turbot fleet, the annual bycatch estimate will be around 20 100 individuals. Therefore, assuming a stable bycatch rate during the last 15 years, the upper range of total bycatch estimate which can be taken into consideration is at least 20 000 individuals per year.

The earlier high overall porpoise bycatch estimates for the Black Sea (Birkun et al., 2009; Birkun et al., 2014) seemed incompatible with the data on overall abundance (65 000) of the Black Sea population of the harbour porpoise. That abundance though was based on an aerial survey that covered only the northwestern Black Sea (29% of total area). However, the CeNoBS aerial survey conducted in summer 2019 as part of ACCOBAMS Survey Initiative (ASI) has covered more than 60% of the basin. It is the most comprehensive basin survey so far providing an overall abundance estimate (uncorrected for $g(0)$) of 94 219 (CV=0.07) porpoises with the highest density in the southwestern part of the Black Sea (Paiu et al., 2021a). Using the correction factor for $g(0)$ of 0.364 for good sighting conditions, calculated for the harbour porpoise in SCANS-III aerial survey of European Atlantic waters (Hammond et al., 2017), the abundance in the Black Sea would be some 258 900 porpoises. Thus, the bycatch rate is between 4.6% and 21.3% of the total abundance estimation, depending on the sources and methods of population and bycatch assessment (Table 3). We consider the lower end of this range as quite a realistic estimate on basis of previous demographic study for the Black Sea harbour porpoise. Therefore, the new data obtained under the ASI have been crucial for reconciling bycatch and abundance estimates.

A previous demographic study (Vishnyakova, 2017) that suggested a long lifespan (23 years) and generation time (7.5 years) for Black Sea harbour porpoises was consistent with a relatively low bycatch rate, which indirectly supports the lower limit of estimates presented here. In terms of assessing impacts of

TABLE 3 Bycatch rate calculation.

Bycatch estimation	Abundance estimation	Bycatch rate
11800	94200	12,5%
20100	94200	21,3%
11800	258900	4,6%
20100	258900	7,8%

bycatch on the Black Sea harbour porpoise populations, this suggests that if a PBR approach is to be considered, a review of the ‘traditionally’ used parameter values with respect to reproduction and recovery must be undertaken given the demographic information available for the Black Sea population which is known for its early maturation and high reproductive rates (Gol’din, 2004; Vishnyakova, 2017). However, it is clear that the information presented, even for the most conservative estimates of total bycatch, reveals population bycatch rates among the highest in the world (Read et al., 2006; Nelms et al., 2021), and greatly exceeds present agreed thresholds for sustainable levels (c.f. 1.7%, ASCOBANS, 2015) and thus poses a significant threat for this endemic subspecies.

5 Conclusions and future work

Despite being a species of high conservation concern and under strict protection, conservation of harbour porpoise is failing in Europe (Carlen et al., 2021) including the Black Sea. The conservative estimates of bycatch levels for the Black Sea harbour porpoise in this paper raise serious concerns about the survival of this subspecies. Whilst there is a scientific need to continue to improve monitoring of bycatch and refining bycatch estimates in the light of abundance estimates and population assessment, it is quite clear that the available data are already sufficient to demand that the primary focus must be on establishing effective mitigation measures (see below) and ensuring that these are implemented and monitored for effectiveness (that will also entail population abundance monitoring). Cooperation with fishermen and fisheries authorities for enhancing bycatch reporting is crucial to increase the sample size and robustness of the estimate and to evaluate the effectiveness of mitigation approaches. More effective implementation of existing regulations and recommendations (ex. EU Habitats Directive, ACCOBAMS Recommendations 2.13, 4.9 and 7.11) is needed to minimize IUU and ghost fishing. Accurate and standardized spatio-temporal recording of fishing effort should be conducted, and spatio-temporal closure of fishing should be considered when necessary (this can be evaluated *via* population dynamics modelling approaches). Further aerial surveys on density, abundance and distribution of cetaceans in the Black Sea are needed to detect trends in population development and seasonal distribution patterns. Support of all Black Sea states for realization of recently adopted ACCOBAMS Resolution 8.10 on implementation of Long-Term Monitoring Strategy is crucial to achieve that. The retrieval of bycaught animals from fishing vessels should be encouraged by the authorities to obtain biological data for population structure assessment.

Thus, as a matter of urgency, bycatch mitigation measures should be further tested and introduced in the Black Sea. Elsewhere, acoustic approaches such as ADDs (pingers) have

been used (Dawson et al., 2013). A few models have been tested in Türkiye, Romania and Bulgaria, some showing good results while others not (Gönener and Bilgin, 2009; Bilgin and Köse, 2018; Popov et al., 2020). In the current study, use of PAL pingers showed promising results. The sample size was small; thus, a large-scale trial is required to confirm these provisional results. Dolphin-safe fishing gears and technology are worth attention e.g., modified nets with acrylic glass spheres to improve acoustical detectability were tested in the Turkish Black Sea (Kratzer et al., 2021). In the implementation of mitigation measures and testing their continued effectiveness, in addition to common problems (habituation, habitat exclusion), local specific features should be considered carefully (e.g., assessment of effectiveness of pingers for the endemic Black Sea harbour porpoises). In conclusion, bycatch poses such a serious threat to the Black Sea harbour porpoises that all riparian countries engaged in turbot fisheries are required to implement urgent measures to reduce it immediately (ACCOBAMS, 2019; CMS, 2020).

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical review and approval was not required for the animal study because No disturbance of animals occurred.

Author contributions

DP, KV, MPai, AT and PG planned the study. DP, GM, KV, JI, MPai, CT, ED and PG collected data. PG led the analysis. DP, KV and PG analyzed data with input from GM, MPan, AAO, AT and ED. DP, GM, KV and PG wrote the initial manuscript draft with input from all the authors. All authors contributed to the article and approved the submitted version.

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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.2023.1119983/full#supplementary-material>

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Shipping noise assessment in the Black Sea: insights from large-scale ASI CeNoBS survey data

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Sighting data deriving from the ACCOBAMS¹ Survey Initiative (ASI), conducted through the CeNoBS² project, enabled the investigation of the habitat preferences for three different cetacean subspecies occurring in the Black Sea waters: the bottlenose dolphins (*Tursiops truncatus*), the common dolphins (*Delphinus delphis*) and the harbour porpoise (*Phocoena phocoena*). ASI aerial surveys, aiming at assessing the distribution and abundance of cetacean populations, were conducted during summer of 2019 in waters in front of Romania, Georgia, Bulgaria, Turkey and Ukraine. The surveys allowed recording of 1716 sightings: 117 bottlenose dolphins, 715 common dolphins and 884 harbour porpoises. The aim of this study was twofold: (i) to develop habitat models, using physical characteristics, such as depth and slope, as covariates, in order to estimate the presence probability of the three cetacean species in the Black Sea; (ii) to demonstrate the usefulness of the habitat models in support of environmental status assessments on marine mammals where the stressor is the shipping noise. The results of this study show the reliability of physical covariates as predictors of the probability of occurrence for the three species of interest in the Black Sea, providing additional knowledge, complementary to abundance estimates, which may support the assessment of the vulnerability of marine areas to different pressures, including noise.

KEYWORDS

large-scale survey, habitat models, marine mammals, Black Sea, impact assessment, continuous noise, shipping noise

1 ACCOBAMS: The Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area.

2 CeNoBS: Support MSFD implementation in the Black Sea through establishing a regional monitoring system of cetaceans (D1) and noise monitoring (D11) for achieving Good Environmental Status.

1 Introduction

The Black Sea is a naturally isolated body of water with a unique marine environment. Three subspecies of cetaceans can be found in there: the Black Sea bottlenose dolphin (*Tursiops truncatus ponticus*) (Barabash-Nikiforov, 1940), the Black Sea common dolphin (*Delphinus delphis ponticus*) (Barabash, 1935), and the Black Sea harbour porpoise (*Phocoena phocoena relicta*) (Abel, 1905). For many years it was legal to catch cetaceans in the Black Sea during commercial fishing (Smith, 1982; Birkun, 2002), causing a decline in the populations of these three cetacean species (Smith, 1982; Zemsky, 1994; Sánchez-Cabanes et al., 2017; BSC, 2008; Tonay and Öztürk, 2012). It was only in 1966 that such activities were banned in the Russian Federation, Bulgaria and Romania, and in 1983 in Turkey (Smith, 1982; Tonay and Öztürk, 2012; Sánchez-Cabanes et al., 2017). Despite these bans, incidental and illegal catches continue to be present and documented (Buckland et al., 1992; Birkun, 2002; Gol'din and Gol'din, 2004). Due to the historical situation outlined above, both the Black Sea bottlenose dolphin and harbour porpoise have been classified as endangered species, while the Black Sea common dolphin is listed as vulnerable on the IUCN Red List of Threatened Species (Birkun and Frantzis, 2008; Birkun, 2012; IUCN, 2012; ACCOBAMS, 2021b; ACCOBAMS, 2023). Besides the incidental catches, the considered species are subject to other major anthropogenic pressures such as collisions, underwater noise and chemical pollution (IUCN, 2012).

Monitoring surveys in the Black Sea have been limited, with most large-scale surveys conducted prior to 1987. Recent efforts have focused on local surveys in coastal areas (Birkun et al., 2004; Birkun et al., 2004; Dede and Tonay, 2010; Birkun et al., 2014; Kopaliani et al., 2015; Panayotova and Todorova, 2015; Gladilina and Gol'din, 2016; Gladilina et al., 2017; Popov et al., 2017; Baş et al., 2019; Paiu et al., 2019).

Collaborative aerial surveys have also been undertaken to gather valuable information about these species (Birkun et al., 2004; Raykov and Panayotova, 2012; Radu et al., 2013; Sánchez-Cabanes et al., 2017). However, prior to the CeNoBS initiative (ACCOBAMS 2021a), a comprehensive assessment of the overall abundance and distribution of these cetacean species in the Black Sea was not available. CeNoBS, with its systematic and synoptic approach, has provided a significant advancement in our understanding of the abundance, distribution, and density of all three cetacean species in the Black Sea during the summer months.

In the European Strategy for Marine and Maritime Research (COM (2008) 534), it is highlighted how important it is to reconcile environmental sustainability with the growth of maritime activities in order to decrease their strong environmental impact. Marine mammals are recognized by the EU Marine Strategy Framework Directive, as flagship species and therefore an essential element of ecosystem sustainability (Hooker and Gerber, 2014), their protection being a priority issue, requiring a robust analysis of existing information and data to identify areas requiring priority conservation actions (Pennino et al., 2013a; Pennino et al., 2016). Modelling the habitat of marine mammal may offer a fundamental

understanding of the ecological processes that determine the population distributions (Redfern et al., 2006; Embling et al., 2010), allowing a better understanding of the ecology of these animals (Sánchez-Cabanes et al., 2017; Hamazaky, 2002) and providing a tool which can support management (Azzellino et al., 2012; Forney et al., 2012; Mannocci et al., 2014; Cribb et al., 2015; Pennino et al., 2017).

As part of the QUIETSEAS project (www.quietseas.eu), a methodological framework was created specifically for the Mediterranean Sea and Black Sea (Azzellino et al., 2023, Deliverable 5.2) and continuous noise, aligning with the guidelines provided by the TG Noise (EU Technical Group on Underwater Noise for providing guidance to the Member States on the implementation of the Marine Strategy Framework Directive (MSFD) as regards Descriptor 11 (Energy including Underwater Noise) of the Directive). This framework enables the quantification of the impact of continuous noise sources on the potential habitat of the primary cetacean species in the Mediterranean Sea and Black Sea. The methodology introduced in this project utilizes habitat models, which make it possible to estimate the likelihood of species presence by considering bathymetric characteristics. Additionally, acoustic propagation models are employed to analyse the noise sources under consideration. By integrating these components, the framework facilitates the assessment of the reduction in potential cetacean habitat caused by noise. Regarding the habitat models, preference was given to models incorporating physical covariates, as they have been widely employed in ecological studies (Frankel et al., 1995; Gowans and Whitehead, 1995; Baumgartner, 1997; Raum-Suryan and Harvey, 1998; Karczmarski et al., 2000; Ferguson and Barlow, 2001; Ferguson et al., 2006a; Ferguson et al., 2006b; Azzellino et al., 2008; Azzellino et al., 2012; Blasi and Boitani, 2012; Marini et al., 2015) and offer greater stability and applicability across various study areas. This choice is based on their proven track record and reliability. In contrast, dynamic predictors like chlorophyll-a or surface sea temperature, exhibit considerable temporal and spatial variations due to factors such as seasonal fluctuations, interannual variability, and localized dynamics.

However, while habitat models based on physical predictors, and built upon a robust, long-term observation time series (Azzellino et al., 2012) have been available for the Mediterranean Sea, a comparable model specific to the Black Sea has not been readily accessible.

The primary objective of this study is to develop presence/absence habitat models for the cetacean species in the Black Sea, utilizing large-scale survey data and employing habitat physical characteristics as predictors.

This endeavour aims to expand the current QUIETSEAS methodological framework, which assesses the impact of continuous noise, to encompass the Black Sea region. By doing so, we aim to enhance our understanding of the effects of continuous noise on the marine environment in the Black Sea and its implications for cetacean species, supporting the implementation of the Marine Strategy Framework Directive (MSFD) and other relevant legal frameworks.

2 Material and methods

2.1 Study area

The Black Sea is a semi-closed basin covering an area of 436400 km² and constituting a unique marine environment, with a maximum depth of 2200 m (Murray et al., 1989). The Black Sea is connected by the Kerch Strait to the smallest and shallowest sea in the world (14 m maximum), called the Sea of Azov, located in the north-eastern part of the basin. Through the Istanbul Strait (Bosphorus), it is connected to the Sea of Marmara, which in turn is connected to the Aegean Sea through the Çanakkale Strait (Dardanelles) (Özsoy and Ünlüata, 1997). The continental shelf, at depths < 200 m, constitutes about 25% of the total area, while the flat abyssal plain, at depths > 2000 m, occupies about 60% of the total area. There are many steep slopes adjacent to the mainland and submarine canyons, such as Sakarya Canyon, in the southwestern area, where the depth suddenly increases from 100 m to 1500 m (Murray et al., 1989). It is important to mention that Black Sea has a positive water balance, where inputs from freshwater sources exceed evaporation losses. The freshwater inflow has great seasonal and inter-annual variability, having the Danube, Dnepr and Dnestr as the main rivers flowing into the North-West shelf (Murray et al., 1989). The water layer above 200 m is well oxygenated, while the deeper layer (between 200 and 2200 m) is anoxic. About 87% of the Black Sea water mass is therefore anoxic and contains high levels of sulphide, making pelagic and benthic organisms largely absent (Sánchez-Cabanes et al., 2017). The Black Sea is also characterized by low salinity levels due to high freshwater outflow from rivers and inflow from the Mediterranean with higher salinity and density, thus creating high water stratification. The temperature has a seasonal and regional variation, with an average annual surface temperature ranging between 16°C in the south and 11°C in the northwest (Balkas et al., 1990; Sánchez-Cabanes et al., 2017). The Black Sea hosts a wide variety of habitats, but relatively low biodiversity resulting in the absence of many local competitors, generating favourable conditions for invasive species which pose a great threat to the biodiversity of the Black Sea (Oğuz and Öztürk, 2011; Selifonova, 2011). The anoxic conditions and strong contrast in temperature and salinity make the ecology of the Black Sea vulnerable to anthropogenic effects compared to the Mediterranean and open seas (Kideys, 2002; Sánchez-Cabanes et al., 2017). The three cetacean subspecies that can be found regularly (Black Sea bottlenose dolphins, Black Sea common dolphins and Black Sea harbour porpoises) are distinct subspecies from the Mediterranean and Atlantic populations and are endemic to the Black Sea and the Sea of Azov (Birkun, 2002). The three species are at the top of the trophic network of the basin and have no natural predators (Kleinenberg, 1956; Jefferson et al., 2008).

2.2 Data set and data collection

The data used for this study were collected through the EU-funded CeNoBS project, in collaboration with and co-funded by ACCOBAMS under the ACCOBAMS Survey Initiative (ASI). The

CeNoBS project (ACCOBAMS, 2021a; Paiu et al., 2021) supported the implementation of the MSFD in the Black Sea through the establishment of a regional cetacean monitoring system and noise monitoring to assess the status of cetaceans in the Black Sea through MSFD descriptors and particularly of Descriptors 1 (D1, Biological Diversity) and Descriptor D11 (Energy including Underwater Noise).

CeNoBS data were collected through regional aerial surveys conducted in the Black Sea between June and July 2019, following specific shared protocols. Data were collected, following the distance sampling method (Buckland et al., 2015), by observers on board small twin-engine aircrafts, equipped with bubble windows, allowing the sight of cetaceans and other marine megafauna below the aircraft. Specific transects were designed and prepared to ensure fair coverage and representation of the study area. The surveys covered the waters in front of Romania, Bulgaria, Turkey, Ukraine and Georgia. The transects were predefined and adapted to the flight limitations in some areas. Six blocks and zigzag traces were drawn for each of them in order to have a minimum coverage of 3% of the study areas (Paiu et al., 2021) (Figure 1). Two observers, a team leader and the pilot were on board. Data were collected according to specific protocols prepared by the project's researchers and scientific collaborators. During the surveys, target altitude was 183 m (600 feet) with target speed of 100 knots. The software used to collect data was SAMMOA dedicated to marine megafauna data collection (SAMMOA 1.1.2, 2017-2018; Pelagis Observatory-La Rochelle University-CNRS), linked to GPS to collect position data (Paiu et al., 2021). Data regarding environmental conditions were collected at the beginning of each transect and whenever a change occurred. Variables considered were sea state (Beaufort scale), glare, cloud cover and sighting conditions. The three cetacean species (common dolphin, bottlenose dolphin and harbour porpoise) were the main target species, and data were collected also on group size and composition [mixed groups and two age classes Adults and Juveniles (calves)], swimming direction and group behaviour (using 8 defined categories) (SCANS II, 2008; Hammond et al., 2013).

The survey was conducted flying along the planned surveys primarily in passive mode, unless it was necessary to confirm species, obtain reliable estimates of school size, composition or behaviour by circling over the sighted animals. The survey was then resumed at the exact point it was left and all the secondary sightings (i.e. the additional sightings made after leaving the predetermined trackline) although recorded have not been used to obtain the abundance and density estimates.

CeNoBS aerial surveys were carried out from 17 June 2019 to 4 July 2019. A total of 15246 km of effort were surveyed: 9354 km on-effort and 5892 km off-effort. A total of 1984 sightings were collected, belonging to the three target species regularly occurring in the Black Sea. Sightings were distributed as follows: Bottlenose dolphins: 117 sightings and 335 individuals; Common dolphin: 715 sightings and 1762 individuals; Harbour porpoises 884 sightings and 1522 individuals; Delphinidae (unidentified species when a common dolphin or bottlenose dolphin was involved, mostly was due to size of the animal (juvenile) or poor sightability conditions such as reflexion, glare, swell etc): 28 sightings and 50 individuals (Paiu et al., 2021). Figure 2 shows the sighting distribution in the study area.

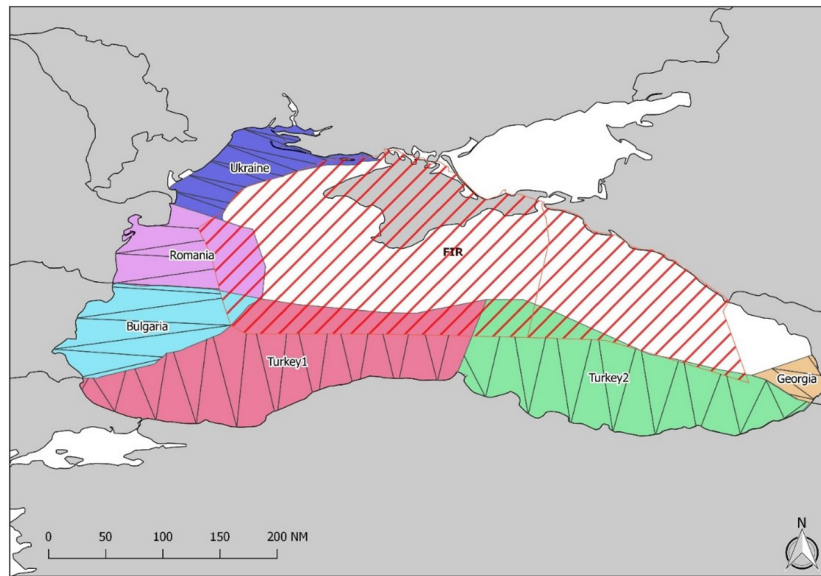


FIGURE 1
The six blocks and tracks covered by planes in the waters of Romania, Bulgaria, Turkey, Ukraine and Georgia (map source: ACCOBAMS-CeNoBS project).

2.3 Data analysis

2.3.1 Data matrix preparation

Using QGIS 3.20.3, a grid was created for the Black Sea with a total of 2069 cells of size 0.16° x 0.16° (10 nm x 10 nm,

corresponding to 18.5 km x 18.5 km). Bathymetric data were obtained through GEBCO with a 2021 model, providing elevation data in metres on a grid at 15 arc second intervals (<https://download.gebco.net/>). Sea bed slope was calculated using QGIS 3.20.3 from the depth layer via the GDAL slope command.

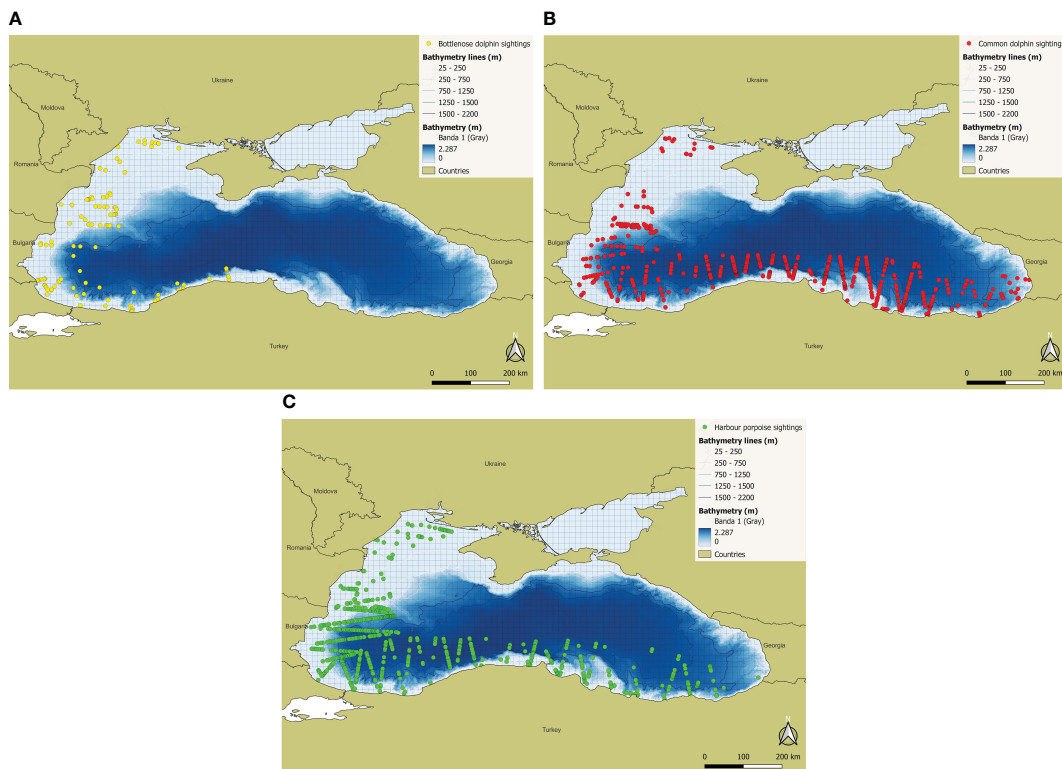


FIGURE 2
CeNoBS sightings: (A) Bottlenose dolphin; (B) Common dolphin; (C) Harbour porpoise.

Bathymetric data were integrated with the grid, enabling the calculation of depth and slope statistics within each cell. Such statistics were later used as potential covariates for the habitat models. The statistics calculated were mean, median, minimum value, maximum value, standard deviation and range (in metres for depth and in percentage value for slope). Moreover, the searching effort (i.e. length of track line in kilometres), and the sightings of each species were associated with the grid cells, and integrated with the data matrix needed for the following analysis.

2.3.2 Habitat model development

Habitat model development and evaluation was conducted by means of a binary logistic regression analysis, used also in similar ecological applications (Guisan and Zimmermann, 2000; Davis et al., 2002; Azzellino et al., 2008; Anderwald et al., 2011; Azzellino et al., 2011). All the analyses were performed using the IBM SPSS Statistics package (version 27). To make the analysis more efficient, the number of absence cells (i.e. pseudo-absence of the target species) in the dataset was balanced with the number of presence cells (presence of 1 or more sightings of the target species). Following Azzellino et al. (2012), the total number of presence cells was in fact maintained, while a number of absence cells (absence of sightings) equal to the number of presence cells was randomly extracted from the overall number of absence cells. Moreover, the binary logistic regression was applied using a stepwise approach, based on Wald statistics (Hosmer and Lemeshow, 2000) in order to select the best set of predictors. Each predictor was tested for entry into the model one by one, based on the significance level of the Wald statistic. After each entry, variables already in the model were tested again for possible removal. The procedure stopped when no more variables met the entry or removal criteria or when the last fitted model was the same as the previous. To prevent the risk of overfitting, each specific model was limited to a maximum of three predictors ensuring a sufficient number of observations, exceeding ten for each predictor. Additionally, the model's accuracy in terms of classification performance was assessed using leave-one-out cross-validation.

2.3.3 Noise magnitude assessment

The noise assessment was conducted following the risk-based methodological approach drafted in Deliverable 3 of the EU TG Noise (TG Noise, 2021). Following the risk-based approach, the probability of having an effect on a population exposed to a given pressure (i.e. continuous noise) can be estimated by considering the magnitude of the pressure (i.e. the noise level) and the effective exposure which depends on the presence of vulnerable species within the study area.

Based on TG Noise framework, the starting point of the methodology is the selection of habitats of the target species, at regional or subregional or at the Marine Reporting Unit (MRU) level. Afterwards, the habitat status is assessed within a reference grid where the condition in each grid cell is evaluated referring to the current state or to a reference condition. The deviation of the current state from the reference condition enables to evaluate whether the cell is non-significantly or significantly affected by the anthropogenic

noise (Annex 7-TG Noise – Sigray et al., 2021). All the grid cells of the habitat will thus be quantified both in time and space as significantly or non-significantly affected. Thus, for a specific time period, a certain fraction of the grid cells will be significantly affected. The potential for adverse effects at population level is assumed to occur when a certain fraction of the habitat is exposed to continuous noise for a certain fraction of time. Area and duration of exposure to anthropogenic sound can be assessed in terms of:

- Tolerable impacted area of the habitat
- Tolerable duration of the noise.

More specifically, based on the TG Noise methodological framework, the noise exposure and the consequent potential impact on the species of interest needs to be assessed following different methodological steps. As a first step a noise exposure map for the study area was generated covering the period from 01.07.2018 to 31.08.2018 using a reference grid of 100 m x 100 m mesh size. Three daily random AIS (Automatic Identification System) images were used to build a 60-days summer scenario of ship traffic which was used as basis to simulate noise levels. AIS data were supplied by Spire Group <https://spire.com/maritime/>. Sinay, who created the noise maps thanks to a partnership with Spire Group, requested AIS data worldwide on-demand through API-calls for a period/time of interest. For the present work, historical data in the study period were accessed. Row AIS data appear, for a given instant t , as a list of vessels for which an AIS message has been received at that instant t , with associated metadata such as the geographical position of each vessel (coordinates X,Y), their identification code-MMSI, speed, size, destination etc. Since it is known that AIS data transmission may fail for some reasons (poor coverage, signal collision, bad weather, etc.), to get a view of ship traffic at a given time it is better to look at a short period of time instead of looking at a single instant. To implement the noise modelling approach, all the single MMSI codes in a 10-minute period, three times/day, randomly during the day were used. This raw list of vessels that are found in a single 10-minute period can be easily transformed into a georeferenced file of points, where each point represents a vessel and can be plotted on a map. From this map of points (vessels) relative to a 10-minute period, the propagation modelling is implemented for each point (vessel), and the contributions of sound pressure from different vessels on a single mesh are summed up. The 180 vessels maps over the study period (3/day x 60 days) were used and hence 180 noise maps were produced, each relative to a 10-minute period. This statistical sample size ($N = 180$) is used to calculate the median and percentiles of sound pressure level (SPL).

The noise levels (i.e. sound pressure level, SPL dB re 1 μ Pa) were later calculated for the surface layer, (i.e. in the first 10 m of depth), for the one-third octave band centred at 63 Hz and considering the 50th and the 95th percentile level. Afterwards, the percentage of the target species habitat affected by noise levels above which negative effects may occur (such as behavioural changes, or changes in the species hearing ability, or in birth rates, or any loss of habitat permanent or temporary) was evaluated.

2.3.4 Habitat exposure assessment

The noise map previously described was overlaid to the bathymetric grid which was used to develop the species habitat models and the SPL zonal statistics (mean, median, maximum value, minimum value and standard deviation (SD) were calculated per each grid cell unit. Subsequently, based on the review by Gomez et al. (2016) in which 79 studies and 195 cases of data concerning the exposure of cetaceans to various noise sources are considered and reviewed, the sound pressure level of 110 dB re 1 μ Pa is assumed as LOBE (i.e. Level of Onset of Biological adverse Effects, see Deliverable 4 of TG Noise; TG Noise, 2023). Therefore, considering this noise level, cells with mean SPL level greater than or equal to 110 dB were identified as a potential habitat loss. It should be noted here that while noise map shows SPL in terms of 50th or 95th percentile and is defined on a grid of 100 m x 100 m mesh size, habitat exposure is instead defined at a much coarser mesh size (18.5 km x 18.5 km). Therefore, when noise level and habitats are combined, the noise levels (either the 50th or the 95th percentile) are averaged assuming the cell mean value as the reference noise level. Finally, to quantify habitat, the Potential Usable Habitat Area (PUHA after Azzellino et al., QuietMED2 D6.2) of each species was calculated (see Table 1 showing PUHA calculation for a single cell unit). Risk maps were generated by superimposing noise maps with Potential Usable Habitat Area maps, and calculating the overall proportion of the area exposed to shipping noise above LOBE noise level for the three target species.

2.3.5 Noise impact comparison between the South-western and South-eastern portions of the Black Sea

The Black Sea South-western and South-Eastern regions are very different in terms of traffic and noise: the South-western region has higher ship traffic and higher noise levels, while the South-eastern region has a lower ship traffic, and is therefore much quieter in terms of noise. Since common dolphin regularly occurs in both regions and is the most frequent species, we compared common dolphin relative distribution in equivalent suitable habitat areas in the two regions, which were different only in terms of noise levels.

The hypothesis we wanted to test was that higher noise levels may make the habitat less suitable and therefore affect the species relative abundance. As an index of relative abundance the encounter rate was used, defined in each grid cell as the number of cell sightings divided by the cell effort in kilometres.

For this purpose, an area of 456 cells (108099 km² or the 23.3% of the Black Sea total area) in the South-western region, and an area

of 401 cells in the South-eastern portion (95051 km² or the 20.5% of the Black Sea total area) were previously identified, and cells with habitat suitability (i.e. species presence probability) greater than 0.6 were selected. Moreover, in order to better compare the suitable habitat, the primary productivity was also considered in terms of remote-sensed concentration of chlorophyll-a (mg m⁻³, spatial resolution 4 km) available for the year 2019 from the <https://giovanni.gsfc.nasa.gov/giovanni/portal>. The gridded chlorophyll-a values, in every cell unit, were used as an offset variable and subdivided into two main chlorophyll classes based on the basin median value (i.e. ≤ 0.593 and > 0.593 mg m⁻³).

The difference of the species relative abundance in the two regions was assessed by means of a Mann-Whitney independent samples U test ($P < 0.05$).

3 Results

3.1 Habitat models

The stepwise Binary logistic regression analysis was applied to the three species presence and absence data using depth and seabed slope statistics per cell as possible covariates. Generic Delphinidae sightings were not considered for habitat model development.

3.1.1 Black Sea bottlenose dolphin

Despite the fact that bottlenose dolphin sightings were lower than those of the other two species and more concentrated in the western part of the Black Sea, the species habitat model is decently accurate. The cell effort and mean depth were selected as best predictors for the species presence probability (see Table 2), being respectively a direct and an inverse predictor. Presence probability was in fact inversely proportional to the mean depth and directly proportional to the cell effort (i.e. sum of transect km). The model accuracy (e.g. percent of correct presence and absence classifications) was 89.3% when predicting presence cells and 90.7% when predicting absence cells (see Table 3).

Bottlenose dolphin habitat was mainly predicted along the coast, ranging from a bathymetry of 25 and 500 m, with a higher probability in the north-western sector of the Black Sea. In the Azov Sea, where the maximum depth is of the order of 14 m, the habitat is optimal, with high presence probability for the species. In the areas where the continental slope begins and in the central part of the basin, where the depth is greater, the probability of occurrence is instead very low. The total PUHA (sum of each cell PUHA) over the entire Black Sea for this species is 190316 km² (Figure 3).

TABLE 1 Example of PUHA calculation over a single cell unit: the target species presence probability is multiplied by the cell area to obtain PUHA.

Species	Presence probability	Cell's Area (Km ²)	Calculation Example	PUHA (Km ²)
Bottlenose dolphin	0.55	342.2	PUHA = (0.55 x 342.2)	188.2
Common dolphin	0.80	342.2	PUHA = (0.80 x 342.2)	273.7
Harbour porpoise	0.72	342.2	PUHA = (0.72 x 342.2)	246.3

TABLE 2 Results of the binary logistic regression analysis modelling the presence/absence of Black Sea species.

Species	Covariates	B	S.E.	Wald	Sign.
Bottlenose Dolphin	Cell effort (km)	0.181	0.030	37.443	<0.001
	Cell mean depth (m)	-0.002	0.000	14.177	<0.001
	Constant	-1.228	0.411	8.917	0.003
Common Dolphin	Cell effort (km)	0.153	0.012	152.556	<0.001
	Cell Mean Depth	-0.001	0.001	5.920	0.015
	Cell Max Depth	0.002	0.001	11.490	<0.001
	Constant	-3.430	0.393	76.170	<0.001
Harbour Porpoise	Cell effort (km)	0.193	0.015	159.891	<0.001
	Cell Mean Slope	0.033	0.016	4.003	0.045
	Constant	-5.106	1.400	13.301	<0.001

The covariates are shown as the regression coefficients (B) and the corresponding standard errors (SE). Wald Statistics, and Significance level of the coefficients are also shown.

3.1.2 Black Sea common dolphin

Sightings of common dolphins were well distributed throughout the study area, providing more data for habitat modelling. The selected predictors for the common dolphin are the cell effort, the cell mean and maximum depth (Table 2). The model exhibited an accuracy of 86.9% when predicting presence cells and 91.2% when predicting absence cells (Table 3). Common dolphin presence was found directly proportional to the effort and to the cell maximum depth, but inversely proportional to the cell mean depth that is the weakest predictor. The results show that the common dolphins are associated with depths between 50 and 2200 m. Along coastal areas, but especially in the north-western zone, between 25 and 100 m, the species probability of occurrence is very low. Likewise, the occurrence probability is low in the Azov

Sea, while it is higher in the central and southern portions of the Black Sea basin. The areas with the maximum presence probability (i.e. values between 0.71 and 0.92) are mainly concentrated in Turkish waters, along the continental slope, at depths between 150 and 1750 m. The total PUHA (sum of each cell PUHA) of the species over the entire Black Sea is 231156 km² (Figure 4).

3.1.3 Black Sea harbour porpoise

Harbour porpoise sightings are well distributed throughout the study area, although more concentrated in the western portion of the Black Sea basin. The selected predictors for the harbour porpoise habitat model are the cell effort and the cell mean sea bed slope (Table 2). The model exhibits an accuracy of 91.6% when predicting presence cells, and of 93.4% when predicting absence cells (Table 3). Harbour porpoises' presence probability is directly proportional to both the cell effort and the mean sea bed slope. Habitat suitability is higher along the coasts of Bulgaria, Turkey, Georgia and Russia, and in the central portion of the basin. On the other hand, in the north-western area, along the coasts of Ukraine, the presence probability is low, reaching its minimum in the Azov Sea, where the probabilities are close to 0. Harbour porpoises are therefore associated with both shallow and deep waters, between 25 and 2200 m, more precisely from the beginning of the continental slope towards the interior of the basin. The results also show that there is a central-eastern zone where the probability of occurrence decreases since depth increases but the slope decreases. For the harbour porpoise, the calculated PUHA (sum of each cell PUHA) is 274220 km² (Figure 5).

TABLE 3 Confusion matrix showing the classification performances of the habitat models for predicting presence and absence cell for the three considered species.

Species		Predicted		Correct Predictions (%)
		Absence	Presence	
Bottlenose Dolphin	Observed Absence	68	7	90.7
	Observed Presence	8	67	89.3
	Overall percentage			90.0
Common Dolphin	Absence	229	22	91.2
	Presence	33	219	86.9
	Overall percentage			89.1
Harbour Porpoise	Absence	254	18	93.4
	Presence	23	251	91.6
	Overall percentage			92.5

The accuracy of the models is evaluated as the percentage of the correct predictions.

3.2 Noise comparison between the South-western and South-eastern portions of the Black Sea and LOBE validation

In order to test the potential impact of noise on the species' relative abundance the species encounter rate was tested between the two regions, having equivalent habitat suitability but different noise levels.

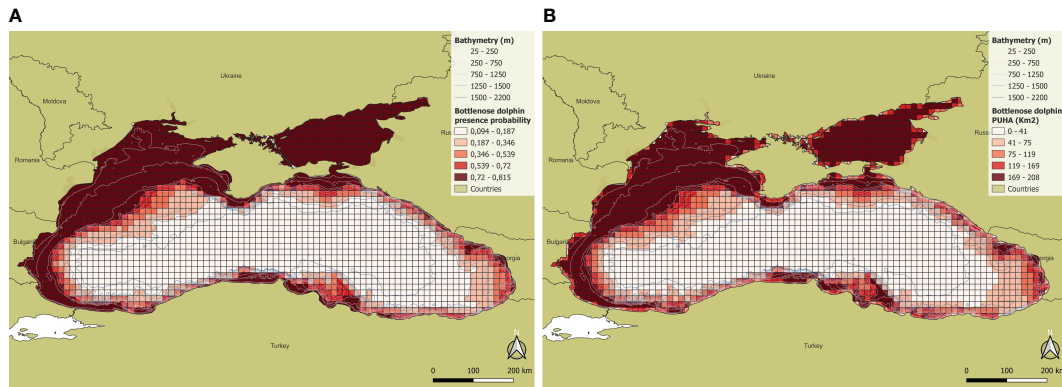


FIGURE 3
(A) Presence probability and (B) PUHA of Bottlenose dolphin.

Therefore, 364 cells of most suitable habitat (presence probability higher than 0.6) were selected in the South-western area, with 218 sightings, and 295 cells in the South-eastern area, with 240 sightings (Figure 6). As explained in the methods, these cells were also divided per chlorophyll-a classes, resulting respectively for the South-western and the South-eastern regions in 193 cells and 269 cells having chlorophyll-a values lower than the Black Sea July median, and 171 and 26 cells having chlorophyll-a higher than the Black Sea July median.

Mann Whitney test showed a significant difference of the encounter rates between the two regions for the lower chlorophyll-a class (Chl-a \leq 0.593; U: 807, N: 100; P-value < 0.05) (Figure 7A) while no significant difference was found for the higher chlorophyll-a class (Chl-a > 0.593; U: 49, N: 32; P-value: 0.670) (Figure 7B).

Particularly, the encounter rate was significantly higher in the South-eastern region when comparing the lower chlorophyll-a conditions (see Table 4).

Figure 8 shows the comparison of the South-Western and South-Eastern regions in terms of monthly chlorophyll-a average levels. With the exception of a few months (e.g. in Spring and in Autumn) the western primary productivity is higher than in the eastern portion.

Table 5 shows the average noise levels of the two subregions subdivided by chlorophyll-a class. It can be observed that there is a difference of about 15-17 dB between the South-Western and the South Eastern regions. It is also worthwhile to observe that the higher encounter rates occur in areas where noise levels do not exceed 110 dB. Therefore, grounding on these results, we could assume as LOBE, Level of Onset of the species Biological adverse Effect (i.e. the encounter rate decrease), an SPL ranging between 110 dB (50th grid cell percentile of the median SPLs) and 120 dB (75th grid cell percentile of the 95th SPLs).

3.3 Noise impact assessment

The amount of habitat negatively impacted by underwater noise for each of the three target species was assessed as described in the Methods, combining the relevant noise maps with the habitat models and the derived PUHA. The simulated noise map shows where the presence of harbours causes higher noise (Figure 9A). The obtained noise maps are coherent with the ship traffic density maps available from the Emodnet portal for the year 2019 (Source: <https://www.emodnet-humanactivities.eu/>), Figure 9B.

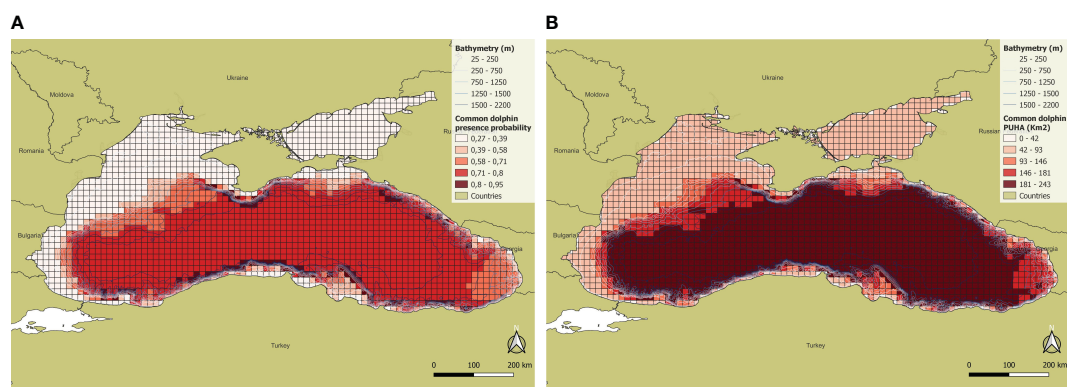


FIGURE 4
(A) Presence probability and (B) PUHA of Common dolphin.

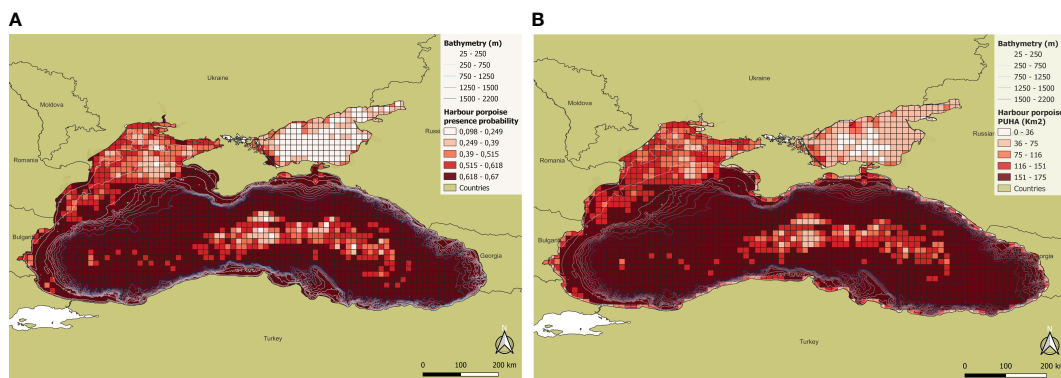


FIGURE 5
(A) Presence probability and (B) PUHA of Harbour porpoise.

As already explained, the areas with the highest noise levels are mainly located in the South-western portion of the basin, while in the South-eastern portion noise is lower. The grid cells (501 cells) having a noise level higher than LOBE (i.e. SPL above 110 dB re 1 μ PA), in the Black Sea occupy 25.16% of the basin area (2069 cells). Most of the “impacted” cells are located along the main traffic routes in the basin, especially in the South-western portion, while no “impacted” cells are present in the South-eastern portion of the basin. Finally, risk maps were created from which the percentages of Potentially Usable Habitat Area (PUHA) exposed to noise levels above LOBE were quantified for the target species (Figure 10).

The results also show that the percentages of impacted PUHA are very similar for the three species considered. As far as the bottlenose dolphin is concerned, the percentage of impacted PUHA was 21.4%, which is not unexpected for a species regularly occurring in the coastal areas, where ship traffic and related noise are generally higher. The pelagic areas in the Black Sea having noise levels greater

than LOBE do not impact too much on the species PUHA since the species presence probability in the pelagic area is very low (Figure 11A). The percentage of impacted PUHA is higher for harbour porpoise (24.46%), since the species’ suitable habitat is concentrated along the southern coasts and in the central areas of the basin where the noise levels more frequently exceed LOBE (Figure 11C). But the highest percentage of impacted PUHA was found for common dolphin (26.47%), being the species habitat suitability higher in the central portion of the basin, corresponding to the area of the main shipping lanes (Figure 11B).

4 Discussion

This study presents a modelling approach for estimating the Potentially Usable Habitat Area (PUHA) of the three cetacean species regularly occurring in the Black Sea. The models were

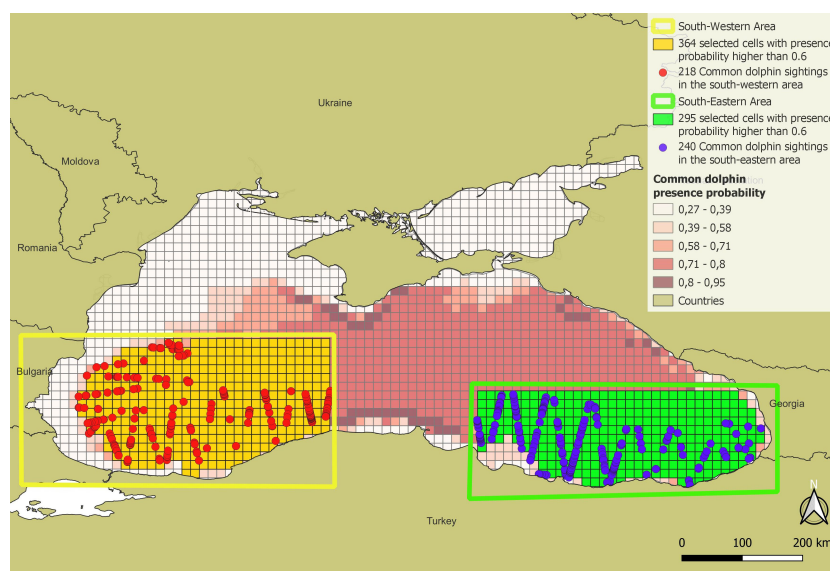
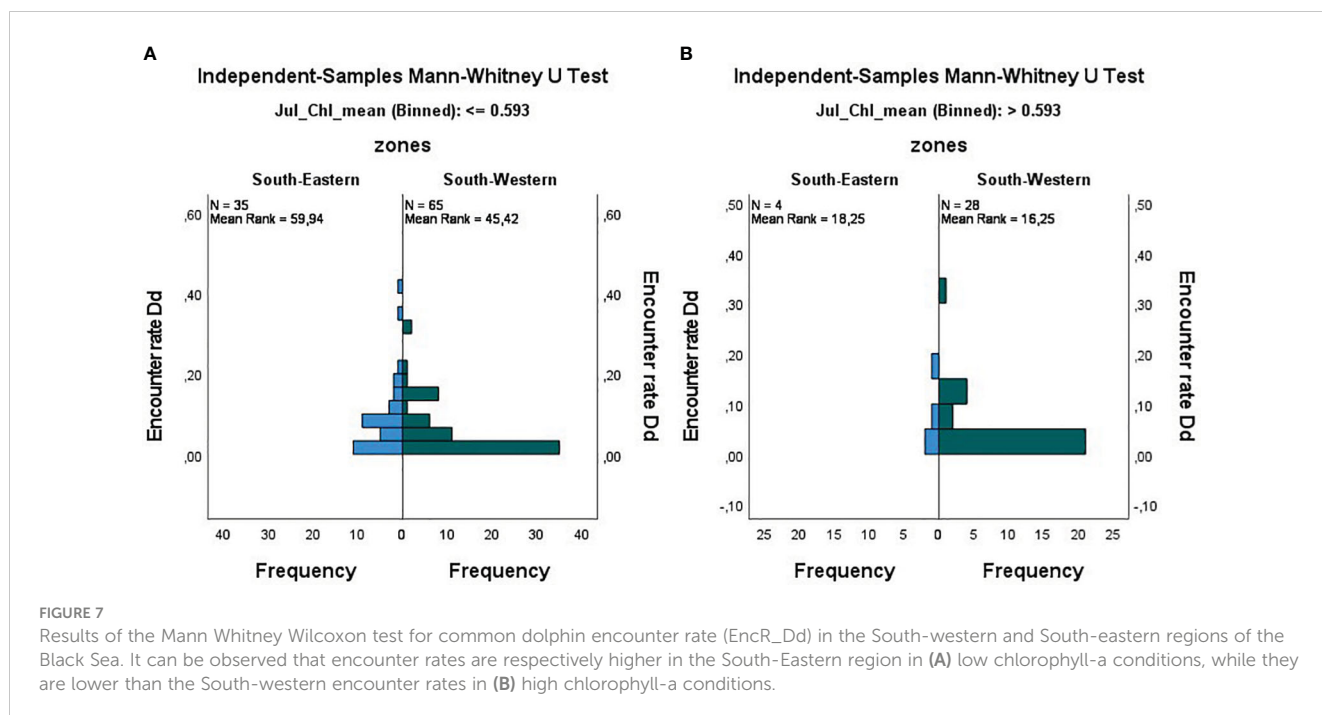


FIGURE 6
Map of the common dolphin sightings in the cells with optimal habitat suitability (species presence probability higher than 0.6) in the South-western (yellow-enclosed) and South-eastern (green-enclosed) subareas in the Black Sea.



developed on the basis of the large scale data collection acquired through the EU-funded CeNoBS project, in collaboration with and co-funded by ACCOBAMS under the ACCOBAMS Survey Initiative (ACCOBAMS, 2021a; Paiu et al., 2021). The study shows how physiographic predictors may play an important role in predicting the potential distribution and habitat preferences of cetaceans.

The developed habitat suitability models exhibited high accuracies (between 89% and 92%) in predicting the presence/absence of the considered species in the Black Sea, demonstrating that these species have very specific habitat preferences.

The bottlenose dolphin is among the best known, most widespread and studied cetacean species in all seas, with habitat preferences in Mediterranean Sea being mostly reported in coastal and shallow waters (e.g. Gnone et al., 2011; Azzellino et al., 2012). Black Sea bottlenose dolphins show habitat preferences in line with this common pattern, the optimal depths ranging between 25 and 500 m depth. The model shows that bottlenose dolphins are more likely to be found in coastal areas, especially in the north-western part, while in the central zone of the basin, at greater depths (over 750 m), and in areas where the continental slope begins, the

probability of their presence is much lower. These results are in agreement with those obtained by Sánchez-Cabanes et al. (2017) in the same area, which showed a preference for habitats along the coast but at slightly shallower water depths, between 50 and 250 m. Paiu et al. (2021) also showed a higher abundance in coastal areas, and in the western part of the Black Sea. Numerous other studies (e.g. Cañadas et al., 2002; Gnone et al., 2011; Azzellino et al., 2012; Marini et al., 2015; Carlucci et al., 2016; Giannoulaki et al., 2017; Affinito et al., 2019; Muckenhirn et al., 2021) show that the areas of greatest bottlenose dolphin presence are associated with coastal areas at depths up to 40-600 m. In the Black Sea, however, the species' higher preference for shallow-water habitats may also be the consequence of the deep anoxic waters and the consequent lack of prey. In deep water, no sightings of bottlenose dolphins have been detected and the predicted presence probability is close to zero, in agreement with the study of Sánchez-Cabanes et al. (2017) which has approximately the same spatial coverage but is based on data deriving from opportunist surveys. The same authors refer that the species distribution changed in time since it was known that in the 1960s and 1970s, bottlenose dolphins were also reported in the deep central part of the basin. Sánchez-Cabanes and colleagues

TABLE 4 Common dolphin encounter rate (EncR_Dd) statistics in the South-western and South-eastern region in lower and higher chlorophyll-a conditions.

Zones	JulChl2cl	N		Mean	Median	Minimum	Maximum	Percentiles		
		Valid	Missing					25	50	75
South-Eastern	<= 0.593	35	44	0.087	0.079	0.00	0.40	0.026	0.079	0.107
	> 0.593	4	9	0.057	0.029	0.00	0.17	0.000	0.029	0.144
South-Western	<= 0.593	65	35	0.054	0.026	0.00	0.33	0.000	0.026	0.075
	> 0.593	28	43	0.043	0.013	0.00	0.32	0.000	0.013	0.050

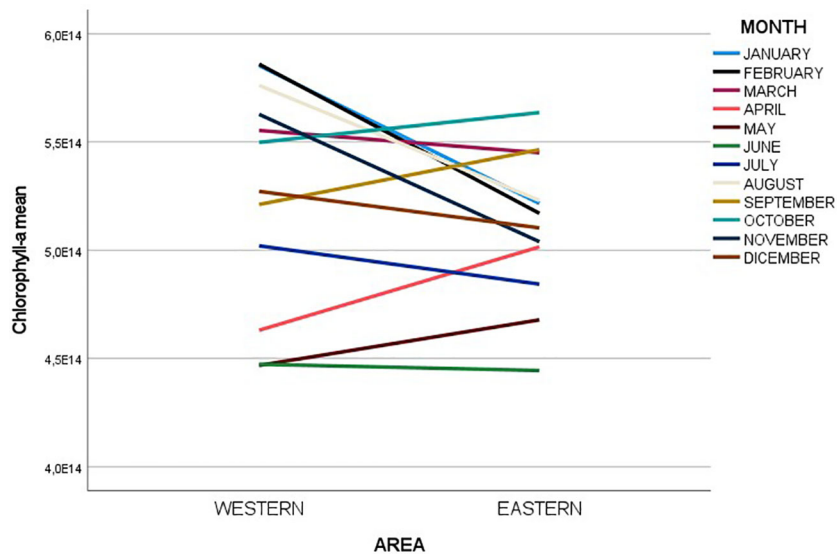


FIGURE 8 Line graph showing how mean chlorophyll-a concentrations vary over the months of the year 2019 in the selected western and eastern Black Sea regions.

TABLE 5 Noise level (SPL re 1 μPa) statistics are shown for the common dolphin suitable habitat in the South-Western and in the South-Eastern regions at the different primary production conditions (i.e. Chl-a).

JulChl2cl			Jul_Aug_10m_Median_mean		Jul_Aug_10m_95perc_mean	
			Zones		Zones	
			South-Eastern	South-Western	South-Eastern	South-Western
<= 0.593	N	Valid	79	100	79	100
		Missing	0	0	0	0
	Mean		93.1	110.7	103.3	127.2
	Median		102.3	110.3	113.7	127.2
	Minimum		31	107	31	122
	Maximum		107	121	133	133
	Percentiles	25	94.7	108.9	99.1	124.8
		50	102.3	110.3	113.7	127.2
75		104.8	111.6	119.9	129.2	
> 0.593	N	Valid	13	71	13	71
		Missing	0	0	0	0
	Mean		57.7	110.3	60.1	127.0
	Median		36.3	109.9	36.4	126.4
	Minimum		33	107	34	122
	Maximum		104	122	112	158
	Percentiles	25	35.3	108.7	35.4	123.7
		50	36.3	109.9	36.4	126.4
75		101.5	111.1	108.3	128.0	

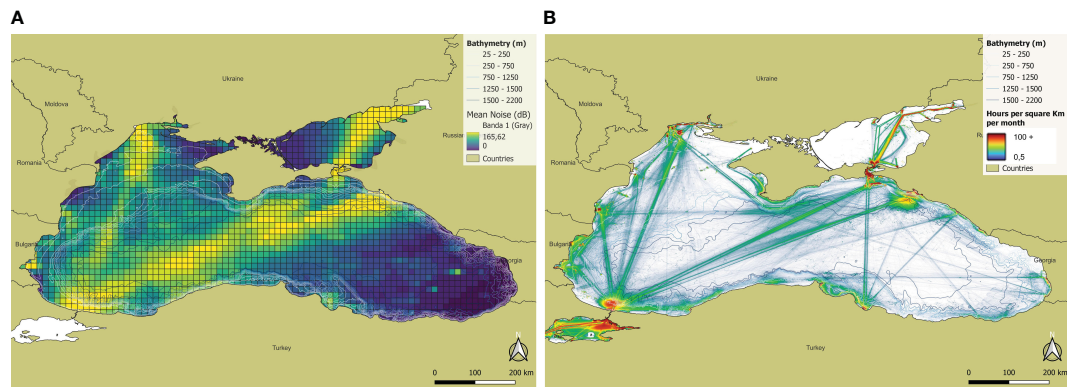


FIGURE 9 (A) Noise map showing mean SPL values (dB re $1\mu\text{Pa}$) per cell using the grid of 10×10 nm; (B) Mean vessel density for 2019 (hours per square Km per month, Source: <https://www.emodnet-humanactivities.eu>).

speculated that the current distribution shift could be the effect of the ecosystem changes occurred in the last decades which may have prompted the species to change its diet and consequently distribution. Another possible explanation might be related to fisheries which, before being regulated, led to a decline in pelagic fish, thus impacting both prey availability and feeding behaviour of bottlenose dolphins. It is known, in fact, that bottlenose dolphins may change their habitat preferences depending on the presence of prey (Hastie et al., 2004).

The common dolphin is another widespread dolphin species with a variety of habitats, showing greater preferences for deep sea areas and continental shelves (Cañadas and Hammond, 2008). Present study shows greater species presence in deeper waters, with high presence probability associated to areas between 150 and 1750 m, relative to the continental slope. In the north-western region and in the Azov Sea, where there are medium- and -shallow-

water depths but minimal slope values, the species probability of occurrence reports very low values (between 0.24 and 0.34). These results also agree with those obtained by Sánchez-Cabanes et al. (2017), whose models showed greater presence for the central part of the basin between the bathymetries of 50 and 2250 m and in waters between $5\text{-}18^\circ\text{C}$, which corresponds to the waters of the southern zone towards the central zone of the basin, away from the north-western continental shelves, where the probability is indeed lower (Shapiro et al., 2010). The results also agree with the abundance studies of the CeNoBS project, which predict a higher abundance towards the central zone and along the continental shelf (Paiu et al., 2021). In addition, other former studies reported a prevalence of the common dolphin mainly in the central part of the Black Sea (Raykov and Panayotova, 2012; Radu et al., 2013; Birkun et al., 2014), suggesting that the species habitat preferences at least in the last two decades have not changed over time. Also in other

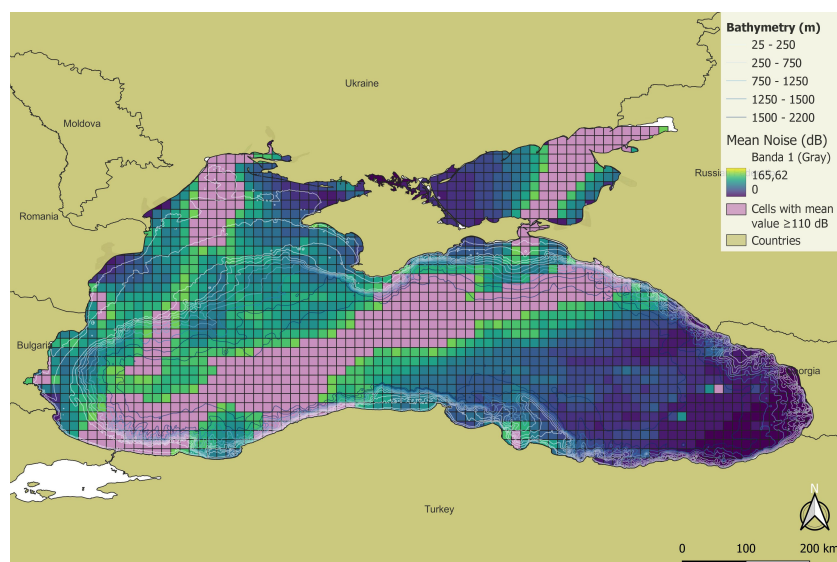


FIGURE 10 Noise Risk maps showing the pink the grid cells where noise levels are higher than LOBE equal to $110\text{ dB re }1\mu\text{Pa}$ (501 cells, corresponding to 25.16% of Black Sea total area).

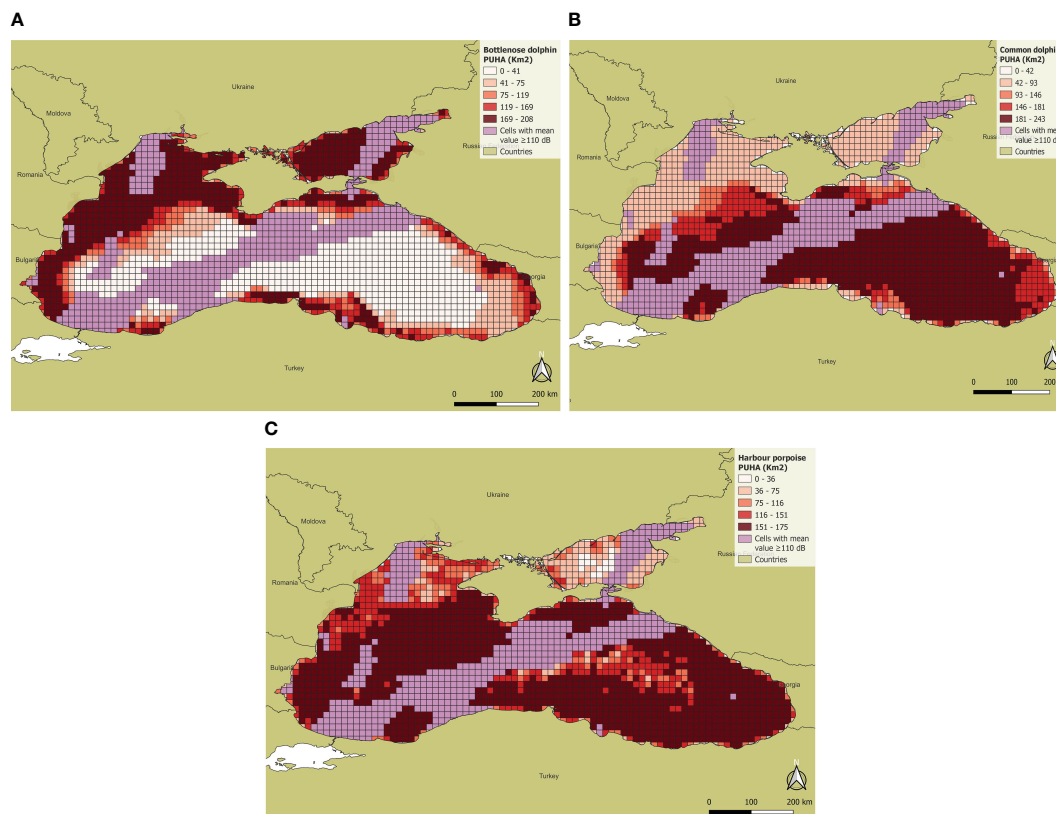


FIGURE 11

PUHA of the three Black Sea cetacean species where pink cells indicate mean noise values equal to or greater than 110 dB: (A) Bottlenose dolphin; (B) Common dolphin; (C) Harbour porpoise.

areas, such as the Northeast Atlantic and the Mediterranean Alboran Sea, this species shows a preference for deep waters, between 400 and 2000 m (Cañadas et al., 2002; Cañadas et al., 2009). As with bottlenose dolphins, common dolphins are also closely linked to the presence of prey. In fact, they prefer pelagic fish in particular Clupeidae and Engraulidae, which are mainly found in the continental shelf areas (Cañadas et al., 2002). Furthermore, the movement and aggregation of fish due to currents and tides attract the common dolphin (Paradell et al., 2019) linking their distribution to prey migrations (Dede and Tonay, 2010; Radu et al., 2013).

The harbour porpoise is a widespread species in the colder, coastal waters of the North Pacific, North Atlantic, and Black Sea (Bjorge et al., 2018). It has also been observed in the western Mediterranean (Cañadas et al., 2005) and northern Aegean Sea (Cucknell et al., 2016). Patterns show a preference for meridional coastal waters, as well as southwestern and southeastern ones, while occurrence values decrease in the northern part of the basin. The slope is an excellent predictor, as the preferences just described refer to the areas where the continental shelf begins and where the mean slope values per cell are highest. For example, within the central eastern part of the basin and in the Sea of Azov, the presence values are lower as the slope tends to decrease. The harbour porpoise is therefore present in both shallower and pelagic waters, with a depth range between 25 and 2200 m. As far as Black Sea Harbour porpoise

is concerned this study habitat models only partially agree with the ones presented by Sánchez-Cabanes et al. (2017), which predict a greater presence in shallow waters below 200 m with preferences for shelf waters between 50 and 150 m, and a predicted habitat similar to that of bottlenose dolphins. However this discrepancy could be the effect of the effort bias deriving from the opportunistic surveys which are the source of their dataset. Birkun et al. (2014) also predicted a secondary habitat in the open sea (deeper than 200 m) in agreement with the results obtained here. In the Azov Sea Birkun et al.'s models also predicted a good probability, agreeing with previously described seasonal migrations in which harbour porpoises occupy the Azov Sea during the warmer months (Kleinenberg, 1956), and abandoning it during the winter (Tzalkin, 1938). Studies in the Atlantic and Pacific Ocean show the presence of harbour porpoise in shallow waters between 20 and 50 m up to a maximum of 150–200 m, bathymetrics corresponding to continental slopes (Read and Westgate, 1997; Raum-Suryae and Harvey, 1998; Marubini et al., 2009; Isojunno et al., 2012). Minor preferences within this range are in waters deeper than 100 m (Carretta et al., 2001; Embling et al., 2010; Isojunno et al., 2012). In Greenland, however, harbour porpoises have been found moving to deeper habitats during winter periods by diving in waters above 400 m (Stalder et al., 2020). In the Black Sea the interspecific competition may have led to a change in harbour porpoise feeding habits favouring more pelagic prey. Harbour porpoises in fact

usually feed on both benthic fish found in shallow waters and pelagic fish (Krivokhizhin and Birkun, 2006; Tonay et al., 2007). Moreover, in addition to the relevance of the habitat models for the better understanding of the species ecology, the possibility to predict the species' potentially usable habitat (PUHA) offers also a great opportunity for assessing the extent of the species habitat which is affected by potentially harmful noise levels, and it may also support the identification of the Level of Onset of Biological adverse Effects, LOBE. By predicting the areas with the highest likelihood of the species presence, habitat models may in fact support their risk exposure assessment to different anthropogenic pressures such as military sonars, shipyard work, vessel traffic, etc. (Azzellino et al., 2012).

In this study, it has been shown how habitat models predictions, combined with noise maps produced through specific shipping noise models, allow to estimate the potential impact of continuous anthropogenic noise on the species potential habitat availability (e.g. PUHA). Vessel traffic is considerably high in the Black Sea, especially in the vicinity of the main ports, along the Western coast and across the basin, as reflected by the corresponding noise maps. Considering as LOBE an SPL of 110 dB re 1 μ Pa for the 63 Hz one-third octave band these noise levels are more concentrated along the routes crossing the basin, connecting the ports of Istanbul, Odessa, Zonguldak, Mariupol and Rostov. It is therefore explainable that the species having the largest portion of impacted habitats are the common dolphin (26.5%) and the harbour porpoise (24.5%), both of which are present in the central part of the basin and in relatively deep waters. The bottlenose dolphin, being more coastal and concentrated in the Western basin shows a slightly lower portion of impacted habitat area, since the species habitat is only limitedly impacted by the shipping routes present in the central part. However, the percentages of impacted habitat area for the three species are overall relatively high, affecting important areas where cetaceans carry out their main survival activities, such as foraging, reproduction and socialisation. Analyses carried out for the western and eastern areas, where traffic and noise data have different values, show that, in any case, common dolphin and harbour porpoise are more present in the western part where noise is higher. This could mean that, although the noise is high, these cetacean species can still manage to use the area. Chlorophyll concentration data clearly show that the Western basin is significantly more productive than the Eastern basin, especially in the summer months (i.e. June and July). The common dolphin has been reported to prefer highly productive areas (Fiedler and Reilly, 1994) and to have larger groups in areas with higher chlorophyll concentrations (Cañadas and Hammond, 2008). As far as harbour porpoises are concerned, several studies have also found a greater species presence in areas with higher chlorophyll and nutrient gradients (Wingfield et al., 2017). In the study by Stalder et al. (2020), anomalies regarding high chlorophyll concentrations in certain areas seem to explain the movements and aggregations of harbour porpoises, as these areas possess oceanographic features that can accumulate primary consumers, making them important foraging areas. In some habitat models built for harbour porpoises in which dynamic

predictors, such as sea state temperature (SST) and chlorophyll concentration, were considered in addition to static predictors (depth and slope), chlorophyll turns out to be a very good predictor of harbour porpoise presence (Gilles et al., 2011). This would seem coherent with our results showing that although noise and traffic levels are high in the Western basin, the target species still use these areas because there is a high primary productivity and they have adapted to tolerate the noise levels present. Several studies have documented cetacean adaptation capabilities to noise, especially with regard to vocalisations and their masking. In fact, many cetacean species appear to have increased frequencies in the emission of vocalisations to continue communicating with conspecifics even in places where noise has become increasingly loud (Lesage et al., 1999; May-Collado and Wartzok, 2008; Parks, 2012). Noise 'tolerance' could be the result of various contexts such as the absence of habitat, excessive costs related to avoidance or, as hypothesised here, the need for individuals and populations to remain in the area (Wright et al., 2007). Such adaptation to loud noise levels is in fact an additional challenge to assess noise effect. The habitat models developed in this study enabled also to design a methodological approach to assess noise impact: equivalent suitable and productive habitats with different noise levels have been in fact compared between the South-Western and the South-Eastern portions of the Black Sea, showing that the noisier habitats have a significantly lower common dolphin encounter rate. It is noteworthy to underline that the statistical difference could be assessed only for suitable habitats having a primary productivity lower than the Black sea median (chl-a \leq 0.539) while there was no statistical difference between high productivity (chl-a $>$ 0.539) suitable habitats.

This result suggests that species have a tendency to avoid environments with high levels of noise when it is not crucial for their foraging activities. However, they demonstrate adaptability when high noise levels coincide with critical foraging habitats. It's important to note that the sample size for the high productivity habitats was significantly limited, particularly in the South-Western basin where cells with such high primary productivity are rare.

It is important to note that noise pollution is not the sole stressor arising from shipping activities. While there is limited documentation on collision risks for dolphins (Schoeman et al., 2020), the avoidance of collisions could be an additional factor that is challenging to separate from noise pollution. Dolphins may tend to avoid areas with high ship traffic to minimize the risk of collisions rather than solely avoiding noise pollution, making it difficult to disentangle the effects of these two factors.

Nevertheless, it is crucial to emphasize that when comparing the noise levels of low productivity cells with the same habitat suitability between the South-Western and the South-Eastern regions (as shown in Table 5), the sound pressure level (SPL) at which the biological response of the species, indicated by a decrease in encounter rate, was presumed to occur ranged from a median noise level of 110 dB to a 95th percentile noise level of 120 dB. This evidence strongly suggests that this noise range can be considered a reasonable Level of Onset of Biological adverse Effects (LOBE).

5 Conclusion

Large-scale synoptic surveys have proven to be valuable and functional for modeling the presence/absence of cetaceans and generating habitat suitability models. Aerial surveys of this nature can effectively bridge data gaps, particularly in areas where even long-term surveys exhibit limitations, thereby providing additional information on Black Sea populations. This study demonstrates the effectiveness of physical predictors, such as depth and seabed slope, in predicting the potential distribution and habitat preferences of cetaceans.

These habitat models are highly valuable for assessing the impact of anthropogenic pressures, such as shipping noise, on specific areas. They can support management efforts by identifying critical habitats that require special protection, thus enhancing local and regional management strategies. Additionally, the application of habitat models facilitates the assessment of species' exposure and risk to noise. It enables the implementation of methodologies, such as the proposed assessment of MSFD D11, and evaluation of the need for mitigation measures such as ship traffic reduction or vessel speed limits in critical areas. These models can also help establish noise levels that should not be exceeded to avoid adverse effects on the populations of the target species (e.g., LOBE).

This study specifically suggests that a sound pressure level (SPL) at a frequency of 63Hz ranging from a median noise level of 110 dB to a 95th percentile noise level of 120 dB can be considered as the threshold for the biological response of the target species (common dolphin), resulting in a decrease in encounter rate. Further investigations and studies are necessary to provide additional evidence supporting this LOBE value and to extend the study to other target species. Nonetheless, the methodology demonstrated in this study, which utilizes habitat models to select equivalently suitable habitats that differ only in noise levels, will be valuable for controlling potential confounding factors when assessing the effects of noise.

Data availability statement

The original contributions presented in the study are included in the article/supplementary material. Further inquiries can be directed to the corresponding author.

Author contributions

Writing—original draft preparation: VF. Writing—review and editing: AM, AP, AA. Designing the case study: AA and VF.

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Provided conceptual details on the realisation of the habitat models: AA. Involved in the realisation of noise maps for the case study: AM. Involved in the organisation and data collection of the CeNoBS project: R-MP. Provided conceptual details on the creation of noise maps and risk assessment: AA, AP, AM and NO. All authors provided comments to improve the manuscript. 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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Matching visual and acoustic events to estimate detection probability for small cetaceans in the ACCOBAMS Survey Initiative

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Estimating the detection probability of small cetaceans using either visual or acoustic surveys is difficult because they do not surface or vocalise continuously and can be imperceptible to an observer or hydrophone. Animals seen at the surface may have lower vocalisation rates, while submerged individuals may be more vocally active. This study aims to estimate visual, acoustic and combined detection probability by using Mark-Recapture Distance Sampling (MRDS) methodology. We used vessel-based visual sightings and acoustic data (based on click identification) collected simultaneously during the ACCOBAMS Survey Initiative in summer 2018 onboard the *R/V Song of the Whale*. This study focused on small cetaceans in the Mediterranean Sea, including the most commonly-encountered species, the striped dolphin (*Stenella coeruleoalba*). We identified duplicate events between visual and acoustic platforms using a decision tree based on time and distance thresholds to estimate $g(0)$ (the detection probability on the trackline) for small cetaceans. A total of 30 duplicate events were identified from 107 and 109 events identified by the visual and acoustic platforms respectively. We tested the models with two key functions. With a hazard-rate key function, the $g(0)$ was estimated at 0.52 (CV=21.0%) for both platforms combined, 0.29 (CV=25.6%) for the visual platform and 0.32 (CV=25.1%) for the acoustic platform. With a half-normal key function, $g(0)$ was estimated at 0.51 (CV = 21.7%) for both platforms combined, 0.29 (CV = 25.6%) for the visual platform and 0.33 (CV = 23.2%) for the acoustic platform. Our results illustrate that passive acoustic monitoring can be used as an independent platform in MRDS to estimate the detection probability. Our estimate of $g(0)$ was well below 1, far from the perfect detection commonly assumed for abundance estimation. Without correction for detection biases, total abundance would be underestimated by a factor of two when using both acoustic and visual data. This highlights the importance of using dual-platform surveys to estimate detection probability in order to improve abundance estimates and conservation efforts.

KEYWORDS

detection probability, duplicate events, mark-recapture distance sampling, dual platform, passive acoustic monitoring, line-transect surveys

1 Introduction

Marine megafauna species, which have long life spans, low fecundity rates and high longevity (McCauley et al., 2015; Sequeira et al., 2019), are particularly sensitive to anthropogenic pressures and consequently face conservation risks (Bossart, 2011). Although positive actions have been taken for the conservation of populations (Alves et al., 2022), they are still exposed to a wide range of anthropogenic threats such as over-exploitation, underwater noise or bycatch, which affect their vital rates and population viability (Bossart, 2011; New et al., 2014; Pirotta et al., 2018). As a result, many populations of marine megafauna currently have a critical conservation status (Lascelles et al., 2014).

Conservation status assessment is based on information on the abundance, population status and natural history of the species, as well as anthropogenic pressures, all of which may vary over time. Estimating cetacean abundance is a difficult task, as they are mobile and elusive species that spend most of their time below the sea surface, over extended home ranges. Due to these difficulties, opportunities to detect cetaceans in a study area (hereafter called events once detected) may be missed, leading to a biased estimate of population abundance. While most cetacean conservation programs use minimum abundance estimates to take conservation measures (Evans and Hammond, 2004), unbiased absolute abundance is essential to identify acceptable levels of human-caused mortality for cetaceans (Punt et al., 2021). For example, bycatch is the most common source of cetacean mortality (Reeves et al., 2013) and assessing the sustainability of cetacean bycatch can allow the development of effective conservation and management measures (Parra et al., 2021).

Line-transect and mark-recapture methods are two well-known techniques for estimating cetacean population abundance (Buckland et al., 2001; Hammond, 2018). Mark-recapture methods can be applied using different techniques such as acoustic devices (Marques et al., 2012), DNA sampling (Mills et al., 2000) or photo-identification (Genov et al., 2008). The latter is the most commonly used technique, using natural markings on animals to identify cetaceans, and patterns of recaptures in the form of capture histories to estimate the number of undetected animals and hence population size (Hammond, 2018). Photo-identification based mark-recapture technique is effective for studying small coastal populations of cetaceans in a limited study area. In contrast, the line transect method, which is based on distance sampling techniques, does not require the identification of individuals and is generally applied over large study areas, while allowing for the simultaneous monitoring of several species (Buckland et al., 2004; Daura-Jorge and Simões-Lopes, 2017). This method relies on the visual and/or acoustic detection of events collected by observers/recorders on a platform moving along a predetermined linear route in a study area. Observers record visual and/or acoustic detection events, i.e. each event in which an individual or group of individuals is encountered, the radial distance of the event from the observer/recorder and the angle between the vessel's bow and the animals. For each event, the radial distance and angle are used to calculate the perpendicular

distance from the transect line. A detection function $g(x)$, which is the probability of detecting an animal given its distance x from the line, is estimated from the distribution of the perpendicular distances. Knowing this probability of detection, the number of events and the size of the covered region, density and abundance of the population and the effective strip width (ESW; the number of objects detected beyond this distance is equal to the number of objects missed before this limit) can be estimated (Buckland et al., 2015). Conventional Distance Sampling (CDS) usually requires three assumptions to be met: (1) all animals on the transect line are detected, usually referred to as $g(0) = 1$, (2) animals are detected at their initial location and (3) distance measurements are accurate. The assumption $g(0)=1$ has been commonly used to estimate cetacean abundance (Barlow, 2006; Øien, 2009; Dick and Hines, 2011; Hildebrand et al., 2015) despite the fact that many animals spend a significant proportion of the time submerged and might hence be missed, potentially inducing a negative bias in abundance estimates.

Abundance estimation is indeed closely linked to the detectability of individuals during surveys (McCarthy et al., 2013). Detectability can be affected by both an availability bias and a perception bias (Laake and Borchers, 2004), constituting together the detection biases. Availability bias corresponds to the unavailability of animals for detection, either because they are below the sea surface, which depends on the time spent at depth by the species and the platform speed, or because they are not acoustically active, which depends on the vocalisation rate of the species. Perception bias accounts for the non-detection of available animals by observers or recorders due to imperfect observer vigilance or unfavourable conditions for detection (e.g. high sea states). Multi-Covariate Distance Sampling (MCDS) partially addresses detection biases using covariates in the detection function (Marques and Buckland, 2003). However, estimating the detection probability of small cetaceans remains difficult and the assumption that all animals are detected on the transect line can be violated due to both availability and perception biases. If they are not accounted for, the estimated abundance will be negatively biased (Saavedra et al., 2018).

To provide more accurate abundance estimates, the Mark-Recapture Distance Sampling (MRDS) method deals with missed detections at distance zero by estimating $g(0)$. This method requires two independent platforms scanning the same area simultaneously, which are used to 'mark' animals (Laake and Borchers, 2004). The $g(0)$ and overall detection probability are estimated by using the number of animals seen by each platform and by both platforms (duplicate events). Most dual platform line-transect surveys use two visual platforms, either with a single survey platform or with two separate survey platforms (Cañadas et al., 2004; Hammond et al., 2013; Laran et al., 2017; Lambert et al., 2019). The number of surveys using an acoustic platform with a towed hydrophone array as a second platform has increased in recent years (Barlow and Taylor, 2005; Boisseau et al., 2010; Richman et al., 2014; Martin et al., 2020; Rankin et al., 2020; Dalpaz et al., 2021). The acoustic platform has the advantage of detecting cetaceans even when they are not visible at the surface, and thus not available to the visual platform. These two methods are complementary and can improve the overall detection efficiency (Verfuss et al., 2018). This kind of

analysis can however be challenging due to the complexity of identifying events; acoustic events are typically validated during post-field analysis, while visual events are recorded directly during the survey.

The ACCOBAMS Survey Initiative (ASI) vessel component, conducted in the summer of 2018 with the *R/V Song of the Whale* in the western and central Mediterranean Sea, was a unique survey using a visual-acoustic dual platform (ACCOBAMS, 2021). The ASI was carried out under the auspices of the Agreement on the Conservation of Cetaceans of the Black Sea Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) to estimate cetacean abundance in the entire Mediterranean Sea. This region is exposed to increasing levels of anthropogenic impacts (Coll et al., 2012) and is home to cetacean populations that are often distinct from their nearby Atlantic counterparts. Numerous studies have been conducted in the Mediterranean Sea to estimate the abundance of cetaceans (Laran et al., 2017; Panigada et al., 2017; Lewis et al., 2018) but survey efforts have been recognised as heterogeneous across the basin (Mannocci et al., 2018). Some species, like the Risso's dolphin (*Grampus griseus*) or the rough-toothed dolphin (*Steno bredanensis*), have received less attention than others (Boisseau et al., 2010; Kerem et al., 2012; Luna et al., 2022) and some areas have been studied more extensively, especially within the western basin (Mannocci et al., 2018). Therefore, the ASI project aimed at filling these data gaps.

In this study, we aimed to use this visual-acoustic dual platform to estimate the detection probability of small cetaceans in the Mediterranean Sea. Small cetaceans studied here include the bottlenose dolphin (*Tursiops truncatus*), the long-finned pilot whale (*Globicephala melas*), the Risso's dolphin, the rough-toothed dolphin, the common dolphin (*Delphinus delphis*), the striped dolphin (*Stenella coeruleoalba*) and unidentified dolphins. This diverse community is largely dominated by the striped dolphin (ACCOBAMS, 2021). We only included transects from the ASI survey where visual and acoustic data (based on echolocation clicks) of small cetaceans were collected simultaneously. In this study, we did not go as far as estimating cetacean abundance because the transects where visual and acoustic data were collected simultaneously did not cover the study area uniformly, and therefore these transects were not a representative sample of the species distribution (Buckland et al., 2004). In addition, we did not estimate group size in our study, which is necessary for absolute abundance estimation. Although group size is a key aspect of abundance estimation, we did not fully address it in this study as it was primarily focused on estimating detection probabilities. To estimate detection probability, we first developed a new methodology to identify duplicate events based on a decision tree (rule-based classification). Then, we implemented an MRDS approach with covariates affecting detectability on the prior identification of visual/acoustic event duplicates. The MRDS analysis allowed the detection probability and ESW of the visual, acoustic and combined platforms to be estimated, taking detection biases into account. We expected to obtain a $g(0)$ lower than 1, since detection cannot be considered as perfect for either platform, with a number of the animals on the transect

line inevitably being missed. This paper aims to provide estimates of detection probabilities to be used to estimate the abundance of small cetacean populations, and to demonstrate the benefits of systematically using a dual platform in multi-species line-transect surveys.

2 Materials and methods

2.1 Studied species

This study focused on small cetaceans in the Mediterranean Sea which included: the bottlenose dolphin, the long-finned pilot whale, the Risso's dolphin, the rough-toothed dolphin, the common dolphin and the striped dolphin. The echolocation clicks of these species share similar frequency ranges (Table 1), making species identification difficult (Soldevilla et al., 2008), hence all studied species were pooled for the MRDS analysis. In addition, there were likely too few encounters with species other than striped dolphin to generate robust detection functions for individual species. Striped dolphins, with 52% of the sightings during the vessel survey (ACCOBAMS, 2021) and 85% during the aerial survey (Panigada et al., in review), were the most prevalent small odontocetes in the Mediterranean Basin. These species do not share the same behaviours (i.e. group size, habitat preference, dive duration, responses to the boat) and by grouping all small odontocetes in the same analysis, we assumed that all species behave in the same way in terms of visual and acoustic events; this simplification will be discussed later on.

TABLE 1 Frequencies of peaks energy characteristics (kHz) for each studied species.

Species	Peak(s) energy (kHz)	References
Bottlenose dolphin	120-130 60-140 35-60 33-109 40-80 24-30 40-120	Au et al., 1974* Akamatsu et al., 1998* Diercks et al., 1971 Wahlberg et al., 2011* Soldevilla et al., 2008 Baumann-Pickering et al., 2010 Finneran et al., 2014*
Long-finned pilot whale Short-finned pilot whale (behaviourally similar sp.)	50 40	Eskesen et al., 2011* Pedersen et al., 2021*
Risso's dolphin	22-39 50	Soldevilla et al., 2008 Madsen et al., 2004*
Rough-toothed dolphin	18-30	Rankin et al., 2015
Common dolphin	40-80 23-67	Soldevilla et al., 2008 Evans, 1973
Striped dolphin	Striped dolphin clicks have not been described but we assumed they produce in the same frequency range as bottlenose and common dolphin	

*Studies that included only "on-axis" signals.

2.2 Survey design

The study area covered the Alborán Sea/Strait of Gibraltar, the Algero-Provençal Basin, the Tyrrhenian Sea/eastern Ligurian Sea, the Strait of Sicily/Tunisian Plateau/Gulf of Sirte, and the Ionian Sea/Central Mediterranean (from 6°W to 36°E and from 30°N to 46°N; Figure 1). The survey area was divided into 21 blocks. It was conducted using *R/V Song of the Whale*, a 21 m acoustically quiet vessel. The vessel operated with minimal disturbance to marine life by using noise-reduction devices, including vibration-damping mounts for the main engine and a five-bladed propeller. Some transects were surveyed using either a visual or an acoustic platform, while others were surveyed using with both platforms. In this study, we focused on data collected with both platforms between May and September 2018 at an average speed of 3.2 m/s (6.2 knots; Figure 1). Transects were designed with the *Distance* software (Thomas et al., 2010) as equal-spaced zig-zag transects to provide uniform coverage probability. The ASI survey used a line-transect sampling protocol and visual observers recorded the location, environmental conditions, group size and perpendicular distance to the animal(s) from the transect line (Buckland et al., 2001). Transects were divided into legs with constant environmental conditions, and each leg was finally divided into 5 km segments for analysis.

2.3 Visual data collection

Visual data were collected during daylight hours, whenever the observation conditions were appropriate (i.e. Beaufort sea state ≤ 4). During the visual effort, two observers were positioned on a 5 m elevated platform to search for cetaceans. Each observer was on either side of the platform: the first observer scanned the starboard side from 340° to 90° and the second observer scanned the port side from 270° to 20° with 0° representing the bow. They observed the respective areas with naked eye and used binoculars to confirm species identity if required. For each visual event, observers

provided information to a dedicated data logger using the Logger software package (www.marineconservationresearch.org). Information about species, group size, bearing angle and distance from the boat (between the observer and the centre of the group) were recorded for each visual event. Bearings and distances were estimated by eye and group sizes were reported as best, high and low estimates of the number of animals for each visual event. In order to improve effectiveness of and consistency between the observers, distance trials and training on the survey protocol were carried out prior to the survey. Additional information was recorded on cue type and animal behaviour. When necessary, the vessel interrupted the transect line to approach animals for species identification or photo-identification before returning to the transect line at the point where it was left. Information on environmental conditions i.e. Beaufort sea state (out of 9), wave and swell heights (m), cloud cover (percentage scale; out of 10), glare, visibility (from 1 low visibility to 3 good visibility), wind direction and speed (m/s) and boat speed (m/s) were logged every hour or whenever conditions changed by the data logger member. The Logger software automatically registered the GPS position, heading and boat speed every 10 seconds. The visual survey team, consisting of at least 5 members, rotated between different roles that lasted no more than one hour: port observer, starboard observer and data logger for three hours, followed at least by two hours of rest to avoid fatigue. Any duplicate sightings made by both observers in the overlap area have been removed.

2.4 Acoustic data collection

The acoustic survey was conducted 24 hours a day with a towed hydrophone array capable of detecting all cetacean species. A 400 m tow cable was used to avoid any boat self-noise; at this distance, any vessel noise, such as propeller cavitation, was imperceptible to both human ear and the detection algorithms described below. Acoustic effort took place when Beaufort sea state was below 5 and local water depths were above 50 m. The hydrophone array was housed

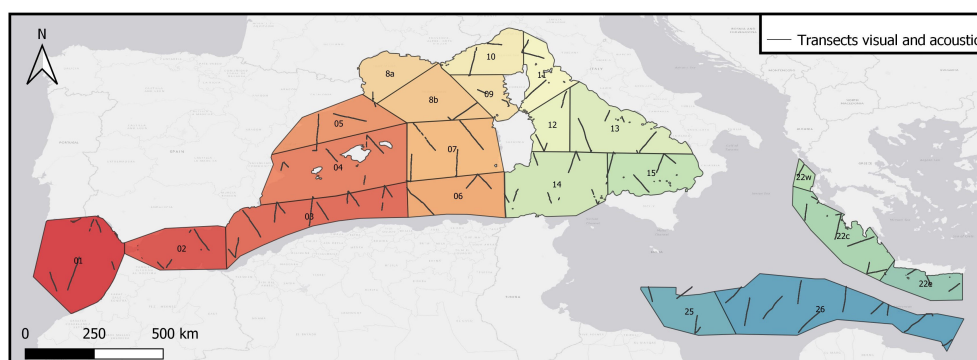


FIGURE 1

Study area covered by the ASI survey in 2018. The area was divided into 21 blocks: 1) Gulf of Cadiz, 2) Alborán, 3) Algeria West, 4) Balears, 5) North East Spain, 6) Algeria East, 7) West Sardinia, 8a) Gulf of Lion shelf, 8b) Gulf of Lion deep, 9) Pelagos South West, 10) Pelagos North West, 11) Pelagis East, 12) Tyrrhenian Central West, 13) Tyrrhenian Central East, 14) Tunisia North, 15) Tyrrhenian South East, 22w) Hellenic Trench West, 22c) Hellenic Trench Central, 22e) Hellenic Trench East, 25) Libya East and 26) Libya East. The black lines represent the transects sampled in dual-platform mode (simultaneous visual and acoustic effort).

in an oil-filled tube and was composed of a pair of hydrophones in a linear configuration. The two hydrophone elements (Magrec HP03), spaced 0.25 m apart, had a sensitivity of -204 dB re 1V/ μ Pa with a flat frequency response (± 3 dB) from 1 to 100 kHz in the frequency band of most odontocete vocalizations. Pre-amplifiers with 29 dB gain were used to prevent voltage drop between the array and the research vessel. The outputs of the pair of hydrophones were digitised at a sample rate of 192 kHz and were monitored in real-time using a click detector in PAMGuard, a passive acoustic monitoring software package (Gillespie et al., 2008). Audio recordings were stored as 16-bit wav files. Outputs from the click detector were compressed into binary storage files in PAMGuard and these files were manually analysed *post-hoc* with a focus on small odontocetes.

2.5 Acoustic data analysis

2.5.1 Echolocation click identification

The small cetaceans considered here have a rich vocal repertoire, producing clicks, whistles, burst pulses and many other vocalisations (Janik, 2009). Here we focused only on echolocation clicks for multiple reasons. Echolocation clicks are produced by all odontocetes and are the predominant vocalizations of toothed whales (Klinck and Mellinger, 2011). Echolocation click trains can be used to derive an estimation of the perpendicular

distance which is key when applying a distance sampling framework (see below). In contrast, whistles are omnidirectional vocalizations (Jensen et al., 2012) without clear onset/offset, and as such are less suitable for estimating perpendicular distances (Amorim et al., 2022).

We defined an acoustic event as a sequence of clicks that was produced by the same animal or group of animals and showed a consistent change in bearing (e.g. when a vocalising cetacean passed from in front to behind the hydrophone array; Figure 2). Sequences of clicks that did not show a consistent trajectory of bearings (e.g. clouds of clicks) or did not cross the 90° line (e.g. detections that did not move past the array) were not considered as acoustic events.

To confirm that an acoustic event was likely produced by a small cetacean, clicks were identified by eye based on peak frequency, the width of the peak frequency, and the number of zero crossings. We hypothesized that impulsive signals between 10 and 80 kHz were most likely produced by a cetacean. Echolocation clicks are short, broadband pulses varying from 10 to 150 kHz for many species (Au, 1993). Odontocetes emit echolocation clicks in a narrow beam projected forward from the melon and the signal energy is more focused in the forward direction (“on-axis”) than to the sides (“off-axis”; Au et al., 1978). Most studies focus on understanding clicks produced “on-axis” (Table 1), i.e. when the animals are facing the hydrophone where the transmission loss of the signal is minimal, which is not typically applicable to the field conditions when recording free-ranging cetaceans (Soldevilla et al.,

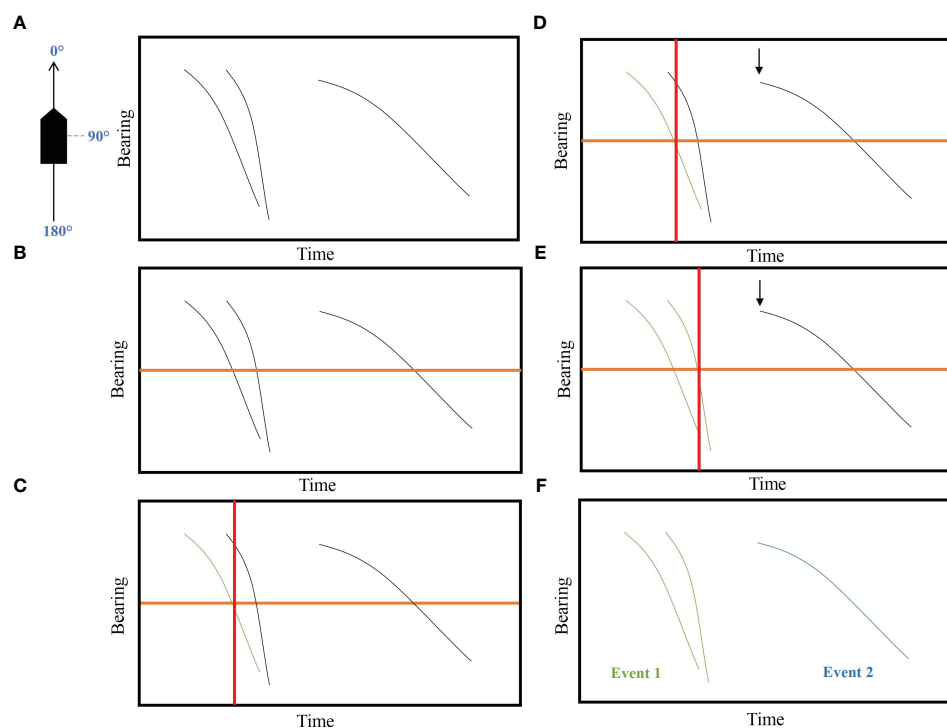


FIGURE 2

Protocol for determining acoustic events using PAMGuard Click detector module. (A) Determination of the number of events. (B) Set a 90° reference line representing the bearing of 90° of the boat. (C) Set a perpendicular line where the first track is crossing the 90° reference line. (D) If the followed track starts before the perpendicular line of the 1st track, it will be considered as the same event. (E) If we set a perpendicular line on the 2nd track, the 3rd track starts after the perpendicular line, this would be considered as a distinct event. (F) In conclusion, the first two tracks are the same event but probably from two different animals and the 3rd one is another acoustic event.

2008; Baumann-Pickering et al., 2010). Signal waveforms can vary strongly between “off-axis” and “on-axis” clicks. As the higher frequencies attenuate faster than lower frequencies, the higher frequency peaks in clicks recorded “off-axis” (or clicks recorded from distant animals) may not be evident. Although acoustic events, in this study, can be either recorded “off-axis” or “on-axis” depending on the swimming direction of the animals relative to the hydrophones, most free-ranging animals are not likely to produce clicks “on-axis” of the hydrophone (Miller, 2002). Therefore, we expected to record echolocation clicks with lower bandwidths than those reported in Table 1 (marked by *), which included only “on-axis” clicks. The frequency range expected from small cetaceans corresponded to those of the ASI hydrophones which recorded sounds up to 92 kHz.

2.5.2 Localisation

A sequence of clicks can be used to derive an estimation of the perpendicular distance using the Target Motion Analysis (TMA) module’s 2D simplex method implemented in PAMGuard. The bearing angle of each detected click was determined using the time delay of arrival of a signal detected by the pair of hydrophones. Convergence of successive bearing lines was used to locate the sound source and allows the estimation of the distance perpendicular from the crossing point. The TMA module attempted to resolve left/right ambiguity using variation in the vessel’s course.

The number of acoustically active individuals per acoustic event was determined as the number of tracks that occurred at the same time. This method allowed the number of vocalising animals to be counted instead of the number of vocalisations (Akamatsu et al., 2018; Richman et al., 2014). As for visual events, acoustic group sizes were reported as best, high and low estimates of the number of animals for each event.

2.6 Identification of duplicate events

For this study, transects where visual and acoustic data were simultaneously collected were retained to identify duplicate events collected by the visual and acoustic platforms. The MRDS analysis is based on the reliable identification of duplicate events between the two platforms. Two events are considered duplicates when the same group of individuals is detected both visually and acoustically. For this purpose, a two-step decision tree (i.e. a rule-based classification system) was elaborated based on a set of temporal and spatial criteria determining how close in time and space a visual and an acoustic event were recorded. A putative duplicate was a pair of events consisting of a visual and an acoustic event to be tested with the decision tree. A putative duplicate became a potential duplicate then a confirmed duplicate when the first and the second step of the decision tree were respectively passed successfully.

2.6.1 1st step of the decision tree: time threshold

The time window in which a pair of visual and acoustic events can originate from the same group of individuals was defined by

considering that the two platforms, visual and acoustic, were 400 m apart, had a specific detection radius and were moving at the vessel speed (Figure 3). The visual buffer was defined as the 270°-90° sector scanned by the observers in front of the vessel with a radius calculated as 95% of the distribution of the visual perpendicular distances recorded during the survey (here 2,107 m). The acoustic buffer was a disk centred on the hydrophone array with a radius calculated as 95% of the distribution of the acoustic perpendicular distances recorded during the survey (here 1,266 m).

When a first event was identified by either platform, we calculated the minimum and maximum time lags (Δt_1 and Δt_2) for an event from the second platform to be identified in the corresponding buffer, assuming that the detected animal was stationary.

The time lag Δt (in s) was determined by:

$$\Delta t = \frac{L}{v}$$

where L is the maximum distance (in m) travelled by the vessel between an acoustic event (v_s visual event) and a subsequent visual event (v_s acoustic event) forming a putative duplicate. It was estimated as 866 m (1,266 m – 400 m) when the acoustic event came first and 3,773 m (1,266 m + 400 m + 2,107 m) when the visual event came first. Based on an average speed boat of 3.2 m/s (6.2 knots), $\Delta t_1 = -271$ s and $\Delta t_2 = 1,179$ s. Hence any pair of records in which the acoustic event occurs within 271 s before and 1,179 s after the visual event was considered as a potential duplicate.

2.6.2 2nd step of the decision tree: distance threshold

Once potential duplicates have been identified with the temporal criteria related to the platform structure and movement, another threshold was applied to account for the potential movement of the target animal(s) between the two events of the potential duplicate. A mobility buffer was thus estimated based on published values of the routine swimming speeds of small cetaceans. This mobility buffer represented the area centred on the first record of the potential duplicate in which the target animal(s) could be at the time of the second record if they were moving at a routine swimming speed, assuming no responsive movement relative to the vessel. If the location of the second record of a potential duplicate fell within the mobility buffer, the potential duplicate became a confirmed duplicate (Figure 4). The radius of the mobility buffer (in m) was calculated as:

$$r = \Delta t \times v_r$$

where Δt was the time lag (in seconds) between a visual and an acoustic event and v_r the routine swimming speed of the animal (in m/s). The routine speed was determined from the literature and the value corresponding to the 95% quantile of the distribution was chosen, i.e. 3.6 m/s (7 knots; Supplementary Table 1; Pilleri and Knuckey, 1969; Saayman et al., 1972; Würsig and Würsig, 1979; Hui, 1987; Tanaka, 1987; Williams et al., 1992; Ridoux et al., 1997; Wood, 1998; Fish and Rohr, 1999; Yazdi et al., 1999). Given the

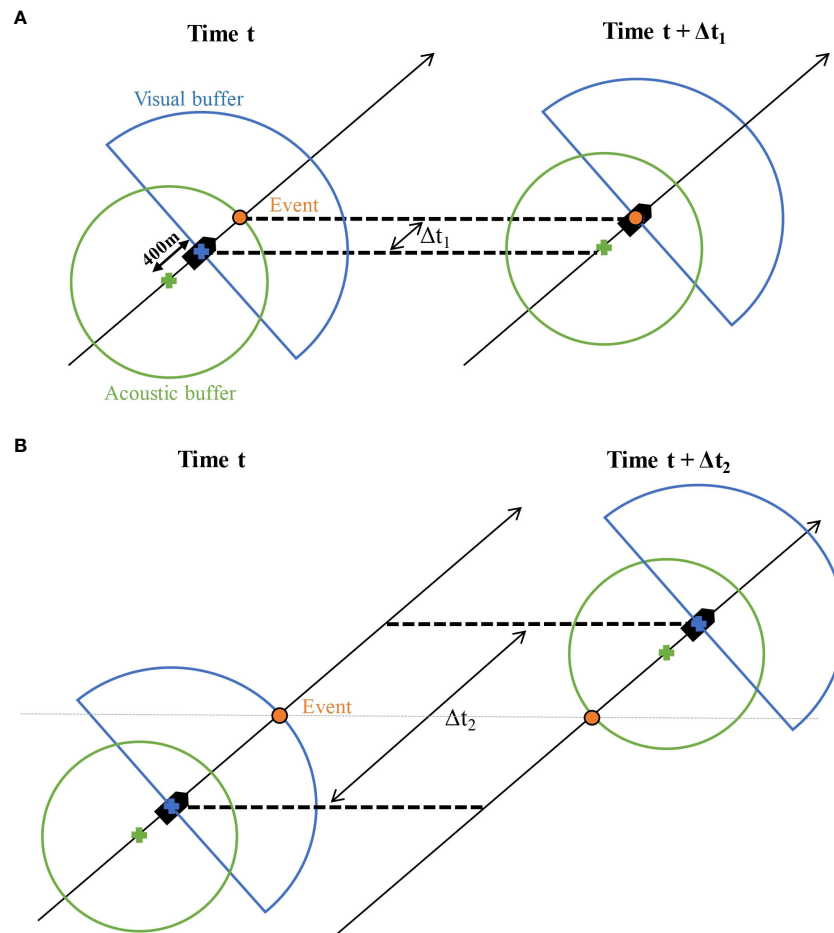


FIGURE 3

First step of the decision tree: the platform-related time lag or dual. The visual semi-buffer (blue) was centred on the vessel (blue cross) and the acoustic buffer (green) was centred on the hydrophone (green cross). A potential duplicate event was considered if a visual and an acoustic event were close in time (between Δt_1 and Δt_2). **(A)** The minimum time lag Δt_1 would correspond to the same group of small cetaceans being acoustically detected as early as possible by the hydrophone array and visually sighted as late as possible by the onboard observers. **(B)** The maximum time lag Δt_2 would correspond to the same group of small cetaceans being visually sighted as early as possible by the onboard observers and acoustically detected as late as possible by the hydrophone array. All intermediate situations between A and B would be accepted as potential duplicates.

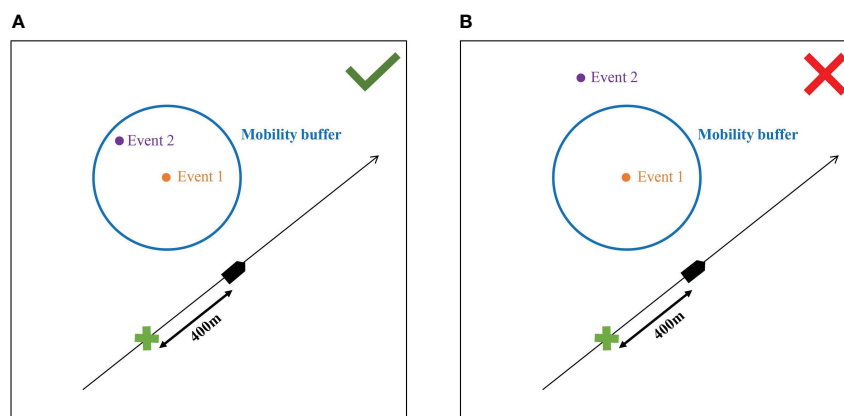


FIGURE 4

Second step of the decision tree: the animal-movement-related spatial lag or mobility buffer. The black line is the surveyed transect. The hydrophone array (green cross) was located 400 m behind the vessel (black pentagon). The mobility buffer is centred on the first event by either platform (blue circle). The status of confirmed duplicate was accepted when the 2nd event was within the mobility buffer **(A)**. The status of confirmed duplicate was rejected when the 2nd event was outside of the mobility buffer **(B)**.

potential left/right ambiguity of the localised acoustic event, we assumed that the acoustic event of a potential duplicate was on the same side as the corresponding visual event.

After passing the two steps of the decision tree, multiple replicate events could be confirmed (e.g. one visual event could correspond to up to three different acoustic events). Among these replicate events, we selected the one with the shortest distance difference to limit the responsive movement of the animal(s) (e.g. if the visual event's distance was 200 m, and the distances for the three acoustic events were 10 m, 100 m and 1000 m, the acoustic event with a distance of 100 m is chosen). The rest of the replicate events were deleted. In the end, a confirmed duplicate event was finally composed of one visual and one acoustic event. For each duplicate event, we selected the highest "best" group size estimate and the larger distance value between the visual and acoustic event.

2.7 Detection function and effective strip width

The detection function and $g(0)$ were calculated using data from both visual and acoustic platforms using the MRDS method (Laake and Borchers, 2004). Events from each platform were set as trials for the other. If events of the same group of animals were made by both platforms (confirmed duplicate, see above), they were considered successful, if not, they were considered failed. There are two possible assumptions in MRDS (Burt et al., 2014): full independence (FI) or point independence (PI). The FI assumption considers the two platforms to be independent at any distance and thus a mark-recapture (MR) model is applied at all distances (including distance zero) to estimate the overall detection probability p . It appears this assumption generates negative biases in abundance estimates in case of failure of the independence assumption (Laake et al., 2011; Burt et al., 2014). The failure can be identified if the abundance estimate is lower than the abundance estimates from the CDS, resulting in the presence of heterogeneity in detection probabilities which generates biases in abundance estimates (Buckland et al., 2010). Attempts have been made to minimise the unmodelled heterogeneity for example by including the effect of covariates in the detection function (Borchers et al., 1998; Laake and Borchers, 2004). To provide a solution to the problem of unmodelled heterogeneity, the concept of PI assumption was introduced by Laake (1999). The PI assumption considers the two platforms to be independent only at distance zero and reduces the impact of unmodelled heterogeneity on detection probability estimates (Buckland et al., 2010). The PI assumption uses a combination of MR and DS submodels. The MR submodel is used to estimate $g(0)$, while the DS submodel is used to estimate the decrease in detectability with distance, and these two are combined to get the overall detection probability p (Burt et al., 2014). In our study, the visual and acoustic platforms were beforehand considered independent but we did not know if this independency was valid for all distances or only at distance zero, so we tested MRDS models with both FI and PI assumptions. Factors affecting the detectability for both platforms were investigated and included in the model to improve precision (Marques and Buckland, 2003; Buckland et al.,

2004). We wanted to assess the effect of the platform on the detectability, so the platform was included as a covariate in all tested models. We also tested the effect of the sea state (0, 1, 2, 3, 4 Beaufort scale) as well as wave and swell height (values in metres grouped into classes: 0 to 0.5 m, 0.6 to 1 m, 1.1 to 1.5 m and 1.6 to 2 m). Single covariates and combinations of two covariates were tested. For the DS model, we tested models with a hazard-rate (HR) and a half-normal (HN) key functions and with or without covariates. The right truncation distance was set at 1,500 m, based on the inspection of the histogram of detection frequencies plotted against distances from the transect line (Buckland et al., 2001). Only segments conducted with good conditions (i.e. Beaufort sea state ≤ 4) were considered in the analysis. The decision was made to exclusively focus on Beaufort sea state due to the fact that visibility, swell and wave height are highly subjective metrics. We tested all models with FI and PI assumptions, with HR and HN key functions for the DS submodel, and combinations of covariates. The Akaike Information Criterion (AIC, Akaike, 1998) and the goodness of fit were used to select the best model fit for each key function. The MRDS analyses were performed in R 4.0.3 using the *mrds* package (Laake et al., 2013). Finally, to estimate the ESW, the overall detection probability was multiplied by the truncation distance (Buckland et al., 2004).

2.8 Sensitivity analysis of the decision tree

Correctly classifying events as unique or duplicate is a critical step in MRDS analysis (Hamilton et al., 2018). We investigated the effect of the thresholds considered in the two-step decision tree on the number of duplicates and on the detection probability. We tested different values of the distance distribution used to set the visual and acoustic buffers in the first step of the decision tree: 70%, 80%, 90%, and 100% based on a fixed value of the routine swimming speed, which was 3.6 m/s (7 knots). For the second step of the decision tree, different routine speeds were found in the literature, ranging from 1.6 to 4.2 m/s (3.1 and 8.2 knots). We analysed the effect of the routine speed by testing different values: 1.60, 2.25, 2.90, 3.55 and 4.20 m/s (3.11, 4.37, 5.64, 6.90, 7.00, 8.16 knots) with a fixed value of the distance distribution of 95%. For each value of the distance distribution and the routine speed, the number of duplicate events and $g(0)$ were estimated, as well as the overall detection probability using half-normal and hazard-rate key functions. This sensitivity analysis allowed comparison of the number of duplicate events and detection probability estimates obtained to assess the effect of using of the values in the decision tree.

3 Results

3.1 Summary of survey data

Transects where acoustic and visual data were simultaneously collected in good conditions (when Beaufort sea state ≤ 4) totalled 6,679 km of effort (Figure 1). The lengths and average speed of surveyed transects for each survey block are given in Supplementary

Table 2. A total of 108 visual and 122 acoustic events of small cetaceans were identified. Species identification based on the visual survey component is shown in [Table 2](#). The striped dolphin was the most frequently observed species and represented 52.8% of the total visual events. The second most observed species was the short-beaked common dolphin (11.1%) followed by the bottlenose dolphin (4.6%). The Risso's dolphin (2.8%), the long-finned pilot whale (0.9%), and the rough-toothed dolphin (0.9%) were occasionally observed during the survey. About a quarter of the visual events could not be identified to the level of species (26.9%). The estimated “best” group size was higher in visual events 9.9 ± 14.0 individuals than in acoustic events 3.8 ± 1.5 individuals.

3.2 Matching visual and acoustic events

A total of 87 potential duplicates were identified after the first temporal threshold was applied, reducing to 44 confirmed duplicates after applying the second spatial threshold. By selecting a single visual and acoustic event for each match, we finally obtained 30 unique duplicate events, which represent 16% of the total events. The visual platform recorded 77 events that the acoustic platform did not. Conversely, 79 events were identified by the acoustic platform only. Among the duplicate events, the species most observed by the visual platform was the striped dolphin (56.7%) followed by the common dolphin (16.7%) and unidentified dolphins (13.3%). Not surprisingly, bottlenose (6.7%), Risso's (3.3%) and rough-toothed (3.3%) dolphins were rarely seen in the duplicate events. Under the assumption that unidentified dolphins have the same species composition as the identified dolphins, about 63% of the duplicate events would be striped dolphins.

3.3 Estimation of the detection probability

A truncation distance was set at 1,500 m, which removed 10 unique visual, 2 unique acoustic and 2 duplicate events,

TABLE 2 Number of visual events for each studied species.

Species	Number of visual events	Part of the total visual events (%)
Bottlenose dolphin	5	4.6
Long-finned pilot whale	1	0.9
Risso's dolphin	3	2.8
Rough-toothed dolphin	1	0.9
Common dolphin	12	11.1
Striped dolphin	57	52.8
Unidentified dolphin	29	26.9

representing 7.5% of the total events ([Figure 5](#)). Thus, in total, 67 unique visual, 77 unique acoustic and 28 duplicate events were used to fit the detection function in the MRDS method.

Based on the AIC and the goodness of fit, the PI model with a HR key function was the best model ([Supplementary Figure 1](#) and [Supplementary Table 3](#)). The MR submodel included perpendicular distance, swell height and platform as covariates. The DS submodel with the HR key function included sea state only. However, the model did not appear to fit the data well and overestimated detectability close to the transect line ([Figure 6](#)). Although the fit of the PI model with a HN key function was not optimal, it had a better fit to the data ([Figure 6](#)) and could therefore be considered as a more conservative model for estimating detection probabilities.

For both key functions, all parameters of the detection function were negative ([Table 3](#)), such that the detection probability decreased with increasing perpendicular distance from the transect, swell height and sea state (the further away from the vessel and the worse the conditions, the more difficult it is to detect the animals). However, $g(0)$ was fixed in all conditions. The detection probability in the MR submodel was slightly higher with the acoustic platform than with the visual platform (parameter estimate for the visual platform equal to -0.14), but the standard error (SE) was high relative to the parameter estimate, so the effect of the platform was marginal ([Table 3](#)) which means their effect on the detectability of small cetaceans was negligible. In contrast, the SE was low for the distance and swell height covariate in the MR submodel and for the sea state in the DS submodel ([Table 3](#)). These results highlighted the strong effect these covariates have on the detectability of small cetaceans on the transect line.

The number of events identified by the visual and acoustic platforms did not differ greatly and duplicate events occurred mainly within 500 m of the transect line. However, the acoustic platform detected more events on the transect line and at a greater distance ([Figure 7](#)). Indeed, the $g(0)$ was slightly higher for the acoustic platform from 0.32 (CV = 25.1%) to 0.33 (CV = 23.2%) depending of the key function ([Table 4](#)). The $g(0)$ of the visual platform was estimated between 0.29 (CV = 25.6%) and 0.30 (CV = 23.8%). Finally, by using combined platforms, we obtained a $g(0)$ of 0.52 (CV = 19.8%) with the HR key function and 0.51 (CV = 21.7%) with a HN key function. The use of the key function had a large effect on the overall detection probability estimations and therefore on the ESW. It was estimated to 0.07 (CV = 28.9%) and 105 m for the HR key function and 0.21 (CV = 20.3%) and 315 m for the HN key function respectively.

If detection biases were not accounted for, i.e. assuming $g(0) = 1$, the estimated overall detection probability and ESW would be 0.14 and 210 m respectively for the HR key function and 0.40 and 600 m for the HN key function.

The sensitivity analysis explored the effect of using different values of the distance distribution and routine swimming speed on the number of duplicate events and the detection probability estimates ([Figure 8](#) and [Supplementary Tables 4, 5](#)). With a distance distribution ranging from 70 to 100% and a routine speed ranging from 1.6 to 4.2 m/s (3.1 to 8.2 knots), the number of duplicate events increases from 14 to 35 and 3 to 37 respectively. Both $g(0)$ and the overall detection probability became stable for

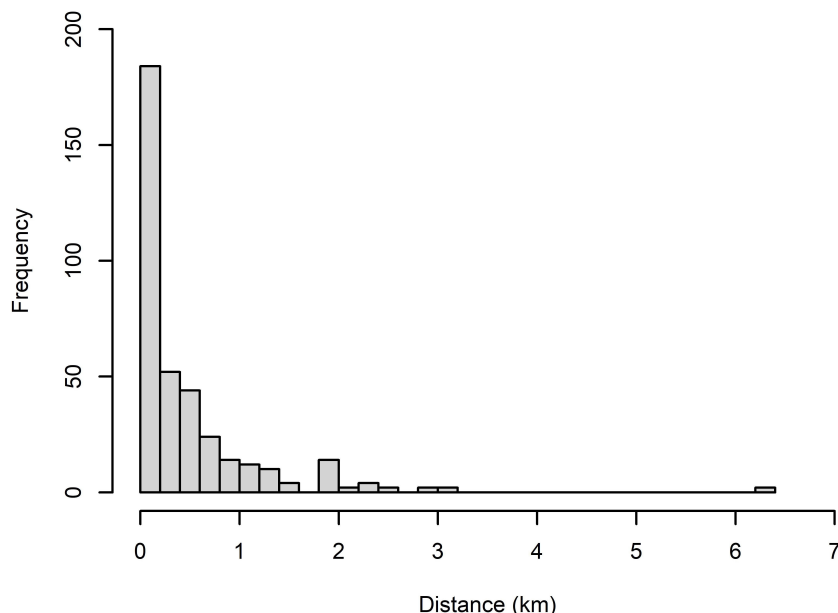


FIGURE 5 Perpendicular distance (km) frequency distributions for small cetaceans identified by the *R/V Song of the Whale* either with the visual or acoustic platforms.

both models from 70% of the distance distribution. On the contrary, $g(0)$ continuously increased as the routine speed increased, and overall probability detection increased more slowly for both models.

4 Discussion

The objectives of our study were to develop a method to match visual and acoustic events and to estimate the detection probability

of small cetaceans accounting for detection biases. Visual and acoustic data were collected simultaneously using a visual-acoustic dual platform in a distance sampling framework. Most studies estimate the detection probability by assuming $g(0)$ is equal to 1 with a single platform (Barlow, 2006; Øien, 2009; Dick and Hines, 2011; Hildebrand et al., 2015). To account for detection biases in abundance estimates, $g(0)$ needs to be estimated. The benefit of using a dual visual-acoustic platform is to quantify the proportion of missed events to estimate $g(0)$ and thus to correctly

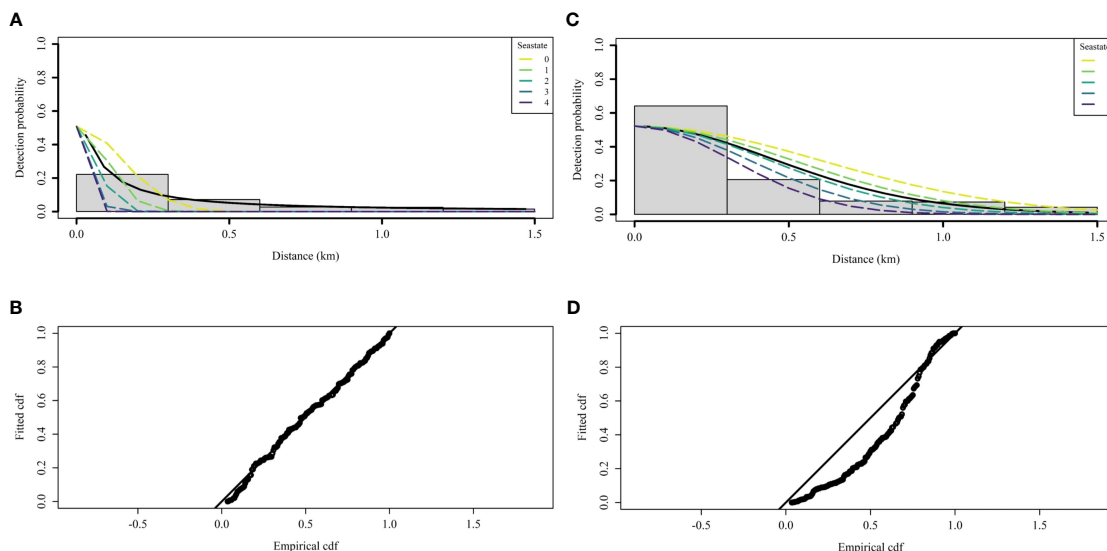


FIGURE 6 Fitted detection function of the best model with a hazard-rate key function (A) with the associated goodness of fit (B) and with a half-normal key function (C) with the associated goodness of fit (D). The selected model is a point-independence model with an MR submodel including the covariate platform and a DS submodel including the covariate sea state. Each line represents the fitted detection function for each class of the sea state covariate.

TABLE 3 Coefficients and standard errors estimated in the PI model with a hazard-rate key function (HR) and a half-normal key function (HN).

	Coefficients (HR)	SE (HR)	Coefficients (HN)	SE (HN)
MR submodel				
Intercept	-0.34	0.32	-0.34	0.32
Perpendicular distance	-1.11	0.72	-1.11	0.72
Swell Height	-0.49	0.30	-0.45	0.30
Visual platform	-0.14	0.17	-0.14	0.17
DS submodel				
Intercept	-1.47	0.44	-0.34	0.12
Sea state	-0.42	0.16	-0.16	0.04

A PI model includes an MR submodel which estimates the $g(0)$ and a DS submodel which estimates the shape of the detection function. SE: standard error.

calculate the detection probability. To do so, we first developed a method to identify duplicate events by taking the movements of the vessel and of the animal(s) into account. Then, we estimated the detection probability through an MRDS analysis by including covariates that can affect the detection.

4.1 Methodological considerations

4.1.1 Responsive movement

Performing distance sampling analysis assumes that animals are moving slower than the speed of the survey platform and are detected before any responsive movement related to the presence of the vessel, which requires detecting animals ahead of the vessel (Buckland et al., 2001). The effect of responsive movement on the detection function can be corrected by using two independent visual teams on the same vessel where one searches further ahead to detect animals before they respond (Borchers et al., 1998). When the second platform is an acoustic platform, it is more complex to account for responsive

movement since small cetaceans are less easy to track with acoustic methods. For example, when they move away, they no longer face the hydrophone and may be less available for detection. Among the species studied, the three most commonly seen species were striped, common and bottlenose dolphin. These species are known to be attracted to the bow of vessels (Würsig, 2018). To reduce the bias due to responsive movement, we first assumed a minimal attraction or avoidance effect from the boat because the *R/V Song of the Whale* is a quiet research vessel designed for this purpose. Second, the responsive movement was partially taken into account in the identification of the duplicate events by setting a mobility buffer based on an average cetacean routine swimming speed. Field observations during the ASI surveys suggested the use of routine speed was appropriate for assessing responsive movement as animals were rarely observed ‘racing’ towards the research vessel. Additionally, a single distance was required for each duplicate event. The distance selected between the matching visual and acoustic events was chosen according to the first event that was detected. In general, animals were first detected visually in front of the

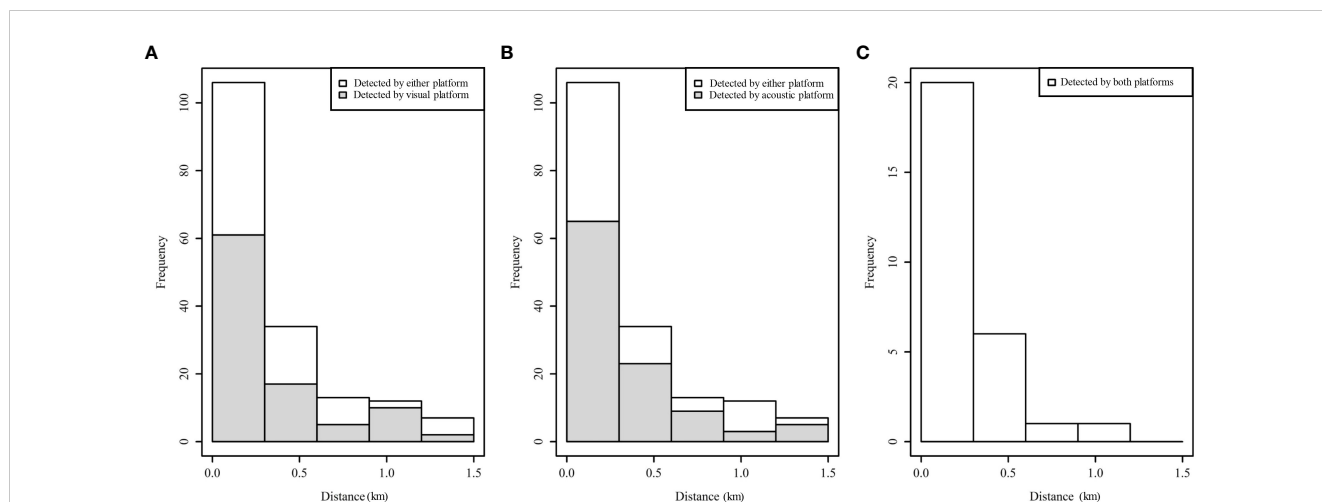


FIGURE 7 Histograms of distances for detections by either platform with the shaded regions show the number for visual platform (A) and for acoustic platform (B). Detections recorded by both platforms are shown in (C).

TABLE 4 Detection probabilities obtained from the MRDS model with a hazard-rate key function (HR) and a half-normal key function (HN).

Probability	Model used	Estimate (HR)	SE (HR)	CV (HR)	Estimate (HN)	SE (HN)	CV (HN)
Probability of detection assuming $g(0) = 1$	DS submodel	0.14	0.03	18.2%	0.40	0.02	4.2%
$g(0)$ of the visual platform	MR submodel	0.29	0.07	25.6%	0.30	0.07	23.8%
$g(0)$ of the acoustic platform	MR submodel	0.32	0.08	25.1%	0.33	0.08	23.2%
$g(0)$ of combined platforms	MR submodel	0.51	0.11	21.7%	0.52	0.10	19.8%
Overall probability of detection	MRDS model	0.07	0.02	28.9%	0.21	0.04	20.3%

The overall detection probability of the MRDS model was obtained by combining the DS submodel and the $g(0)$ of combined platforms from the MR submodel.

boat and then later by the hydrophone towed 400 m behind the boat. By choosing the distance of the first detected event, we minimised the influence of any reactive motion and this partially addressed the responsive movement problem. Martin et al. (2020) assessed responsive movement based on animal orientation and behaviour information based on visual data. They also set the trial configuration (one platform is informed about detections of the other platform) and chose the FI assumption in their model to partially address the responsive movement issue (Laake and Borchers, 2004; Burt et al., 2014). They recommended that responsive movement be properly considered in future vessel-based surveys for abundance assessments. Although responsive movement is difficult to assess and avoid, we believe that taking a mobility buffer into account partially addressed the issue of responsive movement in the MRDS analysis with two independent platforms.

4.1.2 Duplicate events

The number of duplicate events plays an important role in the MRDS analysis when estimating detection probability (Evans and Hammond, 2004; Hamilton et al., 2018). Classifying unique events as duplicates will lead to an underestimation of the detection probability. In the opposite way, classifying duplicate events as unique events would result in an overestimation of the detection probability (Evans and Hammond, 2004). In the present study, only 16% of the total events were confirmed duplicates, while in other studies, they represent more than 30% of total events (35%, Dalpaz et al., 2021; 30%, Rankin et al., 2020; 48%, Richman et al., 2014). We tested the decision tree with different values of the distance distribution in the first step and the routine speed in the second step. The distance distribution was based on the data and was not an arbitrary threshold. Changing the value of the distribution did not

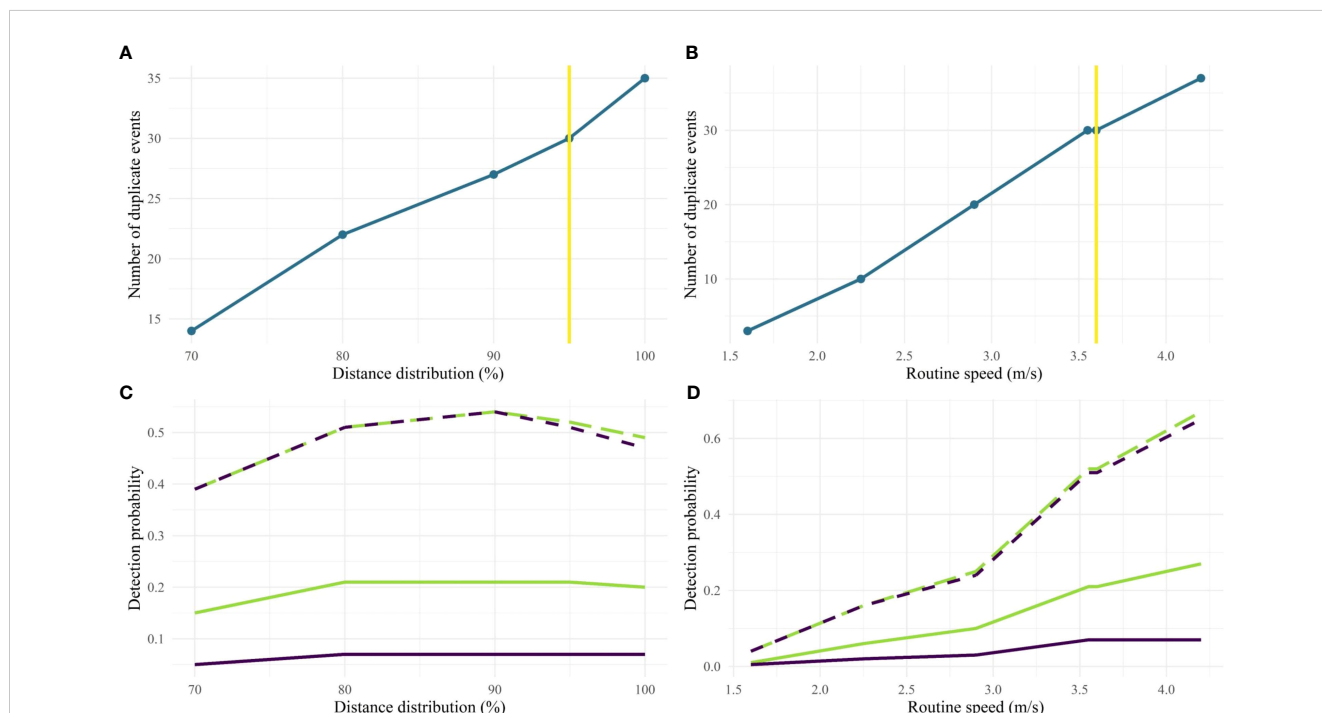


FIGURE 8 Sensitivity analysis of the two-step decision tree. The variation of the number of duplicates was explored with different values of the distance distribution (A) and the routine speed (B). The vertical yellow line indicates the chosen value for this study. The overall detection probability (solid line) and $g(0)$ (dashed line) were estimated for each value of the distance distribution (C) and the routine speed (D). The green and purple lines represent the models used with a half-normal and a hazard-rate key function respectively.

have a great impact on detection probabilities, particularly from 80% to 100%, and this can be explained by the truncation distance value set at 1,500 m. As for the routine speed chosen from values between 1.6 and 4.2 m/s (3.1 and 8.2 knots) found in the literature, it was an arbitrary threshold. The effect of varying this speed on detection probabilities was not negligible: the higher the routine speed, the higher the number of duplicate events and detection probabilities. By choosing the 95% quantile of the swimming speed distribution, i.e. 3.6 m/s (7 knots), we lost 7 duplicate events, which was not insignificant. The choice of a higher speed would probably correspond to an attraction or repulsion movement relative to the vessel, but we were unable to determine whether the animals detected were being attracted or repelled or not. It seemed more appropriate to consider a routine swimming speed, with the aim of including all motivational states, including travelling, socialising, resting and foraging. Our number of duplicate events seemed low compared to other studies, but the approach used to identify duplicates was different from that of other studies. [Dalpaz et al. \(2021\)](#) used a decision tree based on three criteria: (1) less than 30 min between events, (2) less than 6 km between events and (3) taxonomic compatibility between detections of Delphinidae and Ziphiidae. [Rankin et al. \(2020\)](#) identified visual and acoustic detections of rough-toothed dolphins as duplicates when both detections occurred in a close range. However, the authors did not explain in detail how they matched the detections and how “close” was defined. [Richman et al. \(2014\)](#) studied detectability of Ganges River dolphins and used a distance threshold between a visual and an acoustic detection to identify duplicates. They found the value of the distance threshold based on visual inspection of the frequency distribution of number of matched detections over distance; which was 249 m for a single animal and 349 m for a group of more than one animal. In open ocean habitats, such as in our study, hydrophones are towed as far back as possible to avoid self-boat noise. The delay between a visual sighting from in front of the vessel and the subsequent detection of the animal by the hydrophone to the rear of the vessel can be relatively long. As small cetaceans are highly mobile species, it is necessary to take their movement into account during this time. Although routine speed remains a sensitive parameter for the decision tree, the application of the mobility buffer still reduced the number of duplicate events identified by the time buffer by half, which can be considered very restrictive. However, it allowed to identify with more certainty the potentially true duplicate events, reducing the risk of false duplicates.

4.2 Detectability of small cetaceans

4.2.1 Detection process

During the survey and the analytical stage, a consistent protocol was implemented to define distinct events. However, the performance of the models tested was not perfect, as either the goodness of fit was good but the model did not seem to fit the data, or the opposite was observed. The detection function using the HR key function was unable to accurately describe the data observed close to the transect line due to a poor fit at low perpendicular distances. This indicates a possible problem associated with the

detection process. Accurate distance estimation can be an issue in visual surveys ([Marques et al., 2006](#)). In this study, radial distances were estimated by naked eyes without tools, which may bias perpendicular distance estimates. For a small vessel with a low platform like the *R/V Song of the Whale*, the use of reticule binoculars or photogrammetry is complex ([Leaper et al., 2023](#)). Observers during the ASI survey had varying levels of field experience and identification skills. Thus, using the photogrammetry or reticules might have been counter-productive if being used by novice observers. Laser rangefinders can be a useful tool but only under optimal sea state conditions to avoid waves interfering with the laser and/or the motion of the vessel making reliable range-finding challenging. All of these methods would also result in a biased distance estimate. With distance estimation training conducted prior to the survey, naked eye measurements appeared to be the best approach in these conditions.

4.2.2 Group size estimation

During a survey, detectability between individuals may vary for multiple reasons; some animals may surface/vocalize more than others, or some may be more detectable in specific habitats such as shallow or deep waters. Due to this variability, each individual, species or population should have its own detection probability. However, some variables that influence the detection probability cannot be observed, which corresponds to unmodelled heterogeneity and therefore does not allow a reliable estimation of detection probability and abundance ([Buckland et al., 2004](#); [Buckland et al., 2015](#)). The first source of unmodelled heterogeneity could be related to group size estimation. Estimating group size is complex in visual and even more so in acoustic studies because animals are moving and an unknown fraction of the group might be underwater or silent at any given time ([Gerrodette et al., 2019](#)). In visual surveys, estimating the group size of a large school of active dolphins is a difficult task and the estimates can vary considerably between observers ([Gerrodette et al., 2002](#)). The average group size was estimated to be 10 individuals in this study, which was consistent with other studies averaging between 4 and 30 individuals ([Notarbartolo di Sciarra et al., 1993](#); [Bearzi et al., 2003](#); [Cañadas and Hammond, 2008](#); [Boisseau et al., 2010](#); [Notarbartolo di Sciarra and Tonay, 2021](#)) in the Mediterranean, although larger aggregations are known ([Watkins et al., 1987](#); [Boisseau et al., 2010](#); [Notarbartolo di Sciarra and Tonay, 2021](#)). In acoustic surveys, group size cannot be estimated directly during surveys and most studies use visual events to determine group size of the acoustic events ([Barlow & Taylor, 2005](#); [Pirota et al., 2015](#)). Another approach is to use cue counting to estimate the density of cues that animals produce, such as calls, that can be converted into animal density using the cue production rate of the species ([Marques et al., 2009](#)). In our study, we used overlapping clicks trains as an indicator of the number of acoustically active individuals in the group and to estimate the acoustic group size for each event, following [Thomas and Marques \(2012\)](#). With our methodology, the estimated visual group size was approximately three times higher than the acoustic group size. Such a discrepancy between the visual and acoustic group size suggests that the latter was underestimated. The first assumption suggests that our recordings may have captured only a subset of the group, namely those animals

that were echolocating. The second assumption is that we counted individuals moving from the front of the boat to the back, and that all the animals in a group were probably not moving in the same way toward the hydrophone, so that only part of the group was detected and counted, the others having moved away or not in the direction of the hydrophone. The third assumption is a mixture of the two, implying that disentangling availability bias and perception bias from the acoustic method is difficult. Due to probably incorrect estimates of the acoustic group size, we did not include this covariate in the model and the remaining unmodelled heterogeneity can lead to a potential overestimation of the detection probability (Borchers et al., 2006; Burt et al., 2014). With further studies of vocalization rates, it may be possible to scale up the underestimation of acoustic group size, but currently we do not have enough meaningful data on vocal rates for small odontocetes in the Mediterranean Sea.

4.2.3 Pooling species

Pooling species with different behaviours may be another source of unmodelled heterogeneity in detection probability. Indeed, small cetaceans show different group sizes, dive durations, vocalization rates or preferred habitats. For example, the three most observed species in the Mediterranean Sea are distributed differently. The bottlenose dolphin mostly lives on the continental shelf (Bearzi et al., 2009; Cañadas et al., in review), while the striped dolphin is an oceanic species that is usually found in open waters (Cañadas et al., in review; Notarbartolo di Sciara and Tonay, 2021). The common dolphin can be observed in both inshore and offshore waters (Bearzi et al., 2003). By grouping species together, the variation in species occurrence and distribution within a study area would not be significant if the detectability across species were equal. In acoustic studies, the common spectral features of the studied species and the characteristics of clicks (i.e. directionality, high-frequency) contribute to click variability and complexity in classification. Although ongoing research opens avenues for improved classification (Oswald et al., 2003; Soldevilla et al., 2008; Bittle and Duncan, 2013; Pedersen et al., 2021), species identification from echolocation clicks remains limited and we had to pool the different species. By grouping all species together, a single detection function was fitted for species. However, the striped dolphin is a dominant species in the Mediterranean, where our study detected over 63% and Panigada et al., (in review) detected over 85% in an aerial line-transect survey. Pooling species is a common practice in aerial line-transect survey where some species were merged (striped dolphin/common dolphin; ACCOBAMS, 2021). As striped dolphins were highly dominant in the study area, the problem of pooling species would be reduced.

4.3 Comparison of $g(0)$ estimation with other studies

Despite the main limitations of this study, our results were statistically accurate, as shown by reasonably low CVs (20–27%),

and were consistent with other studies, especially for the visual platform. Rankin et al. (2020) estimated $g(0)$ at 0.37 and 0.77 for the visual and acoustic platforms respectively and 0.84 with the combined platforms for rough-toothed dolphins. The $g(0)$ for the visual platform was similar to our results (0.29, CV = 25.7% and 0.30, CV = 23.8%). For acoustics, our results were lower (0.32, CV = 25.1% and 0.33, CV = 23.2%) but Rankin et al. (2020) also included other vocalizations such as whistles and burst-pulses (Rankin et al., 2020), which resulted in a higher number of acoustic events and thus a higher estimate of $g(0)$. Additionally, echolocation clicks are highly directional and therefore if the animals are too far away or off-axis of the hydrophone, the detection probability decreases sharply (Au et al., 2012; Finneran et al., 2014), which could partly explain the low estimate of $g(0)$ from the acoustic platform in our study. Although the appropriate data are lacking for the Mediterranean, it is possible that small odontocetes are more vocal when submerged, as is typically the case for larger odontocete species including sperm whale and Cuvier's beaked whale (Akamatsu et al., 2001). This situation would preclude exact duplicates to occur. However, since dolphins can switch between the two conditions in a matter of a few seconds, and the time window for potential duplicates was within 271 s before and 1,179 s after the visual event, this left ample time for duplicates to be recorded if the individual was acoustically active during this time interval. Therefore, these methods appeared to be complementary and, as expected, we obtained a higher detection probability by combining both platforms (Laake et al., 2011; Rankin et al., 2020).

4.4 Implication for cetacean conservation

In this study, we highlight the importance of using a visual-acoustic dual platform in multispecies line transect surveys to estimate the detection probabilities of small cetaceans with consideration of detection biases, which have a major effect on the detection probability estimates. While recognising that most cetacean conservation programs use minimal abundance estimates (Evans and Hammond, 2004), we emphasise an unbiased abundance estimate to provide a better approach to assessing the sustainability of cetaceans in the face of anthropogenic mortalities. Based on our estimates of $g(0)$ and p , if we were to estimate cetacean abundance in the Mediterranean Sea without correcting for detection biases, this would be underestimated by a factor of two when using both acoustic and visual data for either model (HR and HN key functions). In order to estimate the abundance of small cetaceans from the ASI vessel survey, it would be necessary to consider all transects, i.e. acoustic, visual and combined transects to ensure uniform coverage. A homogeneous distribution of dual platform effort over the study area would be recommended to avoid spatial bias (over-representation of a habitat or species) which could then affect the $g(0)$ estimate. Further research is needed to estimate small cetacean abundance from a visual-acoustic dual platform in the Mediterranean Basin.

Data availability statement

The original contributions presented in the study are included in the article/[Supplementary Material](#). Further inquiries can be directed to the corresponding author.

Author contributions

The present work was designed by CO, AV and VR. OB provided the data. CO and IS developed the decision tree and conducted the analyses. CO wrote the manuscript. All revisions were done by OB, VR and AV. All authors contributed to the article and approved the submitted version.

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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.2023.1244474/full#supplementary-material>

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Summer distribution of the Mediterranean sperm whale: insights from the acoustic Accobams survey initiative

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The Mediterranean sperm whale population, *Physeter macrocephalus*, is listed as endangered due to population decline caused by human activities. To mitigate the impact of these activities, accurate knowledge of their distribution and abundance is crucial. During their long dives, sperm whales are not available to visual observation, but since they produce sounds when they dive, they are available to acoustic detection. Therefore, we aimed to use towed acoustic data to model their habitat and fill the knowledge gap on Mediterranean sperm whale distribution. Generalised additive models were used to link the number of sperm whales detected acoustically during the ACCOBAMS Survey Initiative in 2018 with different environmental variables integrated over different depth classes, encompassing the depth range used by the species for foraging. Sperm whale distribution was influenced by water temperature at the bottom, eddy kinetic energy between 200 and 600 m, as well as gradients of sea surface temperature and chlorophyll-a concentrations. The abundance of sperm whales was estimated at 2,959 individuals [2,077 - 4,265] in the sampled areas of the Mediterranean Sea. We predicted that sperm whales were mainly distributed in summer along the continental slope of the north-western Mediterranean basin from the Balearic Islands to the Ligurian Sea and off the Algerian coast. They were present throughout the western Mediterranean Sea and in the northern Ionian Sea. In contrast, predicted densities were low in the eastern part of the Mediterranean Sea. The use of acoustic data compensated for the main difficulty in studying sperm whales, the unavailability of animals at the surface during visual observation and the paucity of visual data. We thus encourage more systematic use of passive acoustics to study sperm whale distribution. The model highlighted a higher concentration of sperm whales in the western Mediterranean basin than in the eastern basin in summer, opening up avenues to improve the conservation of this endangered Mediterranean sub-population.

KEYWORDS

species distribution model, Mediterranean Sea, passive acoustic monitoring, sperm whale distribution, *Physeter macrocephalus*

1 Introduction

The Mediterranean sperm whale population, *Physeter macrocephalus* (Linnaeus, 1758), genetically different from the Atlantic populations, has been listed as endangered since 1982, due to apparent population decline and habitat loss caused by human activities (Notarbartolo di Sciara et al., 2012; Rendell and Frantzis, 2016). The Mediterranean sperm whale population is particularly exposed to various anthropogenic pressures, such as activities generating high-intensity noise (e.g., military sonar, maritime works or construction), fisheries that can lead to entanglements in drift nets, ship strikes, plastic ingestion, or exposure to chemical pollution (Aguilar et al., 2002; Reeves and Notarbartolo di Sciara, 2006; Notarbartolo-Di-Sciara, 2014). To mitigate the impact of these activities, accurate knowledge of sperm whale and other cetacean distribution and abundance is crucial for planning activities in the marine environment and implementing management measures (Douve, 2008). Previous studies have provided some information on the distribution and relative abundance of sperm whales in the Mediterranean Sea, but knowledge gaps on their distribution persist (Lewis et al., 2007; Virgili et al., 2019). More generally, heterogeneous data collection in marine environments has resulted in large gaps in our understanding of cetacean distributions in the Mediterranean Sea (Mannocci et al., 2018).

Species distribution models (SDMs) are powerful tools for identifying mechanisms influencing species distribution (Elith and Leathwick, 2009). They allow establishing relationships between the number of detected animals and different environmental variables. They also allow predicting hotspots of densities (Elith and Leathwick, 2009). Among SDMs, generalised additive models (GAMs) are commonly used to model marine mammal distributions and study their habitat preferences as they are able to capture non-linear animal-habitat relationships (Mannocci et al., 2014; Tepsich et al., 2014; Roberts et al., 2016; Virgili et al., 2022). Static variables (e.g., bathymetry, slope) and surface dynamic variables (e.g., chlorophyll-a concentration, net primary production, surface temperature, sea surface height) are commonly considered (Praca et al., 2009; Virgili et al., 2017; Virgili et al., 2019). For species that spend much of their time underwater and feed at great depths on mesopelagic and bathypelagic prey, as is the case for sperm whales, it would seem more relevant to use environmental variables integrated over the water column (Brodie et al., 2018; Virgili et al., 2022). In Virgili et al. (2022), the use of such variables increased the performance and the explanatory power of the models and improved the understanding of the distribution of sperm and beaked whales in the Bay of Biscay. Brodie et al. (2018) found that subsurface dynamic variables (isothermal layer depth, a proxy for the depth of surface mixing, and buoyancy frequency, a proxy for the stratification level of the water column) increased the explanatory power and predictive performance of the models for most species (sharks and swordfishes in the study). Therefore, environmental variables other than surface variables, such as those describing deep oceanographic processes, should also be used.

Most SDMs applied to deep-diving cetaceans are fitted to visual sightings (Roberts et al., 2016; Virgili et al., 2019). The sperm whale, however, can be difficult to observe at the surface because it spends much of its time at depth to forage. Sperm whales produce loud vocalisation, emitting series of regular echolocation clicks during dives (with a repetition rate between 0.5 and 2 clicks per second; Goold, 1995) and while foraging (Whitehead and Weilgart, 1991; Andreas et al., 2022). These clicks can be detected using passive acoustic monitoring (PAM) methods. These methods have been used since the First World War to detect underwater sounds, and it is a technology that has been developed, improved, and used by the scientific community to detect and identify marine mammals (Browning et al., 2017). Pirotta et al. (2020) used PAM methods and acoustic monitoring and showed that passive acoustic methods are valuable for studying the distribution of sperm whales. Poupard et al. (2022) were able to obtain sperm whale acoustic data over several seasons and years, showing the year-round presence of the species in the northwestern Pelagos sanctuary. They also highlight the importance of acoustic monitoring in understanding the distribution and activity of sperm whales in the region. PAM can thus be used to study species that have low availability during visual surveys.

Unlike visual sightings, animals can be detected acoustically in any light and weather conditions (e.g., day or night, or in fog; Marques et al., 2013). Hence, it seems more relevant to use acoustic data than visual data to study sperm whale distribution, as it has been done by Pirotta et al. (2020) in the Balearic Sea.

From June to September 2018, the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) Survey Initiative (ASI) was conducted throughout the Mediterranean Sea. The aim of the ASI was to obtain new information on the distribution and abundance of cetaceans in the Mediterranean Sea in order to improve and strengthen measures to reduce the impacts of human activities on cetaceans. The survey had an aerial and a boat-based component. The boat-based component used towed hydrophones allowing the study of sperm whale distribution at a large-scale using PAM methods. The aerial component of the ASI also collected sightings of sperm whales but in limited numbers (Panigada et al., this special issue; Cañadas et al., this special issue).

In the present work, we aimed to study the sperm whale summer distribution in the Mediterranean Sea. We modelled sperm whale habitats using towed passive acoustic data and static variables, such as bathymetry, along with dynamic environmental variables integrated over the water column, including *inter alia* temperature, salinity, currents and chlorophyll-a. First, the detection function and the Effective Strip Widths (ESW) were estimated. Then, we extracted environmental variables and associated them with the effort data. We used GAMs to describe relationships between the number of sperm whales detected acoustically and the different environmental variables as well as to explain the mechanisms leading to a concentration of individuals. We finally predicted sperm whale densities over the whole Mediterranean Sea.

2 Methods

2.1 Study area

The study area encompassed the entire Mediterranean Sea (from 6°W to 36°E and from 30°N to 46°N). The Mediterranean Sea is a distinctive ecosystem with a relatively small continental shelf and a mostly cyclonic circulation. The water column in the Mediterranean Sea is divided into distinct water masses: (1) the surface waters, which extend between 0 and 200 m in depth, originate from the Atlantic and enter the Mediterranean Sea via the Strait of Gibraltar; (2) underneath, the intermediate Levantine water circulates, on average between 200 and 600 m, this is the warmest and saltiest water of the Mediterranean Sea; (3) below 600 m, the deep waters originate in part from the intermediate Levantine water (Millot and Taupier-Letage, 2005). Even if dives made by sperm whales do not generally exceed 1,500–2,000 m (Amano and Yoshioka, 2003; Irvine et al., 2017; Towers et al., 2019), we chose to consider the entire water column, down to a depth of 4,100 m.

2.2 Data collection

The ASI survey is the first survey carried out at the Mediterranean basin scale for all cetacean species. Acoustic surveys were conducted using hydrophone arrays towed by the *R/V Song-of-the-Whale* (Figure 1), a 21 m auxiliary-powered cutter-rigged sailing research vessel. A 400 m tow cable, close to the surface at a depth of less than 10 m, was used to isolate the array from any noise generated by the vessel. Acoustic data were collected 24 hours a day to maximise survey effort when water depth was appropriate (greater than 50 m). The vessel maintained a speed of 5 to 8 knots when surveying sperm whales to avoid bias due to animal movement by exceeding the speed of the target animals by 2–3 times (Buckland et al., 2015). The speed of sperm whales can vary between 2 and 6 knots (Arnbom et al., 1987), but they generally maintain an average speed of 2.1 knots (Whitehead, 2018). A pair of

AQ-4 elements (Teledyne Benthos) was incorporated into the hydrophone array. They were characterised by a receiving sensitivity of -201 dB re 1V/μPa and a flat frequency response (± 1.5 dB) from 1 Hz to 30 kHz. Pre-amplifiers (29 dB gain) were used to prevent voltage-drop between the array and the vessel. A distance of 3 m separated each hydrophone element. A SAIL DAQ card (SA Instrumentation) digitised the array outputs at 48 kHz using a 10 Hz high pass filter and a 12 dB gain added to the signal. A click detector module was used to detect candidate sperm whale clicks in real time using PAMGuard (Gillespie et al., 2008). The click detector identified spectral properties, with most energy at or below 12 kHz (Watkins, 1980; Möhl et al., 2003).

A line transect distance sampling protocol was used during the survey and the perpendicular distances for each detection were estimated in PAMGuard using the target motion analysis tool. Sperm whale clicks were identified, analysed and validated by the Marine Conservation Research (MCR; Boisseau et al., this special issue).

The effort transects were linearised and segmented into 10 km segments for which the detection conditions were homogeneous. Each acoustic detection was then attributed to the effort segment on which the animals were detected (Figure 1).

2.3 Detection functions and ESWs

During an at-sea survey, not all animals are detected acoustically; the probability of detecting an animal decreases with the distance from the transect and as detection conditions degrade. A detection function was therefore fitted to the acoustic data using Multiple Covariate Distance Sampling models (MCDS; Miller et al., 2019). The models were fitted considering two key distributions, half normal (hr) and hazard rate (hz) and different detection conditions (sea state, wave height in meters and wind speed in knots). The best model was selected according to the Kolmogorov-Smirnov and Cramer-von Mises goodness-of-fit tests and the Akaike information criterion (AIC; Buckland et al., 1993). The models were fitted using the 'dfuncEstim' function of the 'Rdistance'

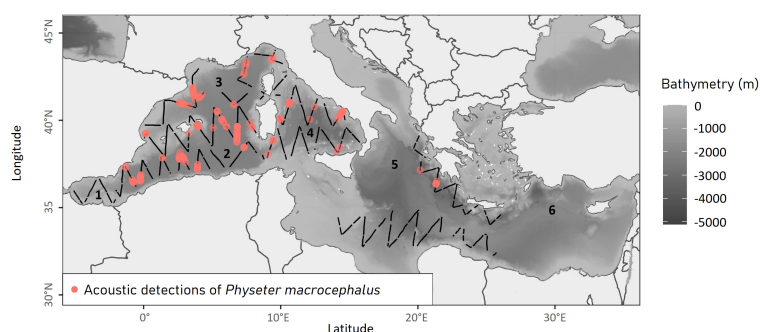


FIGURE 1

Performed effort transects and acoustic detections of sperm whales collected during the ASI survey in the Mediterranean Sea in 2018. The background map represents the bathymetry (in meters; <https://www.emodnet-bathymetry.eu/>), black lines represent the transects and pink points the acoustic detections. The numbers are the names of the different regions in the Mediterranean Sea: 1 Alboran Sea; 2 Algerian Basin; 3 Liguro-Provençal Basin; 4 Tyrrhenian Sea; 5 Ionian Basin; 6 Levantine Basin.

package (McDonald et al., 2019) and the two tests mentioned above were performed using the 'gof_ds' function of the 'Distance' package (Miller et al., 2019). Based on the detection function, ESWs were estimated for each class of the selected detection variable. An ESW is defined so that the number of objects detected beyond this distance is equal to the number of objects not detected before this limit (Buckland et al., 1993). ESWs were calculated using the 'ESW' function of the 'Rdistance' package (McDonald et al., 2019).

The number of individuals detected was determined by using a click detector module in PAMGuard (Gillespie et al., 2008) which was configured to identify potential sperm whale clicks. Field recordings were independently assessed in PAMGuard by two experienced analysts to identify potential sperm whale click sequences. Candidate clicks were identified when they formed part of a click sequence, characterised by consistent bearings and regularly spaced click intervals. Variations in bearing information were used to distinguish individual click sequences (Lewis et al., 2018). Consequently, acoustic detections were made at the individual level rather than at the group level, allowing the number of detected animals to be determined.

Knowing the ESWs and the number of individuals detected, it was possible to estimate the density of individuals along the transects. This density was then multiplied by the total area sampled during the survey (here 1,312,625 km²) to obtain the total abundance of sperm whales and the associated confidence intervals (Buckland et al., 1993).

2.4 Environmental data processing

2.4.1 Delimitation of depth classes

In order to determine whether the presence of sperm whales would be related to processes at the surface or in the water column, four depth classes were delimited according to the water masses of the Mediterranean Sea: the surface, between 0 and 200 m, 200 and 600 m, 600 m and 4,100 m. Environmental variables were extracted for each depth class.

2.4.2 Static and oceanographic variables

We extracted static and dynamic variables from January 1st, 2000 to December 31st, 2021 over the entire Mediterranean Sea with a spatial resolution of 0.042°x0.042° and a monthly temporal resolution (Table 1). We considered variables that can putatively affect the distribution of sperm whales by mediating food availability. Static variables remain stable over time and are independent of depth classes while dynamic variables, which vary with time, were extracted for the four depth classes previously defined.

For static variables, bathymetry was initially extracted from the Emodnet database (<https://www.emodnet-bathymetry.eu/>). From bathymetry, the slope (in degrees), roughness (in m), slope orientation (in m), and topographic complexity index were calculated using the 'terrain' function from the 'Raster' package (Hijmans and Van Etten, 2012).

TABLE 1 Environmental variables used for sperm whale density modelling in the Mediterranean Sea.

Environmental variables (unit)	Source	Interest of variables (Virgili et al., 2019)
Statics		
Bathymetry	A	Shallow waters can be associated with high primary production
Slope (°)	A	In combination with currents, high slopes induce aggregation of prey or primary production
Topographical complexity index	A	
Slope orientation (m)	A	
Roughness (m)	A	High roughness indicates a large escarpment and greater prey richness
Dynamics		
temperature (°C)	B	The variability in horizontal temperature gradients over time reveals the location of fronts, potentially associated with prey aggregation or increased primary production.
Gradients of temperature (°C)	B	
Currents (m ² .s ⁻²)	B	Strong currents induce the mixing of waters and aggregation of prey.
gradients of currents (m ² .s ⁻²)	B	
EKE – Eddy Kinetic energy (m ² .s ⁻²)	B	High EKE is related to the development of eddies and sediment resuspension leading to prey aggregation
Gradient of the eddy kinetic (m ² .s ⁻²)	B	
Chlorophyll-a concentration (mg.m ⁻³)	B	Chlorophyll concentration is an indicator of the resources available for prey, and thus of the availability of prey.
Chlorophyll-a concentration gradient (mg.m ⁻³)	B	
Net primary production (mg.m ⁻³ .j ⁻¹)	B	Net primary production is an indicator of prey availability.
Net primary production gradient (mg.m ⁻³ .j ⁻¹)	B	
Height from sea level (m)	B	High sea level is associated with high mesoscale activity and increased prey aggregation or primary production.
Depth of the mixing layer (m)	B	Indicator of prey availability.
Potential temperature at the bottom of the sea (°C)	B	The hunting depth of deep divers is not really known, the temperature of the sea floor may have an impact on their distribution
Salinity	B	Sudden changes in salinity reveal the location of fronts, potentially associated with prey aggregation or increased primary production.

All dynamic variables were extracted or calculated for each depth class (surface, 0–200 m, 200–600 m and 600–2000 m), from January 1st, 2000 to December 31st, 2021, at a spatial resolution of 0.042°x0.042°. Monthly means and standard deviations were calculated. Source A: Emodnet (<https://www.emodnet-bathymetry.eu/>); Source B: Copernicus (<https://data.marine.copernicus.eu/products?facets=areas%7EMediterranean+Sea>).

Regarding dynamic variables, temperature, salinity, current velocity, sea surface height, mixing layer depth, chlorophyll-a concentration and net primary production were extracted from Copernicus (<https://data.marine.copernicus.eu/products?facets=areas%7EMediterranean+Sea>). From these variables, we computed spatial gradients of temperatures and currents, and eddy kinetic energy ($EKE = 0.5 \cdot (U^2 + V^2)$, where U and V are the current components). Gradients of temperatures and currents were calculated as the difference between the minimum and maximum temperature values found in the eight pixels surrounding any given pixel of the grid (function 'detectFronts' from the R package 'grec'; [Lau-Medrano, 2020](#)). For each depth layer and each variable, climatological means over the 22 years of extracted data and standard deviations were calculated to assess the relevance of the variability of the variables. It means that for each month of the survey, the mean and standard deviation of the month over all years were calculated. Finally, each variable of each depth class was associated with the effort segments ([Appendix 1](#)).

2.5 Habitat-based density modelling

2.5.1 Model selection

In order to determine which environmental variables best explained the distribution of sperm whales in the Mediterranean Sea, GAMs were fitted using the 'gam' function of the 'mgcv' package ([Wood, 2017](#)) with a Tweedie distribution (very close to a Poisson distribution but allowing for some over-dispersion of the data; [Foster and Bravington, 2013](#)). We removed combinations of variables with Pearson correlation coefficients higher than |0.5| and tested all models with combinations of one to four variables to avoid excessive complexity ([Mannocci et al., 2014](#)). The mean number of individuals per segment was linked to the additive predictors with a log function with four degrees of freedom. An offset equal to segment length multiplied by twice the ESW was included. For each tested model, AIC, explained deviance and Akaike weight were calculated ([Burnham and Anderson, 2004](#); [Symonds and Moussalli, 2011](#)). The model with the lowest AIC and the highest explained deviance and Akaike weight was selected.

2.5.2 Prediction and extrapolation

A prediction grid, which covered the entire Mediterranean Sea, was created for each month of the summer (May to September) with a 0.042° spatial resolution. The selected model was used to predict the density of sperm whales in the whole study area with the 'predict' function of the 'mgcv' package ([Wood, 2017](#)). Finally, the monthly predictions were averaged over the entire survey period and standard error associated with the prediction was calculated to estimate the uncertainty of our prediction.

We conducted a gap analysis on environmental space coverage to identify areas where habitat models could produce reliable predictions outside survey blocks, that is, geographical extrapolation, whilst remaining within the ranges of surveyed conditions for the combinations of covariates selected by the models, that is, areas of environmental interpolation ([Mannocci](#)

[et al., 2018](#); [Virgili et al., 2019](#); [Bouchet et al., 2020](#)). We used the 'map_extrapolation' function of the 'dsmextra' package ([Bouchet et al., 2020](#)). The obtained map was represented according to two conditions, the interpolated data (which varied between 0 and 1) and the extrapolated data (which were lower than -1). The concept of nearest neighbour (percentage of data in close proximity) provides an additional measure of the reliability of extrapolations in a multivariate environmental space ([Mannocci et al., 2018](#); [Bouchet et al., 2020](#)). The higher the percentage, the better the prediction.

Finally, for the selected model, the Normalised Root Mean Square Error (NRMSE) was calculated. The NRMSE is an index of the quality of the prediction, it represents the percentage of error related to the prediction ([Dirwai et al., 2021](#)).

3 Results

3.1 Raw data

In total, 13,806 km of transects were conducted by boat during the ASI survey, of which 97.17% were carried out in good conditions (Beaufort sea state ≤ 4). The final dataset included 284 acoustic detections of sperm whales. The majority of these detections were recorded in the western Mediterranean basin and along the Hellenic Trench.

3.2 Estimation of the detection function and ESWs

The wind speed variable associated with a hazard rate key function best explained the distribution of sperm whale detections as a function of distance from the transect ([Appendix 2, 3](#)). The qqplot suggested a good fit.

As the distance from the transect increased, the probability of detecting sperm whales decreased more rapidly as wind speed increased ([Figure 2](#)). Consequently, as the wind speed increased, the estimated ESWs decreased ([Figure 3](#)). When the wind speed was < 5 knots, the estimated ESW was 4.88 km, while when the wind was > 25 knots the estimated ESW was 3.14 km.

Based on the detection function and the ESWs, the abundance of sperm whales in the surveyed blocks was estimated at 2,959 individuals [2,077 - 4,265]. It was a global estimate produced for the sampled areas of the Mediterranean Sea (i.e., in the Western Mediterranean Sea and in the Ionian Basin).

3.3 Habitat modelling

3.3.1 GAM selection

The selected model explained 38.8% of the deviance and the NRMSE calculation resulted in an error of 5.4%. The highest relative densities were estimated for average gradients of temperature at the surface greater than 0.1°C, a standard deviation of eddy kinetic energy between 200 and 600 m around

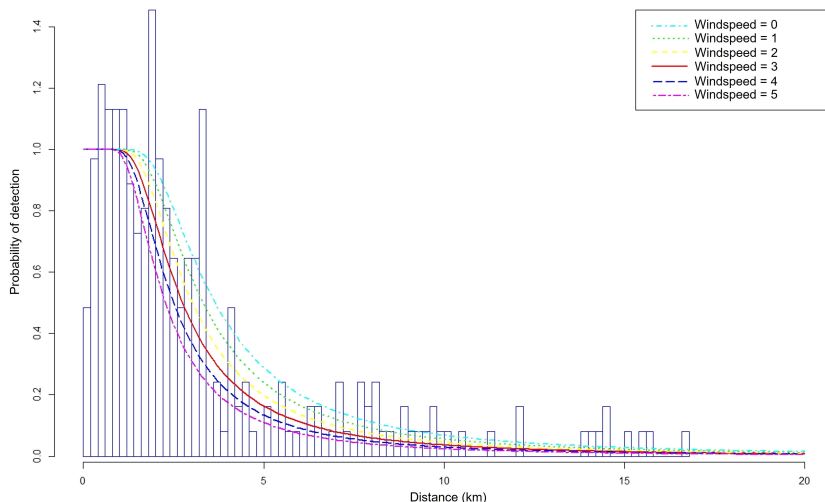


FIGURE 2 Probability of detection as a function of the distance from the transect (in km). A hazard rate distribution was fitted to all detection data and a detection function was estimated for each class of wind speed. class 0: from 0 to 5 knots, class 1: from 5 to 10 knots, class 2: from 10 to 15 knots, class 3: from 15 to 20 knots, class 4: from 20 to 25 knots, class 5: above 25 knots.

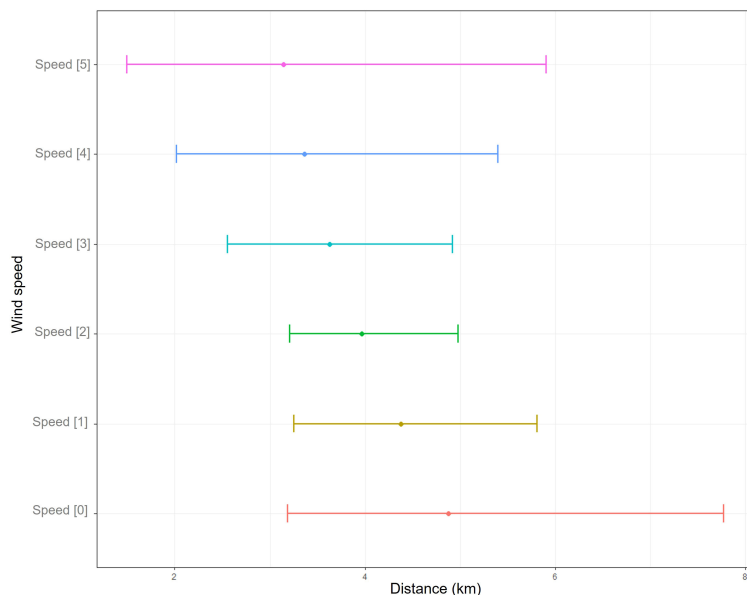


FIGURE 3 Effective strip widths and associated 95% confidence intervals estimated for each class of wind speed. Class 0: from 0 to 5 knots, class 1: from 5 to 10 knots, class 2: from 10 to 15 knots, class 3: from 15 to 20 knots, class 4: from 20 to 25 knots, class 5: above 25 knots.

0.004 m².s⁻², a chlorophyll-a concentration around 0.06 mg.m⁻³ and a low variation in the standard deviation of the bottom temperature (around 0.1°C; Figure 4).

3.3.2 Prediction and extrapolation

The model predicted higher densities of sperm whales in the western Mediterranean basin than in the eastern basin. In the western basin, the most favourable environmental conditions for sperm whales were found in the east of the Corsican coast and in the

north of the Balearic Sea, but also in the Liguro-Provençal, Ionian, and Algerian basins where sperm whale densities reached 0.05 individuals.km⁻². The model predicted average sperm whale densities of around 0.03 individuals.km⁻² in the entire western Mediterranean basin. Almost no individuals were predicted in the Alboran Sea, the Gulf of Lions and along the Spanish coast (Figure 5). In the eastern basin, the highest densities of sperm whales were predicted in the Ionian basin and along the Hellenic Trench and no individuals were predicted in the Adriatic Sea, the

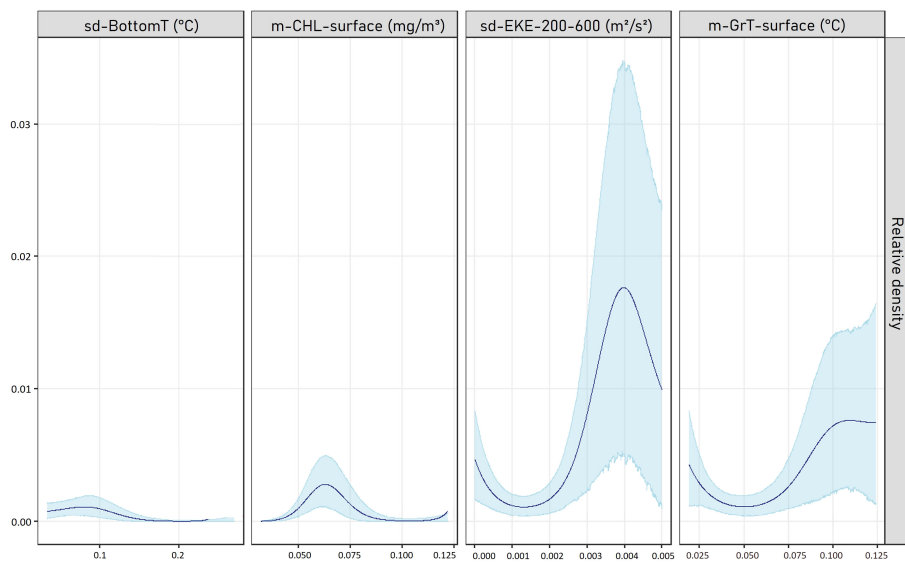


FIGURE 4
 Relationships obtained between the relative density of sperm whales (individuals per km²) and the explanatory variables of the selected GAM. Solid lines represent the estimated smooth functions and blue shaded regions show the approximate 95% confidence intervals. Sd-BottomT: standard deviation of the bottom temperature; m-CHL-surface: mean concentration of chlorophyll-a in the surface layer; sd-EKE-200-600: standard deviation of the eddy kinetic energy between 200 and 600m; m-GrT-surface: mean temperature gradient at the surface.

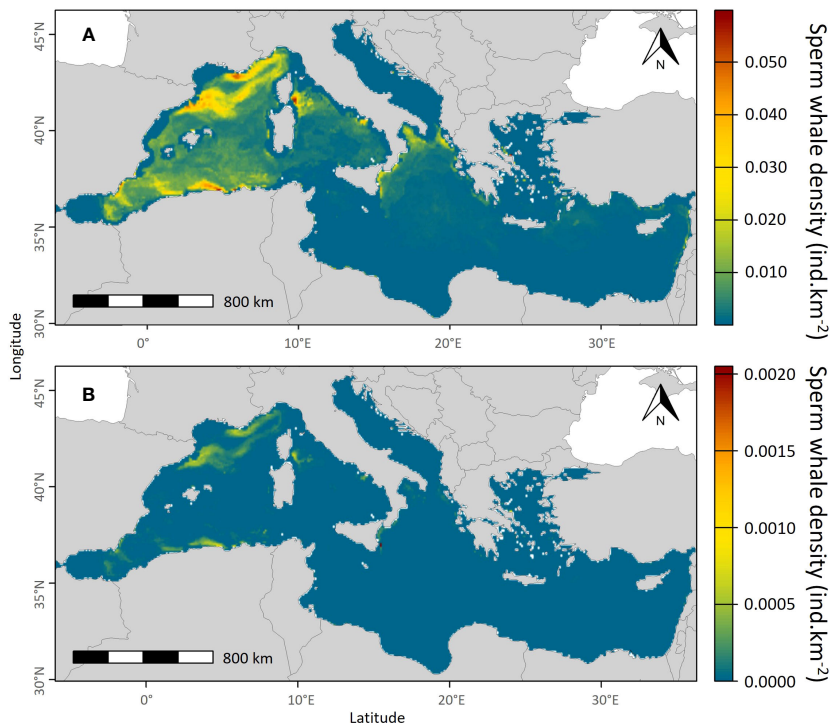


FIGURE 5
 Mean predicted summer densities of sperm whales over the entire sampling period in individuals.km⁻² (June to September; **A**) and the uncertainty associated with the predictions (**B**).

Aegean Sea and the south and the east of the basin. The values of the uncertainties associated with the prediction were very low, mainly around 0.0005 individuals.km⁻² in the Liguro-Provençal and Algerian basins where the predictions were the highest.

Predictions were environmentally extrapolated, and should therefore be taken with caution, in the Adriatic Sea, the Levantine Basin and east of Tunisia (Figure 6). They were interpolated everywhere else. The percentage of nearby data reached 25% in

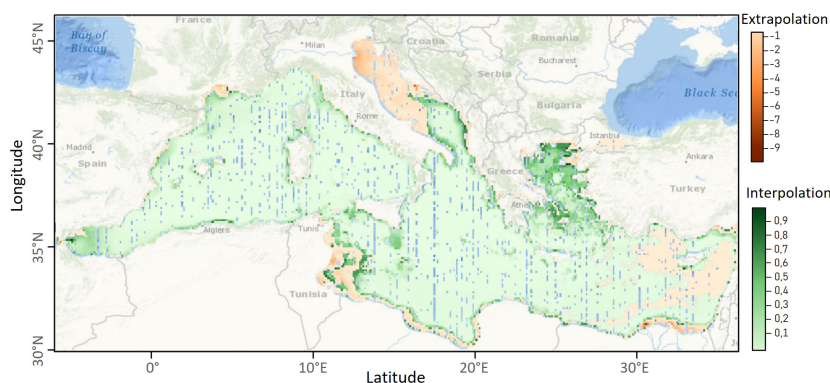


FIGURE 6

Map of extrapolation (in red; the more negative the value, the greater the extrapolation) and interpolation of predictions (in green; the closer the value is to 1, the greater the interpolation). Red areas represent the extrapolation areas where predictions are considered less reliable than in green areas because they were not sampled during the survey. The blue areas represent the absence of information on the extrapolation or interpolation of the data.

the whole of the western Mediterranean Sea and the Ionian Sea south of Italy (Figure 7). The model performs well in all these areas.

4 Discussion

4.1 Methodological considerations

The ASI survey filled gaps in the overall summer distribution of sperm whales in the Mediterranean Sea, as it was the first to be conducted at such a large scale (Mannocci et al., 2018). We obtained maps of the summer distribution with a low error rate.

As sperm whales are deep divers that spend most of their time underwater foraging (dives of up to 138 minutes during foraging; Watwood et al., 2006), they are more easily detected by passive acoustics thanks to the echolocation clicks they emit almost permanently while diving. Indeed, during the vessel survey, only 26 visual detections were recorded compared to 284 acoustic

detections. Although the detection rate was ten times higher using acoustic techniques, all animals were not detected because sperm whales typically do not vocalise when at the surface (Watwood et al., 2006; Fais et al., 2016) and/or individuals may have been beyond the detection range of the hydrophone array. On average, sperm whales dive for 45 minutes and emit sounds and clicks for 81% of the dive, making them easy to detect acoustically (Watwood et al., 2006; Fais et al., 2016). Furthermore, as they emit clicks at relatively low frequencies (≤ 12 kHz), they can be detected over long distances. The ESW was estimated to be between 0.5 and 1.7 km visually (Virgili et al., 2019), whereas acoustically it was estimated to be between 3.14 and 4.88 km. The detection capabilities of the acoustic platform are significantly higher.

The total abundance of sperm whales was estimated at 2,959 individuals [2,077 - 4,265] in the surveyed blocks (i.e., in the Western Mediterranean Sea and in the Ionian Basin). It was similar to or greater than the estimates obtained in previous studies. Lewis et al. (2018) have acoustically detected 194 sperm

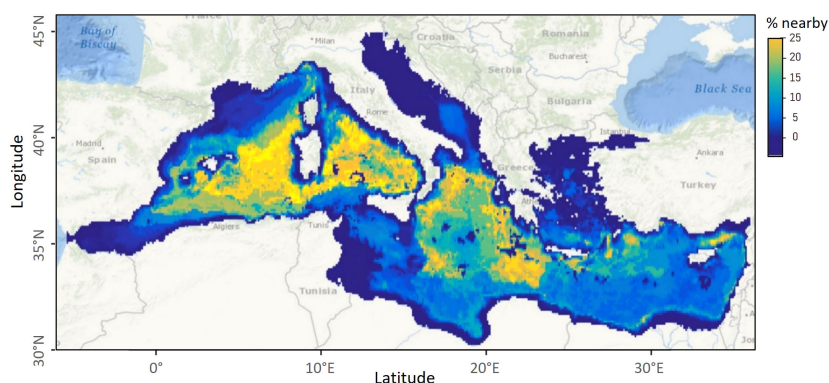


FIGURE 7

Map showing the percentage of nearby data. The areas with the highest percentage of nearby neighbour are the areas with the most reliable predictions because they were properly sampled during the survey. For cells outside the sampled areas, data from neighbouring cells that were sampled are used to inform predictions in the extrapolation areas.

whales in the entire Mediterranean Sea. The various surveys took place in 2004 in the western basin and spread over several years, 2003, 2007 and 2013 in the entire eastern basin. They estimated an abundance of 1,842 sperm whales [842 – 2,842] in the entire Mediterranean Sea. Poupard et al. (2022) have acoustically detected 422 sperm whales in the Pelagos sanctuary over 147 days of recording. They estimated a density of 0.00169 individuals.km⁻² in the sanctuary. Our model predicted average sperm whale densities of 0.03 individuals.km⁻² throughout the western Mediterranean basin. Gannier (2018) have visually (from 1988 to 2012) and acoustically (since 1994) recorded 157 detections of sperm whales in the western Mediterranean basin. They estimated a population size between 200 and 1,000 individuals in the North-Western Mediterranean Sea.

Using acoustics to detect sperm whales is therefore an effective method to estimate their abundance. In the future, it would be interesting to develop more systematic acoustic surveys when targeting deep divers (mainly sperm whales and Cuvier's beaked whale *Ziphius cavirostris*), while continuing to use visual observation for other species.

However, the main limitation associated with the use of acoustic detection is the estimation of the number of individuals (Kimura et al., 2010; Marques et al., 2013). It is difficult to distinguish individuals that emit sounds at similar frequencies in an acoustic recording unless they emit a sound simultaneously. This can lead to an underestimation of the abundance.

In this study, we were able to predict the summer sperm whale distribution throughout the Mediterranean Sea. However, to highlight a change in species distribution throughout the year, the same survey should be conducted at least in winter. Lack of data is a recurrent problem in cetacean habitat modelling; there are very few surveys conducted outside the summer periods creating seasonal gaps (Mannocci et al., 2018). Although we showed that sperm whale detection was influenced by wind speed, the estimated ESW with a wind speed of 25 knots was high (3.14 km), so sperm whales would probably be well detected even in winter conditions. However, detection rates for other species, such as dolphins, may decrease in elevated sea states. Shabangu et al. (2022), for example, suggest that high wind speeds induce a decrease in cetacean acoustic detectability due to increased ambient noise. Such a large-scale survey targeting only sperm whales would therefore not be cost-effective. However, other possibilities exist, such as deploying passive acoustic hydrophones on moorings, buoys, or vessels, which would record sounds throughout the year. Fixed buoys are very effective at assessing the seasonal presence of species at local scales (Mellinger et al., 2007; Stafford et al., 2007). However, they are less suitable for studying animal habitat use, at least at a large scale. This would require the installation of extensive buoy networks, which are very costly and require regular calibration to ensure accurate results (Shabangu and Findlay, 2014). In this case, a large-scale survey using towed acoustics seems more appropriate.

Although we predicted the sperm whale distribution in the entire Mediterranean Sea, the sampling effort was not uniform throughout the area. The gap analysis identified the eastern part of the Mediterranean basin, especially the Levantine basin, as an extrapolation area. This suggests that the sampling effort was not

sufficient in this area and a more intensive sampling effort could help better describe the habitat used by sperm whales in the eastern Mediterranean Sea. More research is needed in the east as anthropogenic pressures are high for sperm whales (e.g., ship strike risk in the Hellenic Trench) and therefore we need to improve our understanding of distribution and seasonal variation.

We expected Beaufort sea state to be selected in the detection function because cetacean detection, notably visual detection, is often affected by sea state. Here, the wind speed was selected as the variable most affecting the acoustic detection; the higher the wind speed the lower the detection probability. This is consistent with the fact that Beaufort sea state is a subjective measure of wind speed as perceived by a visual appreciation of the effect of wind on the water surface (Barlow, 2015). Ambient noise, generated by the boat, but also by all processes other than cetacean sounds, would probably be a more important factor to consider when assessing the acoustic detectability of cetaceans. We encourage the systematic recording of ambient noise levels in future acoustic surveys.

We choose to use GAMs in this study because of their ability to handle non-linear and non-monotonic relationships; GAMs are relevant for modelling ecological relationships (Booth and Hammond, 2014). In GAMS, the explained deviance is an indicator of model quality. Deviance is rarely high for cetaceans because the variables used do not fully explain the distribution of animals, as they are proxies of prey distribution. In contrast, a high deviance would have indicated an over-fitting of the relationships to the data. The value of the selected model was 38.8%, which is a good result, similar to other studies (Bailey and Thompson, 2009; Tepsich et al., 2014; Virgili et al., 2017; Virgili, 2018; Virgili et al., 2019). We were rather confident in the model as the explained deviance was quite high but not too high and the NRMSE calculation gave a result of 5.4% error, indicating a low prediction error rate.

4.2 Model improvement

The good performance of the model may be related to the use of proxies that are probably more direct than the commonly used surface variables. Environmental variables were separated according to the water masses of the Mediterranean Sea, which were assumed to be homogeneous. However, as sperm whales can dive much deeper than 1,000 m (Whitehead, 2018), we might have considered other depth classes. Particularly, the 600-4,100 m layer may not have been sufficiently precise for a deep-diver that feeds in this layer depth. We might consider splitting the deepest layer to be consistent with the depths used by the sperm whales. However, this would lead to a considerable increase in the number of variables and calculation time, as well as a possible lack of variation between depth classes, as water masses tend to be more homogeneous at greater depths.

In addition, other environmental and biological variables could have been considered. Some studies use other variables to study cetacean distribution such as the distribution and concentration of prey species (Pendleton et al., 2020; Virgili et al., 2021) or canyon areas, because submarine canyons are widely recognised as hotspots in cetacean distribution (Tepsich et al., 2014). Pendleton et al. (2020) suggested that using modelled prey availability, rather than

oceanographic proxies, could be important to forecast species distributions. In contrast, in Virgili et al. (2021), the model that used prey distributions obtained from the SEAPODYM model did not accurately model the prey of deep-diving cetaceans, the simulated prey mostly corresponded to the prey of the prey targeted by deep-divers. The SEAPODYM model is a tool for simulating the three-dimensional distributions of prey species in marine ecosystems. It considers various factors such as physical oceanography, prey behaviour, and predator-prey interactions to estimate the spatial distribution of prey. To study the distribution of deep divers, it therefore seems more relevant to use proxies that characterise the water column rather than the distribution of their prey, which is not modelled well enough.

Similarly, it might have been relevant to study anthropogenic pressures such as the impact of noise pollution on sperm whale distribution in order to find out whether these have a direct negative effect on sperm whale distribution. Poupard et al. (2022) analysed the ambient noise recorded by two hydrophones on a 25 m depth buoy to identify the impact of noise pollution (high or low ambient noise) on sperm whales. They showed that sperm whales were present all year round in the Mediterranean Sea, but their abundance decreased when the ambient noise was high due to the presence of ships such as ferries. This is particularly true near the coast where the anthropogenic noise is very high (Buscaino et al., 2016; Pieretti et al., 2020). Poupard et al. (2022) suggested that sperm whales do not come close to ships because ambient noise masks their echolocation when foraging. In addition, many studies have shown that cetaceans would modify their vocalisations in the vicinity of vessels and increase their sound level to maintain acoustic contact with other individuals (Castellote et al., 2012; Melcón et al., 2012; Shabangu et al., 2022).

4.3 Sperm whale summer distribution in the Mediterranean Sea

Sperm whales were predicted in areas associated with high gradients of temperatures at the surface, strong variations in the current velocity between 200 and 600 m, low variations in sea bottom temperatures and rather low chlorophyll-a concentrations in the surface layer. In the literature, sperm whales have often been associated with bathymetric features, such as depth, continental slopes or canyons and seamounts, as well as frontal systems and other mesoscale features such as cyclonic eddies (Virgili et al., 2019; Pirotta et al., 2020). Recently, Virgili et al. (2022) have shown that other parameters influence the distribution of sperm whales at the surface and at depth, such as temperatures and eddies at depth. We also showed that two of the selected variables in the model characterised the water column and not the surface (the standard deviation of the sea floor temperature and the standard deviation of the eddy kinetic energy between 200 and 600 m). For species such as sperm whales, it is therefore relevant to consider the whole environment and not only the surface.

The model predicted average sperm whale densities around 0.03 individuals.km⁻² in the entire western Mediterranean basin, with maximum densities predicted in the Algerian and Ligurian-

Provençal basins, more precisely in the Ligurian Sea and off the Gulf of Lion continental slope. The highest densities were predicted in the protected area of the Pelagos sanctuary (Ligurian Sea). In comparison, Lewis et al. (2018) predicted 2,12.10⁻³ individuals per km² in the southern part of the western Mediterranean Sea and 0,12.10⁻³ individuals per km² in the eastern Mediterranean Sea (with, however, high densities in the Aegean Sea, the Hellenic Trench and the northern Ionian Sea). Our predictions in the southern part of the western Mediterranean Sea coincide with their results.

In summer, sperm whales seemed to concentrate mainly in continental slope areas, whether off the Gulf of Lion, the Liguro-Provençal Sea, the Algerian Coast or around Corsica, frequenting depths above 2,000 m (Gannier, 2018; Laran et al., 2018). This suggests the existence of an east-west gradient of sperm whale densities, with higher abundances in the west. Indeed, from summer to autumn, the northern part of the western basin is a major concentration and feeding area for sperm whales (Drouot et al., 2004). Other studies indicate that the presence of sperm whales extends further east along the Hellenic Trench from south-west Kefallonia Island to central south Crete (Frantzis et al., 2014) and even to the Turkish coast as far as the western part of Antalya Bay (Öztürk et al., 2010). From a single survey performed at the scale of the Mediterranean Sea, we were able to predict hotspots observed at more local scales.

The high presence of sperm whales in the southeast of the Gulf of Lions and in the Ligurian Sea could be explained by the presence of numerous canyons (Harris et al., 2014). Submarine canyons act as a connecting corridor between continental shelf areas and deep waters. As a result, they are widely recognised as cetacean concentration areas (Tepsich et al., 2014). The high densities of sperm whales in the Ligurian Sea can be explained by the presence of numerous canyons in this geographical area. A large-scale study revealed the presence of sperm whales along the Hellenic Trench and the west coast of Greece, and in the north-western Mediterranean Sea from the Gulf of Lion to the Ligurian Sea (Lewis et al., 2018), providing support for the model results.

In addition, sperm whales are often observed in the channel between Mallorca and Ibiza, an area characterised by the presence of three seamounts. The Balearic Archipelago is one of the few areas in the Mediterranean Sea where females, juveniles and single males are regularly observed (Pirotta et al., 2020). This is due to the critical role of the area as a breeding and feeding ground for the threatened Mediterranean population (Rendell and Frantzis, 2016).

Sperm whales were predicted to occur in the Ionian Sea (0.03 individuals.km⁻², mainly off the Italian and Greek coasts), which was consistent with the literature. For example, Lewis et al. (2007); Lewis et al. (2018) found densities 0.24.10⁻³ individuals.km⁻² in the Ionian Sea and 0.12.10⁻³ individuals.km⁻² in the eastern Mediterranean Sea (similar results to Frantzis et al., 2014). Sperm whales appear to prefer the underwater relief of the sides of the Hellenic Trench at depths between 500 and 1,500 metres (Frantzis et al., 2003). However, densities were comparatively lower than in the western basin. There were two possible explanations for this: either there was less effort in the Ionian Sea, or, as suggested by Lewis et al. (2007), density estimates in the Ionian Sea were

underestimated because sperm whales tend to congregate in large groups along the Hellenic Trench, skewing the count results. Even if densities were underestimated, the Ionian Sea appeared to be important for the species as it may be a breeding ground according to [Drouot et al. \(2004\)](#).

The eddy kinetic energy was one of the main variables that explained the distribution of sperm whales in our model. [Biggs et al. \(2000\)](#) showed that sperm whales are mainly distributed in cyclonic eddies, which are proxies for prey distribution. Cyclonic eddies are mesoscale structures that induce a locally increased concentration of plankton in response to the upwelling of nutrient-rich water. The abundance of plankton would induce the strong presence of prey for deep-diving cetaceans, through a trophic cascade mediated by vertically migrating micronekton. Sperm whales are large warm-blooded mammals not physiologically limited by water temperature or other hydrographic features; hence their spatial distribution would be primarily driven by the distribution of their prey, such as squid, supposedly concentrated in cyclonic oases ([Biggs et al., 2000](#)). The eddies present at a depth of 200 m can strongly modify the mixing and dispersion of organic matter and thus lead to a concentration of many prey species ([Pingree and Le Cann, 1992](#); [Koutsikopoulos and Le Cann, 1996](#)).

4.4 Conservation implications

Statistic modelling allows the prediction of the number and distribution of animals, their physiological status, demographic rates and interactions between individuals and species ([Grimm and Railsback, 2013](#)). This information allows more effective conservation measures to be taken.

The Mediterranean sperm whale population is particularly exposed to various anthropogenic pressures, such as high-intensity noise activities, fishing activities, ship strikes, plastic ingestion, or exposure to chemical pollution ([Aguilar et al., 2002](#); [Reeves and Notarbartolo di Sciarra, 2006](#); [Notarbartolo-Di-Sciarra, 2014](#)). To mitigate the impact of these activities, management measures need to be implemented, based on knowledge of the distribution of sperm whales. Our study shows that in the western basin, sperm whales are found in the east of the Corsican coast and in the north of the Balearic Sea, but also in the Liguro-Provençal, Ionian, and Algerian basins where sperm whale densities reached 0.05 individuals.km⁻². A high density of sperm whales has been estimated in the Pelagos sanctuary, mainly in the east of Corsica but also in the south of France in the Ligurian Sea, but a large area where sperm whales were predicted is not protected by the sanctuary, mainly south-east of Gulf of Lion. Since this species has been considered endangered by the International Union for Conservation of Nature's Red List (IUCN; [Notarbartolo di Sciarra et al., 2012](#)) for several years, it may be relevant to extend this marine protected area to provide better protection. High densities of sperm whales were also predicted in the Balearic Sea. This area became a marine protected area (MPA) in 2018, as it has been

recognised as a corridor for cetaceans. In the model, about 0.06 individuals.km⁻² were predicted in the north of the MPA, which is higher than the average density in the whole area (0.03 individuals.km⁻²). It would be necessary to extend this MPA to the east to protect sperm whales more effectively.

Even though the MPAs do not fully encompass the distribution areas of sperm whales, three Important Marine Mammals areas (IMMAs) have been identified within these regions: the Northwest Mediterranean Sea IMMA, the Shelf of the Gulf of Lion IMMA, and the Western Ligurian Sea and Genoa Canyon IMMA. These protected areas represent a discrete portion of crucial marine mammal habitat and have the potential to be earmarked and administered for conservation purposes. The identification of IMMAs is intended to raise awareness among policy and decision makers regarding the urgent need to ensure the favourable conservation status of marine mammals in these specific regions through the implementation of appropriate management measures.

However, the model only predicts the summer distribution and not the annual distribution, which may be different in other seasons. It is therefore essential to conduct winter surveys and incorporate the results from these surveys into our results.

5 Conclusion

We aimed to fill the knowledge gap on sperm whale distribution in the Mediterranean Sea by modelling their habitat using towed acoustic data and static and dynamic environmental variables integrated over the water column. We predicted that sperm whales were mainly distributed in summer off the coast of the north-western Mediterranean basin from the Balearic Islands to the Ligurian Sea and off the Algerian coast. They were present throughout the western Mediterranean Sea and in the northern Ionian Sea. Predicted densities in the eastern part of the Mediterranean Sea were low. This distribution was influenced by several factors, such as the bottom water temperature, eddy kinetic energy at depth, surface temperature gradients and chlorophyll-a concentration. Comparison with the literature showed that their distribution coincides with the presence of canyons. Existing marine protected areas, such as the Pelagos sanctuary, the Balearic MPA and the Strait of Bonifacio generally coincide with predicted hotspots of sperm whale density. These protection zones could be extended to provide optimal protection for the species.

The use of acoustic data compensated for the main difficulty in studying sperm whales, the unavailability of animals at the surface during visual observation and the subsequent lack of data. We encourage more systematic use of towed acoustic data to study the distribution of sperm whales. As the Mediterranean sperm whale sub-population is considered to be closed as a whole, a new survey at the Mediterranean scale in winter could reveal possible changes in the species distribution during the year and enable more appropriate conservation measures to be implemented.

Data availability statement

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because sperm whales were studied using acoustics only.

Author contributions

The present work was designed by CL, AV and VR. OB provided the data. CL and AV conducted the analyses. CL wrote the manuscript. All revision were done by OB, VR and AV. 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.2023.1229682/full#supplementary-material>

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Distribution patterns of marine megafauna density in the Mediterranean Sea assessed through the ACCOBAMS Survey Initiative (ASI)

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The ACCOBAMS Survey Initiative (ASI) is the first synoptic large-scale survey of the entire ACCOBAMS Area and as such it plays a key role in filling the current gaps in our biological and ecological knowledge of large vertebrate species occurring in the region. Data gathered during the ASI were analyzed in a distance sampling surface-modelling framework to assess the summer distribution, densities and patterns, as well as to investigate the correlates of these parameters, for large vertebrate species and taxa in the Mediterranean Basin. Static and dynamic explanatory variables, including water depth (m), distance to depth contours (km), distance to canyons and seabed slope (km), sea surface temperature (°C), mixed layer depth (m) and levels of chlorophyll-a (mg/l), were considered to predict density and compute its variance spatially at a resolution of 10x10 km. A strong longitudinal gradient from low densities in the east to high densities in the west is shared by most taxa. In addition, several taxa also showed a less marked latitudinal gradient varying in direction according to species, and finally, a few of them exhibited patchy distributions.

KEYWORDS

Mediterranean, density surfaces, modeling, cetacean, elasmobranch, large fish, abundance, distance sampling

1 Introduction

In the context of the general decline of biodiversity, the need for monitoring programs at large spatial and temporal scales is widely recognized (Balmford et al., 2005; Pereira and Cooper, 2006; Tiralongo et al., 2019), where the aim is to assess changes in species occurrence to predict long-term responses to anthropogenic pressures and global changes. In this context, measuring distributions and densities of species is crucial to inform conservation and management (Grand et al., 2007).

Historically, the systematic collection of data to assess baseline species population parameters and species responses to stressors has been heterogeneous across the Mediterranean Basin (Bianchi and Morri, 2000) and this is particularly true for cetaceans (Mannocci et al., 2018a). While existing knowledge on the distribution and occurrence of megafauna is abundant during the summer and in the western and northern sectors of the Mediterranean, relatively little information exists for other seasons and for the eastern and southern portions of the basin, where knowledge is primarily anecdotal. This uneven distribution of research effort, limited in both space and time, has led to large gaps in our knowledge of marine megafauna occurrence and distribution. The need for systematic surveys across the entire Mediterranean region has been stressed by many. In response, the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) organized and conducted in 2018 the ACCOBAMS Survey Initiative (ASI) project (Panigada et al., 2023) aimed at monitoring marine megafauna and anthropogenic activities with the ultimate goal of contributing to fill the current gaps in knowledge.

Large scale multi-species monitoring programs for megafauna have been established outside the Mediterranean Sea (e.g., CODA, 2009) with the primary goal to obtain the first comprehensive estimates of abundance of encountered species and to evaluate these estimates in an ecological and conservation context. To date, the continuation of these well-established programs and the planning of new ones is not only justified by their effectiveness to inform management and conservation decisions but is also a legal requirement under the European Union Habitats and Marine Strategy Framework Directives (HD and MSFD respectively) and, albeit in a less prescriptive way, by the Barcelona Convention.

The Mediterranean Sea, although in general considered an oligotrophic sea, is a hotspot of biodiversity, hosting several endemic and rare species. At the same time, it is also subject to ever increasing anthropogenic pressures, with the potential to afflict critical habitats, Important Marine Mammal Areas (Tetley et al., 2022) and the species therein, therefore posing a serious threat to their persistence.

Amongst cetaceans, eleven species, considered sub-populations of their north Atlantic counterparts, are known to occur regularly in the Mediterranean Sea (ACCOBAMS, 2021a): the fin whale *Balaenoptera physalus* (Linnaeus, 1758), the sperm whale *Physeter macrocephalus* (Linnaeus, 1758), the Cuvier's beaked whale *Ziphius cavirostris* (Cuvier, 1823), the common dolphin *Delphinus delphis* (Linnaeus, 1758), the long-finned pilot whale *Globicephala melas*

(Traill, 1809), the Risso's dolphin *Grampus griseus* (Cuvier, 1812), the killer whale *Orcinus orca* (Linnaeus, 1758), the striped dolphin *Stenella coeruleoalba* (Meyen, 1833), the rough-toothed dolphin *Steno bredanensis* (Cuvier, 1823), and the common bottlenose dolphin *Tursiops truncatus* (Montagu, 1821). For most of these populations, the Mediterranean Basin is the relevant unit for monitoring, assessment and conservation actions.

Alongside cetaceans, in the Mediterranean Sea, 73 species of sharks, rays and chimaeras have been recorded (Dulvy et al., 2016), with 31 species endangered or critically endangered. Some shark species, particularly large predatory ones, have suffered a steep decline from >96 to >99.99% in the last decades, often due to overfishing and bycatch (Ferretti et al., 2008). Similarly, of 32 species of rays that are found in the Mediterranean, half are facing extinction risk (Dulvy et al., 2016). Together with species of conservation concern, the Mediterranean is home to commercially lucrative species such as the swordfish (*Xiphias gladius* (Linnaeus, 1758)) and the bluefin tuna (*Thunnus thynnus* (Linnaeus, 1758)), whose stocks have dramatically reduced due to overexploitation and whose fishery has been subjected to strong restrictive and rebuilding measures introduced by the International Commission for the Conservation of Atlantic Tunas (ICCAT)¹.

The patterns of occurrence and distribution of species are influenced by a variety of physical, biological, and environmental parameters operating at different spatial and temporal scales (e.g., Hillebrand, 2004; Whitehead et al., 2008; Tittensor et al., 2010; Torreblanca et al., 2022), as well as by the variability of these parameters due to natural fluctuations and human induced changes (e.g., MacLeod, 2009; Lu et al., 2023; Snell et al., 2023).

The presence of decreasing eastward longitudinal gradients in the Mediterranean Sea has been reported for primary productivity, as well as for the distribution and abundance of benthic and pelagic species (Danovaro et al., 1999; Quignard and Tomasini, 2000; Ben Rais Lasram et al., 2009; López-Sandoval et al., 2010; Bonnet et al., 2011; Danovaro et al., 2020). Bonnet et al. (2011) described the Western Mediterranean as oligotrophic and the Eastern Mediterranean as ultra-oligotrophic, reporting that planktonic N₂ fixation has a clear decreasing trend from West to East. The authors also found that the N₂ fixation in the western basin can sustain up to 35% of new primary production, while it seems negligible in the eastern basin. In the Strait of Gibraltar, there are overlapping, and inverse flows of water, whereby Atlantic waters enter at the surface and Mediterranean waters leave at depth. As a result, the surface water currents produce a general flow of Atlantic waters towards the east with many eddies and diversions of branches on its way, resulting in the formation of cyclonic circuits due to the Coriolis effect. There is no return system at the surface; the return occurs in the intermediate layers coming from the Levantine basin which has a general movement towards the west but also following large cyclonic circuits (Rodríguez Martínez, 1982; Miller and Ketchum, 1983).

¹ <https://www.iccat.int/Documents/Recs/compendiopdf-e/2022-09-e.pdf>

The purpose of this paper is to explore if the same prevailing longitudinal distribution gradients from west to east, and other less marked or less prevalent patterns, are observed particularly in cetaceans, but also in some elasmobranchs and large fish of both conservation and commercial importance. We discuss the results alongside the significant variables that likely explain the predicted estimates and that can help to understand the observed patterns and gradients. The present work, focused on spatial patterns in densities of marine megafauna and their possible ecological significance, is paralleled by another paper dealing with basin-wide abundance estimates of cetaceans and their monitoring and conservation implications (Panigada et al., 2023).

2 Materials and methods

2.1 Study area

The entire Mediterranean expands up to 2.6 million km² (of which we covered around 73%) with an average depth of 1,460 m, and a maximum depth of 5,267 m along the Hellenic Trench in the Ionian Sea (Rodríguez Martínez, 1982). In general, the Mediterranean Sea has a quite narrow continental shelf, with a few exceptions such as the eastern coast of Tunisia and the Adriatic Sea, and large areas of open deep sea (Coll et al., 2010) (Figure I.1 in Supplementary Materials). The morpho-bathymetry, the water circulation (Figure I.2 in Supplementary Materials) and the general oceanographic conditions of the Mediterranean Sea are complex (e.g., Bethoux, 1979; Bas, 2009; Brosolo et al., 2012). The basin is characterized by several regional and local features and strong environmental patterns (Danovaro et al., 1999) with both longitudinal and latitudinal, as well as depth gradients, affecting primary and biological productivity. It is also considered as a concentration basin, where evaporation is higher particularly in the eastern basin, increasing salinity and decreasing sea level, which in turn promotes the flow of Atlantic water into the Mediterranean (Rodríguez Martínez, 1982; Miller and Ketchum, 1983). The sea surface temperature (Sst) in the Mediterranean has two very different regimes, in winter (typically December to March) and summer (usually June to September) with spring and autumn being transition periods (Pastor et al., 2018). During summer, when this survey was carried out, the highest Sst values are found in the south-eastern Mediterranean, the Lybian coast and, in some years, the south Tyrrhenian Sea. While the coolest areas are in the Alboran Sea/Strait of Gibraltar and the Gulf of Lyon (Pastor et al., 2018).

2.2 Survey design and data collection

The Mediterranean region (excluding the waters off Lybia and Egypt) was divided in 32 blocks to best accommodate logistical needs, presence of airports, jurisdictional boundaries and robustness of the sampling design. Transects were designed systematically with equal coverage probability, with an equal-angle zig-zag configuration, using the software Distance 7.3 (Thomas et al., 2010). The survey, conducted in passing mode

(Dawson et al., 2008), was planned with a ratio effort/area varying between 2.7 and 3.6% in each block. Specific details on survey design are provided in Panigada et al. (2023).

A distance-sampling approach was used for marine mammals and large fish, such as elasmobranchs. Accordingly, for these taxa, the declination angle to the sighting was collected when the animal or group of animals were abeam to the plane, and subsequently used to calculate the perpendicular distance to the transect used to account for animals/groups detectability via distance sampling methods (Buckland, 2001). On the other hand, a strip-sampling approach was used for sun fish (most probably *Mola mola* (Linnaeus, 1758), as it is the most common sunfish in the Mediterranean, over *Mola alexandrine* (Ranzani, 1839) (Sawai et al., 2018), although definitive species could not be distinguished from the airplane).

Environmental conditions potentially affecting the detectability of the animals (e.g. Beaufort sea state, glare severity and angle, turbidity, cloud coverage and an overall subjective assessment of the detection conditions) were recorded at the beginning of each transect and whenever they changed. Data collected during sightings included species (identified to the lowest possible taxonomic level), school size and declination angle (measured with a hand-held clinometer) in the case of species for which a line transect approach was used.

More specific details on data collection are provided in Panigada et al. (2023), while a description of the field procedures and protocols for data collection are described in Lambert et al. (2019) and followed well established procedures in aerial distance sampling surveys (e.g., Laran et al., 2017).

2.3 Data analysis

2.3.1 Areas and subareas

The study area has been subdivided into 5 areas for the analyses (Figure I.3 in Supplementary Materials), defined as the Atlantic (33,720 km²), the Western Mediterranean (MedW – 499,002 km²), the Central Mediterranean (MedC – 601,262 km²), the Adriatic Sea (135,783 km²), and the Eastern Mediterranean (MedE – 632,983 km²), totaling 1,902,749 km². Although the study area was originally divided in 32 strata, for the purpose of this study, larger sectors were used for the final analysis (referred to as “Areas” in Figure I.3 in Supplementary Materials) to better reflect existing knowledge on the ecology and distribution of the observed species.

2.3.2 Model-based analysis

Design-based analyses of the dataset arising from the ASI are presented and discussed by Panigada et al. (2023). Data on Chelonids (e.g., *Caretta caretta*) were integrated with existing datasets in the region and are presented and discussed by (DiMatteo et al., 2022); consequently, they are not included in this study. For the aims of this paper, density surface models within a distance sampling framework were used to predict species' density as a function of environmental, oceanographic and physiographic covariates (the list of tested covariates is presented in Table III.2 in the Supplementary Materials). A spatial grid at a resolution of

10x10 km was overlaid to the survey area to associate explanatory covariates values to on-effort segments within each grid cell to predict density spatially. The resolution of the grid cells was chosen as the finest consistent resolution that captures all available covariates. Covariate values were then assigned to the centroid of each grid cell.

The count of groups in each segment was used as the response variable. The density of groups was modelled using a Generalized Additive Model (GAM) with a logarithmic link function, and a Tweedie error distribution, very close to a Poisson distribution but allowing for some over-dispersion. The general structure of the model was:

$$\hat{N}_i = \exp \left[\ln(a_i) + \theta_0 + \sum_k f_k(z_{ik}) \right]$$

where the offset a_i is the effective search area for the i^{th} segment (calculated as the length of the segment multiplied by twice the esw), θ_0 is the intercept, f_k are smoothed functions of the explanatory covariates, and z_{ik} is the value of the k^{th} explanatory covariate in the i^{th} segment. The esw was obtained for each species or species group from their detection function, according to the covariates included in it. The abundance is then estimated by multiplying the density by the survey area.

Abundance for each taxon in each grid cell was estimated by multiplying the abundance of groups, predicted from the best fitting model, by the mean group size estimated for each area or the modelled group sizes if spatial variation was observed. In the case of modelled group sizes, the observed group size of each sighting was taken as a response variable, no offset was used, and the distribution family was negative binomial. For fin whales, however, given the very small group sizes, the number of animals was modelled directly (instead of two steps). The same framework was used as the one used for the model of groups but using the number of individuals instead of the number of groups as the response variable.

Variance of abundance was estimated by a parametric bootstrap procedure, also called “posterior simulation” (Miller et al., 2022). The delta method was used to combine the coefficient of variation (CV) from the bootstrap with the CV from the detection function and from the model. The 95% Confidence Interval (CI) was obtained using the final CV and assuming the estimates were lognormally distributed. All modelling was carried out using the statistical software R.4.0.3 (R Core Team, 2020) using the *mgcv* 1.8-33 package (Wood, 2017).

For some small areas not surveyed (highlighted in Figure I.3 in Supplementary Materials), predictions were extrapolated from surrounding areas and according to their environmental covariates. However, no extrapolation was produced for the large un-surveyed area in the south-eastern portion of the Mediterranean Sea, as the environmental covariates in such a large area in the edge of the study area might be too different and outside the ranges measured in the surveyed areas.

For those species for which robust model-based analyses could not be performed due to a limited sample size, density estimates based on Conventional Distance Sampling (CDS) analyses were

calculated per Area. Additionally, to highlight potential latitudinal or longitudinal gradients and to help compare the distribution of sighting location by species or functional group, a plot of staggered distribution of sightings coordinates was created. For each species or functional group, the ridges in the plot show the probability density function of the sightings’ latitude and longitude estimated via a kernel density method. Plots were prepared using the `stat_density_ridges()` function in the package `ggrridges` (Wilke, 2022) for the software for statistical analyses R (R Core Team, 2023) with an automatic scaling of the ridgelines computed at 2.77.

3 General results

About 91% (55,167 km) of the planned transects (60,808 km) were monitored on effort (Figure I.3 in Supplementary Materials). Table III.1 in the Supplementary Materials summarizes sightings’ information of cetaceans, elasmobranchs and large fish; among cetaceans, striped dolphins were the most commonly observed species, followed by bottlenose dolphins and to a lesser extent, Risso’s dolphins. In 18% of sightings (n= 146) it was not possible to discriminate between striped and common dolphins. Among elasmobranchs, the spinetail devil ray (*Mobula mobular* (Bonnaterre, 1788)) was the most sighted species, followed by unidentified sharks. Sunfish was the most sighted large bony fish in terms of number of encounters, although tunas represented the larger number of individuals observed.

Table 1 shows the parameters and selected covariates for the density surface modelling for each species or group of species for cetaceans, and Table 2 for elasmobranch and large bony fish. Figures 1–12 present the results of model-based abundance estimates for those species with sufficient sightings to allow running models. The maps present predicted numbers of animals per 100 km². Associated uncertainty maps (CVs) are shown in the Supplementary Materials.

All results provided here, including Table IV.1 to Table IV.13 in the Supplementary Materials, which show the abundances estimates per area (Figure I.3 in Supplementary Materials) for the species considered in this paper come from the model-based analysis. Results from the design-based analysis are given in Panigada et al. (2023). Figure 13 shows the density distribution of recorded latitude and longitude at sighting location by species or functional group.

4 Species account

For each taxon, the overall density and its distribution pattern derived from the present analyses are highlighted. These results are followed by taxon-specific comparisons with previous literature on density estimates and ecology. The interpretation of the broad picture about marine megafauna distribution patterns in the Mediterranean Sea is left for the general discussion. The results for the Atlantic contiguous area are not discussed in this paper. The plots of the smooth functions for all models are shown in Supplementary Materials.

TABLE 1 Parameters and selected covariates for cetaceans.

Species	Resp. Var.	Groups/Individuals				Group size			
		Covariates	edf	p	Dev. exp. (%)	Covariates	edf	p	Dev. exp. (%)
Fin whales	Individ.	Lat	1.15	<0.001	45.2				
		Lon	1.16	<0.001					
		mlt_0608	0.89	0.0038					
		distshelf	1.06	<0.001					
Risso's dolphins	Groups + Gsize	distcanes	0.86	<0.001	19.6	Lat	0.64	0.1	38.9
		Lon	1.04	<0.001		mlt_0608	0.75	0.047	
		mlt_month	0.89	<0.001		ssh_0608	1.81	<0.001	
		CI	1.50	<0.001					
Striped dolphins	Groups + Gsize				26.9	Aspect	1.28	0.106	13.2
		Lat-Lon	13.67	<0.001		Lat	0.62	0.126	
		depthmax	4.18	<0.001		Lon	0.82	0.019	
						SD_sst_month	5.74	<0.001	
Striped or common dolphins	Groups + Gsize	Lon	5.62	<0.001	27.9				7.8
		sst_0608	1.01	<0.001		Lat-Lon	1.62	<0.001	
		distslope	0.93	<0.001					
Small dolphins	Groups + Gsize	distescar	0.94	<0.001	27.3				4.6
		Lat	1.27	<0.001		Lat	0.32	0.248	
		Lon	6.23	<0.001		Lon	1.03	<0.001	
		depthmax	3.99	<0.001					
Bottlenose dolphins	Groups + Gsize				15.3	depthmax	2.5	0.004	18.6
		Lat-Lon	20.06	<0.001		distcany	0.66	0.079	
		CI	2.39	<0.001		mlt_0608	0.72	0.069	
						sst_0608	0.92	<0.001	

edf, estimated degrees of freedom; p, significance of the covariate. See Table 2 in Supplementary Materials.

4.1 Fin whale

Overall density estimate for fin whales was $0.8 \cdot 10^{-3}$ ind.km⁻² (CV=0.29) (Table IV.1 in the Supplementary Materials) and mean group size was 1.5 animals per group. The highest density was found in the Western Mediterranean area ($2.3 \cdot 10^{-3}$ ind.km⁻², CV=0.29), notably in pelagic waters from the Ligurian Sea to north of the Balearic Islands (Figure 3). Model-based estimated densities were one order of magnitude lower (Adriatic Sea and Central Med) or nil (Eastern Med) in the other areas.

This general pattern of the distribution of fin whales in the Mediterranean basin is in accordance with previous papers (e.g., Forcada et al., 1995; Notarbartolo di Sciara et al., 2003; Notarbartolo di Sciara et al., 2016; Panigada et al., 2017a). The species strong preference for pelagic habitats, with several groups detected at depths of 2000 metres or more, is also in line with previous works (e.g., Cotté et al., 2009; Panigada et al., 2017b).

While long distance latitudinal movements of fin whales within the Mediterranean have been reported (Panigada et al., 2017b), these movements primarily occur at the beginning of the spring season, while during the summer the species shows a rather limited distributional range, concentrated in the waters of the Corso-Ligurian-Provençal Basin (Geijer et al., 2016; Notarbartolo di Sciara et al., 2016). The species has been reported in the eastern basin, but in a practically negligible way (Stephens et al., 2021). The same pattern was found in (Mannocci et al., 2018a).

4.2 Risso's dolphin

The basin-wide density of Risso's dolphins was estimated at $13.6 \cdot 10^{-3}$ ind.km⁻² (CV=0.25) (Table IV.2 in the Supplementary Materials), with highest values in the Western and, to a lower extent, Central Mediterranean. Modelled densities in the other two

TABLE 2 Parameters and selected covariates.

Species	Resp. Var.	Groups/Individuals			Deviance explained (%)
		Covariates	edf	p	
All rays	Individuals	Lat-Lon	19.27	<0.001	33.83
		mlt_month	3.83	<0.001	
		ssh_0608	5.89	<0.001	
		sst_0608	4.58	<0.001	
Spinetail devil ray	Individuals	Lat	3.58	<0.001	29.71
		Lon	1.10	<0.001	
		ssh_0608	4.79	<0.001	
Sharks	Individuals	Lat	6.18	<0.001	38.81
		Lon	6.99	<0.001	
		distshelf	0.96	<0.001	
		mlt_0608	0.88	<0.001	
Sunfish	Individuals	Lat-Lon	21.12	<0.001	36.36
Swordfish	Individuals	distescar	4.48	<0.001	20.86
		Lat	0.95	<0.001	
		Lon	7.01	<0.001	
		CI	1.06	<0.001	
		SD_sst_month	0.91	<0.001	
Tuna	Groups	Lat-Lon	22.55	<0.001	31.37
	Group size	Lat-Lon	23.7	<0.001	87.81

The meaning of the covariates can be consulted in Table 1; edf, estimated degrees of freedom; p, significance of the covariate. See Table 2 in Supplementary Materials.

areas were only $3 \cdot 10^{-3}$ ind.km⁻². The mean group size was 6 animals per group (CV=0.18). Highest densities were in oceanic waters from the Alborán Sea, north of the Balearic Islands to off the central Algerian slope (Figure 4). Smaller concentrations were found off the slope of Provence and Liguria.

Our results agree with previous knowledge supporting a higher occurrence of this species in the Western part of the Mediterranean Sea (Gómez de Segura et al., 2008; Laran et al., 2017). Although the species is often considered to be associated with continental slopes, during the ASI it was sighted mainly in offshore oceanic environment.

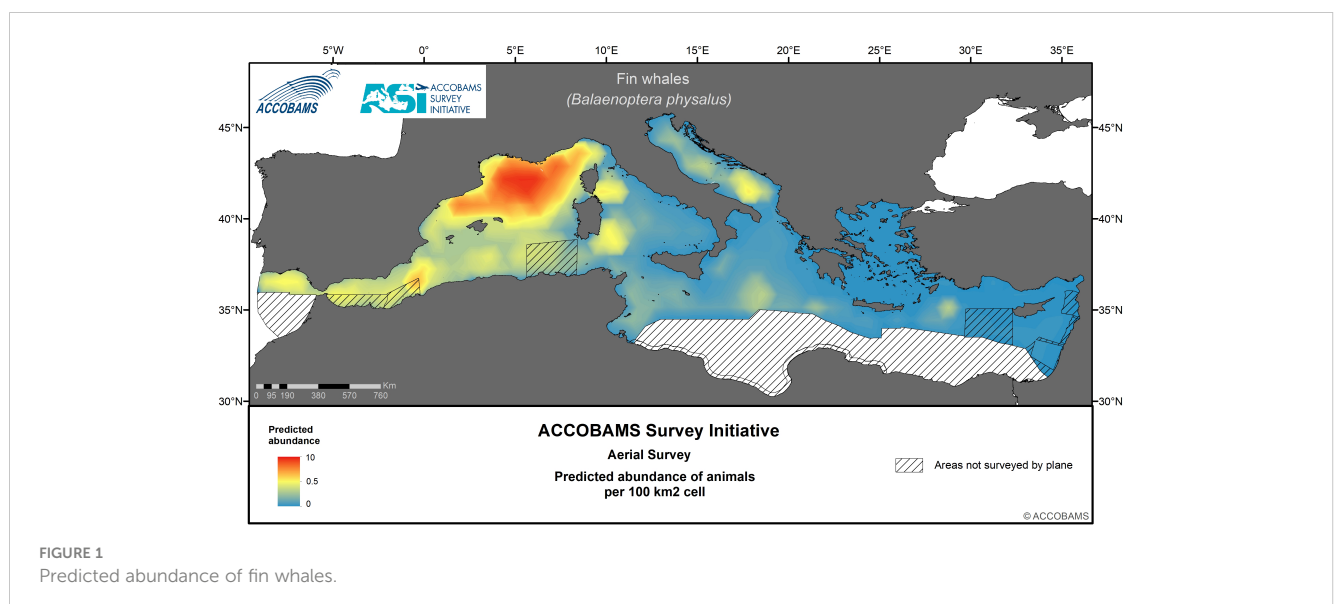


FIGURE 1 Predicted abundance of fin whales.

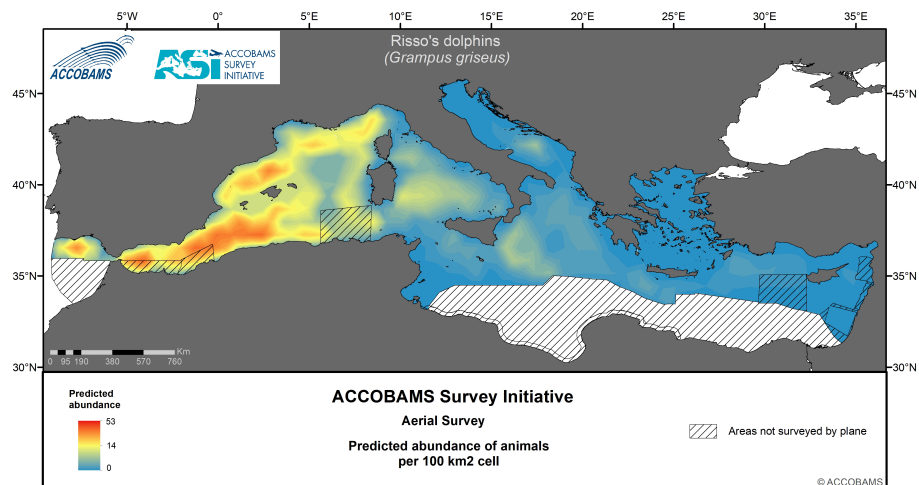


FIGURE 2 Predicted abundance of Risso's dolphins.

The Risso's dolphin in the Mediterranean basin is one of the least-known cetacean species and has been the subject of only few dedicated studies (e.g., Azzellino et al., 2016; Borrell et al., 2021; Cipriano et al., 2022; Luna et al., 2022; Minoia et al., 2023). The species is known for its strong habitat preferences, with individuals primarily encountered in relatively small groups, favoring slope areas, mainly in the north-western Basin (Bearzi et al., 2011). They are also regularly reported in the Alborán Sea, the Gulf of Vera and the Provençal basin, where their range includes deep offshore waters (Cañadas et al., 2002; Cañadas et al., 2005; Laran et al., 2017). Mannocci et al. (2018a) showed higher densities over the continental shelf across the Mediterranean Sea (only depth was included in their models), with most of their observations occurring in the Western Mediterranean and only a few in the Central Mediterranean and southern Adriatic.

4.3 Common bottlenose dolphin

The overall density of bottlenose dolphins was estimated as $28.4 \cdot 10^{-3} \text{ ind.km}^{-2}$ ($CV=0.15$) (Table IV.3 in the Supplementary Materials), with a mean group size of 7.4 animals ($CV=0.17$). Highest densities were in the Adriatic area ($60 \cdot 10^{-3} \text{ ind.km}^{-2}$) and lowest densities in the Eastern Mediterranean area. More specifically, the distribution was patchy, with favorable sectors in predominantly shelf and slope habitats of the Alborán, northern Balearic Islands-to-Gulf of Lion, northern Tyrrhenian, northern Adriatic, southern Sicily-Malta-Tunisian shelf, and the Aegean subareas, separated by extended stretches of low densities (Figure 5).

The present results are in gross agreement with existing knowledge on the coastal and shelf preferences of this species (Bearzi et al., 2009; Mannocci et al., 2018a). However, the

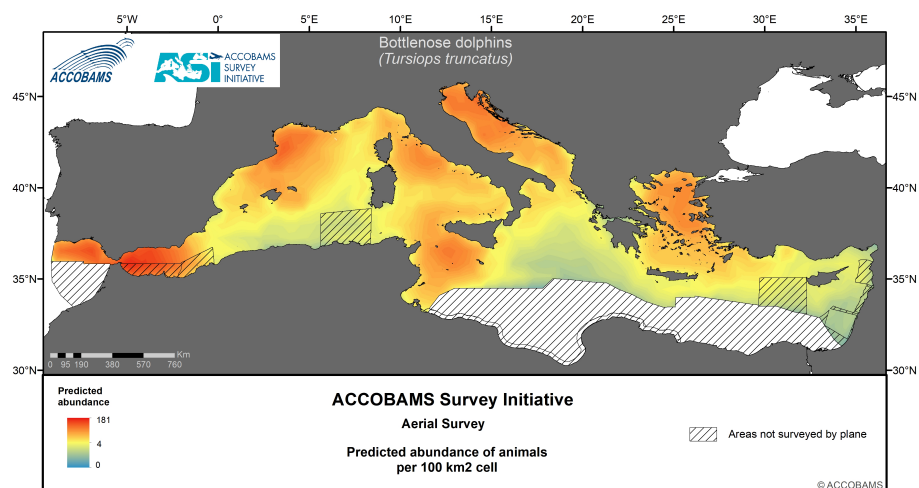


FIGURE 3 Predicted abundance of bottlenose dolphins.

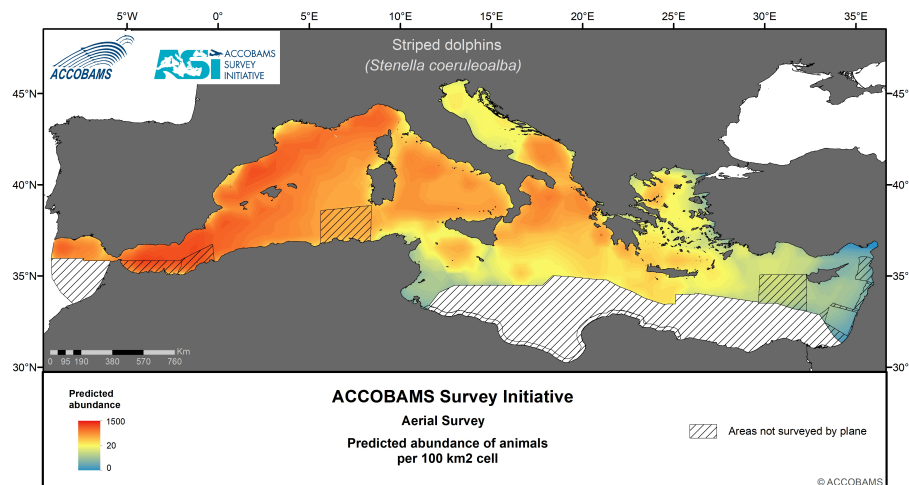


FIGURE 4
Predicted abundance of striped dolphins.

bottlenose dolphin is also present in oceanic waters, albeit at lower densities, and this seems to be more notable in winter (Laran et al., 2017).

4.4 Striped and common dolphins

The two species are considered jointly because an uncertainty often lies in distinguishing common and striped dolphins from a plane during an aerial survey. Therefore, a specific species assemblage (striped or common dolphin) was used when a clear distinction between both species was not possible. The overall estimated density of this category was $111.7 \cdot 10^{-3} \text{ ind.km}^{-2}$ (CV= 0.19) (Table IV.4 in the Supplementary Materials). Another assemblage was called “small dolphins”, which included striped, common and unidentified small dolphins (which were most probably one of these two species), with an estimated density of

$390.2 \cdot 10^{-3} \text{ ind.km}^{-2}$ (CV=0.09) (Table IV.5 in the Supplementary Materials). Regarding this last assemblage, the Western Mediterranean area displayed densities of $972.9 \cdot 10^{-3} \text{ ind.km}^{-2}$, about one order of magnitude above the other three areas; lowest densities were in the Adriatic and Eastern Mediterranean areas. More specifically, the vast majority of sightings of both groupings were recorded from the Alborán Sea, the Balearic Sea, the Gulf of Lions to the Ligurian Sea (Figure 5, Figure 6, Table IV.4 and Table IV.5 in the Supplementary Materials). Almost no sightings of this guild were recorded in the northern Adriatic Sea and the Levantine Basin.

Striped dolphins could be unequivocally identified in 258 encounters. The estimated basin wide density for striped dolphin was $234.7 \cdot 10^{-3} \text{ ind.km}^{-2}$ (CV= 0.15) (Table IV.6 in the Supplementary Materials), with densities in the Western Mediterranean about 5-10 times higher than anywhere else. The highest densities were mostly found in slope and oceanic waters along the northwestern part of the

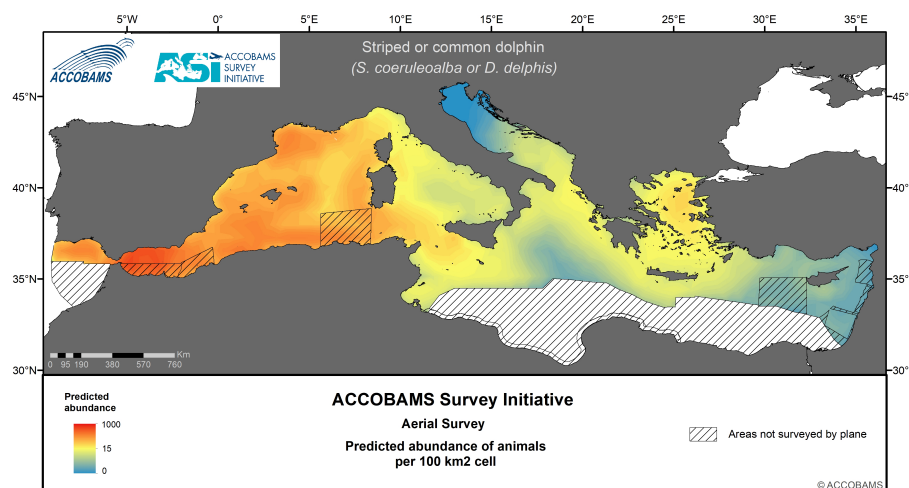
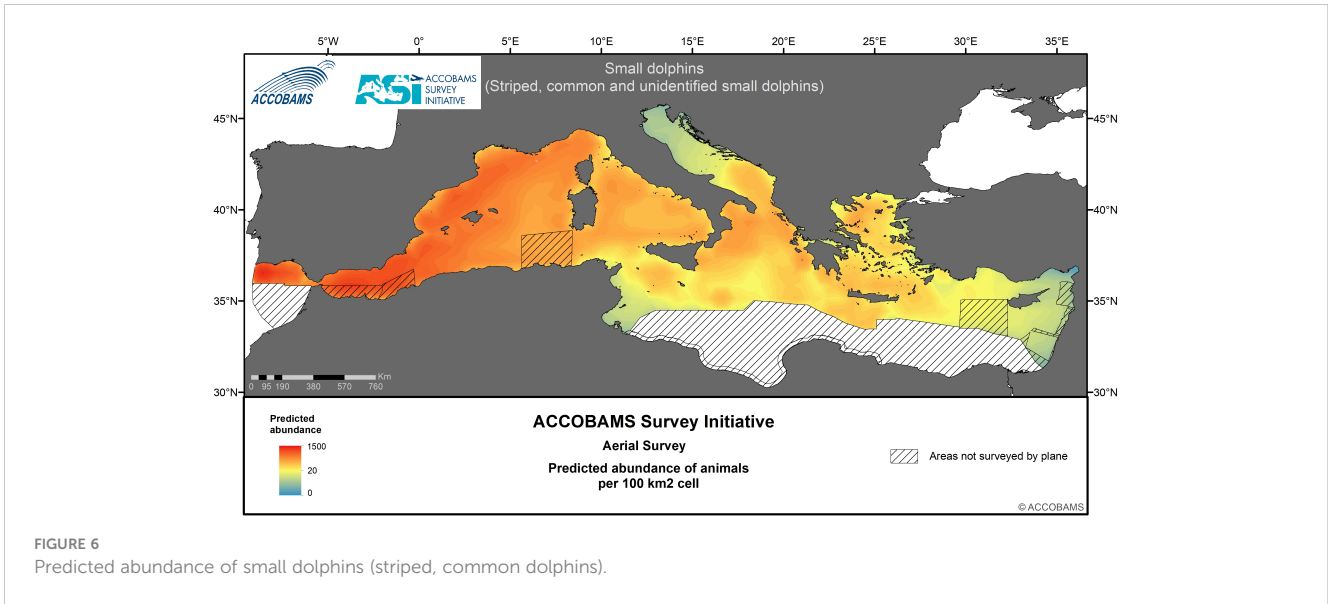


FIGURE 5
Predicted abundance of undetermined striped or common dolphins.



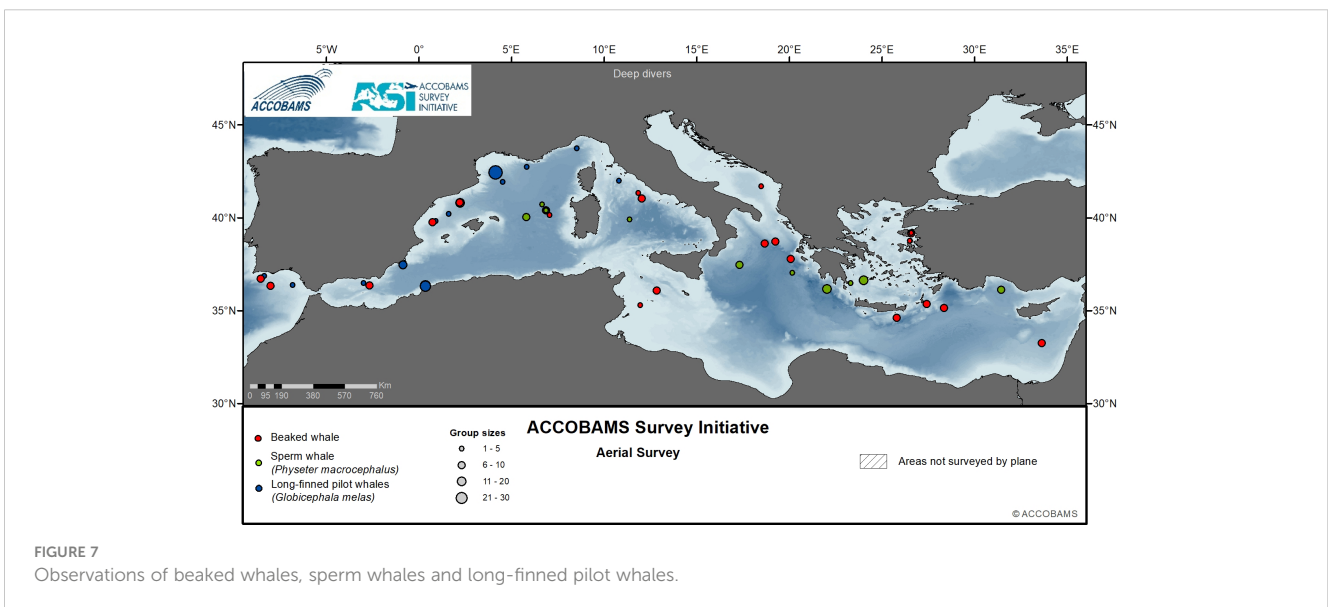
Western Mediterranean area, from the Alborán Sea to the Ligurian Sea, all the way through the Balearic Islands and off the Gulf of Lion. The Tyrrhenian Sea, the Moroccan and Algerian plateau and the waters of the Ionian Sea and the southern Adriatic Sea showed slightly lower densities (Figure 4).

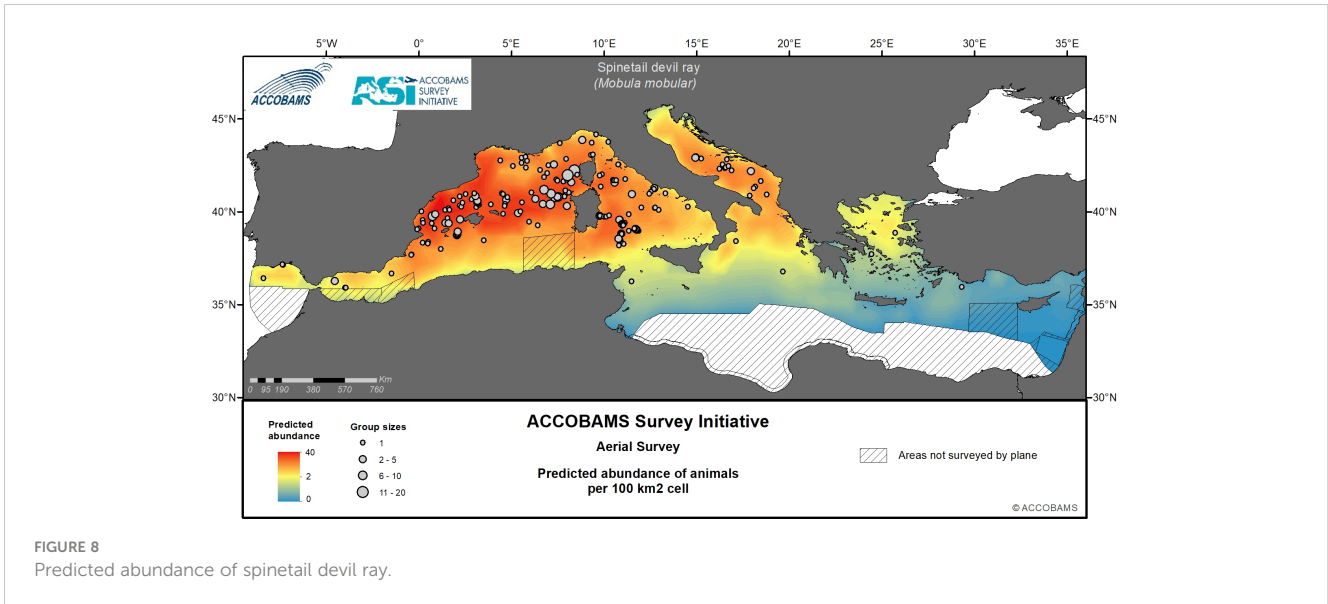
Overall, only 32 sightings of common dolphins, mostly encountered in the western portion of the Basin and in the Strait of Sicily, have been recorded during the aerial surveys (Table III.1 in the Supplementary Materials), mainly between latitude 33-38° North. No spatial modelling could be done for this species due to the small sample size.

The striped dolphin has previously been observed primarily in the offshore waters of the Mediterranean Sea, where the largest groups were also observed, indicating a strong preference for deep pelagic waters (e.g., Azzellino et al., 2008). Overall, the present data

support the existing evidence that the western basin represents the most important striped dolphin habitat in the region (Mannocci et al., 2018a).

Regarding the common dolphin, Mannocci et al. (2018a), with a much larger dataset, showed an extreme gradient in which the density was high in the Alborán Sea, being very low or close to zero in the rest of the Mediterranean, except for a small patch in the north-eastern section of the Aegean Sea and northern Adriatic Sea. Vella et al. (2021) did a compilation of knowledge on this species in the Mediterranean, reporting similar distribution patterns. Therefore, this survey corroborates previous knowledge that common dolphins have a strong longitudinal gradient, with most of their density concentrated in the West and very little as we progress to the East. This result shows the strongest pattern in the staggered distribution plot in Figure 13.





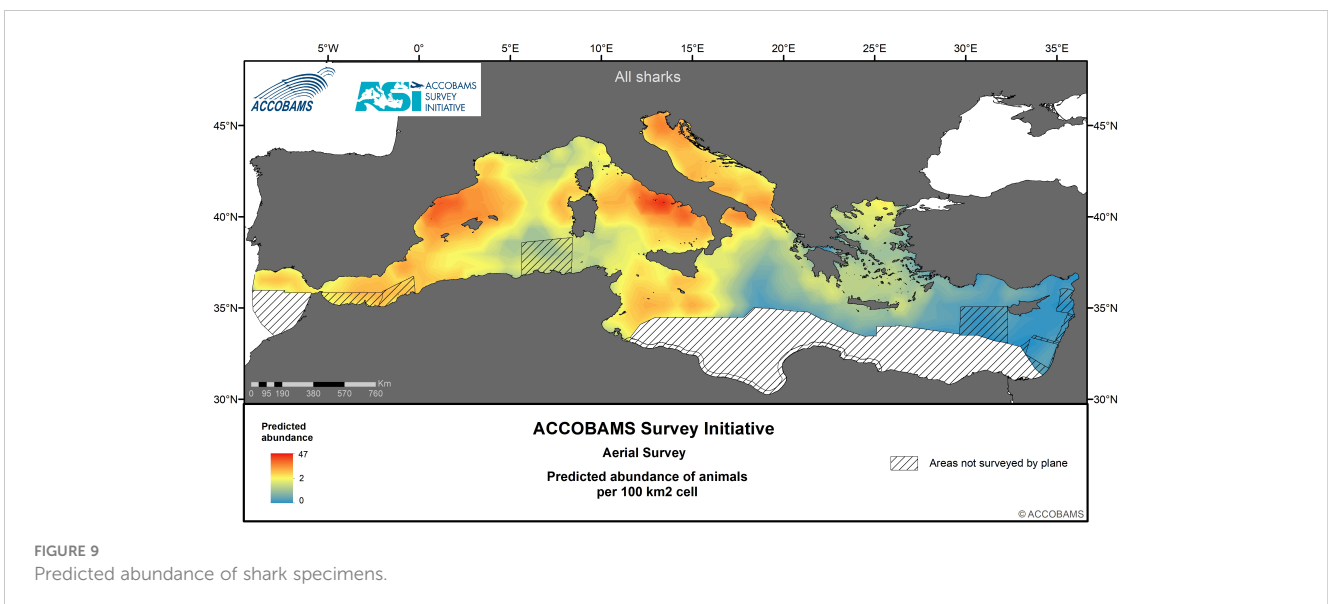
4.5 Sperm, pilot and Cuvier’s beaked whales

Sperm whales, being deep divers, are difficult to detect from airplanes, and only 10 groups were detected (Table III.1 in the Supplementary Materials). They were encountered in both the eastern and western basin, with sightings only recorded within 35° to 40° of latitude North, mainly along the Hellenic Trench and in the offshore waters of the Sea of Sardinia (Figure 7). With such low number of observations, it was not possible to create a detection function and therefore estimate abundance from the aerial survey. A density estimate is provided, however by Boisseau et al. (submitted) from acoustic survey of the ASI vessel-based survey. Mannocci et al. (2018a), which had enough observations to model sperm whale density, found the highest densities in the Western Mediterranean and western Ionian Sea, in agreement with the abundance estimates from acoustic surveys (Lewis et al., 2018)

and data from the vessel-based component of the ASI (Boisseau et al., submitted).

Long-finned pilot whales were only encountered west of 12° E of longitude, with a strong preference for deep pelagic waters. Largest groups of this species were observed in the Alborán Sea, along the coast of Morocco and in the Gulf of Lion. Relatively smaller pods were observed in the Ligurian Sea within the waters of the Pelagos Sanctuary (Figure 7). The distribution of sightings and estimated abundance confirms the almost exclusive presence of this species in the Western Mediterranean Sea (Canadas and Sagarminaga, 2000; Notarbartolo di Sciara, 2016; Verborgh et al., 2016; Mannocci et al., 2018b).

Cuvier’s beaked whales have been mostly sighted in areas rich in canyons in the Ionian Sea and the Hellenic Trench, the deep southern Adriatic Sea, the Central Tyrrhenian Sea, the Balearic and the Alborán Seas (Figure 7). The ASI results confirm existing knowledge on the basin-wide presence of the Cuvier’s beaked whale



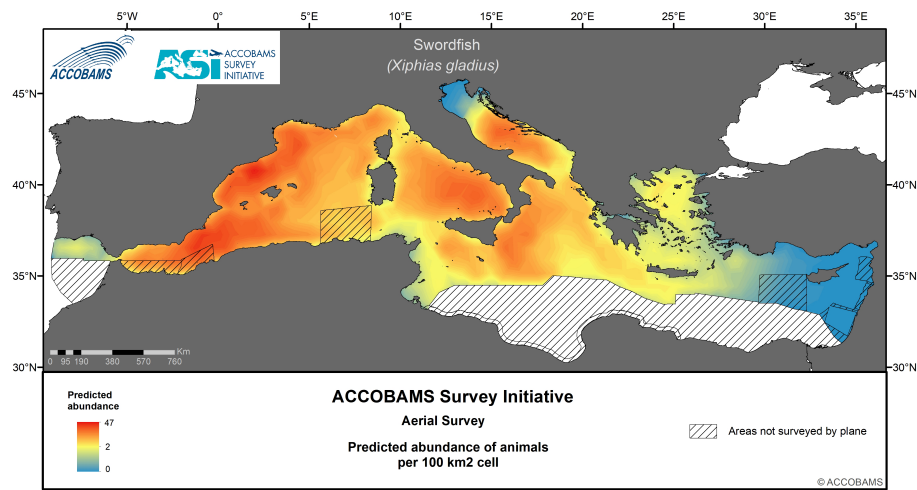


FIGURE 10 Predicted abundance of swordfish.

and, at the same time, confirm that the species occurs in relatively small patches at low densities. Mannocci et al., 2018a predicted higher densities in areas between 1000 and 2000 m depth across the whole basin using only static covariates (depth and slope), despite most of the observations being in the Alborán Sea. Cañadas et al., 2018 found similar patterns as the observations registered here, with the highest densities in the Alborán Sea, and other patches of high density in the Ligurian Sea, Central Tyrrhenian Sea and Ionian Sea. Being a deep diving species, ship-board surveys with an acoustic array represent an important complement to study the distribution of this species (Boisseau et al., submitted).

IV.7 and IV.8 in the Supplementary Materials) and the western and Central Mediterranean areas had the highest densities at about $20 \cdot 10^{-3} \text{ ind.km}^{-2}$. The central part of the Western Mediterranean, from Valencia and the Balearic Islands to Corsica and Sardinia, shows the highest density values (Figure 8 for spintail devil rays).

Notarbartolo di Sciara et al. (2015) estimated a summer density of $25.7 \cdot 10^{-3} \text{ ind.km}^{-2}$ ($Cv=0.13$) in the northern part of the Western Mediterranean between 2009 and 2013, in the same order of magnitude as the estimates reported here for the Western Mediterranean ($0.0285 \text{ individuals per km}^2$).

4.6 Rays

Rays, most of them being identified as spintail devil rays, had an overall surface density of $13.2 \cdot 10^{-3} \text{ ind.km}^{-2}$ ($Cv=0.15$) (Tables

4.7 Sharks

Sharks, only about 10% of which were identified as blue shark *Prionace glauca* (Linnaeus, 1758), had an overall surface density of $13.9 \cdot 10^{-3} \text{ ind.km}^{-2}$ ($CV=0.26$) (Tables IV.9 and IV.10 in the

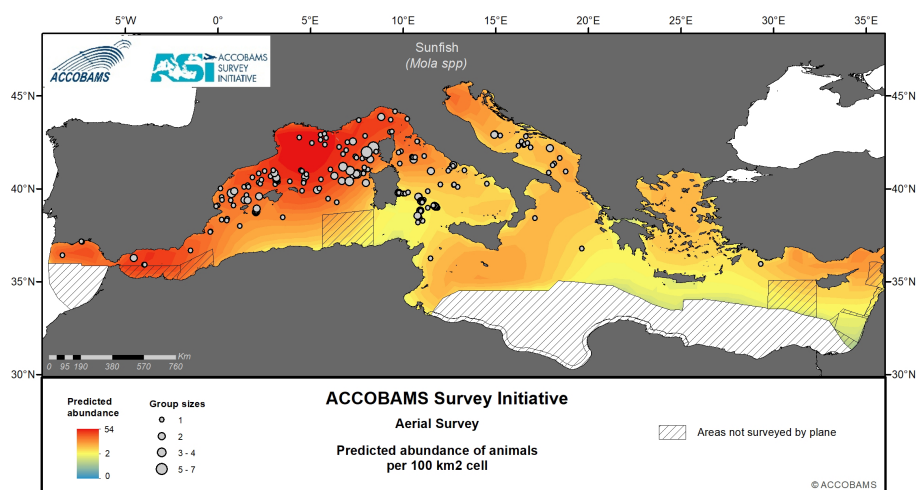


FIGURE 11 Predicted abundance of ocean sunfish.

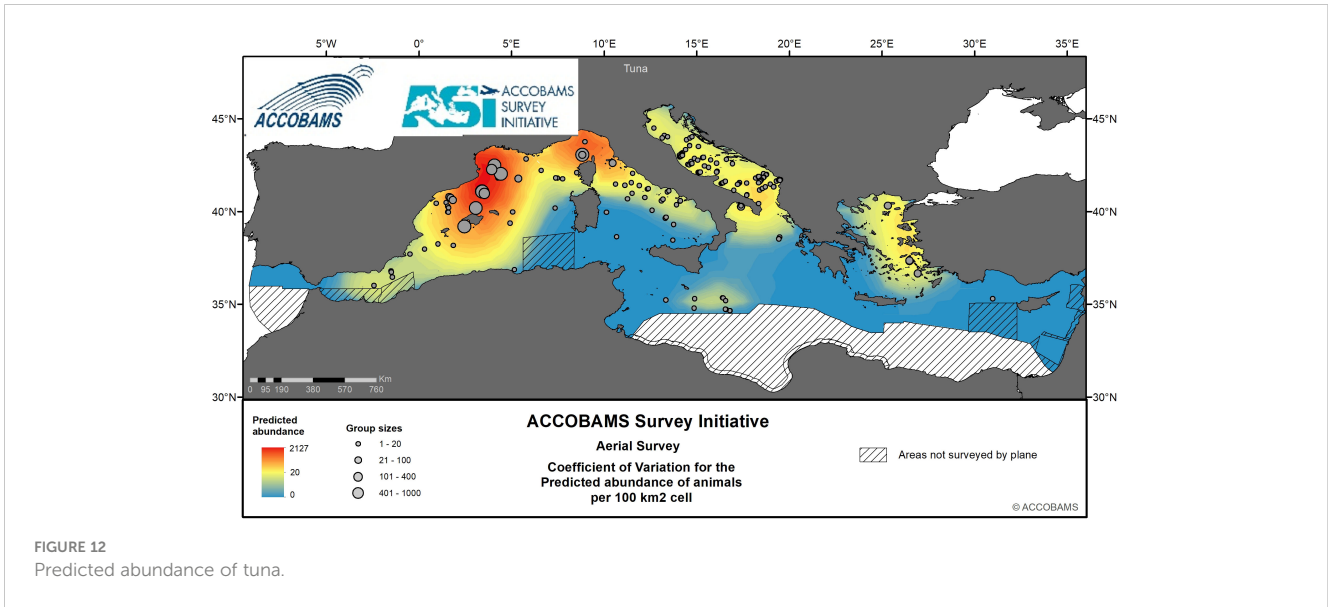


FIGURE 12
Predicted abundance of tuna.

Supplementary Materials), with the highest densities found in the Adriatic Sea, followed by the western and Central Mediterranean areas. The distribution pattern for sharks was fairly patchy, with spots of high densities around the Balearic Islands and along the Tyrrhenian coast of Italy, and other patches in the northern and southern Adriatic, on the Tunisian plateau and in the Alborán Sea (Figure 9). The Eastern Mediterranean area was uniformly low in shark density.

4.8 Swordfish

The basin-wide swordfish surface density was estimated at $14 \cdot 10^{-3}$ ind.km⁻² (CV=0.11) (Table IV.11 in the Supplementary Materials), with highest figures found in the Western Mediterranean ($24.4 \cdot 10^{-3}$ ind.km⁻²), and values one order of magnitude lower in the Eastern Mediterranean. Hotspots of

swordfish densities were from the Alborán Sea and the Algerian coast to the Gulf of Lion, and to a lesser extent in the Tyrrhenian, the western Ionian and the central Adriatic (Figure 10).

Similarly, Lauriano et al. (2017) estimated a summer density of $10 \cdot 10^{-3}$ ind.km⁻² in the same area between 2009 and 2010, in the same order of magnitude as the estimates from ASI for the Western Mediterranean (0.024 individuals per km²).

4.9 Sunfish

The ocean sunfish was present at the surface at an overall density of $14.9 \cdot 10^{-3}$ ind.km⁻² (CV= 0.36) (Table IV.12 in the Supplementary Materials), with up to $47.9 \cdot 10^{-3}$ ind.km⁻² in the Western Mediterranean and low to very low densities everywhere else. The hotspot of highest density was in the north of the Western Mediterranean, from the shelf of the Gulf of Lion all the way to

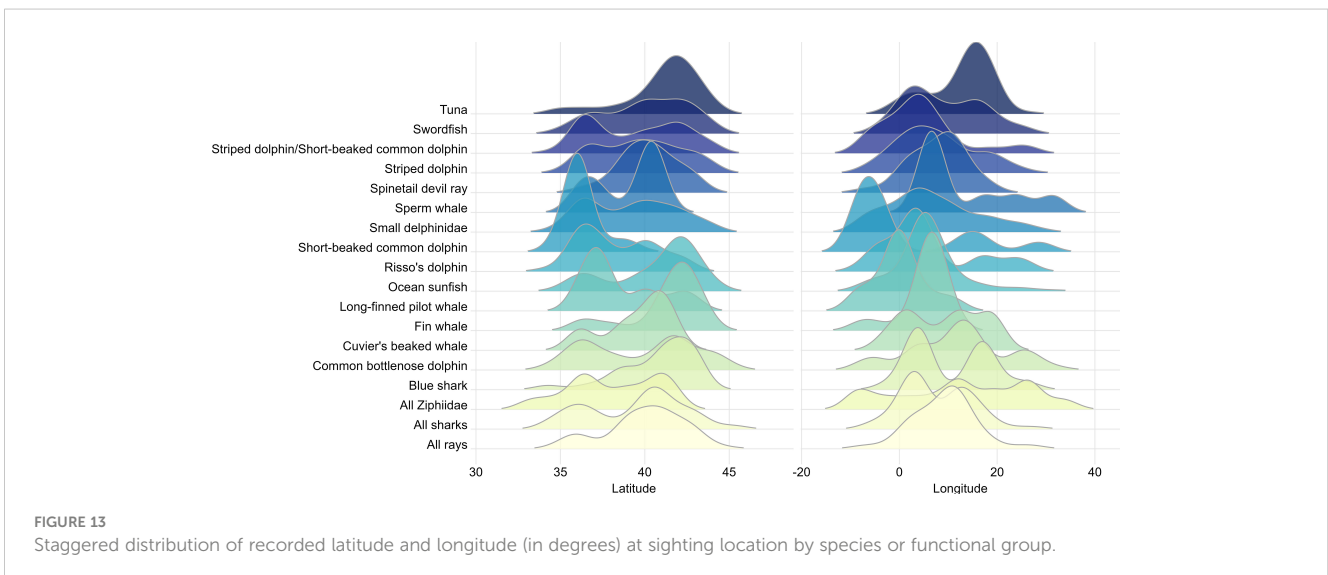


FIGURE 13
Staggered distribution of recorded latitude and longitude (in degrees) at sighting location by species or functional group.

Ligurian Sea through oceanic waters of the northern Algero-Provençal Basin (Figure 11). Patches of intermediate densities were found in either shelf or oceanic waters, in the northern Adriatic Sea, the Alborán Sea and the north-eastern Levantine Basin.

By comparison, densities of $1000 \cdot 10^{-3} \text{ ind.km}^{-2}$ were estimated in the North Western Mediterranean during the summer with hotspots in the Gulf of Lion and along the Provençal slope in the summer, and further offshore in the middle of the north-Western Mediterranean in the winter (Grémillet et al., 2017). This estimate is much higher than the present one for the same area, even when one consider that Grémillet et al.'s estimate was corrected for availability bias by using a $g(0) = 0.4$ to account for time spent at the surface by ocean sunfish.

4.10 Tuna

Tunas (all species observed) were present at the surface at an overall density of $252.7 \cdot 10^{-3} \text{ ind.km}^{-2}$ ($CV=0.39$) (Table IV.13 in the Supplementary Materials), with up to $898.3 \cdot 10^{-3} \text{ ind.km}^{-2}$ in the Western Mediterranean and much lower densities everywhere else. Tunas were encountered mostly in the north-Western Mediterranean (Balearic Sea, Gulf of Lyon and Ligurian Sea, Figure 12), where they had the larger number of individuals per group. They were also encountered, with smaller group sizes, in the eastern Thyrrenian Sea and the Adriatic Sea, and to a lesser extent in the Aegean Sea and South of Sicily. Only one group was observed in the easternmost part of the basin.

5 General discussion

5.1 Strengths and weaknesses

This paper presents the model-based results of the first large-scale synoptic aerial survey of the entire Mediterranean region conducted in the summer of 2018, and provides the baseline density estimates and maps for several large vertebrates occurring in the basin, including cetaceans, elasmobranchs and large teleosts.

Although the values presented here are underestimates of the actual densities, as they have not been corrected for availability and perception biases, known to negatively affect estimates, we consider this limitation not to significantly affect the possibility of comparing the observed spatial patterns. With the exception of sperm whales, we can assume consistent diving behavior of each species across the study area, and therefore the availability biases are likely to be consistent over the monitored area. Male and female sperm whales segregate at different latitudes across most of their range when non breeding, with single males occurring at higher latitudes primarily engaging in feeding activities, and females occurring at lower latitudes within family units. This social structure is accompanied with striking differences in the use of habitats between the two sexes as well as different diving and feeding behaviors (e.g., Pirota et al., 2011; Pirota et al., 2020a; Pirota et al., 2020b). As a result, availability bias for males and females observed during aerial

surveys might be different. During the ASI, most of sperm whale sightings were recorded at lower latitudes where family units rather than adult males are known to occur (e.g., Gannier et al., 2002) and as such the estimates presented here might be more relevant to groups or family units of this species. For this species, abundance estimated from visual aerial line transect distance sampling surveys is likely to be negatively biased because of the long dive times of these species, and passive acoustic approaches might produce more robust estimates (e.g., Barlow and Taylor, 2005; Boisseau et al., submitted; Lewis et al., 2018). The perception bias, although more variable across the region because of the differences in observers' experience, was mitigated by a pre-survey training for all observers and teams. It should also have been minimized by the use of similar airplanes, all implementing identical standardized protocols, including the compulsory use of bubble windows and the same flight speed and altitude. In addition, it is unlikely that the possible differences in observer experiences and perception biases could have any spatial trend, potentially biasing the results presented here.

It is important to note that Mediterranean Sea basin-wide estimates for cetaceans and other mega vertebrates, with the exception of Cuvier's beaked whales (Cañadas et al., 2018) and loggerhead turtles (DiMatteo et al., 2022), from visual surveys have never been obtained before, thus making comparisons with existing knowledge, for some species and geographic areas, rather difficult. Furthermore, the ASI is the only existing snapshot of the whole basin (all surveys in all areas done almost simultaneously over a short period of time), while the aforementioned estimates for Cuvier's beaked whales and sea turtles merge datasets arising from surveys conducted over several years, different areas and different survey platforms. Historically, most of the effort in the region has been allocated along coastal areas and, despite research on cetaceans has been going on for well over three decades, several portions of the Mediterranean have never been, or at best very minimally, monitored in the past (Mannocci et al., 2018b). At sea monitoring has been more substantial in the Western Mediterranean Sea, in particular during the last decade, with several aerial surveys focusing on marine megafauna, with a main focus on cetaceans, and taking place in particular in its central and north-western sectors (e.g., Gomez de Segura et al., 2006; Panigada et al., 2011; Panigada et al., 2017b; Fortuna et al., 2014; Lauriano et al., 2014; Bauer et al., 2015a; Bauer et al., 2015b; Notarbartolo di Sciara et al., 2015; Laran et al., 2017; Lauriano et al., 2017; Fortuna et al., 2018). It is also noteworthy the ICCAT aerial surveys focusing on bluefin tuna conducted annually, in the last decade, in the Levantine Sea, Ionian Sea, Tyrrhenian sea and Algerian-Provençal Sea (e.g., Cañadas and Vazquez, 2020).

Moreover, some areas in the eastern and southern portions of the Basin remain unexplored to date. In fact, some portions of the easternmost and southernmost Mediterranean were not surveyed during the ASI due to logistic constraints, geopolitical issues and lack of research permits. Accordingly, in these areas it is still difficult to assess the occurrence, distribution and abundance of cetaceans and marine megafauna therein. Despite pre-existing knowledge in these areas, direct comparisons cannot be made with the present results and further data collection and analyses are necessary to eventually assess local trends.

All the species considered in this study show geographical patterns that reflect their ecological, biological and life-history traits as well as strong eastward longitudinal gradients, with density being relatively higher in the western portion of the Mediterranean Sea and decreasing towards the eastern basin. Similarly, the uncertainty on the predictions is higher in the eastern basin, and in most cases also in the southern portions of the study area where density has been estimated.

5.2 Dominant spatial patterns

The highest values of densities for most species presented here were obtained for the Western Mediterranean. For eight of the 11 taxa considered here, a longitudinal gradient with highest densities at the western end of the basin was the most prominent feature of their distributions. Other patterns were visible in bottlenose dolphins, which showed a predominantly patchy distribution generally associated to shelf habitats. Similarly, although the current data were too limited, Cuvier's beaked whales are considered to exhibit a patchy distribution in areas of slope with extensive canyons. Sharks showed a combination of strong longitudinal gradient combined with a strong patchiness of the highest density hotspots. Finally, a latitudinal component of the distribution was also clearly visible in fin whales, rays and sunfish, and to a lesser extent in striped dolphins and sharks as well.

The main aggregations of fin whales were in the deeper waters of the Corso-Liguro-Provençal Basin and the western part of the Pelagos Sanctuary. Striped dolphins were found to have their highest densities (>1 individual per km²) in the Alborán Sea, with high densities also estimated across the Corso-Liguro-Provençal Basin. The highest densities of bottlenose dolphins were encountered west of 18°E (eastern tip of Italy). Although Risso's dolphins were seen in both the west and east, highest densities were apparent in the Algerian, Corso-Liguro-Provençal and Balearic Basins. Sperm whales exhibited clustering throughout the survey blocks, with highest densities in the Eastern Mediterranean, which includes the known important area for this species of the Hellenic Trench in Greece.

According to the knowledge of decreasing longitudinal gradients in the Mediterranean Sea from the west to the east at the levels of primary productivity (López-Sandoval et al., 2010; Bonnet et al., 2011), distribution and abundance of benthic and pelagic species (Tecchio et al., 2011; Danovaro et al., 2020) and fish richness (Quignard and Tomasini, 2000; Ben Rais Lasram et al., 2009), it seems reasonable to think that the distribution of the cetacean, elasmobranch and large fish species described in this work is a reflection of the distribution patterns of the primary productivity and of their prey.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession

number(s) can be found below: <https://accobams.org/asi-data-access-request/>.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because this research did not include handling of any animal. It was just observation from airplanes.

Author contributions

AC: Conceptualization, Data curation, Formal Analysis, Investigation, Methodology, Validation, Writing – original draft, Writing – review & editing. NP: Conceptualization, Writing – review & editing. HA: Investigation, Writing – review & editing. LD: Investigation, Writing – review & editing. ND: Investigation, Writing – review & editing. GD: Investigation, Writing – review & editing. JG: Investigation, Writing – review & editing. DH: Investigation, Writing – review & editing. SL: Investigation, Writing – review & editing. GL: Investigation, Writing – review & editing. MP: Writing – review & editing, Investigation. VR: Writing – review & editing. JV: Investigation, Writing – review & editing. SP: Conceptualization, Investigation, Supervision, Writing – review & editing.

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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.2023.1270917/full#supplementary-material>

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PelaSIG, a QGIS plugin for marine megafauna census: application to the aerial ACCOBAMS Survey Initiative dataset

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The distribution of wild animals and their monitoring over large areas raises many logistical and technical difficulties that hinder the collection of observation data. The use of Geographic Information Systems (GIS) has increased significantly in recent years. QGIS, an open-source GIS software dedicated to the processing of geospatial data, enables the development of dedicated plugins for specific workflows. The open-source PelaSIG plugin has been developed in Python for QGIS 3 to facilitate and standardise the different steps before and after distance sampling surveys. It brings together a set of tools for survey preparation, automatic data checking, visualisation and presentation of survey effort and sightings to provide an adapted workflow. This plugin is currently designed to process dedicated aerial datasets collected with the SAMMOA software during marine megafauna surveys (i.e., marine mammals, seabirds, elasmobranchs, sea turtles, etc.). Here, we first describe the different tools already available, and then, we present an application with the dataset from the aerial survey of the ACCOBAMS Survey Initiative (ASI) conducted in 2018 over the Mediterranean Sea and using a multi-target protocol.

KEYWORDS

QGIS, plugin, tools, line transect survey, spatial ecology, GIS, marine megafauna, Mediterranean Sea

1 Introduction

Line transect distance sampling is a widely used technique for the monitoring, mapping distribution, and estimating the density or abundance of marine megafauna. The heterogeneity of users and use cases requires the development of standardised procedures and tools to facilitate and improve the processing of these spatial datasets (Castro et al., 2013). To address these needs, the European Union has adopted the INSPIRE Directive (Infrastructure for Spatial Information in the European Community—2007/2/EC). The aim of this Directive is to establish a geographic information infrastructure to promote environmental protection. To this end, it requires public authorities to publish and share their geographical environmental data.

The adoption of the INSPIRE Directive reinforces the role that Geographical Information Systems (GIS) and spatial data can play in making decisions relevant to species conservation (Del Campo, 2012). In particular, “GIS software”, which refers to software for the creation, management, analysis, and visualisation of geospatial data (Steiniger and Weibel, 2010), can be enhanced with dedicated *ad hoc* plugins. Plugins are extensions that allow users to add any functionality that they need without modifying the source code of the GIS software (Sela et al., 2019). They provide an opportunity to automate tasks, as facilitating workflows has become a necessary process to manage the large amounts of data collected by saving significant amounts of time. Plugins can also support multi-user workflows by standardising data and analysis methods (Palomino et al., 2017).

Aerial surveys have been carried out by the *Observatoire Pelagis* (UAR 3462, La Rochelle University—CNRS) since the early 2000s. Since 2008, a multi-target protocol has been implemented in several large aerial surveys (e.g., Mannocci et al., 2013; Lambert et al., 2017; Pettex et al., 2017; Laran et al., 2017a; Laran et al., 2024). With the technical support of the data processing office, Code Lutin, a dedicated open source software has been developed to collect data during aerial surveys. This includes an automatic GPS connection and an audio recording system in order to fill in complementary information missed during the flight during the post-validation process (SAMMOA, 2019). In order to optimise and standardise the processing of these datasets, SAMMOA has been used for aerial surveys conducted by Pelagis since 2013. More recently, SAMMOA has been used for large European aerial surveys of marine mammals or marine megafauna [e.g., ASI (Panigada et al., 2023), ObSERVE II, and SCANS IV (Gilles et al., 2023)] and occasionally for ship surveys (but not well adapted). The use of this software improves data exchange and provides an opportunity to standardise analysis method through the use of geospatial tools, to check, map, and report survey data. Therefore, the workflow steps were designed in a dedicated plugin to prepare a planned survey design for SAMMOA, then return from the survey, check the data with automatic quality control before exploration, and map or prepare the dataset for further spatial analysis, including export as Basic Data Exchange. All the geospatial tools to carry out these different steps have been pooled in a QGIS 3 (Open Source Geospatial Foundation Project, <http://qgis.org>) plugin, called PelaSIG. Currently consisting of nine modules, the PelaSIG plugin aims to complete the process of standardising the

representation of marine megafauna monitoring data. The aim of this paper is to detail the entire processing chain of PelaSIG and to illustrate its use with a case study, the aerial survey dataset of the ACCOBAMS Survey Initiative (ASI) conducted in the Mediterranean Sea in summer 2018 to assess the distribution of cetaceans, seabirds, and other marine megafauna species and the abundance of key cetacean species (see Cañadas et al., 2023; Panigada et al., 2023).

2 Plugin description

2.1 Required input

Data collected with SAMMOA during a survey are exported to three different files: an observation file containing all the collected observations and associated parameters; an effort file containing all the effort points, such as the start and end of each transect, the location of each change in associated parameters according to the protocol; and finally, a GPS file with positions automatically recorded at regular intervals, depending on the initial settings in SAMMOA.

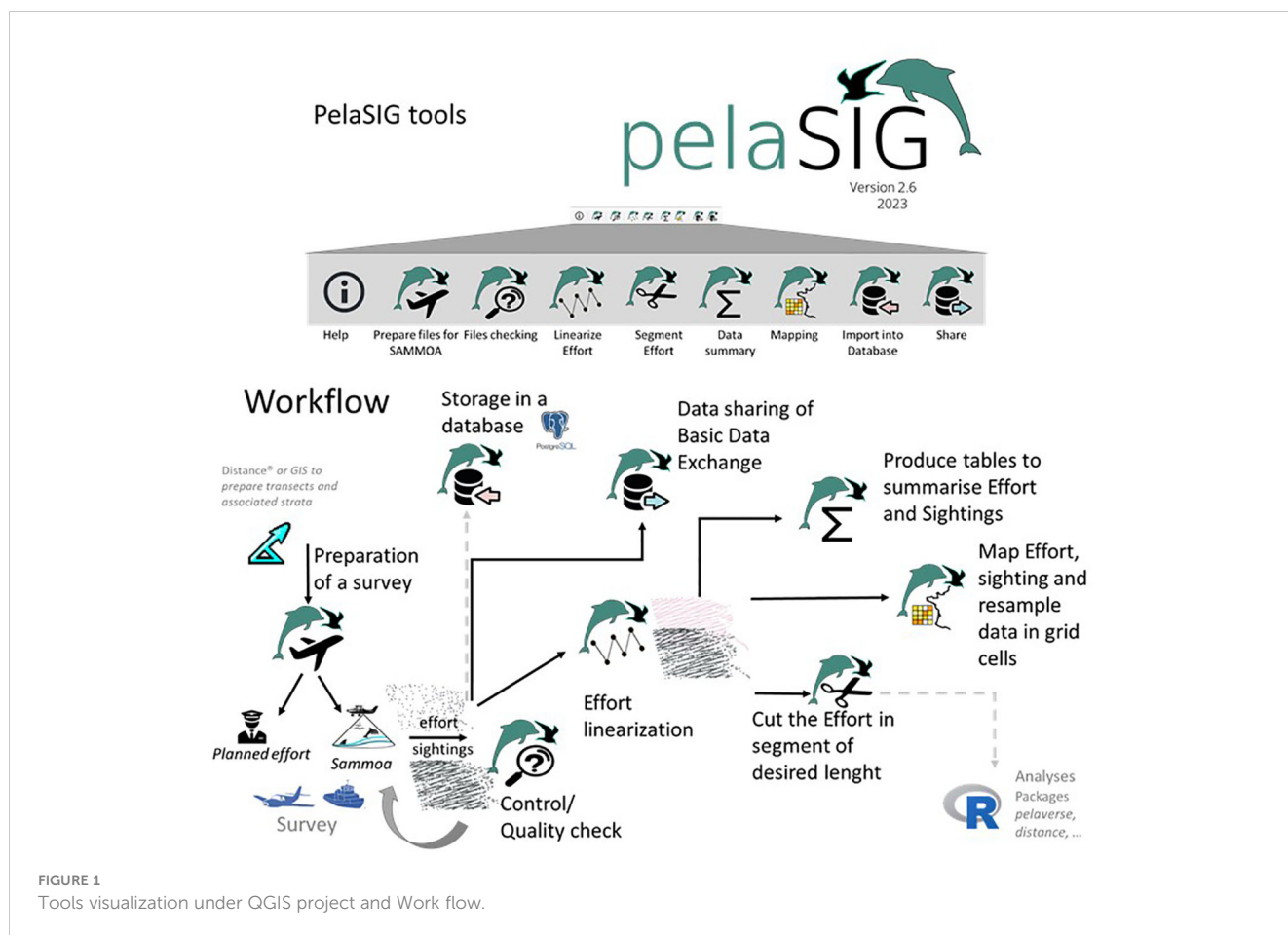
PelaSIG has been designed to process the output files directly after data collection on SAMMOA, using effort and observation shapefiles. The use of other formats (e.g., SeaScribe, www.briwildlife.org/seascribe/) requires prior transformation.

2.2 Environment of development/ software architecture

PelaSIG is integrated with QGIS software, a popular open-source GIS that runs on various operating systems including Linux, Mac OSX, Windows, and Android. Thanks to its large community of developers and a robust Python plugin framework, this software can be easily extended with external plugins. The graphical user interface has been built using Qt Designer (<https://doc.qt.io/>), and the associated functions have been written in Python 3.7. PelaSIG is now compatible with QGIS version 3 (3.16 up to 3.28 checked).

2.3 Plugin’s workflow

The PelaSIG plugin consists of nine modules (Figure 1). The modular structure of the plugin allows the operator to use each tool independently. 1) A comprehensive manual of the plugin is available through the first informative module of the toolbar. 2) The second tool is dedicated to the preparation of an aerial survey, using a shapefile of both the strata and the planned design of the transects (such as those produced by Distance software; Thomas et al., 2010); files are generated in the format required by the SAMMOA software to prepare a survey. 3) After the flights, the data are exported from SAMMOA to proceed to the quality control of the dataset, dedicated to the specific multi-target protocol used by the *Observatoire Pelagis*. 4) The following tool is then used to



linearise the effort between points and associated sightings, either directly from the SAMMOA shapefiles export or from an export of a dedicated Postgres SQL database. This step allows the files to be prepared in the required format for the planned subsequent analysis (e.g., analysis and mapping tools of pelaSIG, R-Pelaverse (Genu and Authier, 2020), Distance with R packages or software, ...). 5) The next module allows to cut this linearised effort in different segments, choosing the length, and then export as line centroids, and again with sightings associated to these new effort segments by an index to allow them to be matched. 6) The sixth tool produces several tables of descriptive statistics on the effort and observation files. 7) The next tool provides the ability to create different categories of maps and to export them as a shapefile or image according to a predefined layout. 8) Once the data have been validated, the shapefiles can be saved or stored directly in a PostgreSQL database using the next tool. 9) The last tool prepares the data for sharing in a lightweight format.

2.3.1 User manual

Developed using the Sphinx Python library, the user manual is available online from the plugin's first module (Help) on the left (Figure 1). The installation and activation of the plugin is explained in details, as in the user manual for each module (<https://www.observatoire-pelagis.cnrs.fr/tools/pelasig/>).

2.3.2 Survey planification and preparation for Sammoa software

This module is part of the survey preparation phase. The block survey (or multiple strata) and transects are designed in advance using Distance software (Thomas et al., 2010) or other tools. Once the transect shapefiles (as polylines) and associated strata (as a single or multiple polygon shapefiles) have been designed, they can be automatically prepared for SAMMOA using the Prepare files for SAMMOA tool. The transect shapefile needs to contain a *linkID* field and optionally the *transect* and *plan* (this later to distinguish multiple design runs for example), while the strata shapefile needs only the name field. This tool results in two shapefiles prepared for SAMMOA and additionally generates in kml, and gpx converted files, as a csv table including the location of the start and end points of each transect and the length in nautical miles (Figure 2) in order to facilitate survey implementation with pilots and authorities.

2.3.3 Data checking

Once the survey has been carried out and the dataset validated, possibly using the available audio recordings synchronised with sightings on SAMMOA, effort and sightings are exported as shapefiles. The Sammoa files checking allows the automatic checking of several parameters, taking into account the multi-target protocol (Dorémus et al., 2020). A list of potential errors is

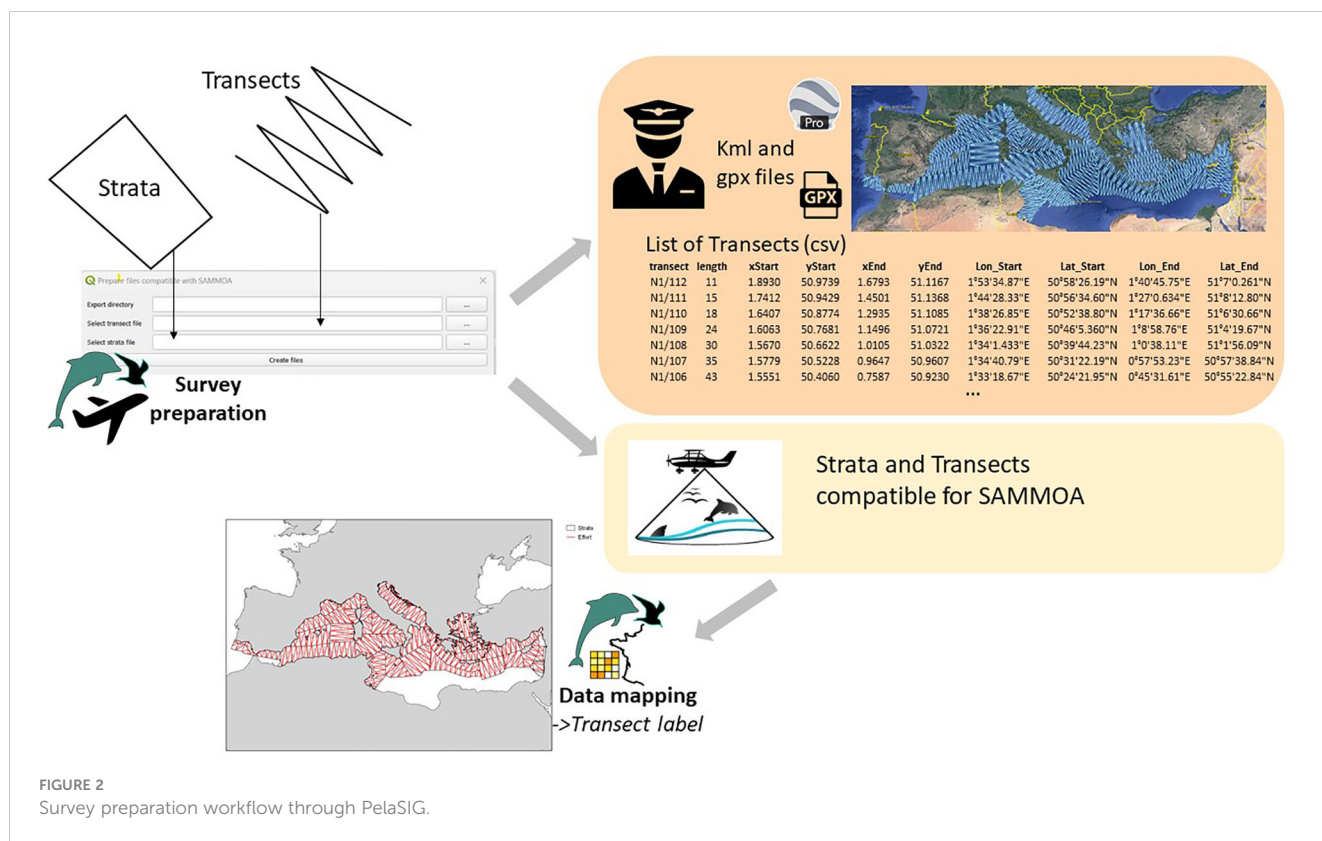


FIGURE 2 Survey preparation workflow through PelaSIG.

generated as log files (in text format) for both effort and sightings. Some of these checks are generic; others are specific to our protocol and therefore may not be appropriate for all surveys. For now, the listed errors need to be corrected directly in SAMMOA, and the shapefiles need to be re-exported. This step is optional.

2.3.4 Effort linearisation

Once the effort and sighting shapefiles have been exported from SAMMOA, the Linearize effort tool allows the trajectory of the survey platform to be visualised by creating lines between effort points and calculating their length in kilometres and duration in minutes. Only trajectories where observers were in standardised protocol are linearised (i.e., on effort). Each line (i.e., leg) is associated with all parameters collected with SAMMOA and has a homogeneous collected value. The date and time of the start and end of these legs are added as two new fields: *DATE_TIME1* and *DATE_TIME2*. Linking the sighting shapefile is not mandatory, but could be associated and thus obtain a new field *legID*, which allows matching between sightings and the numerical identifier of the effort line. Considering an altitude of 600 ft (182 m) and using declination angles measured on board (*angle* field in sighting shape file), *pDistTemp* is estimated as the perpendicular distance for the aerial survey mode. Both new effort and observation files prepared at this step could be used for the next tools or directly for conventional distance sampling (CDS) analysis, also called design-based analysis using the Distance software (Thomas et al., 2010) or R packages (Miller et al., 2019; Genu and Authier, 2020).

2.3.5 Prepare data for analysis: clip effort in regular segments

In order to perform density surface modelling, or kriging analysis, for example, data must be segmented into regular portions of effort and sightings must be associated with each corresponding new segment. The Segment effort tool allows the user to split each leg of the previously linearised effort file into segments of the desired length. Beforehand, the linearised effort must be projected into the desired system, function of the location. The user defines the length of the segments, which is adjusted by dividing the remaining length to each segment. Three files are produced as output, with all initial parameters retained: a polyline of the effort segments resulting from the clipping, a point file with the centroid of each segment, and, if selected, the sighting file (optional). All are with a new field to identify each segment (i.e., *segID*) and to link sighting to the corresponding part of the effort. Several fields are also calculated: *segLengKm* (length of the new segment in km), *DTstart*, and *DTend* (the estimated date and time of the start and end of the leg).

2.3.6 Data summary

The Data summary tool provides a summary of observation and linearised effort data. The user can obtain a summary of the cumulative effort duration or the number of observations and individuals based on taxa or strata. The obtained results can be exported in csv format.

The filters available on the effort table are sampling plan, session, computer (when several platforms are working together), or strata, and effort is summarised by length (in km), duration (in min), and mean sea state condition, taking into account effort in each condition.

2.3.7 Data mapping

The Mapping module allows the user to spatially represent the data through different types of maps. On each map, the addition of a global coastal contour or other shape file is possible to customize the project. For the effort, the use of Transects labels allows to map the effort planned before the survey with labels and effort transects to be carried out, while Context map visualise the effort achieved, and the latter can be coloured according Beaufort scale using the Beaufort index map. The sighting file could be represented as dots coloured by taxa using the Observations map, or usually for a selected species (or group of species), or group size could be automatically plotted using the PodSize proportional circles map. Then, the data could be resampled on a regular grid, with the cell dimension chosen by the user (unit depends on the projection of the linearised effort), combining the number of encounters per kilometre per grid for a selected species or group of species, using the Detections rate map, while the Individuals rate map estimates the number of individuals counted per kilometre in each cell. Finally, the PodSize rate map gives the mean pod size of the selected species/taxon or other, in each grid cell. Sightings not recorded in protocol, as off-effort or only detected by the pilot or secretary, are not considered by the tool in this grid treatment. For the mapping module, a projected coordinate reference system is set up as default EPSG 4326. However, there is an option to change projection (especially for the grid size unit) or to import a suitable shape for the coastline.

For all these maps, the user can choose to apply filters to the sighting data (Observation) to select one or more species/date... (any field of the effort file). To customize, the title, source, and file name could be updated by the user, and a logo could be added to the final export available in pdf, png, or shape files. To generate multiple maps automatically, in a group of species, whatever, the attribute field is ticked, "Generate map" should be selected. All the map will be exported automatically in the selected format (png, pdf, and/or shapefile).

2.3.8 Data sharing

The availability of comprehensive, accurate, and relevant data is central to the effectiveness of rapid assessment, collaboration, and data sharing. The Share tool allows data to be exported directly from a database in a lightweight exchange format, called Basic Data Exchange.

Similarly, the Import into database tool allows shapefiles exported from the SAMMOA software to be stored in the Observatoire Pelagis survey campaign PostgreSQL database. This tool is dedicated to the Pelagis database and may not be suitable for all databases.

2.4 Software availability

Latest software version: 2.6 (2023).

Hardware required: PC (2 GB RAM, 2 GHz CPU recommended).

Software required: Windows XP or later recommended, Linux, Mac; QGIS 3 (3.16 up to 3.28.5 optional but recommended).

Program languages: Python, PyQGIS.

Program size: 138 Mo.

Availability: www.observatoire-pelagis.cnrs.fr/tools/pelasis/

Cost: free, licensed under the GNU GPL.

3 Study case: the aerial survey of ACCOBAMS Survey Initiative

The workflow of the survey preparation is illustrated on [Figure 2](#). Unfortunately, the Survey preparation tool was not ready to be used for the preparation of the ASI survey.

3.1 Data collection using SAMMOA

Surveys were conducted using a standard line-transect method ([Buckland et al., 2001](#)), similar to previous large-scale aerial surveys dedicated for marine mammals in European waters ([Hammond et al., 2014](#)) or to megafauna ([Pettex et al., 2017](#); [Laran et al., 2017b](#)). The aircraft speed was 185 km h⁻¹/100 knots, and the altitude was 182 m/600 ft ([Cañadas et al., 2023](#); [Panigada et al., 2023](#)). The survey crew consisted of two naked-eye observers and a third person collecting data on a laptop computer equipped with SAMMOA, developed for the aerial survey; the version 1.1.2 was used at the time of the ASI aerial survey ([SAMMOA, 2019](#)). The position of the aircraft was automatically recorded (set to a 2-s interval using an on-board GPS device connected to the computer). Environmental conditions were recorded at the beginning of each transect and whenever any of these parameters changed ([Panigada et al., 2023](#)). For marine mammals, elasmobranchs and sunfish, perpendicular distances measured by clinometers were recorded by the observers, while for seabirds, turtles, other large fish, marine debris, and boats, all encounters within 200 m of the aircraft trackline (500 m for boats) were considered detected.

All flights collected by different teams could be combined on a single computer using SAMMOA and then exported as shapefiles. Given the large number of teams and flights for ASI aerial survey, shapefiles were exported for each team and then merged in QGIS before being processed using the PelaSIG plugin.

3.2 Data summary and mapping

Once the linearisation of effort (and association with sightings) is achieved, the Data mapping tool is used with the Context map option to visualise the effort achieved ([Figure 3](#)). Specific sea state representation is also possible using the Beaufort index option to represent weather conditions along the survey ([Figure 4](#)). Using the Data summary, it is possible to create a table of the cumulative effort by stratum ([Table 1](#)) or number of sightings and individuals per taxon ([Table 2](#)).

Considering the distribution of sightings, the three groups of marine megafauna taxa collected during the ASI aerial survey were

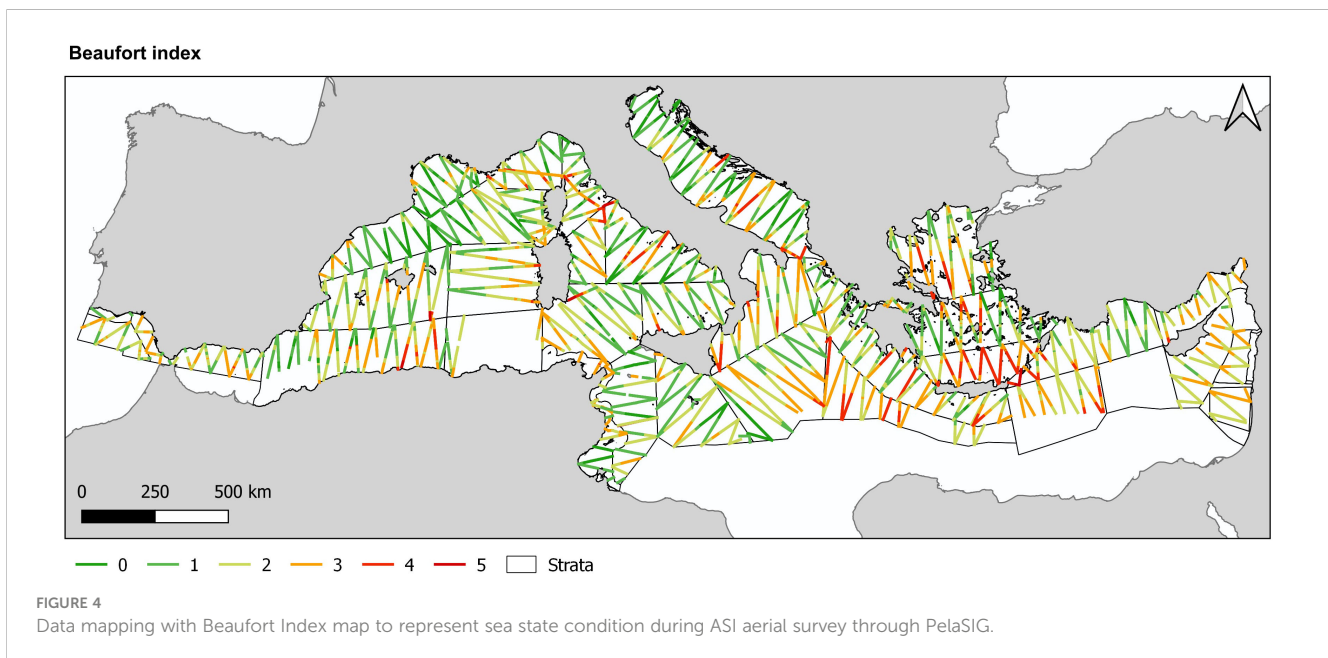
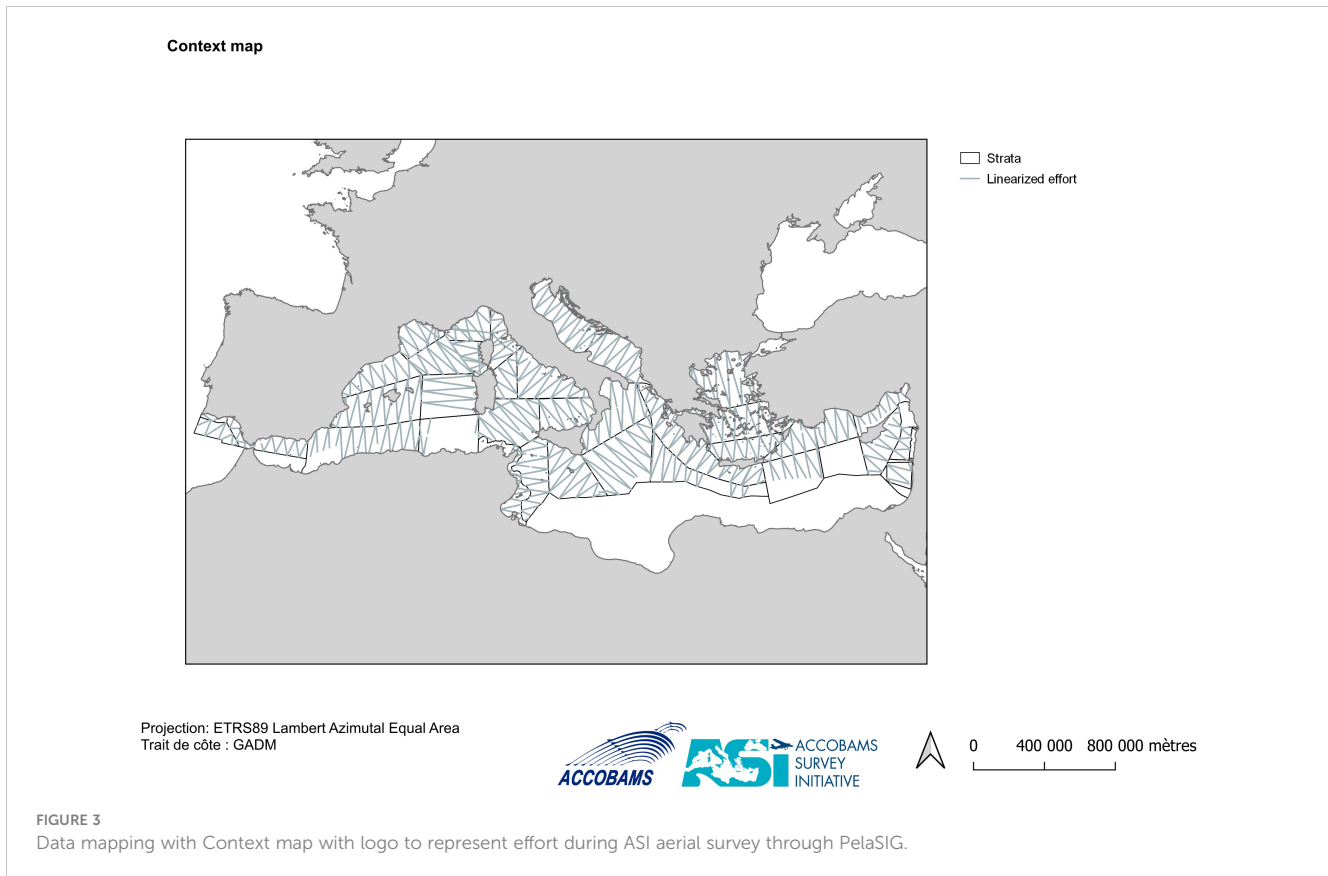


TABLE 1 Cumulative effort by strata during ASI aerial survey through the Data Summary tool of the PelaSIG. See Panigada et al., 2023 for the location of strata.

Strata	Leg Length (km)	Mean Sea state	DeltaTime (min)
01c	1,753	2	542
01d	334	2	102

(Continued)

TABLE 1 Continued

Strata	Leg Length (km)	Mean Sea state	DeltaTime (min)
01e	226	1	75
2	1,783	2	570
3	10,728	1	3,443
4	11,395	1	3,575
5	8,042	0	2,496
6	1,511	1	442
7	8,796	1	2,674
08a	4,756	0	1,533
08b	11,057	1	3,527
9	3,709	1	1,170
10	7,151	1	2,297
11	3,163	1	1,036
12	3,441	2	1,105
13	4,340	1	1,348
14	9,688	1	3,091
14b	952	2	300
15	6,264	1	1,894
16	5,522	1	1,747
17	6,204	1	1,924
18	10,466	2	3,357
19	17,539	1	5,489
20	10,968	1	3,410
21	7,264	1	2,348
21b	1,696	1	548
22a	6,335	3	2,026
22b	11,338	2	3,939
22c	4,383	2	1,452
22d	6,721	2	2,195
23a	8,581	2	2,833
23b	6,589	1	2,218
24	11,204	2	3,853
29a	5,223	1	1,759
29b	2,604	2	909
29c	6,895	2	2,431
30	5,722	2	2,050
31	2,049	2	730
Total	236,393	1	76,438

TABLE 2 Number of sighting and individuals per taxon during ASI aerial survey through Data Summary tool of the PelaSIG.

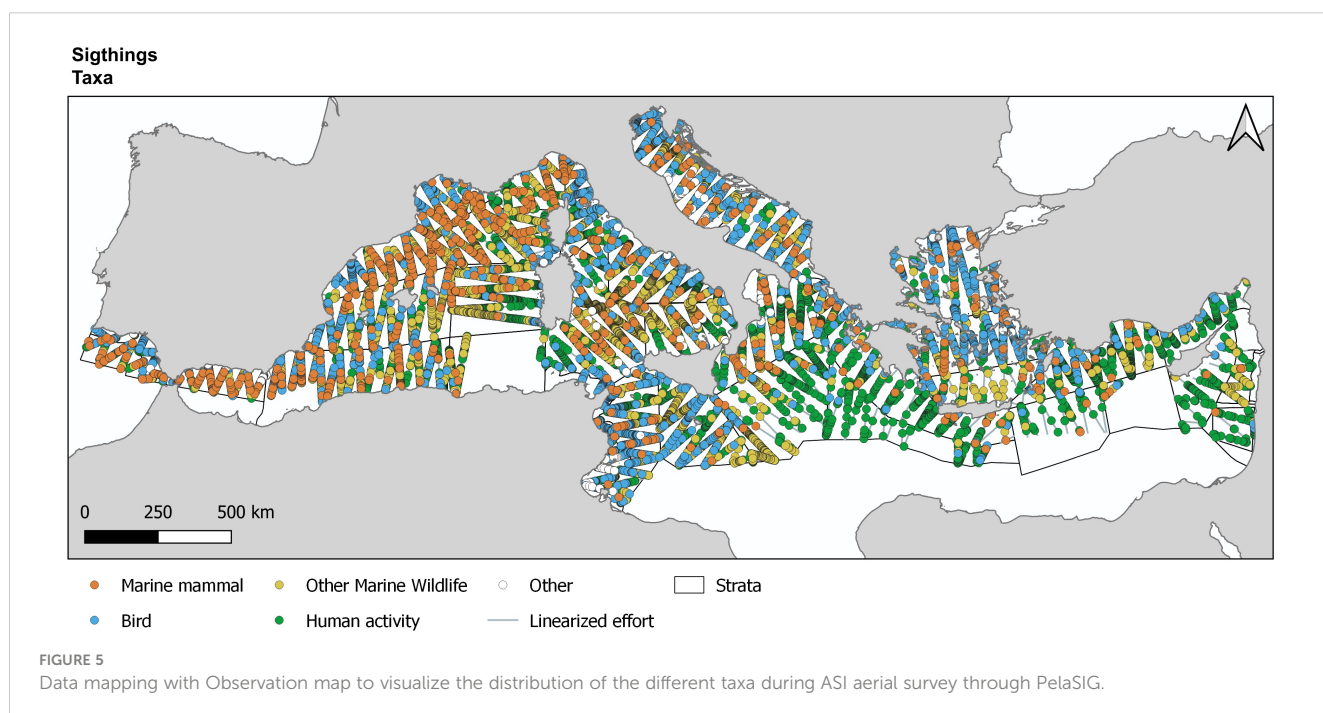
Taxon	Number of sightings	Number of individuals	Number of sightings (off effort)	Number of individuals (off effort)
Marine mammal	836	11,217	91	2,263
Seabird	2,567	10,923	29	618
Land Bird	40	551	1	1,000
Coastal Bird	3	26	1	3
Bird unidentif.	104	491	5	7
Other Marine Wildlife	5,496	23,183	82	1,197
Human activity	19,048	43,427	115	410
Other	176	278	1	1
Total	28,270	90,096	325	5,499

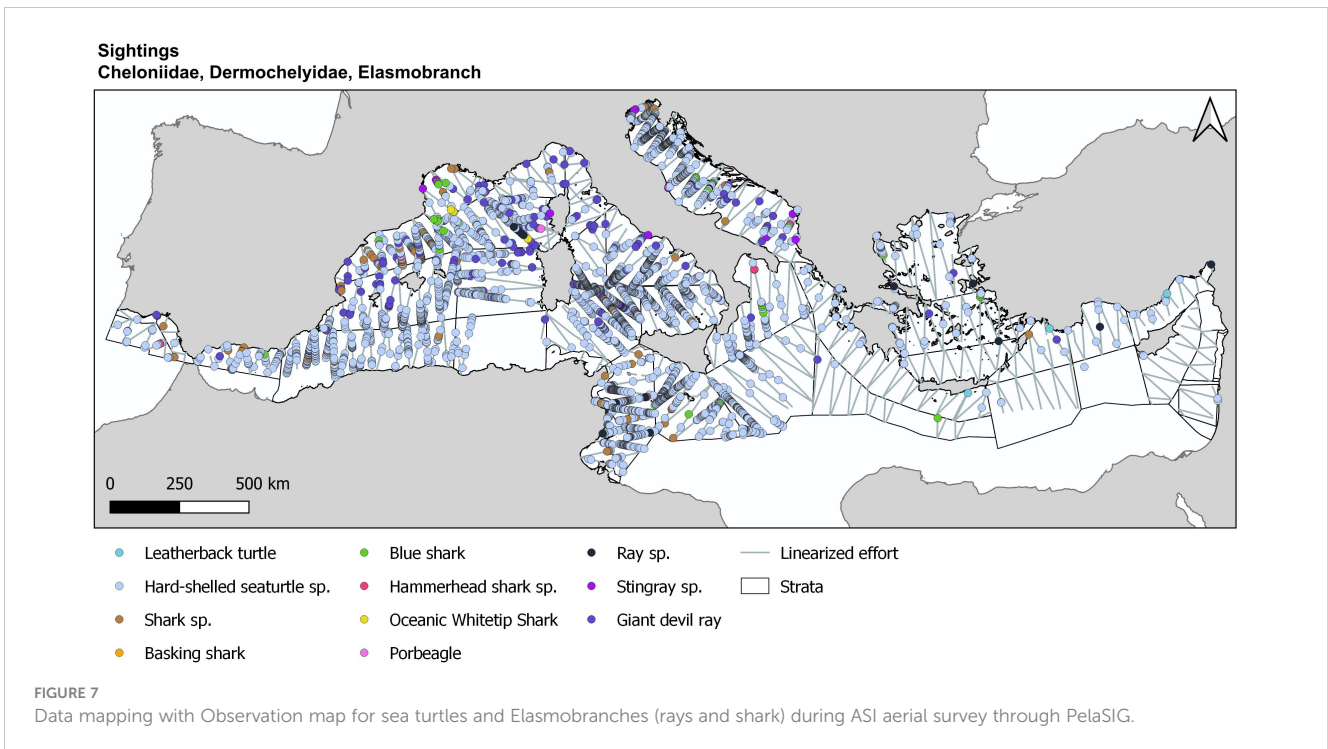
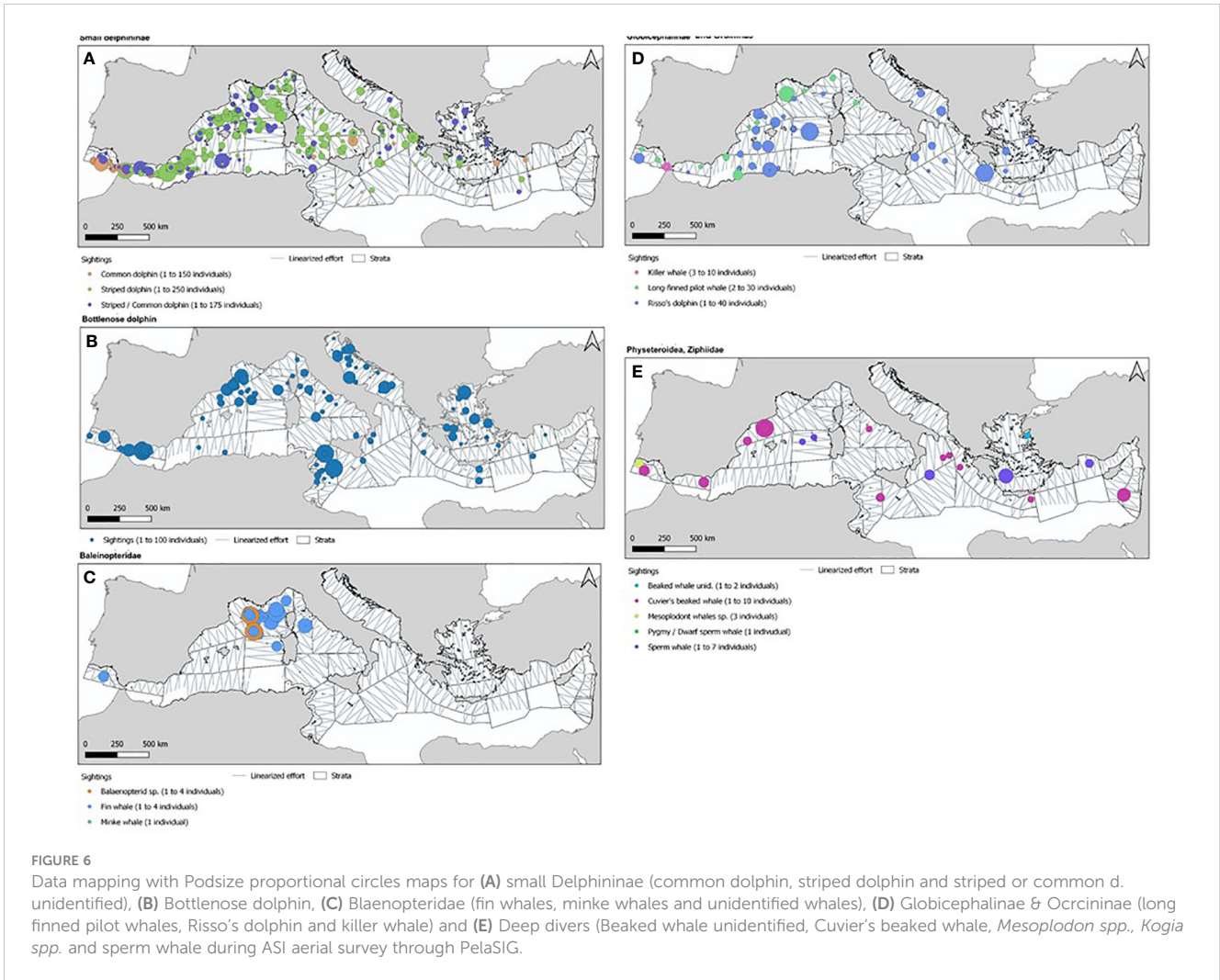
plotted using Observation map (within Data mapping; Figure 5), while for marine mammals' species, they are presented using Podsize proportional circles maps (Data mapping) and generated by family (Figure 6). Sea turtles and elasmobranchs (Figure 7) and seabirds (Figure 8) were also presented using Observation map, due to the large number of sightings.

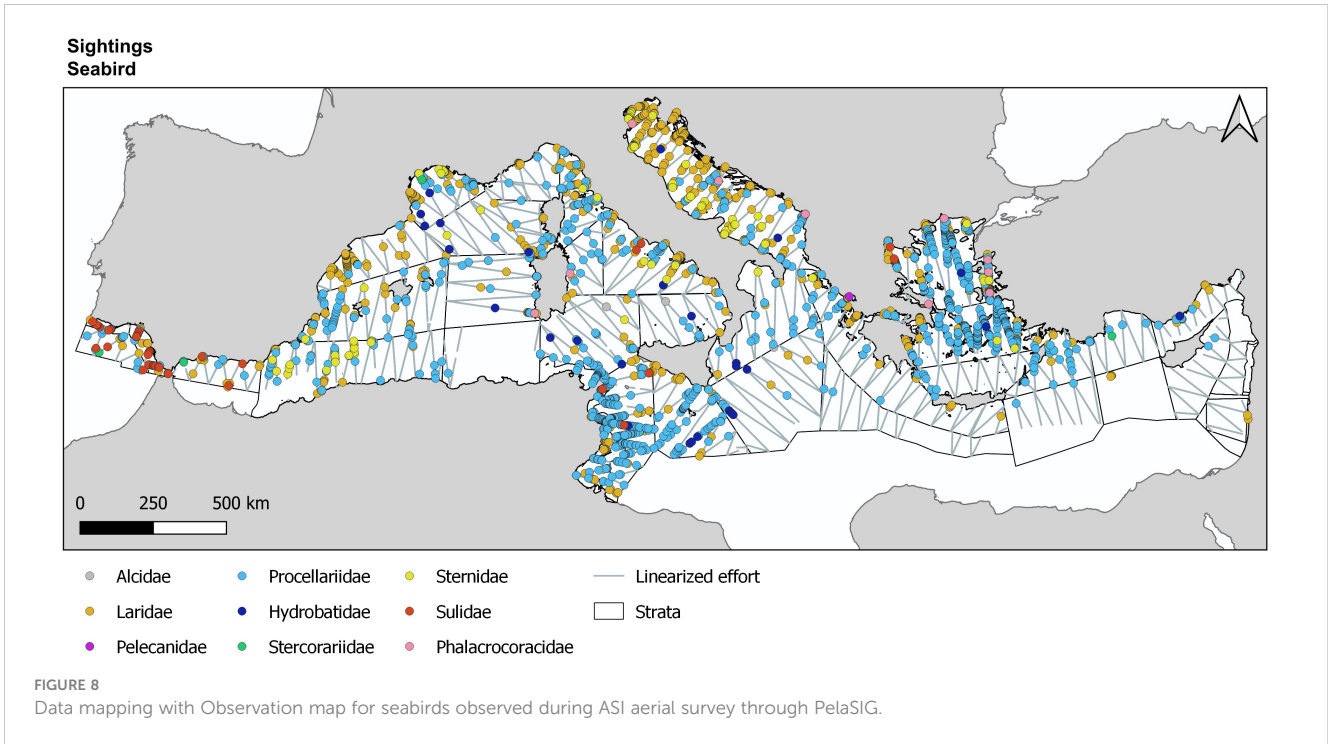
Considering now the resampling in the grid cell in order to better compare the distribution through the two Mediterranean Basin, the Detection rate was presented for the four marine megafauna taxa collected: marine mammals, seabirds, sea turtles, and elasmobranchs (Figure 9), while for the index of anthropogenic activities, the individual rate was presented for marine debris and ship (Figure 10). Finally, the PodSize rate map was presented for the small Delphininae (striped and common dolphins, including undifferentiated) over the entire basin (Figure 11).

4 Conclusion

All steps using the PelaSIG plugin are summarised in Figure 12. The potential applications of GIS in the development of effective and sustainable conservation management strategies are many, but to fully realise the benefits of GIS, time and expertise must be allocated. The development of a tool to automate steps in a scientific workflow reduces the processing time, standardises methods and outputs, and facilitates multi-user collaboration. Choosing open-source technologies facilitates use by third parties and enables long-term collaborative development. In addition, the obligation of public agencies to make data available requires efforts to achieve data homogeneity and interoperability. This standardisation of data facilitated by the development of automation tools allows data to be used by several organisations.

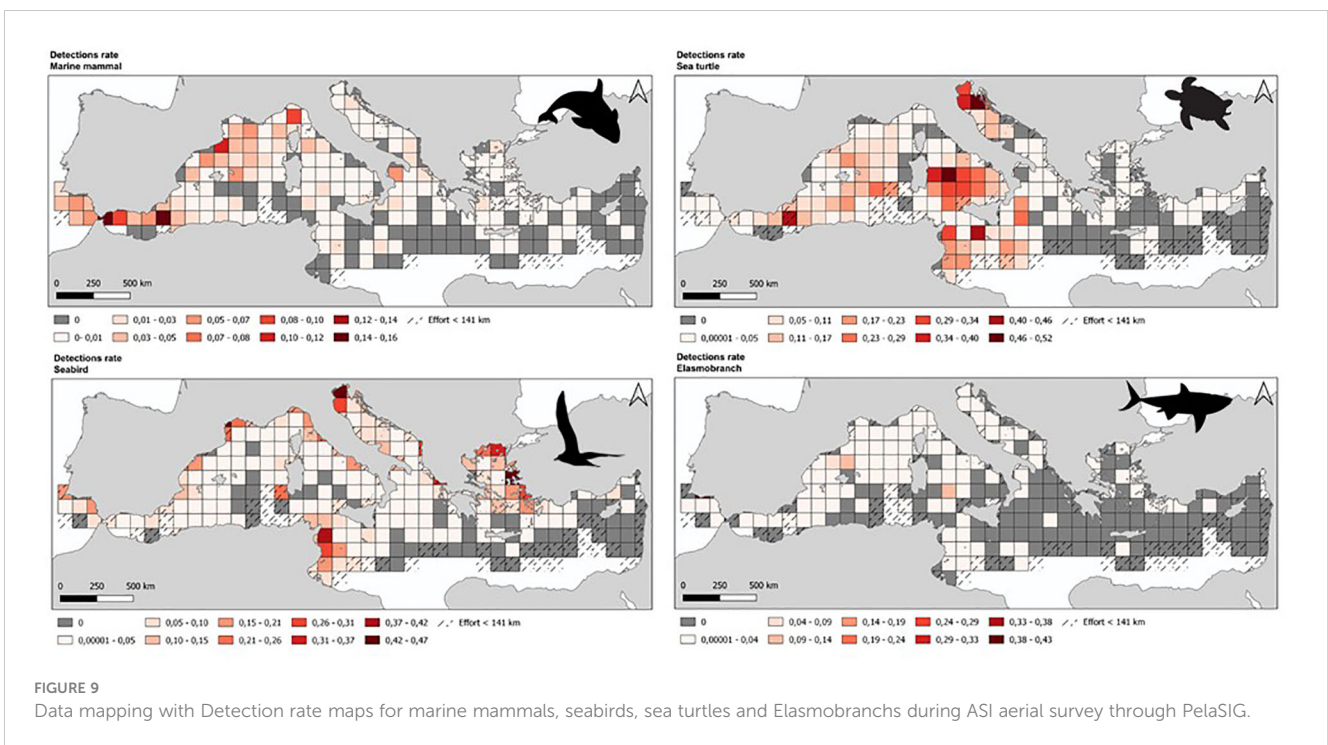






The PelaSIG plugin was initially developed for internal use, but its modular structure allows external use of most of the tools that make up the plugin, especially for teams already using SAMMOA. The linearisation tool and the mapping tool, which have already been tested by several colleagues from external

organisations, are the most widely used, allowing for easy exploitation and visualisation of the data. The PelaSIG plugin is based on the SAMMOA data collection software, which is already used by several European partners, and will therefore be jointly distributed.



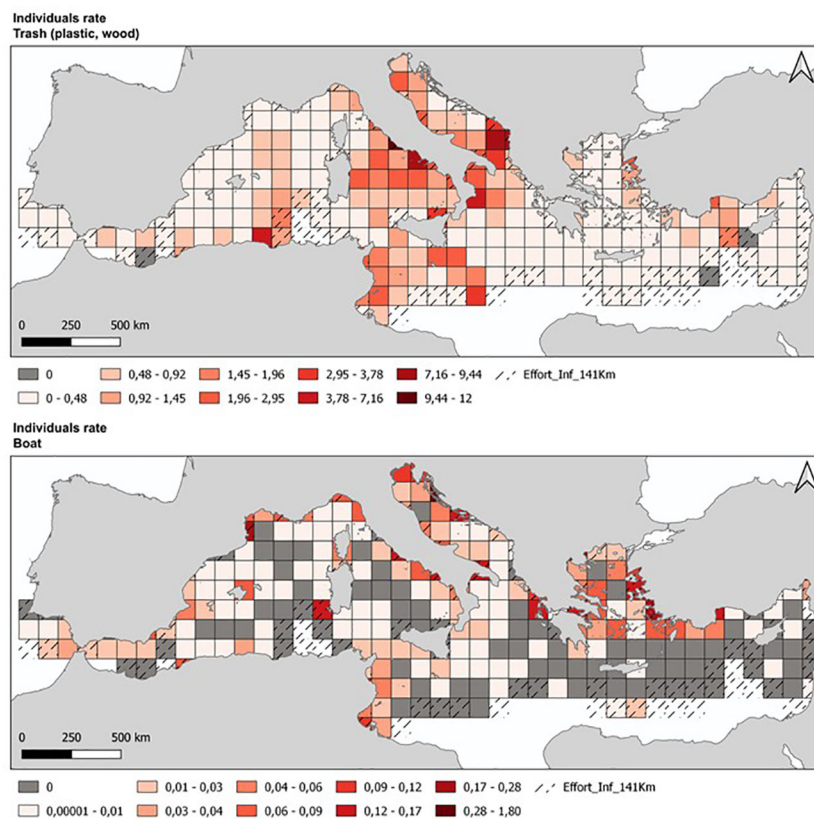


FIGURE 10 Data mapping with Individuals rate maps for marine debris (plastic and wood trash) and boats during ASI aerial survey through PelaSIG.

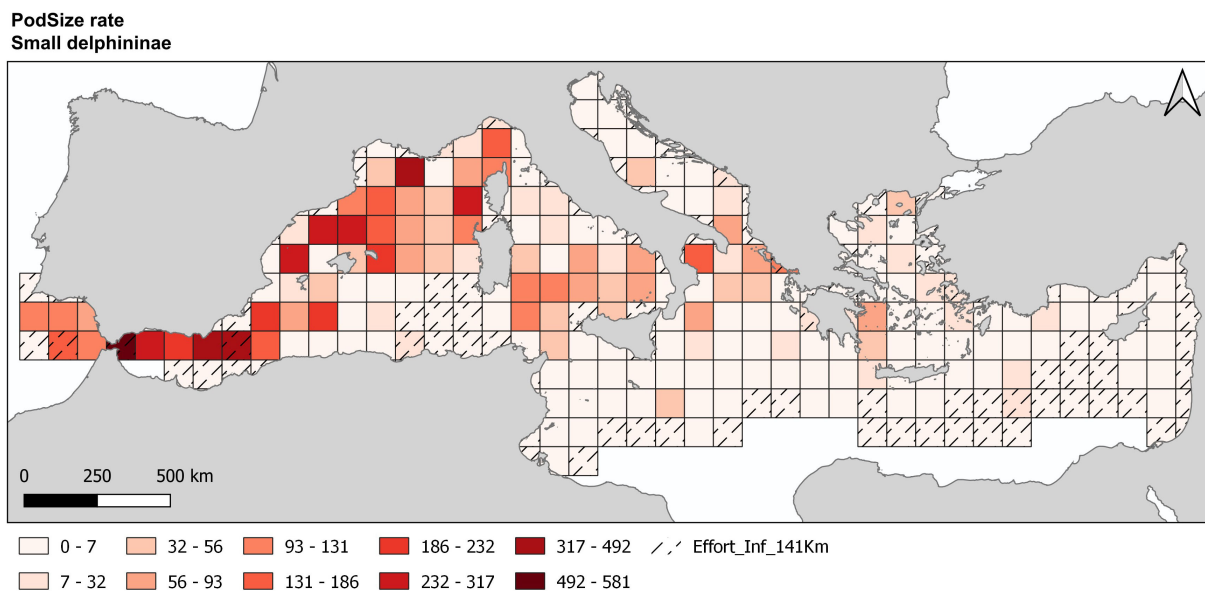


FIGURE 11 Data mapping with PodSize rate map for small delphininae sightings during ASI aerial survey through PelaSIG.

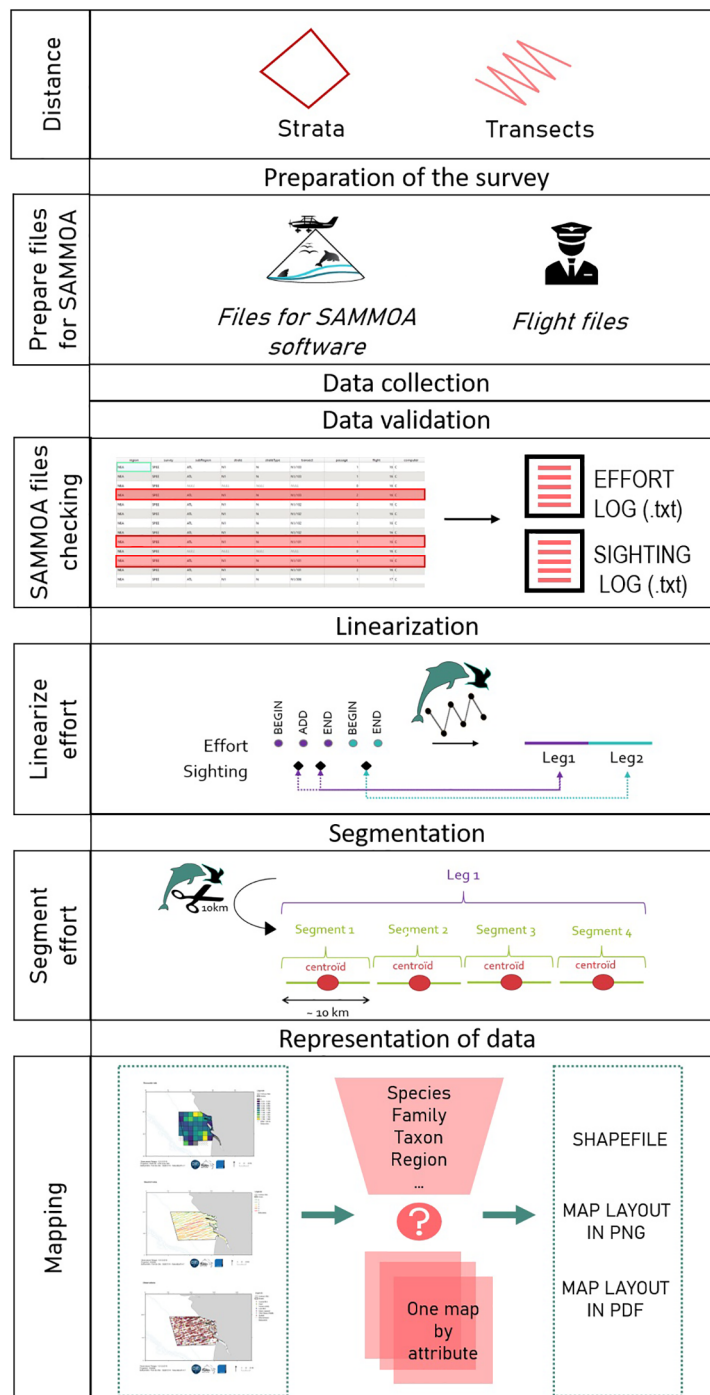


FIGURE 12 Steps of the entire processing chain of PelaSIG from survey preparation to mapping.

Current environmental challenges, such as biodiversity loss and climate change, require multi-scale and multi-temporal surveys and the need to standardise protocols as much as possible and especially in terms of data format. Here, we have proposed a set of open-source tools, the SAMMOA and the PelaSIG, to facilitate their collection, visualisation, processing, and dissemination.

Data availability statement

The PelaSIG plugin is available on <https://www.observatoire-pelagis.cnrs.fr/tools/pelasig/>. The dataset presented in this study is available on demand to ACCOBAMS (<https://accobams.org/asi-data-access-request/>).

Author contributions

MN: Conceptualization, Data curation, Investigation, Methodology, Project administration, Resources, Software, Validation, Visualization, Writing – original draft. AB: Validation, Visualization, Writing – review & editing. OJ: Software, Writing – review & editing. OC: Resources, Writing – review & editing. GD: Resources, Writing – review & editing. JS: Funding acquisition, Supervision, Writing – review & editing. BM: Resources, Writing – review & editing. SP: Resources, Writing – review & editing. SL: Conceptualization, Data curation, Methodology, Project administration, Resources, Software, Supervision, Validation, Visualization, Writing – original draft, Writing – review & editing.

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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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Acoustic estimates of sperm whale abundance in the Mediterranean Sea as part of the ACCOBAMS Survey Initiative

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Acoustic surveys for sperm whales (*Physeter macrocephalus*) were conducted in the Mediterranean Sea in summer 2018 as part of the vessel-based component of the ACCOBAMS Survey Initiative (ASI). Equal-spaced zigzag transects provided uniform coverage of key sperm whale habitats and were surveyed using a towed hydrophone array deployed from a research vessel at speeds of 5–8 knots. A total of 14,039 km of tracklines were surveyed in the western basin, Hellenic Trench and Libyan waters, with an acoustic coverage of 10% realised for sperm whales. During these surveys, 254 individual sperm whales were detected on the trackline, with an additional 66 individuals off-track. Sperm whales were only seen ten times on-track, with an additional 16 off-track sightings. Estimates of slant range to echolocating whales were used to derive density estimates through both design- and model-based distance sampling methodologies. An acoustic availability of 0.912 (sd = 0.036) was derived from via published models. When correcting for availability bias, a design-based abundance estimates of 2,673 individuals (95% CI 1,739–4,105; CV = 0.21) was derived for the surveyed blocks, which incorporated most known sperm whale habitat in the Mediterranean Sea. The equivalent model-based estimate was 2,825 whales (2,053–3,888; CV = 0.16). Over 97% of detected whales were in the western basin, with highest densities in the Algerian and Liguro-Provencal Basins between Algeria and Spain/France. In the eastern basin, detections were sparse and concentrated along the Hellenic Trench. A density surface modelling (DSM) exercise identified location and benthic aspect as being the most instructive covariates for predicting whale abundance. Distance sampling results were used in a power analysis to quantify the survey effort required to identify population trends. In the most extreme scenario modelled (10% per annum decline with decennial surveys), the population could have dropped by 90% before the decline was identified with high statistical power. Increasing the regularity of surveys would allow population trends to be detected more expediently. Mediterranean sperm whales are listed as Endangered on the IUCN's Red List and the need for urgent conservation measures to reduce injury and mortality remains paramount for this unique sub-population.

KEYWORDS

passive acoustic monitoring, density estimation, sperm whale, Mediterranean Sea, density surface modelling, *Physeter macrocephalus*

Introduction

Sperm whales (*Physeter macrocephalus*, Linnaeus, 1758) are the largest of the toothed whales (Whitehead, 2017), and are found in all deep waters of the Mediterranean Sea, from the Strait of Gibraltar to the Levantine Basin (Ryan et al., 2014; Rendell and Frantzi, 2016; Lewis et al., 2018). Although it is unclear how a viable breeding population of sperm whales first established itself in the region, sperm whale vertebrae have been discovered in excavations of a Phoenician colony in western Sicily dating from the sixth to fifth centuries BCE (Reese, 2005). The first written account of sperm whales appears to come from Aristotle's description in the fourth century BCE of a whale with the "air passage in its forehead" (Balme, 2011). Although the Mediterranean Sea is connected to the neighbouring North Atlantic Ocean, the shallow Camarinal Sill to the west of the Strait of Gibraltar may act as a significant barrier to the passage of sperm whales, essentially containing the Mediterranean individuals as a discrete sub-population. This is supported by genetic studies (Drouot et al., 2004a; Engelhaupt et al., 2009) that indicate little genetic flow between the Mediterranean Sea and Atlantic Ocean. Additional evidence of population segregation comes from acoustic studies investigating the variation of 'codas', stereotyped patterns of broadband clicks used in communicative contexts. Mediterranean sperm whale codas are distinctive compared with those in other regions: they are broadly dominated by the 3 + 1 type (67–98% of all codas recorded; Pavan et al., 2000; Drouot et al., 2004b; Teloni, 2005). As codas appear to be acquired via cultural transmission (Rendell et al., 2012), the relatively homogeneous repertoire in the Mediterranean provides further evidence of an isolated population.

Robust baseline information on the abundance and density of sperm whales in the Mediterranean is required to ensure they are protected appropriately. Although the presence of sperm whales in the Mediterranean has been established for several centuries, estimating the size of the population has proved challenging. This is partly due to their routine deep-diving behaviour (to 800 m, Zimmer et al., 2005), prolonged submergence time (97% of the time, Watwood et al., 2006), and widespread distribution (across at least 21 separate national jurisdictions, Notarbartolo di Sciara and Tonay, 2021). Where they do exist, density estimates are typically confined to sovereign waters (e.g. Frantzi et al., 2014). There have been few large scale, multi-jurisdiction surveys. Gannier et al. (2002) conducted surveys from 5°W to 30°E over four years and derived acoustic and visual encounter rates for sperm whales. Rendell et al. (2014) used photo-identification to estimate the abundance of individuals (approximately 400) in Balearic, French, and Italian waters in the northwest Mediterranean. Laran et al. (2017) derived abundance estimates (95% CI 80–2,600) using aerial surveys for the waters of France, Monaco and Italy in the northwest Mediterranean. Lewis et al. (2007) estimated acoustic abundances (95% CI 24–165) from line-transect surveys in the northern Ionian Sea, Sicilian and Malta Channels. The only estimate of total population size in the Mediterranean comes from a series of acoustic line-transect surveys within the eastern and western basins that were extrapolated to unsurveyed areas to derive an estimate of 1,842

individuals (Lewis et al., 2018). These studies, in conjunction with inferred population declines due in part to bycatch and ship strike, have contributed to the Mediterranean sub-population of sperm whales being assessed as Endangered C2a(ii) on the IUCN Red List (Pirota et al., 2021).

Deep-diving cetaceans may be under-recorded by traditional visual surveys as they have proportionally low surface availability to observers (Barlow and Taylor, 2005). Passive acoustic techniques can offer several advantages over visual methods for detecting submerged individuals, including extended strip widths, and detection at night or during periods of bad weather (Leaper et al., 1992; Barlow and Taylor, 2005). Sperm whales are particularly well suited for acoustic surveying as they generate regular loud clicks that can be detected up to 20 km away (apparent source levels up to 236 dB re: 1µPa rms; Möhl et al., 2003; Zimmer et al., 2005). Furthermore, they are vocal throughout 60–80% of their dive cycles (Douglas et al., 2005; Watwood et al., 2006; Teloni et al., 2008; Fais et al., 2016). As sperm whale clicks have rapid onsets, the time-of-arrival differences between two or more hydrophone elements can be used to derive bearing information; the triangulation of bearing lines for successive clicks in a click train can allow robust distance estimates to be derived (Leaper et al., 1992; Matthews, 2014). Thus, acoustic detections of sperm whales lend themselves well to distance sampling techniques for estimating density. Such estimates have been derived for the central islands of the Azores (Leaper et al., 1992), waters of South Georgia (Leaper et al., 2000), the Faroes Shetland Trough (Hastie et al., 2003), a section of the Eastern North Pacific (Barlow and Taylor, 2005), a naval range in the Bahamas (Ward et al., 2012), the Canary Islands (Fais et al., 2016), the Mediterranean Sea (Lewis et al., 2007; Lewis et al., 2018), offshore Irish waters (Gordon et al., 2020) and the northern Gulf of Mexico (Li et al., 2021).

As several cetacean populations in the Mediterranean and Black Seas are threatened by human activities (Reeves and Notarbartolo di Sciara, 2006), robust information on population trends is necessary to evaluate the effectiveness of conservation measures. For sperm whales, entanglement in nets (Notarbartolo di Sciara and Tonay, 2021) and collisions with ships (Frantzi et al., 2019) continue to be significant causes of mortality. In addition, pollution (including chemical and noise), ingestion of plastic debris and disturbance from vessels all contribute to the species' assumed decline in the region (Rendell and Frantzi, 2016; Pirota et al., 2021). Responding to the urgent need for improved knowledge of cetacean populations in the region, the ACCOBAMS (Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and continuous Atlantic area) Secretariat coordinated the first ever large-scale survey of marine megafauna in the Mediterranean Sea during the summer of 2018. In light of increasing human activities at sea, the ACCOBAMS Survey Initiative (ASI) was organised with the participation of range states to generate robust assessments of the status of cetacean populations. Approximately 75% of the Mediterranean basin was surveyed by aerial teams (Cañadas et al., 2023; Panigada et al., 2023), while simultaneous vessel-based surveys prioritised areas not surveyed by plane and known to be important for deep-diving cetaceans that may be under-

represented by aerial surveys. The majority of the vessel-based component of the ASI was conducted from the research vessel *Song of the Whale* and the results of these combined visual and passive acoustic surveys are presented here. The primary aim of this work was to enable improved detection of sperm whales during the ASI and generate both design- and model-based estimates of local density and basin-wide abundance.

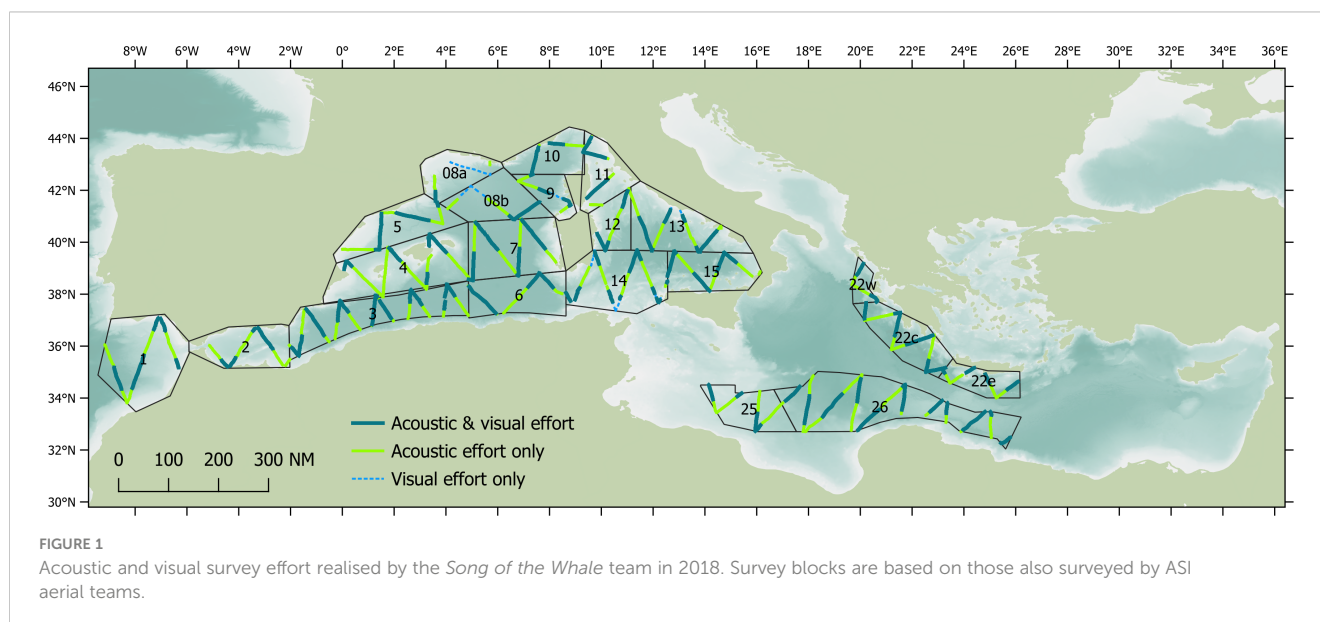
Methods

Survey design

Distance sampling methodologies can provide robust estimates of the density and abundance of a species in a defined space and time (Buckland et al., 2004) and can also detect potential trends (Taylor et al., 2007). Standard line transects methods assume the density of animals on the surveyed tracks is representative of the density in the entire study area; this will typically be true if the transects are designed systematically with a random component (such as a random start) and each part of the study area has an equal probability of being surveyed. Therefore, transects for the ASI vessel surveys were designed as equal-spaced zigzags using *Distance 7.3* software (Thomas et al., 2010) to provide almost uniform coverage probability. Transects were designed within the same survey blocks used for the ASI aerial surveys, with minor modifications made due to logistical constraints (such as security considerations and permit restrictions). Transects were designed to provide acoustic coverage of at least 6% based on an estimated strip half-width (ESHW) of 10 km for sperm whales (based on similar research conducted from the same research vessel; Lewis et al., 2018). A total of 17,272 km of transects were designed for the vessel-based surveys conducted by the *Song of the Whale* team (Figure 1).

Acoustic surveys

Surveys were conducted from R/V *Song of the Whale*, a 21 m auxiliary-powered cutter-rigged sailing research vessel. Acoustic effort was conducted 24 hours a day when the depth was sufficient to tow a hydrophone array (> 50 m). Survey speeds of 5 to 8 knots were optimal for both minimising cable strain and biases related to animal movement, being 2-3 times faster than the speed of the target animals (Buckland et al., 2015); the mean speed of sperm whales is typically 2.1 knots (Whitehead, 2017). The towed array consisted of a 400 m tow cable attached to multiple hydrophone elements in an oil-filled tube. The array incorporated a pair of AQ-4 elements (Teledyne Benthos) with a flat frequency response (± 1.5 dB) from 1 Hz to 30 kHz and receiving sensitivity of -201 dB re 1V/ μ Pa. Pre-amplifiers with 29 dB gain were used to prevent voltage drop between the array and the research vessel. Each hydrophone element was separated by 3 m. The array outputs were digitised at 500 kHz by a SAIL DAQ cards (SA Instrumentation) after a 10 Hz high pass filter and 12 dB gain had been added to the signal; the high sample rate was chosen to allow for the detection of the ultrasonic clicks of beaked whales (see ACCOBAMS, 2021). Signals from the two elements were decimated to 48 kHz using a low pass 4th order Chebyshev filter with a cut off frequency of 20 kHz (i.e. approximately 0.8 times the Nyquist frequency to avoid aliasing) and monitored in real-time using a click detector module in PAMGuard (Gillespie et al., 2008) configured to detect candidate sperm whale clicks. Recordings were written to disk as 16-bit wav files. The 400 m tow cable provided typical array depths of 29-33 m (see Figure 7 in Boisseau et al., 2023 for the towing profile). As the mixed layer depth is typically shallower than 20 m in summer (Houper et al., 2015), the array was assumed to tow below any thermocline which could refract upwelling clicks from sperm whales.



Visual surveys

Visual effort was conducted by two dedicated observers from an elevated observation platform (mean eye height of 5.4 m). Effort was separated in two quadrants, with observers primarily scanning the trackline ahead of the vessel with the naked eye; a starboard observer scanned the sector from 340–90° and a port observer from 270–20° degrees. Observers used 7x50 binoculars to confirm details of sightings. Observers reported species identity, range (estimated by eye), bearing (from angle boards) and group size to another team member acting as a dedicated data recorder. The data recorder saved the information to a survey database using *Logger* software (www.marineconservationresearch.org). *Logger* also logged the vessel's GPS stream with the heading from a GPS gyro sensor to the database; various other parameters, including wind speed and direction measured by masthead instruments, were also logged automatically every 10 seconds. Environmental information (including sea state, wave and swell height, cloud cover and glare) were logged manually every hour, or when there was a significant change in conditions (Lewis et al., 2018).

Acoustic analysis

Recordings made in the field were independently re-examined in PAMGuard by two experienced analysts (OB and JR) to identify candidate sperm whale click trains. Sperm whale clicks have stereotypical spectral properties (with most energy at or below 12 kHz), waveforms (with rapid onset and offset and evidence of multiple pulses within each click) and inter-click intervals (a regular click being produced every 1–2 seconds (Leaper et al., 1992; Møhl et al., 2003)). Candidate clicks were identified as forming part of a click train, i.e. with similar bearings and regular inter-click intervals. Differences in bearing information were used to identify individual click trains (Lewis et al., 2018); the standard deviation of consecutive clicks from a focal animal is typically less than one degree (when bearings are greater than 15°; Rankin et al., 2008). Thus, acoustic detections were made at the individual level rather than the group level. Estimates of slant range to individual whales were made in PAMGuard using the target motion analysis (TMA) module. A towed array will detect multiple sequential clicks from a focal animal; if the source is assumed stationary, then each click will be detected with a time differential on the two hydrophone elements. Successive sets of time delays can be visualised as 2D bearings converging on the likely sperm whale location. To overcome the left/right ambiguity inherent in a linear array, PAMGuard calculates a chi-squared goodness-of-fit between the expected and observed bearings, and the side with the smaller value is considered the best convergence point.

Acoustic density estimation of sperm whales

Slant ranges to sperm whale clicks detected when the survey vessel was 'on-track' and following the survey protocol (i.e. traveling at 5–8 knots) were used to generate acoustic detection functions and

density estimates, using multiple covariates distance sampling (MCDS) in *Distance* 7.3. Without information available to determine the depth of vocalising animals (for example through the use of time-depth recorder tags), slant ranges could not be accurately converted to perpendicular distances. Thus, as most sperm whales are detected at depth, using uncorrected slant ranges does not incorporate the vertical component of their location (Westell et al., 2022). To increase the robustness of the analysis, the distance data were right-truncated where the probability of detection was estimated to be approximately 0.15 (Buckland et al., 2001). Detection functions were modelled using a key function (either half-normal or hazard rate) with an adjustment term (null, cosine, simple polynomial or Hermite polynomial). Covariates that could modify the noise field around the hydrophone array, and thus affect the likelihood of detecting clicks, were included in the analysis to modify the scale of the detection function without affecting its shape. These covariates were logged at least every hour in the field and included sea state (Beaufort scale), wave height (m), swell height (m) and rain condition (heavy, light or none); in addition, instruments on the research vessel logged wind speed (knots), sea surface temperature (SST; °C), engine speed (rpm), vessel heading (° true) and vessel speed (knots) every 10 seconds. These covariates were investigated for collinearity using Pearson's correlation coefficient to remove any redundancy; all remaining covariates were subsequently incorporated into model generation. Models were initially generated with single covariates; the best-fitting detection function was selected using Akaike's Information Criterion (AIC). Forward stepwise selection was then conducted by adding one additional covariate at a time to a model containing the one(s) already selected until there was no decrease in AIC. Densities could then be estimated using traditional design-based approaches (Lewis et al., 2018), both with and without a correction for availability. Availability for detection is influenced by both whale behaviour (specifically the proportion of time sperm whales spend clicking) and by survey protocol (as survey speed affects the length of the time window during which whales can be detected). In the absence of detailed information of the vocal behaviour of individual sperm whales during the ASI surveys, for example via the application of suction-cup tags, the acoustic availability of sperm whales was taken from a Monte Carlo simulation performed for tagged sperm whales in the Azores (Fais et al., 2016). ESHW and mean vessel speed were used to determine availability bias.

In addition to design-based estimates, the survey transects were sub-divided into short segments of homogeneous effort type and detection probabilities for individual sperm whales were used in subsequent density surface modelling (DSM). To derive model-based estimates of density, the encounter rate data were fitted to density covariates using a generalised additive model (GAM; Wood, 2006), assuming local density varied in space and in response to specific environmental covariates. In addition to latitude and longitude, several bathymetric and oceanographic parameters were used to generate the DSM (Table 1) and were selected on the basis of their potential to influence sperm whale distribution and their availability for the whole survey area. These parameters have been linked to sperm whale distribution in other studies, and

TABLE 1 Summary of all bathymetric and oceanographic parameters used in DSM.

Parameter	Description	Source	Resolution (km)
Depth (m)	Water depth	NOAA ETOPO1 (ice)	1.4
Slope (°)	Seabed slope (angle relative to horizontal)	NOAA ETOPO1 (ice)	1.4
Aspect (°)	Orientation of slope (degrees magnetic)	NOAA ETOPO1 (ice)	1.4
Distance to shore (m)	Distance to 0 m isobath	QGIS	0.1
Distance to 200 m contour (m)	Distance to 200 m isobath	QGIS	0.1
Distance to 1000 m contour (m)	Distance to 1000 m isobath	QGIS	0.1
SST (°C)	Sea surface temperature	NASA MODIS (Moderate Resolution Imaging Spectroradiometer)	4.0*
Chlorophyll (mg m ⁻³)	Near-surface concentration of chlorophyll a	NASA MODIS (Moderate Resolution Imaging Spectroradiometer)	4.0*
Ocean mixed layer thickness (m)	Thickness of the surface layer for which physical parameters vary little	CMEMS GLORYS2V4 GLOBAL-REANALYSIS-PHY-001-031	27.8 ⁺
Water layer velocity (ms ⁻¹)	Speed of water movement in a given direction	CMEMS GLORYS2V4 GLOBAL-REANALYSIS-PHY-001-031	27.8 ⁺
Water layer direction (°magnetic)	Direction of water movement at a given speed	CMEMS GLORYS2V4 GLOBAL-REANALYSIS-PHY-001-031	27.8 ⁺
Distance to nearest canyon (m)	Distance to “steep-walled, sinuous valleys with V-shaped cross sections”	www.bluehabitats.org	1.0
Distance to nearest escarpment (m)	Distance to “elongated, characteristically linear, steep slope”	www.bluehabitats.org	1.0
Distance to nearest ridge (m)	Distance to “elongated narrow elevation (s) of varying	www.bluehabitats.org	1.0

(Continued)

TABLE 1 Continued

Parameter	Description	Source	Resolution (km)
	complexity having steep sides”		
Distance to nearest seamount (m)	Distance to “large isolated elevation(s), >1000 m in relief above the sea floor”	www.bluehabitats.org	1.0
Distance to nearest shelf (m)	Distance to “depth at which there is a marked increase of slope”	www.bluehabitats.org	1.0
Distance to nearest slope (m)	Distance to “deepening sea floor out from the shelf edge to the upper limit of the continental rise”	www.bluehabitats.org	1.0
Distance to nearest terrace (m)	Distance to “surface(s) bounded by steeper ascending and descending slopes”	www.bluehabitats.org	1.0
Distance to nearest trough (m)	Distance to “long depression of the sea floor characteristically flat bottomed and steep sided”	www.bluehabitats.org	1.0

Parameters denoted with an asterisk (*) are composites of all data collected during boreal summer 2018 (21 Jun - 20 Sep); those denoted with a plus sign (+) are composites of monthly datasets collected in 2018 (16 Jun - 15 Sep). The chlorophyll a measure uses a band difference approach at low chlorophyll concentrations with a band ratio approach (log-transformed) at higher chlorophyll concentrations (Hu et al., 2019).

included depth (Cañadas et al., 2005; Pirotta et al., 2011; Mannocci et al., 2017b; Pace et al., 2018; Pirotta et al., 2020), slope (Cañadas et al., 2005; Praca and Gannier, 2008; Pirotta et al., 2011; Pirotta et al., 2020), aspect (Pirotta et al., 2011; Pirotta et al., 2020), SST (Cañadas et al., 2005; Praca and Gannier, 2008; Pirotta et al., 2011; Pirotta et al., 2020), chlorophyll (Jaquet et al., 1996; Praca and Gannier, 2008; Mannocci et al., 2017b), distance to isobath (including 0, 200 and 1,000 m; Praca and Gannier, 2008; Pace et al., 2018; Sahri et al., 2020; Avila et al., 2022), distance to bathymetric features (such as canyons, escarpments, ridges, seamounts, shelves, slopes, terraces and troughs; Mannocci et al., 2017b; Sahri et al., 2020; Vachon et al., 2022), mixed layer thickness (Avila et al., 2022) and local currents (Vachon et al., 2022). Dynamic oceanographic parameters, such as SST, chlorophyll, depth of mixed layer and water speed/direction, can vary at time-scales from seconds to decades. As animal associations with large scale and persistent oceanographic features are best modelled with climatological covariates (Mannocci et al., 2017a), composites of

dynamic covariates were used that approximately overlapped with the duration of the survey. ‘Instantaneous’ covariates were not used, as these are typically more useful for modelling associations with ephemeral and/or fine-scale features.

In addition to generating density estimates for the surveyed blocks, DSM allowed extrapolation to the entire Mediterranean Sea. To achieve this, a prediction grid was generated for the Mediterranean Sea by dividing the region into 3,634 grid cells with a resolution of 8 km latitude by 8 km longitude (Lambert azimuthal equal-area projection); grid resolution was selected to correspond with the approximate lowest resolution of the available covariates. Segments should be small enough such that neither whale density nor covariate values vary markedly within a segment (Miller et al., 2013); making segments approximately square is usually sufficient to achieve this. As each segment is no wider than twice the truncation distance (4 km in this study), using a segment length of 8 km ensured segments were approximately square. Increasing the size of segments can reduce the number of ‘empty’ segments (i.e. those without detections); however, if segment size becomes too large, the ability of the model to identify associations with persistent oceanographic features can become compromised. The segment length of 8 km in this study was larger than that used in similar modelling exercises in nearby regions (e.g. means of 6.96 and 5.84 km respectively for the North-East Atlantic; Cañadas et al., 2009; Rogan et al., 2017), but was deemed unlikely to reduce model performance as it approximately matched the resolution of the covariates. Only regions with waters deeper than 200 m were considered for the prediction grid to exclude those regions not likely to provide suitable sperm whale habitat (Pirotta et al., 2011; Lewis et al., 2018). The covariates described in Table 1 were averaged over each grid cell. Survey effort was sub-divided into segments approximately twice the truncation distance of the dataset (i.e. 8 km), making the two-dimensional outline of a segment approximately square. The centroid of each segment was assigned to a cell in the prediction grid, and the average values of all covariate in that cell were assigned to that segment. The response variable used to model sperm whale distribution was the count of individuals in each segment, once corrected using the detection function generated during MCDS; the effective area of each segment (defined as the actual area multiplied by the estimated probability of detection using the selected detection function) served as an offset in the model. When taking availability bias into account, this offset was divided by the correction factor for availability. Spatial location was included in the model as a bivariate smooth of x and y (metres east and north respectively). As smoothing over areas with complicated boundaries, such as islands and peninsulas, can lead to the inappropriate linking of different regions (Wood et al., 2008; Miller et al., 2013), a realistic spatial model should be fitted to the data to provide valid inference. A soap film smoother was used to allow boundary conditions to be estimated for the complex study area and to be incorporated in to a bivariate smooth function of location (Wood et al., 2008); the complexity of the soap film was set to 10 knots. Smooth functions of the environmental covariates were constructed using thin plate regression splines with shrinkage, except for the circular variables aspect and water direction which

used cyclic cubic regression splines. The Tweedie distribution with logarithmic link function was assumed for the response variable, an approach that adequately handles zero-inflated spatial models (Miller et al., 2013). GAMs were fitted using the “dsm” R package (R Core Team, 2021). Model selection was conducted by adding one candidate explanatory variable at a time in a forward approach. The model selected at each step was chosen by looking for an improvement in the Restricted Maximum Likelihood (REML) score and percentage of variation explained; randomised quantile residuals and quantile-quantile plots were also examined for normality, auto-correlation and homoscedasticity. REML was used for model selection as it derives less variable estimates of the smoothing parameter than other criteria (Wood, 2011); comparing REML scores is appropriate for models that use shrinkage in the smoothing penalty. Maps showing extrapolated densities for the whole Mediterranean were created in QGIS using the outputs from the DSM procedure for the surveyed study area. Variance estimates of abundance were derived by combining the variances of the GAM and detection function using the delta method.

Power analysis to determine required survey effort

Repeated survey effort allows population trends to be identified; the greater the survey effort, the more rapidly any changes can be identified. To investigate the power of repeated surveys to detect significant changes, the general inequality model of Gerrodette (1987) was used, whereby:

$$r^2 n^3 \geq 12CV^2(z_{\alpha/2} + z_{\beta})^2$$

where r is the annual rate of population change, n is the number of surveys, CV is the coefficient of variation for the population estimate, $z_{\alpha/2}$ is the one-tailed probability of a Type I error (false positive) and z_{β} is the probability of making a Type II error (false negative). For subsequent calculations, the corrected population size estimated by model-based distance sampling was used, along with the attendant CV. The influence of different levels of survey effort was investigated by varying the inter-survey interval from one year (i.e. annual surveys) to 10 years (i.e. decennial surveys). Following Taylor et al. (2007), statistical power was assessed at both a high level (i.e. 0.95) and an acceptable level (i.e. 0.80).

Results

Between 28th May and 29th September 2018, the *Song of the Whale* team completed almost 22,000 km of survey effort in both the eastern and western basins as part of the ACCOBAMS Survey Initiative (Figure 1). Approximately 14,039 km (66%) was “on-track”, following pre-determined survey transects at 5-8 knots with acoustic effort; visual effort was conducted during daylight hours when weather conditions were appropriate. A total acoustic coverage of 8.3% was realised, based on an ESHW of 3.5 km. Sperm whales were detected acoustically throughout the western basin of the Mediterranean Sea, with additional detections in the

Hellenic Trench in the eastern basin, and in the Atlantic approaches to the Strait of Gibraltar region (Figure 2). A total of 254 individual sperm whales were detected on-track, with an additional 66 individuals detected off-track (i.e. when off a transect or faster/slower than 5–8 knots). Sperm whales were seen only ten times on the trackline, with 16 sightings made off-track; observed group sizes ranged from one to seven individuals.

Design-based acoustic density estimation

Slant ranges estimated via TMA in PAMGuard were imported into the Distance software to generate acoustic detection functions and density estimates using MCDS. Only the 254 on-track detections were used. Distance data were right-truncated where the probability of detection was approximately 0.15 (Buckland et al., 2001); this excluded detections beyond 5000 m prior to analysis, representing 19% of the largest distance estimates. Prior to including covariates in subsequent analysis, they were first investigated for correlation using Pearson's correlation. Wind speed and wave height were found to be strongly correlated ($r^2 = 0.566$, $p < 0.001$); as wind speed was logged by a sensor on board R/V *Song of the Whale*, it was used in MCDS in lieu of the subjective estimates of wave height. The remaining covariates (vessel heading, vessel speed, engine revs, wind speed, wind direction, sea surface temperature, sea state, swell height and rain condition) were used to modify the detection function. A hazard rate key function without an adjustment term generated a detection function with the closest fit to the slant range estimates based on AIC scores. Inclusion of wind speed had the most pronounced effect on the detection function, deriving the lowest AIC score. Inclusion of additional covariates did not improve the fit of the model and thus only this covariate was included in the final model. A goodness-of-fit test suggested the detection function incorporating wind speed

adequately represented the slant ranges ($\chi^2_{(3,205)} = 1.158$, $p = 0.763$). The ESHW was 3,442 m (Figure 3). A quantile-quantile plot suggested model fit was adequate and randomised quantile residuals did not exhibit heteroscedasticity.

MCDS was used to generate density estimates for those blocks with a sufficient number of on-track detections (Table 2). Without an adjustment for $g(0)$, the uncorrected total estimate was 2,439 whales (95% CI 1,598–3,717) which included most of the known habitat for sperm whales in the Mediterranean Sea. The acoustic availability of sperm whales was taken from a Monte Carlo simulation performed by Fais et al., 2016 for tagged sperm whales recorded in the Azores. An estimate for $g(0)$ of 0.912 (sd = 0.036) was derived using an ESHW of 3.5 km and average survey speed of 6 knots. By incorporating this estimate of availability bias, a corrected abundance estimate of approximately 2,673 individual sperm whales was derived for the blocks surveyed (95% CI 1,739–4,105; CV = 0.212) (Table 3).

Model-based acoustic density estimation

The DSM procedure applied the detection function generated during MCDS (i.e. a hazard rate key function without an adjustment term incorporating wind speed as a covariate) to 720 segments of homogeneous effort type. As sperm whales tend to aggregate in clusters, and the study area was orders of magnitude larger the average segment size, many segments were 'empty' ($n = 678$). However, if larger segments were used, many may have had very similar covariate values, which could have reduced the utility of the model. The Tweedie distribution used in the model can be useful when modelling count data with a high proportion of zeros in the dataset (Miller et al., 2013). Using a simple bivariate smooth of location showed signs of 'leakage', particularly between the Tyrrhenian, Adriatic, and Aegean. To help address this leakage,

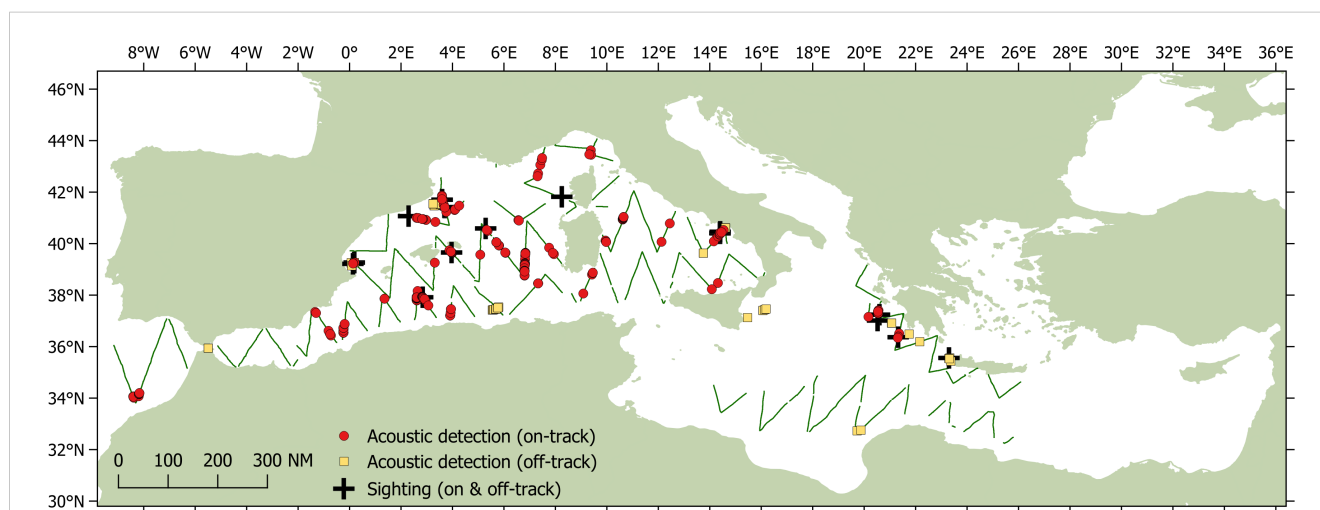


FIGURE 2

Sperm whale acoustic detections from *Song of the Whale* during the ASI survey. Individual whales detected on the track line are shown as red circles ($n = 254$); whales off-track are shown as orange squares ($n = 66$). Both on- and off-track sightings are shown as black crosses. Those sections of track survey using acoustic effort are shown as green lines.

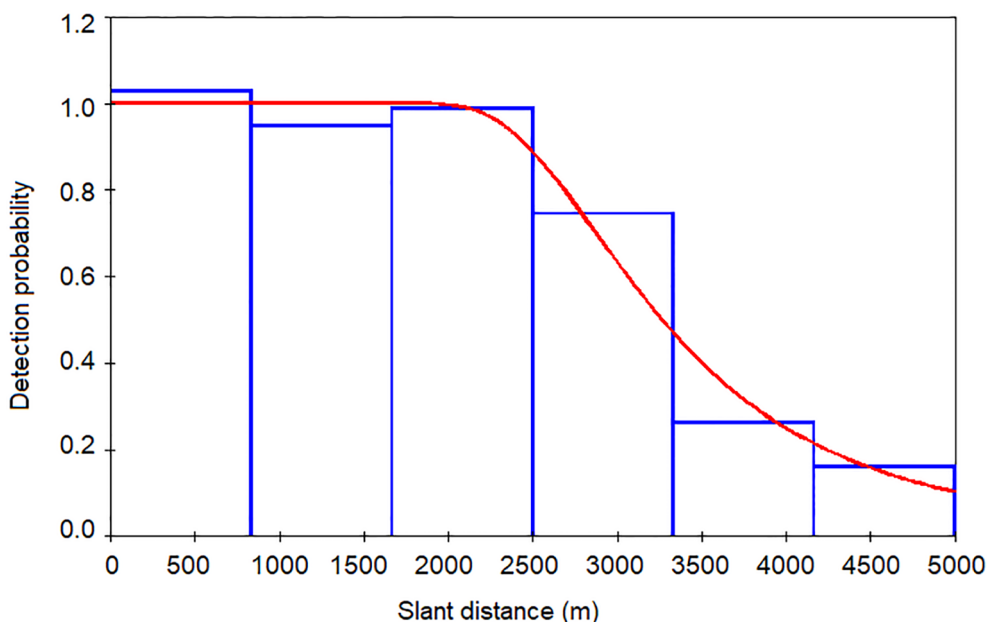


FIGURE 3 The detection function generated using MCDS (hazard rate key without adjustment). The covariate wind speed was used in the final model. Effective strip half-width was estimated as 3,442 m.

TABLE 2 Density (*D*) derived from design- and model-based approaches for each survey block expressed as the number of individuals per 1000 km².

Block	Design-based <i>D</i>			Model-based <i>D</i>		
	<i>g</i> (0)=1.000	<i>g</i> (0)=0.912	CV	<i>g</i> (0)=1.000	<i>g</i> (0)=0.912	CV
01: Gulf of Cadiz	2.82 (0.51-15.57)	3.10 (0.57-16.73)	0.43	1.85 (0.64-5.38)	2.03 (0.7-5.89)	0.58
02: Alborán Sea	-	-	-	1.56 (0.42-5.88)	1.71 (0.46-6.45)	0.76
03: West Algeria	6.58 (2.61-16.63)	7.22 (2.85-18.27)	0.44	3.23 (1.84-5.67)	3.54 (2.01-6.22)	0.29
04: Balearics	2.4 (0.39-15.31)	2.66 (0.42-16.78)	0.82	5.52 (3.17-9.61)	6.05 (3.47-10.54)	0.29
05: Northeast Spain	2.03 (0.31-13.18)	2.22 (0.34-14.43)	0.76	2.47 (1.19-5.15)	2.71 (1.3-5.64)	0.39
06: East Algeria	1.04 (0.06-18.07)	1.14 (0.07-19.58)	0.75	3.67 (1.61-8.37)	4.02 (1.76-9.18)	0.44
07: West Sardinia	5.38 (1.48-19.56)	5.90 (1.63-21.39)	0.50	4.02 (2.19-7.4)	4.41 (2.4-8.12)	0.32
8a: Gulf of Lion (shelf)	4.57 (0.13-156.46)	5.02 (0.18-140.05)	0.32	1.64 (0.58-4.66)	1.80 (0.63-5.11)	0.57
8b: Gulf of Lion (deep)	2.60 (0.17-39.38)	2.85 (0.19-42.64)	0.71	2.71 (1.26-5.81)	2.97 (1.39-6.37)	0.40
09: Pelagos	-	-	-	4.85	5.32	0.51

(Continued)

TABLE 2 Continued

Block	Design-based D			Model-based D		
	$g(0)=1.000$	$g(0)=0.912$	CV	$g(0)=1.000$	$g(0)=0.912$	CV
(southwest)				(1.89-12.43)	(2.08-13.63)	
10: Pelagos	0.67	0.74	0.89	3.72	4.07	0.57
(northwest)	(0-9520)	(0-9631)		(1.31-10.52)	(1.44-11.53)	
11: Pelagos	0.43	0.47	1.04	1.86	2.04	0.53
(eastern)	(0.03-6.56)	(0.03-7.17)		(0.7-4.92)	(0.77-5.4)	
12: Tyrrhenian	6.53	7.16	0.22	1.31	1.43	0.48
(northwest)	(2.83-15.05)	(3.20-16.02)		(0.54-3.19)	(0.59-3.5)	
13: Tyrrhenian	3.82	4.19	1.05	1.57	1.73	0.45
(northeast)	(0.35-41.59)	(0.39-45.54)		(0.67-3.68)	(0.74-4.04)	
14: Tyrrhenian	–	–	–	1.64	1.80	0.55
(southwest)				(0.6-4.51)	(0.66-4.95)	
15: Tyrrhenian	–	–	–	1.71	1.88	0.53
(southeast)				(0.65-4.52)	(0.71-4.96)	
22w: Hellenic Trench	–	–	–	0.99	1.09	0.86
(western)				(0.23-4.26)	(0.25-4.67)	
22c: Hellenic Trench	1.14	1.25	0.80	0.47	0.51	0.69
(central)	(0.19-6.94)	(0.20-7.60)		(0.14-1.6)	(0.15-1.75)	
22e: Hellenic Trench	–	–	–	0.20	0.22	1.55
(eastern)				(0.02-1.76)	(0.03-1.93)	
25: Libya	–	–	–	0.20	0.22	0.98
				(0.04-1)	(0.04-1.1)	
Total	2.10	2.30	0.21	2.22	2.43	0.16
	(1.38-3.20)	(1.50-3.53)		(1.62-3.00)	(1.78-3.32)	

MCDS included wind speed as a covariate; detection functions were derived with a hazard rate key without adjustment. DSM assumed a Tweedie distribution with logarithmic link function for the response variable. Estimates are presented as both corrected and uncorrected for acoustic availability [$g(0) = 0.912$].

the final DSM model selected included a soap film bivariate smooth of location (xy) along with a cyclic cubic regression of mean aspect, the former having the most pronounced effect on the model. Both covariates were considered significant ($p < 0.01$) and explained 32.2% of the deviance in the model. Densities were highest in the Algerian and Liguro-Provencal Basins (Table 2) and in regions of west-facing aspect ($>180^\circ$; Figure 4). The DSM derived an uncorrected abundance estimate of 3,268 (95% CI 2,499-7,540; CV = 0.287) sperm whales for Mediterranean waters deeper than 200 m; the estimated abundance was 3,583 (95% CI 1,881-5,677) if corrected with a $g(0)$ of 0.912 (Figure 5). If considering only the blocks surveyed by the *Song of the Whale* team, the uncorrected abundance estimate was 2,577 (95% CI 1,872-3,546; CV = 0.164); the estimated abundance was 2,825 (95% CI 2,053-3,888) if corrected with a $g(0)$ of 0.912 (Table 3). The coefficients of variation associated with the DSM predictions are shown in Figure 6.

Power analysis

Repeated surveys are required to detect statistically robust population trends. The power analyses suggested that the shorter the interval between surveys, the sooner that significant declines can be detected (Table 4). Although larger population declines (such as 10% per annum) can be detected more quickly than smaller population declines (such as 1% per annum), by the time they are detected with sufficient statistical power, the population could have dropped by up to 90% (for example, decennial surveys identifying a 10% per annum decline with high power). Although annual surveys would be considered extremely effective, they are financially and logistically unfeasible. Other large-scale survey efforts for cetaceans have been conducted decennially (e.g. SCANS; Hammond et al., 2013), and the modelled outputs for decennial surveys for sperm whales in the Mediterranean are shown in Figure 7. For comparative purposes, outputs for sexennial surveys are also shown, as suggested

TABLE 3 Outputs from design- and model-based approaches to abundance (N) estimation for each survey block using wind speed as a covariate; detection functions were derived with a hazard rate key without adjustment.

Block	Design-based N		Model-based N		Other estimate
	$g(0)=1.000$	$g(0)=0.912$	$g(0)=1.000$	$g(0)=0.912$	
01: Gulf of Cadiz	264	290	174	190	
	(48-1,457)	(54-1,566)	(60-503)	(66-552)	
02: Alborán Sea	–	–	75	83	
			(20-284)	(22-311)	
03: West Algeria	585	641	287	314	
	(231-1,477)	(253-1,622)	(163-504)	(179-552)	
04: Balearics	224	246	509	558	~400; June-October
	(36-1,412)	(39-1,622)	(292-886)	(320-971)	(Rendell et al., 2014)
05: Northeast Spain	110	121	134	147	
	(17-715)	(19-783)	(64-279)	(71-306)	
06: East Algeria	57	62	200	220	
	(3-987)	(4-1,069)	(88-457)	(96-501)	
07: West Sardinia	393	431	294	323	
	(108-1,430)	(119-1,564)	(160-541)	(175-593)	
8a: Gulf of Lion	158	173	56	62	161; May-August
(shelf)	(5-5,391)	(6-4,826)	(20-161)	(22-176)	(Laran et al., 2017)
8b: Gulf of Lion	122	134	127	139	209; May-August
(deep)	(8-1,845)	(9-1,998)	(59-272)	(65-299)	(Laran et al., 2017)
09: Pelagos	–	–	106	117	
(southwest)			(42-273)	(46-299)	
10: Pelagos	23	25	127	139	
(northwest)	(0-325,090)	(0-328,860)	(45-359)	(49-394)	
11: Pelagos	14	15	59	64	
(eastern)	(1-207)	(1-226)	(22-155)	(24-170)	
12: Tyrrhenian	178	195	36	39	
(northwest)	(77-410)	(87-437)	(15-87)	(16-96)	
13: Tyrrhenian	256	280	105	116	
(northeast)	(23-2,782)	(26-3,046)	(45-246)	(49-270)	
14: Tyrrhenian	–	–	130	142	
(southwest)			(47-356)	(52-390)	
15: Tyrrhenian	–	–	83	91	
(southeast)			(31-218)	(34-239)	
22w: Hellenic Trench	–	–	11	12	
(western)			(2-46)	(3-50)	
22c: Hellenic Trench	55	60	23	25	200-250; June-October
(central)	(9-334)	(10-366)	(7-77)	(7-84)	(Frantzis et al., 2014)
22e: Hellenic Trench	–	–	6	6	

(Continued)

TABLE 3 Continued

Block	Design-based <i>N</i>		Model-based <i>N</i>		Other estimate
	<i>g</i> (0)=1.000	<i>g</i> (0)=0.912	<i>g</i> (0)=1.000	<i>g</i> (0)=0.912	
(eastern)			(1-49)	(1-54)	
25: Libya	–	–	36	40	
			(7-181)	(8-198)	
Total	2,439	2,673	2,577	2,825	~1800; May-November
	(1,598-3,717)	(1,739-4,105)	(1,872-3,546)	(2,053-3,888)	(Lewis et al., 2018)

Abundance estimates are corrected for acoustic availability (*g*(0) = 0.912). Abundance estimates from other studies are also presented (with the months for which data were analysed); the study regions used in the other studies do not necessarily align closely with survey blocks used for the ASI surveys.

by the Long Term Monitoring Programme adopted at the ACCOBAMS Meeting Of Parties (Malta, November 2022).

Discussion

This study presents acoustic density estimates for sperm whales in the Mediterranean Sea and contiguous Atlantic region

derived using both design-based and model-based methods. Although the precise estimates varied between the two approaches, they generally agreed on approximately 2,500 whales in the surveyed blocks (2,439 and 2,577 whales using design- and model-based approaches respectively), rising to 2,800 individuals if a correction for acoustic availability is applied (2,673 and 2,825 whales respectively). Although the distribution of sperm whales in the Mediterranean may vary by

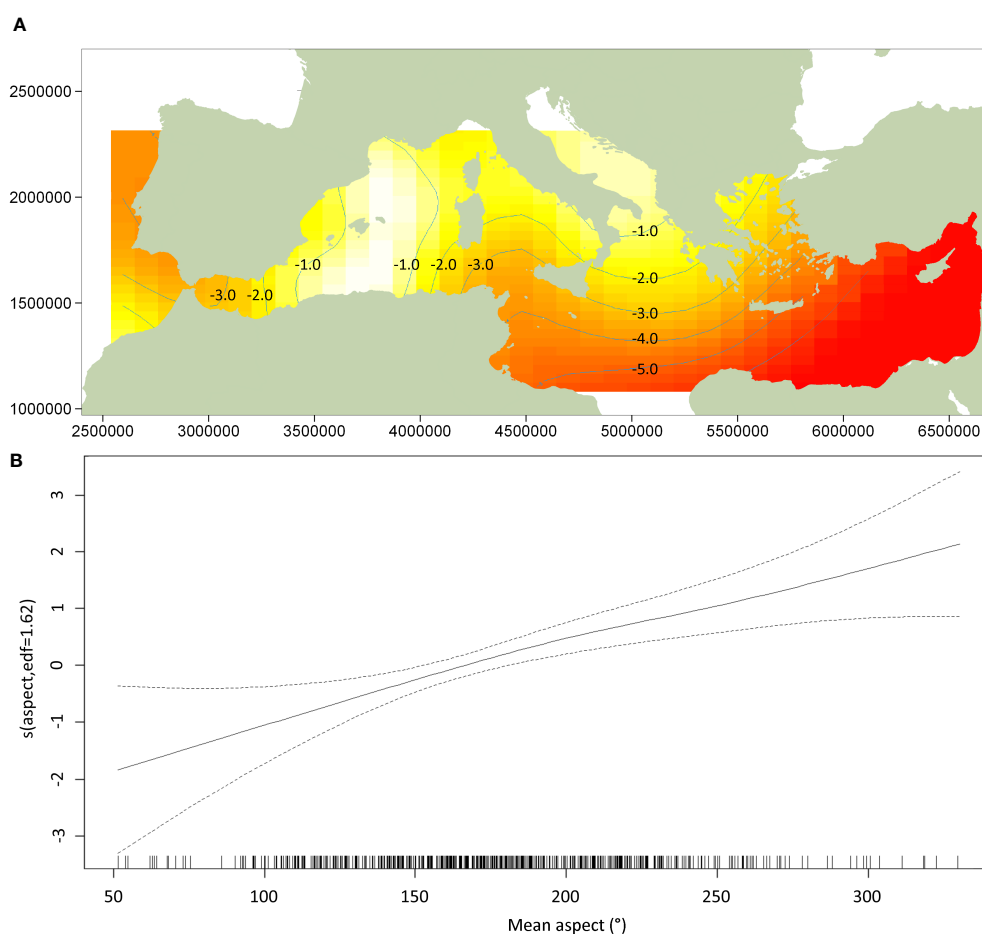
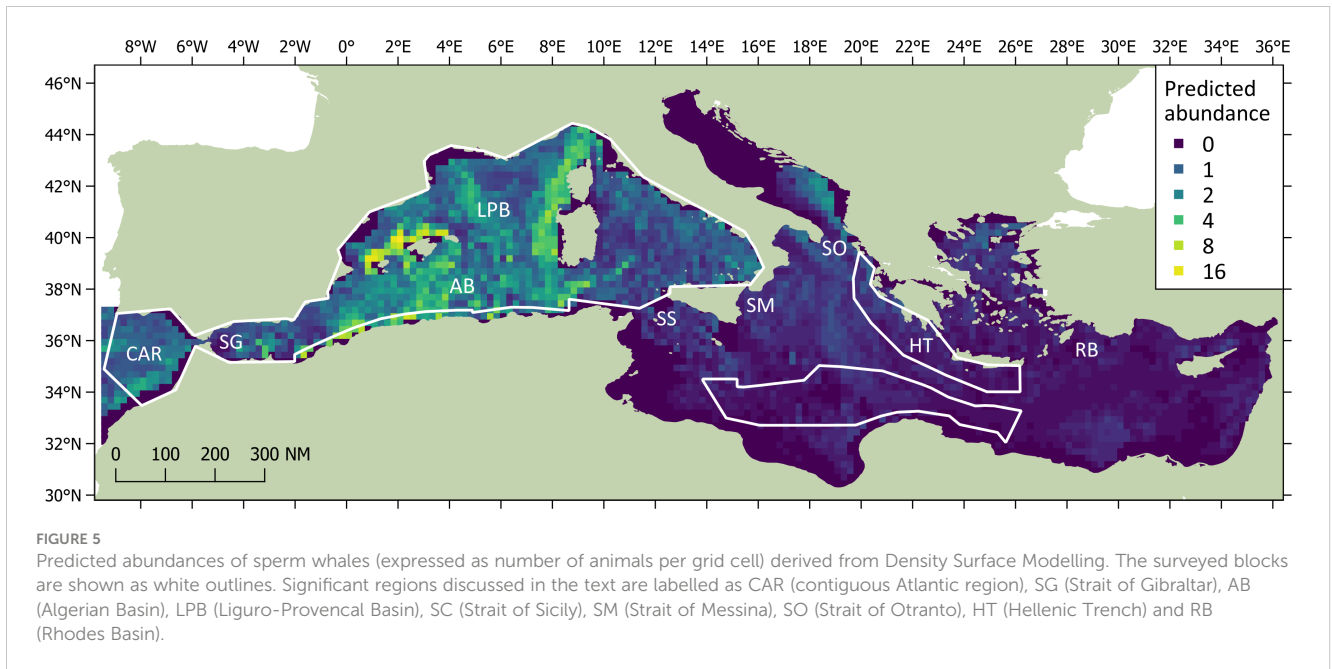


FIGURE 4 Plot of the GAM smooth fit of density across (A) location and (B) mean aspect. Values in plot (A) of the soap film spatial smooth are relative abundances; yellow indicates high values, red low indicates low values (Lambert azimuthal equal-area projection). In plot (B) of mean aspect, the solid line represents the best fit with dashed lines representing 95% confidence intervals; vertical lines on the x-axis are observed data values.



sex and age (Caruso et al., 2015; Reid, 2019), these abundance estimates include all individuals, regardless of sex, age or size.

Western basin

In keeping with other studies (Rendell et al., 2014; Laran et al., 2017; Lewis et al., 2018), the western basin was found to provide habitat for the majority of sperm whales in the Mediterranean, with blocks 1-15 accounting for over 97% of total abundance. Both design- and model-based approaches found highest densities in the Algerian and Liguro-Provencal Basins between Algeria and Spain/France. Densities were generally 2-6 whales per 1,000 km² in the western basin, although they declined close to the Straits of

Gibraltar, Sicily and Messina. These straits are characterised by relatively shallow water sills that may restrict the movements of deeper water cetaceans. The Strait of Gibraltar provides the only natural connection between the Mediterranean Sea and the North Atlantic Ocean; however, the low densities encountered near the 290 m deep Camarinal Sill supports the theory that it acts as a migratory barrier to sperm whales (Drouot et al., 2004a; de Stephanis et al., 2008; Engelhaupt et al., 2009). The Strait of Sicily provides a 160 km wide interface between the western and eastern basins, and the maximum depth of the area is only 316 m; the nearby Strait of Messina is only 3 km wide with a maximum depth of 80 m. Despite the noted year-round presence of sperm whales in the Ionian Sea (Pavan et al., 2008; Caruso et al., 2015), it is likely that both straits may also act as a significant deterrent to

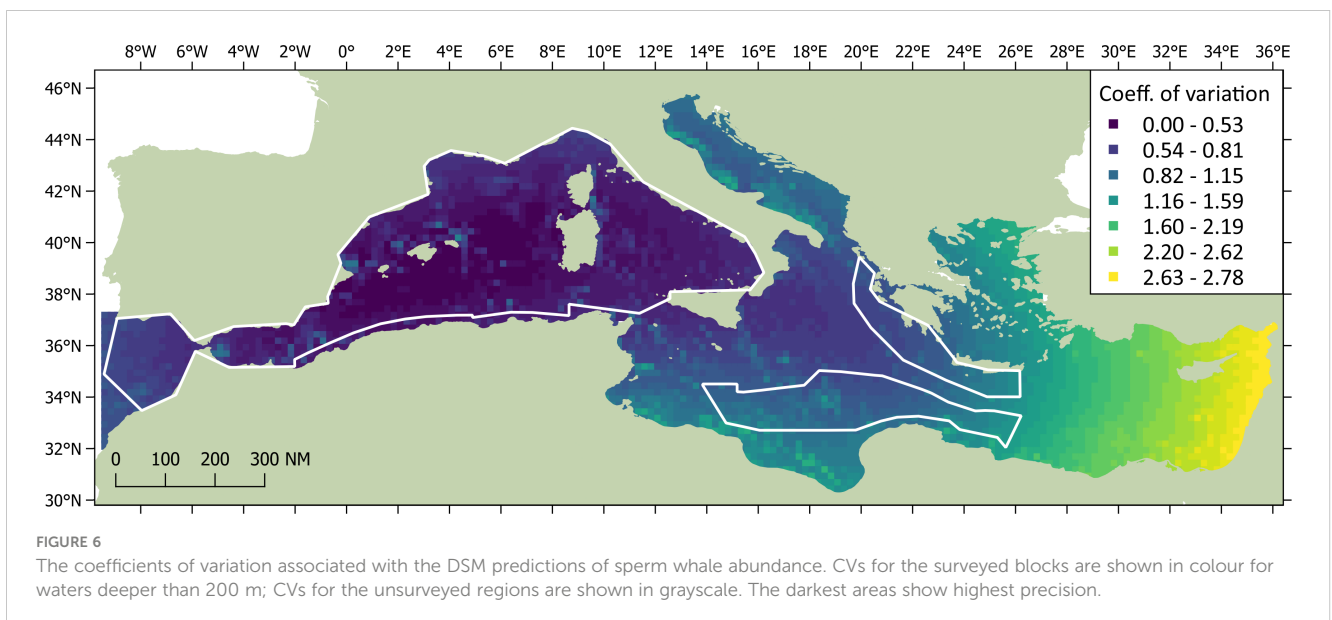


TABLE 4 The ability to detect population decline with two separate levels of statistical power; high (0.95) and acceptable (0.80).

Power level	Years between surveys	Years to detection				Total % change at detection			
		$r = -0.01$	$r = -0.02$	$r = -0.05$	$r = -0.10$	$r = -0.01$	$r = -0.02$	$r = -0.05$	$r = -0.10$
High (0.95)	1	34	21	11	7	-0.29	-0.35	-0.43	-0.52
	2	42	26	14	8	-0.34	-0.41	-0.51	-0.57
	3	48	30	15	9	-0.38	-0.45	-0.54	-0.61
	4	52	32	16	12	-0.41	-0.48	-0.56	-0.72
	5	55	35	20	10	-0.42	-0.51	-0.64	-0.65
	6	60	36	18	12	-0.45	-0.52	-0.60	-0.72
	7	63	42	21	14	-0.47	-0.57	-0.66	-0.77
	8	64	40	24	16	-0.47	-0.55	-0.71	-0.81
	9	72	45	27	18	-0.52	-0.60	-0.75	-0.85
	10	70	40	20	20	-0.51	-0.55	-0.64	-0.88
Acceptable (0.80)	1	28	18	9	6	-0.25	-0.30	-0.37	-0.47
	2	36	22	12	8	-0.30	-0.36	-0.46	-0.57
	3	39	24	12	9	-0.32	-0.38	-0.46	-0.61
	4	44	28	16	8	-0.36	-0.43	-0.56	-0.57
	5	50	30	15	10	-0.39	-0.45	-0.54	-0.65
	6	48	30	18	12	-0.38	-0.45	-0.60	-0.72
	7	56	35	14	14	-0.43	-0.51	-0.51	-0.77
	8	56	32	16	8	-0.43	-0.48	-0.56	-0.57
	9	54	36	18	9	-0.42	-0.52	-0.60	-0.61
	10	60	40	20	10	-0.45	-0.55	-0.64	-0.65

Outcomes are presented with t (years between consecutive surveys) varying from annual to decadal. The number of years required until a given decline is detected ($t[n-1]$), and the corresponding total decline in population size ($(1+r)^{t(n-1)}-1$), are presented for annual decline rates (r) of 1, 2, 5 and 10%. The corrected model-based CV of 0.16 is used for calculations; n is calculated as per Gerrodette's (1987) general inequality model.

the movement of sperm whale groups between east and west (Lewis et al., 2007; Boisseau et al., 2010), with inter-basin movements possibly restricted to adult males (Frantzis et al., 2011).

Most detections, and the highest modelled densities, were in an approximately rectangular region bounded by Algeria, Spain, France and Sardinia. All sightings except one made by the aerial component of the ASI in the western basin were also made in this

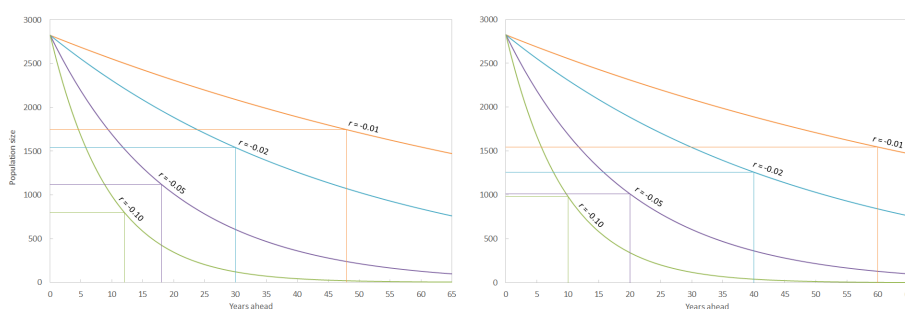


FIGURE 7 The time taken to detect different rates of decline of the sperm whale population in the Mediterranean Sea with an acceptable level of power (0.80) if estimates of population size are made sexennially (left) and decennially (right). The vertical lines represent the time taken for a significant decline to be detected, with the corresponding population size marked by the horizontal lines, for each hypothetical rate of decline (r). The corrected model-based abundance of 2,825 whales ($CV = 0.16$) is used for calculations. As an example, running surveys every six years might detected a 10% per annum decline in the population after two surveys with acceptable statistical power (by which point the total number of animals would have reduced by 72%).

region (Cañadas et al., 2023; Panigada et al., 2023). The region has long been noted for its importance to Mediterranean sperm whales (Pavan et al., 2000; Gannier et al., 2002; Praca and Gannier, 2008; Rendell et al., 2014; Lewis et al., 2018), although there had previously been little systematic survey effort off the Algerian coast. Numerous detections were made in regions of steep slope, such as off the Spanish mainland, the Balearic Islands and Sardinia. However, sperm whale encounter rates were just as high in expanses of open water with relatively uniform bathymetry, such as in the Algerian and Liguro-Provençal Basins. Outside of the central region of highest density, predicted abundances were also high in the eastern Tyrrhenian and off the Moroccan Atlantic coast. Although regional patches of high sperm whale density have been noted off Italy's west coast (e.g. Mussi et al., 2014; Pace et al., 2018), only two vessel surveys covering the whole Tyrrhenian Sea had been conducted prior to the ASI (Gannier et al., 2002), both being conducted over 20 years ago. In keeping with these previous studies, sperm whales were clustered near the Italian coast to the south of Ischia. Likewise, surveys for sperm whales in the contiguous Atlantic region have been rarely undertaken, in part because of challenging swell and weather conditions. As in a similar previous acoustic/visual study (Boisseau et al., 2010), sperm whales were encountered off Morocco's Atlantic coast but not towards the Iberian peninsula. These waters once supported seemingly high densities of sperm whales (Sanpera and Aguilar, 1992; Aguilar and Borrell, 2007), but 19th and 20th century whaling removed significant numbers of animals over several decades and it is not clear to what extent this has affected the local distribution patterns seen today.

Eastern basin

The surveys conducted in the eastern basin found very low densities of sperm whales except for the Hellenic Trench; both design- and model-based approaches estimated only 40-60 animals present in block 22 during the ASI survey. This finding is supported by other studies that have found moderately high year-round densities of sperm whales in the Hellenic Trench, Rhodes Basin and south Aegean Sea (Öztürk et al., 2013; Frantziş et al., 2014; Lewis et al., 2018; Akkaya et al., 2020), but in few other places in the eastern basin. Lower densities have been reported around the Republic of Cyprus and reported group sizes are typically small (Boisseau et al., 2010; Ryan et al., 2014; Boisseau et al., 2017; Lewis et al., 2018; Snape et al., 2020); however, a larger social unit has been encountered in Cypriot waters at least once (Kerem et al., 2012). Although there was one off-track detection of a single sperm whale in Libyan waters during the ASI, it seems this area may only be used occasionally by sperm whales, with only a single individual encountered in a similar acoustic/visual survey conducted in 2007 (approximately 500 km east of the 2018 detection; Boisseau et al., 2010). Likewise, visual surveys conducted from other vessels during ASI rarely documented sperm whale encounters, with only two groups seen off Egypt (a group of two plus a group of three to five) and a solitary whale seen off Syria (ACCOBAMS, 2021). It should be noted, however, that the species identification for all three of these encounters was not considered 'definite'. The aerial surveys conducted during ASI

documented sperm whale sightings in the Hellenic Trench and southern Aegean Sea, but also a group of four in the Ionian Sea and a group of three in Turkey's Gulf of Antalya (Cañadas et al., 2023; Panigada et al., 2023). Although strandings have been documented for most eastern basin states, including Italy (Bearzi et al., 2011; Pace et al., 2019), Greece (Frantziş et al., 2019), Turkey (Tonay et al., 2021), Syria (Gonzalvo and Bearzi, 2008), Israel (Kerem et al., 2012), Egypt and Libya (Farrag et al., 2019), and Tunisia (Karaa et al., 2016), aggregations of live sperm whales are rarely encountered outside of Greek, Turkish and Cypriot waters. In Israeli waters before 2012, for example, there had only been a single acoustic detection documented plus seven sightings by non-experts of unsexed animals of which most were solitary (Kerem et al., 2012). Since 2017, there have been at least 28 sightings of unsexed sperm whales in the waters of Israel, Lebanon and Cyprus, most of which were of solitary animals with occasional pairs being reported (D Kerem & O Galili, pers. comm. August 2022)¹. These patchy encounters and the results from DSM modelling reinforce the theory that the core sperm whale habitats in the eastern basin are concentrated near the Hellenic Trench (Frantziş et al., 2014). Although during the ASI some sperm whale sightings were made in deep waters, a large dataset of sightings collected over the last two decades indicates that sperm whales and particularly social units have a strong preference for waters close to the 1000 m contour of the Hellenic Trench where the density of marine traffic is often highest (Frantziş et al., 2014; Frantziş et al., 2019).

Density surface modelling

The selected model used for the DSM analysis suggested location and aspect were the most instructive covariates for predicting sperm whale abundance. The GAM soap film smooth fit of density using latitude and longitude highlighted the central region in the western basin as having notably high sperm whale densities. In the eastern basin, the highest-density region identified by the soap film smooth of location was the southern Adriatic Sea. Although this region was not surveyed by vessel during the ASI, the aerial surveys did not encounter any sperm whales in the Adriatic (Cañadas et al., 2023; Panigada et al., 2023). Previous surveys have similarly not found evidence that the Adriatic Sea provides suitable habitat for sperm whales (Bearzi et al., 2009; Lewis et al., 2018), and it is therefore incongruous that the model suggested high densities in this region. DSM outputs that make predictions outside the range of the input data should be treated with caution (Miller et al., 2013). The Adriatic Sea connects to the Ionian Sea via the relatively narrow (72 km) yet deep (780 m) Strait of Otranto (Širović and Holcer, 2020). Although deeper than the other notable straits in the Mediterranean, and therefore unlikely to present a barrier to the free movement of sperm whales, the deepest point of the southern Adriatic Sea is 1,233 m (Širović and Holcer, 2020). As the northern Adriatic is essentially a shallow continental shelf, it is unlikely that sperm whales can find enough suitable habitat in the broader

¹ D Kerem and O Galili was made on 25th August 2022, the contact with A Frantziş and P Alexiadou was made on 13th Dec 2022.

Adriatic Sea, particularly when considering those in the nearby Hellenic Trench may be found in waters 2,500 m deep (Frantzi et al., 2014). In addition to providing sub-optimal foraging conditions, anthropogenic pressures in the Adriatic Sea may also prevent the region from supporting significant numbers of sperm whales. Illegal, unreported and unregulated fisheries (IUU), including driftnets, have been reported for Italian waters (Piroddi et al., 2015), and it is not clear to what extent these may have affected Adriatic sperm whales. As the Adriatic Sea is essentially a shallow, enclosed basin, it is susceptible to noise; as an important shipping route with high densities of recreational boating (Širović and Holcer, 2020), this region may present considerable risks to sperm whales in terms of both noise and ship-strike risk (Bearzi et al., 2011) and may account for their local absence.

The other covariate retained in the final DSM model was mean aspect. The smooth fit of aspect suggested areas with west- to south-facing slopes were of particular importance to the sperm whales encountered during the ASI. This was particularly evident off the Atlantic coast of Morocco, Sardinia, the Ligurian Sea, the Tyrrhenian Sea and the Hellenic Trench. However, in other regions, such as the Alborán Sea, Algerian Basin and Liguro-Provençal Basin, this did not appear to be the case. It should be noted that although considered a significant smooth term, including mean aspect with location in the final model only explained an additional 0.4% of deviance. The importance of mean aspect in the DSM output should therefore not be over-interpreted. Other studies have found aspect to be an important covariate; for example, analysis of a long-term dataset from the Balearic Islands suggested sperm whales were encountered less often when the seafloor was oriented west to northwest (Pirota et al., 2011; Pirota et al., 2020). It is likely that the orientation of slope aspect preferred by Mediterranean sperm whales varies by region in response to local bathymetry, currents and prey density, and a snapshot DSM analysis is not granular enough to capture this heterogeneity. Where slope aspect does play a role in sperm whale distribution, it is likely to interact with the local circulation of water to drive downwelling/upwelling events that influence the availability and density of bathypelagic cephalopods that predominate the diet of Mediterranean individuals (Foskolos et al., 2019).

Abundance estimates

The analysis process involved generating detection functions using slant ranges to vocalising individuals as a proxy for perpendicular distances. Although this approach can lead to overestimation of perpendicular distances which may in turn lead to underestimation of abundance, a previous modelling exercise for Mediterranean sperm whales found that for hazard rate detection functions with high values (i.e. > 1,000) of the scale parameter, σ , and values of β between 1 and 5, this bias is negligible (Lewis et al., 2018). As the hazard rate detection function used in the final ASI model had parameter estimates of $\sigma = 3,000$ and $\beta = 4.3$, it is likely that any errors introduced in to the estimate of detection probability were minimal. The addition of wind speed as a covariate improved the fit of the detection function. Higher wind speeds tended to be associated with more

distant detections of sperm whales (i.e. a broadening of the detection function). Although high winds at the sea surface increase ambient noise levels, and thus may make it harder to detect sperm whale clicks, they may also promote mixing of the water column. This mixing action may remove any thermoclines that could have the potential to reflect or refract clicks produced at depth, thus modifying estimated slant ranges.

The uncorrected abundance estimate derived from a design-based approach was 2,439 whales for the surveyed regions; the model-based approach derived a slightly higher number of 2,577 whales. When correcting for availability bias, these estimates rose to 2,673 and 2,825 respectively. A noticeable difference in the two approaches was that the design-based approach could not be used in those survey blocks without detections, whereas the model-based approach derived estimates for these regions. An uncorrected model-based estimate that excludes the 'blank' blocks of the design-based approach (namely blocks 2, 9, 14, 22w, 22e and 25) is 2,466, a number much closer to the design-based estimate of 2,439. Although these 'blank' blocks all had detections of sperm whales, these were typically removed from the design-based analysis as the right-truncation distance was set as 5,000 m (i.e. probability of detection > 0.15). This truncation distance was used to avoid a resulting long tail of low detectability in the detection function, as detections a long way from the line contribute little to abundance estimates (Buckland et al., 2001).

Abundance estimates increase with the inclusion of corrections for availability. The estimate of 0.912 for acoustic $g(0)$ was based on the diving behaviour of seven tagged whales off the Azores in the mid-Atlantic (Fais et al., 2016). Although dive data exists for five sperm whales tagged in the Ligurian Sea (Miller et al., 2004), that dataset only generated 21 complete dive cycles compared with the 80 in the Azorean dataset. For that reason, the Azorean dataset was used; however, considering the published summaries (Zimmer et al., 2005; Fais et al., 2016), it does not appear that Mediterranean whales perform radically different foraging dives to those in the Azores. If the results from DSM were extended to include those regions not surveyed by the *Song of the Whale* team in 2018, the uncorrected abundance estimate rose by almost 40% to 3,268 whales. Although this estimate excluded shallow water habitats (only 2% of all encounters were in waters shallower than 200 m), it included 56 individuals in the seemingly unsuitable habitat in the Adriatic Sea. As discussed above, this figure does not seem realistic. The total estimate also included 197 whales in the contiguous Atlantic region outside block 1. This block was surveyed during ASI in part due to its importance to smaller odontocete species (such as killer whales *Orcinus orca* and long-finned pilot whales *Globicephala melas*; Cañadas et al., 2023; Panigada et al., 2023); as sperm whales along Morocco's Atlantic seaboard are likely to belong to a separate sub-population, it is not appropriate to consider these animals as part of the core Mediterranean assemblage as there is little evidence of movement between the two populations (Drouot et al., 2004a; Engelhaupt et al., 2009). Therefore, the estimate for the surveyed regions alone (i.e. a corrected value of 2,825) is likely to provide the most accurate approximation of the total sperm whale population size for the Mediterranean Sea.

Comparison with other studies

The ASI results showed some agreement with the few regional abundance estimates that exist from previous survey effort. Aerial surveys in 2011/12 derived corrected summer estimates of 161 (95% CI 44-583; CV = 0.74) and 209 (95% CI 39-1,108; CV = 1.03) sperm whales for regions approximately equivalent to ASI blocks 8a and 8b (Laran et al., 2017). Despite the high CVs for these estimates, the figures are little higher than the ASI model-based figures for blocks 8a (62 whales; 95% CI 22-176; CV = 0.57) and 8b (139 whales; 95% CI 65-299; CV = 0.42). A photo-identification study conducted in the Balearic Islands and Ligurian Sea from 1990 to 2008 estimated no more than 400 sperm whales in the western Mediterranean basin (Rendell et al., 2014). This figure is radically different from the corrected ASI estimates of 1,833 (design-based) and 2,102 (model-based) for blocks 2 to 10. However, if considering the Balearic and Ligurian individuals as separate populations, the respective estimates of 320 (95% CI 241-541) and 112 (95% CI 76-180) were closer to the model-based ASI estimates for the analogous blocks 4 (558; 95% CI 320-971) and 10 (139; 95% CI 46-299). A comparison of this nature is perhaps more appropriate as distance sampling approaches assume whales are stationary and do not move between survey blocks. In addition, the discovery curve for the photo-ID study did not show signs of becoming asymptotic, suggesting the population had not been fully characterised. In the Hellenic Trench, a photo-ID study from 1998 to 2009 derived “an advisable working” estimate of 200-250 sperm whales (Frantzis et al., 2014). However, more recent estimates by the same research team suggest numbers in the Hellenic Trench have decreased to below 200 (A Frantzis & P. Alexiadou, pers. comm. December 2022). The corrected ASI estimates of 60 whales (design-based) in block 22c and/or 43 whales (model-based) in blocks 22e, 22c and 22w are less than the more recent photo-ID estimates for the region; however, as the Hellenic Trench may provide at least temporary habitat for all sperm whales in the eastern basin, a snapshot survey in this region may be expected to estimate fewer individuals than a multi-year photo-ID study.

Other survey effort in the Mediterranean has derived density estimates for sperm whales. The northwest Pelagos Sanctuary has received a great degree of research effort over the last few decades; visual density estimates have ranged from 0.39 (CV = 0.39; Laran et al., 2010) to 1.0 sperm whales per 1000 km² (Gannier, 1995) for summer months, while an acoustic density estimate of 1.69 whales per 1000 km² (Poupard et al., 2022) has been derived from a static recorder deployed from 2015-18. The ASI results for block 10 showed some variability, with a corrected design-based estimate of 0.74 whales per 1000 km² (CV = 0.89) contrasting a model-based figure of 4.07 (CV = 0.57). The high CVs for these ASI results suggests caution should be taken when interpreting these densities, but when considering the neighbouring Pelagos Sanctuary blocks 9 and 11, densities were similarly high (5.32 and 2.04 respectively for model-based estimates). It is possible sperm whale densities have been increasing in the Pelagos Sanctuary over recent years, i.e. from 0.39 in 2001-04 (Laran et al., 2010) to 1.69 in 2015-18 (Poupard et al., 2022) to 2.04 in 2018 (this study). However, as the estimates from the other studies were not corrected for availability, it is likely

they represent underestimates, and as such direct comparisons are challenging. Juxtapositions such as these are useful for detecting any potential trends, but it should be borne in mind that the survey areas under discussion often do not closely align, either spatially or temporally.

Prior to the ASI project, the most comprehensive effort to characterise the population size of Mediterranean sperm whales was conducted using the same field and analysis protocols from the same research vessel (Lewis et al., 2018). This multi-year survey used design-based methods to derive a corrected total estimate of 1,842 whales (95% CI = 1,173-2,892 if using CV = 0.23 reported for vessel surveys) when extrapolating density estimates to unsurveyed regions. Although the equivalent design-based ASI estimate (2,673 whales) fell within the confidence interval of the composite study by Lewis et al., the ASI estimates of density and abundance for all blocks tended to be higher than the composite study. One interpretation of this difference could be that the number of sperm whales in the Mediterranean is increasing. However, it is unlikely that the results from the two studies actually provide evidence of this. The study by Lewis et al. used the best available data at the time that had been collected over several years (2003-2013), and used extrapolation and/or aerial survey data to characterise densities in unsurveyed regions. The long dive time of sperm whales led to high uncertainty in the aerial survey estimates for the Ligurian Sea, for example, with CVs of 0.76-1.05 reported (Laran et al., 2017). Pooling together surveys conducted over a decade may mask any shifts in distribution or introduce biases in to models exploring habitat preferences. Photo-identification studies have suggested the area between the Strait of Gibraltar and the Liguro-Provençal Basin is characterised by the fluid movements of individuals (Carpinelli et al., 2014; Rendell et al., 2014). Thus the ASI snapshot survey, incorporating the entire western basin over the course of several weeks, may be more likely to faithfully characterise the population of the western basin than episodic surveys conducted over non-contiguous periods and locations. Any perceived population increase since the Lewis et al. (2018) estimate may rather be the result of the above confounding factors.

Power analysis

When investigating the amount of survey effort required to identify population trends, it is assumed not only that the population is closed, but also that surveys are taken at regular intervals, the field protocols are the same, and the abundance estimates are independent. Some of these assumptions may be logistically difficult (e.g. ensuring repeat surveys take place at the same interval) but the analysis is robust to mild violations of these assumptions (Gerrodette, 1987). The power analysis suggested that conducting regular surveys every six years would detect significant population declines much sooner than surveys every 10 years, with the exception of the most precipitous decline modelled (10% per annum detected after 12 years vs. 10 years under sexennial and decennial surveys respectively; Figure 7). These anomalies are rare however, and more intense effort will normally detect significant

trends sooner than less intense survey regimes. The Mediterranean sub-population of sperm whales is currently listed as endangered C2a(ii) (Pirota et al., 2021). The related listing of Endangered C1 pertains to small populations declining by 20% in five years or two generations. Using the former parameter (i.e. an annual decrease of 4.4%), sexennial surveys would only detect this decline with acceptable power (0.80 after Taylor et al., 2007) after three surveys (i.e. after 18 years), at which point the population would have more than halved to 1,265 whales. Decennial surveys would take 20 years to detect this decline, at which point the population would have declined by approximately 60% to 1,157 whales. Currently, any trajectory in the population size of Mediterranean sperm whales is unknown. A regular censusing regime is essential to characterise any trends, with more frequent surveys (e.g. every six years) more powerful than less frequent surveys (every ten years plus). Additional survey effort, such as by vessel or via static acoustic recorders, can also be important for indicating changes in distribution at a finer temporal and regional resolution.

Sperm whales in the Mediterranean are exposed to direct human-induced mortality risks, such as bycatch in illegal driftnets and ship strike, as well as cumulative stressors, including underwater noise, chemical and plastic pollution, prey depletion and the effects of climate change (Rendell and Frantzis, 2016; Notarbartolo di Sciara and Hoyt, 2020). In part due to these threats, sperm whales are protected by their listing on the Bonn Convention, (CMS Appendices I and II), the Bern Convention (Appendix II), CITES (Appendix I), ACCOBAMS (a priority species for conservation action) and the Protocol on Specially Protected Areas and the Biological Diversity in the Mediterranean of the Barcelona Convention (Annex II) (Pirota et al., 2021). In addition, the EU's Marine Strategy Framework Directive requires Member States to achieve or maintain 'Good Environmental Status' (GES) of their waters. In 2017, six areas within the Mediterranean region were designated as Important Marine Mammal Areas (IMMAs) as they provide discrete portions of habitat of particular importance to sperm whales; the Alborán Corridor and Alborán Deep, the Balearic Islands Shelf and Slope, the North West Mediterranean Sea, Slope and Canyon System, the Campanian and Pontino Archipelago, and the Hellenic Trench (IUCN Marine Mammal Protected Areas Task Force, 2017). An additional candidate IMMA has been proposed for East Sicily and the Strait of Messina due to evidence of the routine presence of sperm whales (Pavan et al., 2008; Caruso et al., 2015). Although IMMAs do not confer any legal protections, they provide impetus for marine mammal and wider ocean conservation measures (Tetley et al., 2022). Despite these various designations, there is an inferred continuing decline in the Mediterranean sub-population of sperm whales (Pirota et al., 2021), and the threats listed above may therefore threaten their continued survival in the region. As an example of how these mechanisms may fail, the Hellenic Trench IMMA has recently been impinged upon by a large area granted by the Greek government as a concession to the oil and gas industry for hydrocarbon exploitation (Notarbartolo di Sciara and Hoyt, 2020). In the west, a security area limiting maximum vessel speeds to 13 knots was established in the Strait of Gibraltar in 2007

(Notarbartolo Di Sciara, 2014), with the aim of reducing collisions with sperm whales. However, the measure is only implemented from April to August, despite evidence that sperm whales use the area year-round (Gauffier et al., 2012), and there is little evidence of compliance by mariners (Silber et al., 2012). Oversights such as these highlight the inconsistency with which protective measures are implemented and enforced. The ASI results suggest many regions of core sperm whale habitat, such as the waters off Algeria and the Atlantic seaboard of Morocco, may remain excluded from any targeted protection such as those provided through the European Union. A wider network of effectively managed and monitored protected areas is required to improve conservation outcomes for sperm whales in the Mediterranean Sea, in tandem with further population censusing to determine trends in abundance.

Conclusions

The ASI vessel surveys in 2018 allowed a snapshot survey to determine the density of sperm whales using acoustic techniques, as deep-diving cetaceans may be under-represented by aerial surveys. Both design- and model-based approaches broadly agreed on a total estimate of approximately 2,800 individuals using a correction for acoustic availability. As for previous research effort, density was not homogenous, with model results suggesting most sperm whales detected were in the western basin. Densities were highest in the Algerian and Liguro-Provencal Basins between Algeria and Spain/France. Although few whales were detected in the eastern basin, the Hellenic Trench, Rhodes Basin and south Aegean Sea appeared to provide the core habitat, as noted in previous studies. Although comparisons with previous surveys are challenging, the ASI results were broadly in keeping with other density estimates. Importantly, the ASI project allowed a synoptic survey to be conducted of all key sperm whale habitats within the same year and same season, thus overcoming any biases introduced by the long-range movement of individuals. Repeat survey effort is required to determine any population trends, and using the parameters estimated in this study, undertaking systematic surveys every six years allows a much faster identification of any significant population decline than other regimes (e.g. decennially). As Mediterranean sperm whales are currently listed as Endangered on the IUCN's Red List, and they are known to suffer significant mortality risk from anthropogenic stressors including fisheries interactions and ship strike, there is an urgent need to reduce anthropogenic mortalities to improve the conservation status of this vulnerable population.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://accobams.org/asi-data-access-request/>.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because this research was not conducted through a University or governmental organization; as such, no relevant animal ethics committee was available. However, all research was conducted under permits issued by the relevant countries involved, and every measure was taken to minimize disturbance to the animals studied (for example, via the use of passive acoustic detection techniques deployed from a research vessel specifically designed to be quiet).

Author contributions

OB, AM, RM and SP contributed to the conception and design of the study. OB, AM and RM prepared field protocols and secured relevant permissions. OB, JR, CR and RM conducted fieldwork. OB, JR and CR performed acoustic analysis; subsequent analyses were conducted by OB. Manuscript preparation was overseen by OB. 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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The ACCOBAMS survey initiative: the first synoptic assessment of cetacean abundance in the Mediterranean Sea through aerial surveys

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The “ACCOBAMS Survey Initiative” (ASI) is a pilot programme aimed at establishing an integrated and coordinated monitoring system for cetaceans across the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic (hereafter “ACCOBAMS”) area. Conducted in coordination with Mediterranean coastal countries, it supports the implementation of European and regional policies, in particular the EU Marine Strategy Framework Directive and the Ecosystem Approach process. In summer 2018, a synoptic survey was conducted across the Mediterranean Sea and contiguous Atlantic area, combining visual monitoring from aircrafts with visual and passive acoustic monitoring from vessels. Species density and abundance were estimated through design-based approach in a line-transect sampling framework. Based on data arising from the aerial survey only, uncorrected design-based abundance was obtained for striped ($N=426,744$, $CV=0.13$), common ($N=65,359$, $CV=0.4$), bottlenose ($N=63,333$, $CV=0.17$), and Risso's dolphins ($N=26,006$, $CV=0.3$), Cuvier's beaked whales ($N=2,929$, $CV=0.4$) and long-finned pilot whales ($N=5,540$, $CV=0.4$). A merged category of either striped or common dolphins resulted in 212,828 individuals ($CV=0.26$). Fin whales abundance of 1,749 animals ($CV=0.3$) was corrected for both availability and perception biases and resulted in 3,282 ($CV=0.31$). The ASI survey offers an overall picture of the distribution and abundance of cetaceans throughout the Mediterranean basin, providing robust estimates to be considered as a baseline for future regional systematic monitoring programmes. The ASI survey is the first

step towards establishing a long-term monitoring program across the entire ACCOBAMS area, and, as such, it sets the basis for further future basin-wide monitoring efforts using systematic, shared, coordinated and comparable methods. The information gathered will further enhance knowledge on cetacean status, facilitating the development of informed conservation and mitigation measures, as well as supporting the implementation of international obligations. Furthermore, the outcomes of this survey will support both place- and threat-based conservation efforts in the ACCOBAMS area, through the identification of Important Marine Mammal Areas and Cetacean Critical Habitats. Here the results of the ASI survey are presented and discussed alongside proposed management and conservation actions aimed at ensuring the persistence of cetacean populations in the region.

KEYWORDS

Mediterranean, density and abundance, aerial surveys, cetacean, conservation, large-scale surveys

1 Introduction

The need for monitoring programmes at large spatial and temporal scales aimed at assessing changes in species distributions and abundances, and to predict long-term biological responses to anthropogenic pressures and global changes is widely recognized (Balmford et al., 2005; Green et al., 2005; Pereira and Cooper, 2006). These programmes are also crucial to inform and implement conservation actions and to evaluate the efficacy of management (Grand et al., 2007). However, it is difficult to obtain robust estimates of these population parameters and assess changes for highly mobile and cryptic species, such as cetaceans, whose ranges extend over large areas and occurrence can show strong temporal and spatial patterns (Hughes et al., 2011; Thomas et al., 2015; Guerra et al., 2019; Nykänen et al., 2020).

In the Mediterranean and Black Seas, hotspots of biodiversity (Coll et al., 2010) overlap with high volumes of human activities. The Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) has historically recognized the need for robust baseline data on the conservation status of cetaceans across the Agreement Area, where the overall human impact on the environment is believed to be high (Micheli et al., 2013; Halpern et al., 2015; Stock et al., 2018; Halpern et al., 2019) and where cetacean populations are threatened (Notarbartolo di Sciara, 2016). The ACCOBAMS Secretariat and Scientific Committee therefore proposed and developed the ACCOBAMS Survey Initiative (hereafter 'ASI'), as the first synoptic survey of the entire ACCOBAMS Region aimed to establish an integrated, collaborative and coordinated monitoring system of the status of cetacean populations across the ACCOBAMS Area (ACCOBAMS Resolutions 6.13, 8.10). The ASI also contained a strong capacity building, sharing and training component that will ultimately strengthen conservation and management in the Region.

Whilst primarily developed to further knowledge of cetaceans, the ASI also makes an important contribution to the fulfilment of European Union (EU) Regulations and Directives, specifically the Habitats and the Marine Strategy Framework Directives (Authier et al., 2017), as well as other relevant policy frameworks such as the "Protocol concerning Specially Protected Areas and Biological Diversity in the Mediterranean" of the Barcelona Convention, the "Convention on the Conservation of Migratory Species and Wild Animals" (also known as CMS or Bonn Convention), and the "Berne Convention on the Conservation of European Wildlife and Natural Habitats".

The ASI officially started at the Sixth Meeting of the Parties to ACCOBAMS (Monaco, 22-25 November 2016) whereas data collection took place in summer 2018 and 2019. Following well-established large-scale monitoring initiatives, such as the Small Cetaceans in European Atlantic waters and the North Sea (SCANS; Hammond et al., 2013), the primary approach was to undertake visual line-transect distance sampling aerial surveys, complemented by ship-based visual and acoustic distance sampling surveys (Boisseau et al., 2023), to maximize survey effort and area coverage. Data were collected for all the cetacean species occurring in the Agreement area (ACCOBAMS, 2021a). Data on other megafauna taxa were also recorded including marine turtles (DiMatteo et al., 2022), and fish (Cañadas et al., 2023). In addition, information on the presence, distribution, and levels of human activities (e.g., floating macro-marine litter, marine traffic, etc.) was collected (Lambert et al., 2020; Nivière et al., 2024).

The ASI is not the first example of such large-scale synoptic surveys, but the inherent and geopolitical complexity of the Mediterranean Sea, with 21 countries bordering its basin, make the ASI a unique case worldwide. Moreover, the fact that the ASI survey spanned across the entire Mediterranean ecoregion, recognized to be a single biogeochemical province (Longhurst, 2007), makes the programme unique in that it attempts to assess

and gain knowledge on all cetacean species and their suitable habitats in the entire ecological region.

In this context, the ASI plays an important collaborative role in gathering baseline data for the wide range of cetaceans known to inhabit the Mediterranean Sea, a region where systematic research effort is spatially limited (Mannocci et al., 2018), and gaps exist in the current knowledge of species occurrence, distribution and abundance (Cañadas et al., 2023).

This paper presents uncorrected – apart for fin whales (*Balaenoptera physalus*) – design-based abundance and density estimates (i.e., estimates are not corrected for perception and availability biases) of cetacean species in the Mediterranean Sea and contiguous Atlantic area (Gulf of Cádiz), based on data collected through the aerial component of the ASI survey. The results are discussed considering their relevance towards informing conservation and management decisions, as well as an example of how a coordinated effort between countries is crucial to raise knowledge and ultimately to achieve and maintain good conservation status of species and habitats in a diverse and heterogeneous biogeographic and geopolitically complex region.

2 Material and methods

2.1 Survey design

A large portion of the Mediterranean (77% of its entire area totaling 1,9 million Km²) and Gulf of Cádiz was divided in 32 strata based on the best compromise between oceanographic and physiographic characteristics, as well as the overarching political or jurisdictional constraints and logistic issues such as fuel availability, planes' endurance, location of airports and issuing of flight permits. Equally-spaced zig-zag transects (Buckland, 2001; Strindberg and Buckland, 2004) were designed through the dedicated software Distance 7.3 (Thomas et al., 2010), to obtain an equal coverage probability within each stratum and to optimize effort. Transects were planned to be flown once with the ratio Effort/Area varying between 2.7 and 3.6% coverage, assuming an effective strip width of 0.5 km. The survey design, with transects and strata, is shown in Figure 1. Areas that could not be monitored by plane in the southern basin were instead largely covered by the ship-based component of the ASI and are presented in Boisseau et al. (2024).

2.2 Data collection

The survey was conducted between June and August 2018. Eight planes of three different models – 4 Partenavia (P68), 2 Britten Norman Islander (BN-2) and 2 Cessna 337 Skymaster O-2 (push-pull) – all equipped with bubble-windows to allow for direct observations on the track-line, were used for the survey. Each aircraft accommodated two primary observers, scanning the sea surface on both sides of the aircraft, and one data operator, in addition to the pilot. Surveys were conducted in passing mode (Dawson et al., 2008), i.e., the plane did not leave the track-line to approach sightings, unless it needed to estimate group size of large

groups of animals or to identify species, based on *ad-hoc* decisions by the observers and cruise leader. The flight altitude was kept constant at 183m (600 feet), in accordance with similar surveys where target species were small cetaceans (Hammond et al., 2013) or marine megafauna (Laran et al., 2017a; Laran et al., 2017b; Pettex et al., 2017; Rogan et al., 2018), and ground speed was maintained at 100 knots (185 km/h).

The software SAMMOA 1.1.2¹ was used for data collection, storage, validation and management. Teams were created according to previous experience in leading and participating in aerial surveys, with researchers taking part in both theoretical and practical training sessions to prepare for field work activities, and familiarize with protocols.

Data were collected in 'line transect mode' by recording the declination angle when the animal or group of animals were abeam to the plane (Buckland, 2001). Sea and weather conditions (i.e., Beaufort wind scale, glare severity and angle, water turbidity, cloud coverage and presence of glint) were recorded at the beginning of each transect and at any time they changed. An overall subjective assessment of the detection conditions (i.e., "excellent", "good", "moderate" or "poor", hereafter referred to as "Sightability score") for each observer was also recorded where, as an instance, "good" conditions corresponded to an observer perceiving the likelihood of spotting a small *delphinid* within the searching area (within approx. 300 m from the track line) to be good (e.g., Beaufort wind scale ≤ 2, turbidity < 2 and glare moderate, good or absent). These approximate limits were identified by marks on the bubble-windows corresponding to a theoretical sighting angle of 31°, which at the survey flight altitude would equate to a perpendicular distance of 304.7 m. Survey was halted when conditions were deemed "poor" with at least Beaufort wind scale and water turbidity greater than 3 and 1, respectively. Details on the 'environmental condition fields and codes' used during the surveys are presented in the Supplementary Materials.

Data collected during sightings included the species (identified to the lowest possible taxonomic level), school size, number of calves, behavior, swimming direction and possible reaction to the aircraft. The declination angle to the sighted animal/group of animals, measured with a hand-held clinometer, was used to calculate the perpendicular distances to the track line. For sightings with species or school size initially uncertain, primary search effort was stopped, and a specific circling maneuver – similar to the "race-track" when the aircraft circles back to resurvey a defined segment of the transect (Hiby, 1999; Scheidat et al., 2008; Gilles et al., 2009) – was implemented only to gain insight of group size or species identification. Data were validated after each flight, all flight data were collated and merged into a single dataset and sightings were associated to corresponding effort using ArcGIS 10.6 and MGET tool (Roberts et al., 2010) for final review prior to the analysis.

¹ SAMMOA 1.1.2. Système d'Acquisition des données sur la Mégafaune Marine par Observations Aériennes, Software developed by UMS 3462 Pelagis LRUniv-CNRS and Code Lutin (2012-2019).

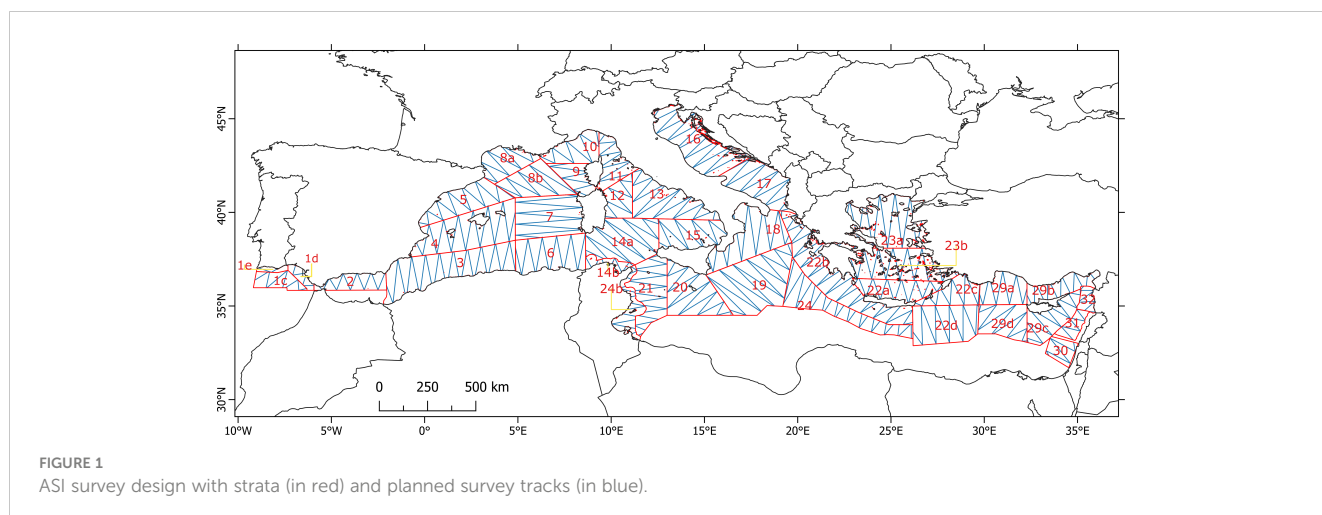


FIGURE 1
ASI survey design with strata (in red) and planned survey tracks (in blue).

2.3 Data analysis

Before performing density and abundance estimation analysis, data were used to provide explorative descriptive statistics and calculation of encounter rates (sightings per unit of effort), using a grid cell of 50x50 km, through a dedicated plugin PelaSIG. The results of this exploratory analyses and the details on the plugin are presented in [Nivière et al. \(2024\)](#).

2.3.1 Abundance and density estimates

Although the study area was divided into 32 strata ([Figure 1](#)), these were later merged into larger sectors referred to as “Sub-Areas” ([Figure 2](#)) to better reflect distributional ranges of species, and to prevent having sectors with little effort or too few observations to produce robust estimates. This post-stratification was done in the Distance software by specifying the Sub-Area as stratum within the model definition properties. These larger sectors were also identified to reflect existing spatial extents defined under the MSFD and EcAp framework² and to facilitate reporting. Although the results of design-based analysis have been performed for each original block (see [Supplementary Materials](#)), this paper presents the results as obtained for the above-mentioned Sub-Areas.

2.3.2 Design-based analyses

Analysis, conducted in the software Distance 7.3 Release 1 ([Thomas et al., 2010](#)), followed standard multiple covariate distance sampling approach (MCDS; [Buckland, 2001](#); [Marques and Buckland, 2004](#)), where additional explanatory variables are considered along with the perpendicular distance to the sightings to estimate the detection function ([Buckland et al., 2015](#)). Density of individuals was calculated as:

$$\hat{D} = \frac{n\bar{s}}{2eswL}$$

where \hat{D} is density, n is the number of sighted groups, \bar{s} is mean group size, L is the total length of transect searched, and esw is the effective half strip-width (i.e., width of the strip multiplied by the average probability of detection within that strip), which provides a simple measure of detectability. The quantity $2eswL$ is thus the area effectively searched. Population density is calculated as the number of individuals/groups counted divided by searched area.

From the estimated density, the abundance \hat{N} is calculated as the product of \hat{D} and the searched area A :

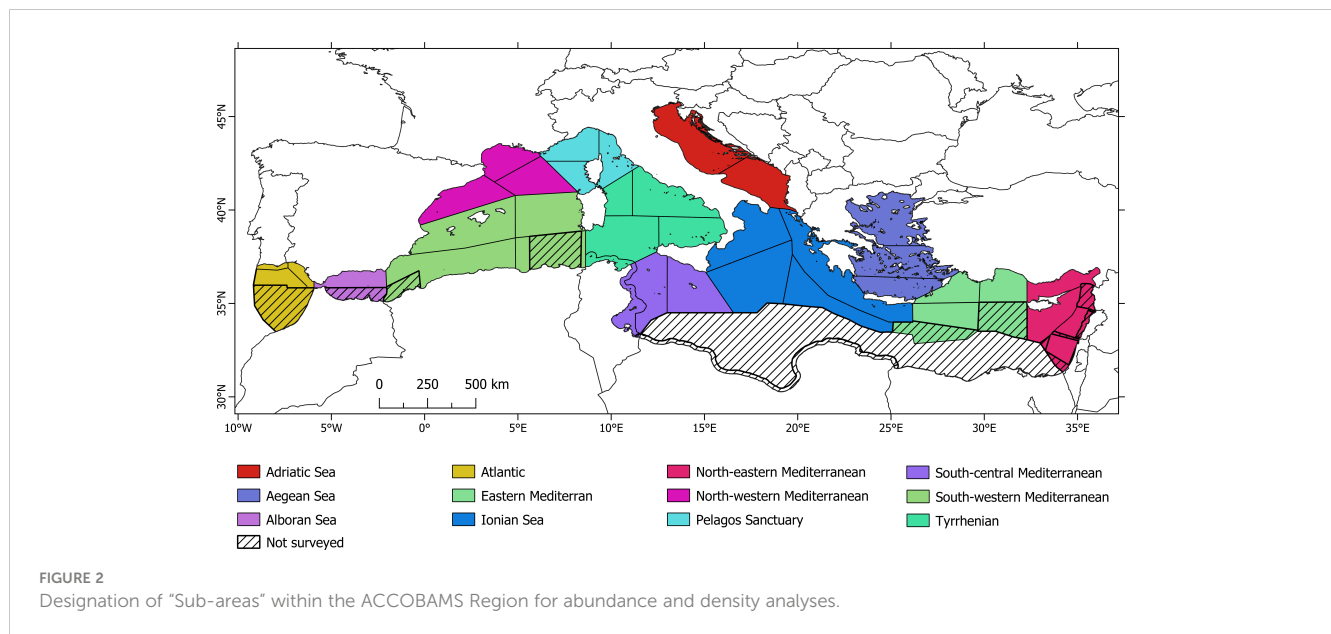
$$\hat{N} = A\hat{D}$$

Detection functions were fitted to the perpendicular distance data to estimate the esw . When the number of observations for some species was too low to build a reliable detection function, species with similar characteristics (therefore potentially similar detectability) were pooled together to derive their abundance estimates. The Mark-Recapture Distance Sampling engine (MRDS) available in Distance 7.3 was used with the configuration of “single observer” for these analyses, so estimates could be derived for each species within a guild (when a guild was modelled). However, by using the “single observer” configuration, it worked practically as an MCDS (multiple covariate distance sampling).

A species-specific detection function was obtained for bottlenose, striped and Risso’s dolphins. Considering the wide variation of *Delphinidae* body lengths, with adults ranging between 1.5 to over 9 m ([Jefferson and LeDuc, 2018](#)), and considering the within-species geographical clines in body-length and morphological traits (e.g., [van Aswegen et al., 2019](#)), for the purpose of this paper we consider any species larger than striped or common dolphins to be a large *Delphinidae*. Accordingly, a pooled detection function, mainly based on body size, was calculated for the following groups:

- Small dolphins (including common, striped and unidentified small dolphins), to derive estimates for common dolphins.

² https://ec.europa.eu/environment/marine/images/MSFD_regions.jpg



- Large dolphins (including bottlenose and Risso's dolphins and long-finned pilot whales), to derive estimates for pilot-whales.
- Baleen whales (including fin whales, one single minke whale and unidentified *Balaenopteridae*).
- Beaked whales.

For fin whales, estimates were corrected by a factor of 0.538 ($CV=0.13$) based on an average group size of 1.6 whales from the Western Mediterranean Sea (Panigada et al., 2021). Concerning sperm whales, although size is comparable to other species such as fin and minke whales, their diving-surfacing patterns strongly differ. The differences between these similarly sized species would therefore imply strong differences in the probability of detection and related availability biases. Also, for sperm whales, abundance estimated from visual aerial line-transect distance sampling surveys is likely to be negatively biased because of the long dive times, and passive acoustic approaches might produce more robust estimates (e.g., Barlow and Taylor, 2005; Lewis et al., 2018; Boisseau et al., 2023). Accordingly, sperm whales were not included in the analyses of aerial survey data and estimates are presented, instead, in Boisseau et al. (2024) based on ship-based visual and acoustic surveys.

Covariates used in the MRDS analyses were selected for their potential role in the process of cetacean detection by visual observers from the air (Table 1). The final model selection for each species or guild was done on the base of several parameters, including Delta AIC, K-S and CvM goodness of fit tests, qq-plots, and visual inspection of the shape of the detection function. QQ-plots and detection functions are provided in the Supplementary Materials as well as fit statistics for the selected models (the latter provided as Rdata file with extension *.Rdata).

3 Results

Overall, more than 55,000 km were monitored along predetermined transects, from the Gulf of Cádiz in the West to the Israeli coast in the far East, over a surface of almost 1.9 million km^2 . Approximately 91% of the planned transects were surveyed (Table 2; Figure 3). Nine species of cetaceans, identified to the lowest taxonomical level, were encountered (Table 3): bottlenose dolphins (*Tursiops truncatus*), common dolphins (*Delphinus delphis*), Risso's dolphins (*Grampus griseus*), striped dolphins (*Stenella coeruleoalba*), long-finned pilot whales (*Globicephala melas*), Cuvier's beaked whales (*Ziphius cavirostris*), sperm whales (*Physeter macrocephalus*), fin whales and minke whales (*Balaenoptera acutorostrata*), for a total of 577 sightings and 8,348 individuals. For 234 sightings (3,460 individuals) it was not possible to identify the species.

3.1 Search effort and sightings

Table 3 presents a summary of sightings and related information. Striped dolphins were the most observed species in terms of both the number of recorded sightings and the number of observed individuals, followed by bottlenose dolphins. In 18% of the sightings, it was not possible to discriminate between striped and common dolphins.

3.2 Design-based abundance estimates

Uncorrected density and abundance were estimated for all the species with a sufficient number of observations and for 4 guilds,

TABLE 1 Covariates collected during effort and tested in MRDS models and their ranges or factor levels.

Covariate	Type	Levels
Sighting related		
School size	Numerical	
Observer	Categorical	Observers' names
Effort related		
Beaufort scale	Factor & numerical	0 (calm) 1 (very light) 2 (light breeze) 3 (gentle breeze) 4 (moderate breeze)
Beaufort2	Factor	0-1 2-3 4
Swell	Factor & numerical	0 1 (presence without affecting the detection) 2 (presence + affecting detection)
Water turbidity	Factor & numerical	0 (clear) 1 (moderately clear) 2 (turbid)
Silvery shine (glint)	Factor	0 (no glint) 1 (glint)
Glare severity	Factor & numerical	0 (null) 1 (slight) 2 (moderate) 3 (strong)
Glare under the plane	Factor	0 (clear) 1 (glare)
Clouds	Numerical	0 to 8 from clear to totally cloudy
Clouds2	Factor	0-2 3-5 6-8
Sightability score	Factor	E (Excellent) G (Good) M (Moderate) P (Poor)
Time day (in UTC)	Factor	am (6-12am) noon (12-2pm) pm (2-8pm)
Aircraft	Factor	Aircraft's marks
Team	Factor	Team's code

alongside their Coefficients of Variation (CV) and 95% Confidence Intervals (95% CI) (Table 4). The full results of the design-based analyses are presented in the [Supplementary Materials](#), alongside information, for each species/group of species and Sub-areas, on mean group, expected group size, the encounter rate of groups, as well as the parameters and results of the final detection functions and q-q plots.

Truncation distances and goodness-of-fit test results for cetaceans are summarized in Table 5. Only for two species right truncation was not necessary, and the maximum detected distance was used instead.

TABLE 2 Summary of survey effort by Sub-Area.

Sub-Area	Stratum Id	Extent	No. Transects	Length
Adriatic	16	78,503	15	2,084
	17	57,279	11	1,742
Total	2	135,782	26	3,826
Aegean	22a	42,635	13	1,326
	23a	78,529	11	2,228
	23b	70,009	15	2,175
Total	3	191,173	39	5,729
Alboran	2	28,123	12	855
Total	1	28,123	12	855
Eastern Mediterranean	22c	34,434	10	1,105
	22d	68,066	10	1,250
	29a	33,631	8	1,147
Total	3	136,131	28	3,502
Ionian	18	76,134	12	2,146
	19	110,086	15	3,184
	24	63,467	15	1,765
	22b	95,148	29	3,519
Total	4	344,835	71	10,614
North-Eastern Mediterranean	30	18,140	5	512
	31	14,553	6	445
	29b	31,598	11	754
	29c	43,368	13	1,152
Total	4	107,659	35	2,863
North-Western Mediterranean	5	53,216	14	1,514
	08a	34,726	12	1,319
	08b	47,021	9	1,785
Total	3	134,963	35	4,618
Pelagos	9	22,644	9	1,039
	10	34,098	11	1,293
	11	31,069	11	1,036
Total	3	87,811	31	3,368
South-Central Mediterranean	20	69,226	14	2,104
	21	59,014	13	1,711
	21b	24,568	12	866
Total	3	152,808	39	4,681
South-Western Mediterranean	3	110,147	19	2,691
	4	93,068	15	2,671
	6	67,045	3	419

(Continued)

TABLE 2 Continued

Sub-Area	Stratum Id	Extent	No. Transects	Length
	7	73,499	7	1,893
Total	4	343,759	44	7,674
Tyrrhenian	12	27,265	9	977
	13	66,632	15	2,061
	14	77,041	14	2,452
	15	49,836	8	1,409
	14b	10,553	3	227
Total	5	231,327	49	7,126
Grand-total	32	1,806,560	378	51,488

“Stratum Id” is the unique identified for each Stratum as output from the design phase. “Extent” is the extent of each Stratum expressed in km² calculated on a Lambert Azimuthal Equal Area projection, “No. Transects” is the number of transects surveyed per stratum and “Length” is the total length of transects monitored per Stratum and expressed in linear km. Totals are calculated for each Sub-Area and for the overall survey. Bold text highlights the total values.

4 Discussion

4.1 Strengths and weaknesses

The 2018 ASI has provided a first overall picture of the abundance of cetaceans throughout a large portion of the Mediterranean region and the Gulf of Cádiz, providing robust estimates for several species, as well as important information on their occurrence. These represent a baseline for further regional systematic monitoring programmes, coordinated and comparable amongst all areas. They greatly improve current knowledge on cetacean status and facilitate the development of targeted conservation and mitigation measures, as well as facilitate international obligations (EU, UNEP-MAP). Moreover, together with the spatial approach developed by Cañadas et al. (2023), they can contribute towards both place- and threat-based conservation

efforts in the Agreement area, with the identification of Important Marine Mammal Areas (IMMA; Hoyt and Notarbartolo di Sciara, 2021; Tetley et al., 2022) and Cetaceans Critical Habitats (CCH), as well as areas of major overlap between cetaceans and human activities in the summer.

It is important to note that this effort represents the first synoptic basin-wide estimates for cetaceans in the Mediterranean Sea and contiguous Atlantic area, thus making quantitative comparisons with existing knowledge more difficult. Most of the past effort in the region has been allocated along coastal areas and – over three decades of research on cetaceans – several portions of the Mediterranean have never or only minimally been surveyed in the past (e.g., Mannocci et al., 2018). The easternmost and southernmost Mediterranean basin remain currently unsurveyed by systematic effort either by plane or vessel. By contrast, coverage of the western Mediterranean Sea has been more substantial during the last decade, with aerial surveys taking place in its central and north-western sectors (e.g., Gómez de Segura et al., 2006; Fortuna et al., 2014; Lauriano et al., 2014; Notarbartolo di Sciara et al., 2015; Laran et al., 2017b; Lauriano et al., 2017; Panigada et al., 2017; Fortuna et al., 2018), as well as wide scale vessel based monitoring (e.g., Forcada et al., 1994; Forcada et al., 1996). Work is ongoing to enable more direct quantitative comparisons between these and the ASI results.

We do acknowledge the need for a comprehensive integration of the design- and model- based estimates (and comparisons with relevant estimates at a more ‘local’ scale, where possible) and the spatial modelling distributional information. This aspect, as well as the issue of stock structure within the region, will be the subject of future dedicated effort. Model-based results and distribution across the Mediterranean Sea are presented and discussed in Cañadas et al. (2023).

With the exception of fin whales, the abundance estimates provided here are underestimates of the true numbers, in that they have not been corrected for availability nor perception biases. Although the perception bias was not estimated, it was assumed to be constant throughout the whole survey given that same protocol and type of planes were used, and survey teams (created during the training phase) remained unchanged throughout the entire survey.

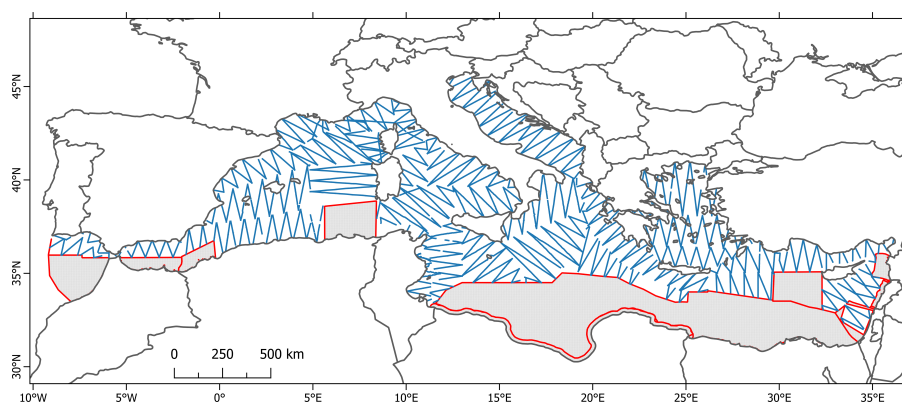


FIGURE 3
Map with the total realized effort within the ACCOBAMS Region.

TABLE 3 Sightings of cetaceans encountered while 'on effort' during the aerial survey.

Taxonomic groups		Species or taxa	Sightings	Individuals
Balaenopteridae	Large Balaenopteridae	Balaenoptera spp.	3	6
		Fin whale	43	65
	Small Balaenopteridae	Minke whale	1	1
Cetacea	Cetacean unidentif.	Cetacea	2	3
		Large Cetacean	3	12
		Medium Cetacean	6	32
		Small Cetacean	14	48
Delphinidae	Delphinid	Delphinid spp.	40	692
Delphininae	Large Delphininae	Bottlenose dolphin	152	1128
		Large delphinidae spp.	4	10
	Small Delphininae	Common dolphin	32	842
		Small delphinidae	13	121
		Striped dolphin	262	5819
		Striped dolphin/Common dolphin	146	2532
Globicephalinae	Large globicephalinae	Long-finned pilot whale	14	96
	Small globicephalinae	Risso's dolphin	58	350
Ziphiidae	Other beaked whale	Cuvier's beaked whale	15	47
		Ziphiidae sp. (Beaked whale)	3	4
Total			811	11,808

Bold text highlights the total values.

Perception and availability biases are not mutually exclusive and can negatively affect the estimates, unless accounted for (e.g., Pollock et al., 2006; Fuentes et al., 2015). Perception bias varies with the target species and can be minimized by using experienced observers and accounted for in a double-platform framework (Buckland et al., 2010; Burt et al., 2014), while availability bias is

linked to the activity periods and habitat types of the species of interest (Smolensky and Fitzgerald, 2011) and the integration of tagging data (i.e., data on dive-surfacing patterns) with aerial surveys datasets can help accounting for availability bias in the estimation of abundance (Nykänen et al., 2018). Correcting for such biases, whilst important to obtain estimates of absolute abundance,

TABLE 4 Summary of design-based abundance estimates of cetaceans for sub-areas with associated Coefficients of variation (CV) and Confidence intervals at 95% (95% CI).

Species/Guilds	Detection function	Abundance	CV	95% CI
Fin whales	Baleen whales	1,749	0.3	979 – 3,123
Cuvier's beaked whale	Beaked whales	2,929	0.4	1,407 – 6,096
Long-finned pilot whales	Large dolphins	5,540	0.4	2,497 – 12,295
Risso's dolphins	Risso's dolphins	26,006	0.29	14,851 – 45,540
Bottlenose dolphins	Bottlenose dolphins	63,333	0.17	45,331 – 88,484
Striped dolphins	Striped dolphins	426,744	0.13	327,944 – 555,310
Common dolphins	Small dolphins	66,359	0.4	31,054 – 141,810
Striped or common dolphins	Small dolphins	212,828	0.26	127,483 – 355,307
Small dolphins	Small dolphins	730,074	0.13	567,565 – 939,113
Large dolphins	Large dolphins	95,847	0.15	72,192 – 127,253

For both the Species and the guild items are sorted ascending based on estimated abundance.

TABLE 5 Parameters and results of the detection functions for cetaceans.

Species/ group	Truncation (m)	Max. Distance (m)	Lost n	n	Key function	Covariates	p	CV p	Esw (m)	CvM p
Whales	1303	1741	1	6763	HN	Glare severity (as factor)	0.3677	0.1674	479	0.3383
Baleen whales	None	1741	0	52	HN	Glare severity	0.371	0.2108	646	0.7437
Beaked whales	None	359	0	20	HR	null	0.7554	0.1638	271	0.871
<i>Tursiops truncatus</i>	800	1490	2	169	HR	Sky glint	0.3269	0.0824	262	0.9053
<i>Grampus griseus</i>	None	503	0	60	HR	Seastate2	0.5056	0.1305	254	0.3092
<i>Stenella coeruleoalba</i>	700	2617	1	263	HN	Glare severity (as factor)	0.3174	0.0438	222	0.1408
Small dolphins	700	5240	4	515	HN	Aircraft – Turbidity	0.3417	0.0350	239	0.3520
Large dolphins	800	2092	3	256	HR	Seastate – Sky glint	0.3355	0.0634	268	0.9921

Codes: Truncation = right truncation in m; Max. distance in m = largest perpendicular distance observed; Lost n = number of groups discarded with truncation; n = number groups in detection function after truncation; key functions: HN = half-normal, HR = hazard-rate; p = average probability of detection; CV p = coefficient of variation of the probability of detection; esw = effective half-strip width in m; CvM p = p-value of the Cramer von Mises goodness of fit.

is not essential for trend analyses, for which the estimates can be treated as indices of abundance, provided that the levels of bias remain constant over time.

For fin whales, estimates have been corrected by a factor 0.538 (CV=0.13), assuming that perception bias for a trained and expert observer to be 1. This assumption is based on the fact that, in general, perception bias for large whales is thought to be relatively small. Hansen et al. (2019) estimated perception bias to range from about 0.86 – 0.99 for fin whales from aerial surveys off Greenland. The same authors provided an availability bias ranging between 0.2 – 0.3. Heide-Jørgensen et al. (2010) estimated a factor of around 0.86 for fin whales from an aerial survey off West Greenland but they did not provide an estimate for availability bias. Here, we used an average group size (g) of 1.6 whales from the Western Mediterranean Sea (Panigada et al., 2021), to obtain a correction factor of 0.538 (CV=0.13).

Generally, cetaceans found in the Mediterranean Sea can be easily distinguished at the species level. However, during aerial surveys the flight altitude and speed, as well as the sea and weather conditions, amongst other factors, can hamper discriminating species when small delphinids are observed. This is exacerbated by the occurrence of sympatric species often in mixed associations. These mixed-species groups mostly include striped and common dolphins (Frantzi and Herzing, 2002; Frantzi et al., 2003; Bearzi et al., 2011b), and to a lesser extent striped, common and Risso's dolphins (Frantzi and Herzing, 2002). Furthermore, animals with intermediate striped-common dolphin pigmentation have been reported (Frantzi and Herzing, 2002; Bearzi et al., 2011b), providing evidence of introgressive hybridization between these two species (Antonioni et al., 2018). In future campaigns, image acquisition by digital cameras could be used to improve small cetaceans' species identification (e.g., Żydelis et al., 2019; Garcia-Garin et al., 2020; Raudino et al., 2022; Bigal et al., 2022).

In a line transect distance sampling framework, the probability of detecting an object is a function of many factors other than

distance of the object from the track-line, including group-size, species and habitats, as well as sea state (e.g., Holt and Cologne, 1987; Thomas et al., 2012; Fandel et al., 2020). Numerous studies have demonstrated that detection varies among species, over time, and among habitats, and there may be serious consequences when this variability is ignored. Failure to correct for imperfect detection (by observers and/or because of changes in the animal behavior over time or with habitat), as an instance, may result in spurious estimated relationships with ecological covariates and inaccurate abundance estimates that could mask trends and improper selection of indicator species (Kellner and Swihart, 2014). In this context, during the ASI survey, when it was not possible to discriminate between striped and common dolphins, sightings were attributed to a “small dolphin” or “common/striped dolphin” guild. Furthermore, it is important to note that the ASI dataset can be re-analyzed *a-posteriori* by applying a correction factor related to the observed proportion of one species vs the other (Cañadas, personal communication). While this approach could not be implemented at this stage, the re-classification of ambiguous species detections will be applied during the future replicates of the survey.

4.2 Species accounts

4.2.1 Striped dolphin, common dolphin and striped/common dolphin guild

At the species level, the ASI confirms that the striped dolphin is the most abundant species in the Mediterranean Sea (n = 426,744; 95% CI = 327,944 – 555,310) in agreement with previous research (e.g., Forcada et al., 1994; Forcada et al., 1995; Cotté et al., 2010; Laran et al., 2017b; Panigada et al., 2017). Recently, Laran et al. (2017b) obtained an uncorrected density of striped dolphins for a large portion of the north-western Mediterranean, including the Pelagos Sanctuary (corresponding to ASI strata 8a, 8b, 9, 10 and 11;

see Figure 1), for the summer months of 2012, of 0.484 animals/km². Gómez de Segura et al. (2006), for the waters off eastern central Spain, corresponding to the westernmost portions of ASI strata 3-5, estimated an overall summer density of 0.522 striped dolphins/km², while the ASI average estimate for these strata is 1 animal/km² (range= 0.53 – 1.44) individuals/km². Similarly, Panigada et al. (2017) estimated an uncorrected density of 0.41 animals/km² from an aerial summer survey conducted in 2010 over an area roughly corresponding to ASI strata 9, 10, 11, 12, 13 and the eastern sectors of strata 8b and 7 (Figure 1). For the Adriatic Sea (ASI area 17 and part of 16) Fortuna et al. (2011) provided an estimate of uncorrected density of 0.208 animals/km² (N= 15,343, CV=29.8%; 95% CI= 8,545-27,550) from an aerial survey conducted in summer of 2010.

The uncorrected density estimates obtained during the ASI are not directly comparable to these earlier surveys due to the different extent of the surveyed areas, but are within the same range as their results with an average density for those strata of 0.269 animals/km² (range= 0.0127 – 0.5219).

The Mediterranean sub-population of common dolphin has undergone a drastic reduction in the past decades as a consequence of ever-increasing human pressures on the species range of distribution (Bearzi et al., 2021). Habitat loss and fragmentation, alongside the indirect effects of overfishing, unintentional captures during fishing operations, and take, have strongly contributed to the decline of dolphin numbers across the entire region (e.g., Bearzi et al., 2003; Bearzi et al., 2004; Bearzi et al., 2008; Cañadas and Vázquez, 2017; Mussi et al., 2019). Overall, 32 primary sightings of common dolphins, mostly in the western portion of the basin and in the Strait of Sicily (see Nivière et al., 2024), were recorded during the ASI aerial surveys, with a total estimate of 66,359 individuals (95% CI=31,054 – 141,801).

During the ASI a specific sighting guild of unidentified small dolphins was used when a clear distinction between striped and common dolphins was not possible. A total of 148 sightings were assigned to this category, leading to an overall estimated abundance of 212,828 animals (95% CI=127,483 – 355,307).

4.2.2 Bottlenose dolphin

Bottlenose dolphins were the second most abundant species (n=63,333; 95% CI=45,331 – 88,484) observed during the ASI. The distribution showed 'patches' of relatively high density (Cañadas et al., 2023). Lauriano et al. (2014) obtained an overall uncorrected density of 0.006 animals/km² over a large portion of the central and north-western Mediterranean corresponding to ASI strata 9-13, 14a, 15 and portions of strata 7, 8a and 8b (see Figure 1). The average density of bottlenose dolphins over these strata, obtained during the ASI, was 0.032 animals/km² (range= 0 – 0.143). Gómez de Segura et al. (2006), for the waters off eastern central Spain, corresponding to the westernmost portions of ASI strata 3-5 (see Figure 1), obtained an uncorrected density estimate for the summer of 0.041 animals/km² while the average ASI estimated density for these strata is 0.023 (range= 0.003 – 0.063). Fortuna et al. (2018) obtained uncorrected density for the whole Adriatic (2010 and 2013 aerial surveys pooled dataset) of 0.042 animals/km² corresponding to the strata 16 and 17, with the average ASI estimated density for

these strata of 0.074 (range= 0.05 – 0.1). Fortuna et al. (2018) showed there was a notable difference between regions of the three Adriatic sub-basins; North 0.052, Central 0.034 (ASI Strata 16) and South 0.032 (ASI Strata 17). Finally, Laran et al., (2017b) report summer uncorrected density of 0.016 (CV=53%) for an area corresponding to ASI Strata 8a, 8b, 9, 10 and 11. Although also in this case the results are not directly comparable due to differences in the extent of the monitored areas, they agree with previous surveys and show how this species tend to generally occur at low density with patchy areas of higher abundance.

4.2.3 Fin whale

The fin whale was the only species for which a corrected abundance estimate was derived. At 3,282 individuals (CV=0.31), the ASI estimate is comparable to previous estimates of absolute abundance based on shipboard surveys of 3,583 individuals (95% CI=2,130 – 6,027; Forcada et al., 1996). Past abundance and density estimates arising from summer aerial surveys conducted in the central and north-western Mediterranean Sea show that the density of fin whales is higher in the north-western Basin and that abundance decreases at lower latitudes and higher longitudes (Panigada et al., 2017). Laran et al. (2017b), Panigada et al. (2011), Panigada et al. (2017), Bauer et al. (2015) show higher densities of fin whales in the north-western portion of the Mediterranean and this pattern is confirmed by the ASI, as well as by different approaches based on long-term photo-identification and microsatellite genotyping (Zanardelli et al., 2022; Tardy et al., 2023). The north-western Mediterranean is a well-known fin whale summer feeding ground where about 1,000 fin whales aggregate (e.g., Notarbartolo di Sciarra et al., 2016; Panigada et al., 2006). The ASI confirms fin whales summer distribution and habitat use and highlights the necessity of seasonal large scale monitoring programmes aimed at understanding spatio-temporal patterns of fin whales occurrence and distribution at the Basin scale to inform conservation and management decisions. In this respect, the ASI has highlighted no appreciable trends in the size of the sub-population of Mediterranean fin whales. It is however necessary to stress that the basin is visited by North-Eastern North Atlantic fin whales (Gejjer et al., 2016) and therefore the obtained estimates for true Mediterranean whales might be biased upwards. According to the IUCN, total abundance of fin whales should be greater than 5,200 individuals in order for the sub-population to exceed the limit of 2,500 mature individuals (48% of the total population being mature; Taylor et al., 2007). This, in conjunction with the fact that the sub-population experiences an inferred continuing decline in number of mature individuals, and that all mature individuals are in one subpopulation, further stresses the need to identify and implement targeted measures that would ensure the persistence of the species in the basin (Panigada et al., 2021).

4.2.4 Risso's dolphin

The Risso's dolphin of the Mediterranean Sea is one of the least-known cetacean species in the region and it has been the subject of few dedicated studies (Bearzi et al., 2011a). With a total of 58 sightings, abundance for this species has been estimated at 26,006 animals (95% CI=14,851 – 45,540), providing the first abundance

estimates for this species at the basin level, in particular for the north-western African coast. Laran et al. (2017b) provide corrected density estimates of 0.006 (CV=47%) for an area corresponding to ASI strata 8a, 8b, 9, 10 and 11. After aerial survey in the Adriatic in 2010, Fortuna et al. (2011) obtained uncorrected density estimate of 0.007 (CV=78.1%) with uncorrected population estimate of 510 individuals (CV=78.1%; 95% CI=124-2,089) present in the Central and South Adriatic (corresponding to ASI strata 17 and southern part of 16). Gómez de Segura et al. (2006), for the waters off eastern central Spain, corresponding to the westernmost portions of ASI strata 3-5 (Figure 1), obtained an uncorrected density estimate of 0.015 animals/km² while the average ASI estimated density for these strata is 0.044 (range= 0.037 – 0.052). Azzellino et al. (2016) using photographic mark–recapture methods provided a population size estimate for the western Ligurian Sea (corresponding to the north-western ASI stratum 10; Figure 1), for the period from 1998 to 2012, of about 100 individuals (95% CI= 60–220) and detected a decline in population size from an average of about 120–150 from 2000 to 2005, to an average of 70–100 during 2010 to 2014. Furthermore, the same authors highlight how the species occurrence has shifted from coastal and continental slope waters to more pelagic areas. No sightings of Risso's dolphins were recorded in this stratum during the ASI aerial survey; abundance was estimated for the adjacent southern stratum 9 (Figure 1) at about 200 animals (95% CI= 44 – 894), confirming the possible distributional shift observed by Azzellino et al. (2016). Azzellino et al. (2016) suggest that environmental variability, depletion of resources by fisheries and possibly interspecies competition could have contributed to the changes in Risso's dolphin habitat use and occurrence in the western Ligurian Sea. Recent studies show that shifts in cetacean distribution can be attributed to changing environmental conditions (Becker et al., 2022) or arising from the impacts of climate changes (van Weelden et al., 2021). However, the correlates to the observed shift in the occurrence of Risso's dolphins at the Mediterranean level have not been investigated yet.

4.2.5 Pilot and Cuvier's beaked whales

Cuvier's beaked whales are known for their elusive behavior, characterized by long diving times and relatively short surfacing periods (e.g., Quick et al., 2020), making them particularly affected by availability bias during aerial surveys (Thomson et al., 2012). To a lesser extent, this also applies to deep-diving pilot whales (Heide-Jørgensen et al., 2002). Overall, sightings of these two species accounted for less than the 2.5% of all cetacean sightings recorded during the ASI, with pilot whales being observed 14 times and beaked whales 15 times. Long-finned pilot whales' overall abundance was estimated at 5,540 individuals (95% CI= 2,497-12,295). Cuvier's beaked whales' abundance was estimated at 2,929 animals (95% CI=1,407 – 6,096), confirming existing knowledge on the basin-wide presence of the species and, at the same time, confirming how Cuvier's beaked whales typically occur at low densities in relatively small patches. A previous study by Cañadas et al. (2018) estimated the abundance of Cuvier's beaked whales at 5,600 (CV=0.24, 95% CI= 4807 – 7254). While the ASI

aerial component provides useful information on the occurrence, distribution and abundance of deep-diving species in the Mediterranean basin, the results indicate that more targeted studies using different methods may provide more robust estimate abundance of deep diving species.

4.3 Toward a strategy for monitoring cetacean populations in the Mediterranean Sea

Cetacean monitoring programmes aimed at evaluating populations and their distribution in the ACCOBAMS area are implemented within the framework of several initiatives at national and multilateral levels. Member countries of the European Union need to regularly undertake cetacean monitoring programs to collect the information required for their reporting under Article 17 of the Habitats Directive (HD), as well as in relation to the assessment of the Environmental Status under Descriptor 1 (Biodiversity) of the EU Marine Strategy Framework Directive (MSFD).

In the Mediterranean Sea, other cetacean monitoring programmes are expected within the framework of the Integrated Monitoring and Assessment Programme (IMAP) of the Ecosystem Approach process under the framework of the Barcelona Convention. A similar initiative is also expected for the Black Sea within the framework of the Bucharest Convention.

ACCOBAMS is now working to implement a Long-Term Monitoring Programme (LTMP; ACCOBAMS Resolution 8.10), aimed at collecting reliable data to obtain robust estimates of abundance and distribution of cetacean species in the ACCOBAMS area. This effort should also involve the collection of information on human activities at sea (both directly as part of survey protocols, and from relevant organizations collecting such data and indirectly using remote methods) which represent a known or emerging threat to cetaceans (e.g., maritime traffic, leisure boating, fishing, marine litter, chemical pollutants), to facilitate a spatial risk assessment based on pressure data and cetaceans distribution. This information will feed into the ongoing ACCOBAMS process to identify high-risk areas of interaction between cetaceans and human activities, where dedicated conservation measures should be identified and implemented. The ultimate objective is to provide a robust scientific basis for the setting and the regular adjustment of management measures that allow achieving a favorable conservation status for cetaceans in the Agreement area. Through such an achievement, the Parties will fulfil their obligations under ACCOBAMS, as well as their commitments towards other relevant Multilateral Agreements.

The periodic implementation of the ACCOBAMS LTM will allow trend assessments in species population size and distribution and to assess and evaluate conservation measures in place. While we acknowledge that there are high margins of error when detecting trends of small populations or when sample size is small (e.g., Wilson et al., 2011; Porszt et al., 2012), we recognize that the data collected by the ASI in the Mediterranean and Black Seas (see Paiu

et al., under review) have established a reliable baseline from which to measure future changes in the monitored parameters.

ACCOBAMS has agreed that conducting synoptic surveys of the whole ACCOBAMS area on a six-year frequency would provide the right balance between monitoring costs and regular updating of information on abundance and distribution of cetacean species and would be in line with the general recommendations from the EU and the Barcelona Convention, as well as with previous large-scale efforts elsewhere in the world (e.g., Hammond et al., 2021).

The main objective of ACCOBAMS is to achieve and maintain a favorable conservation status for cetaceans in the Black Sea, Mediterranean Sea and Contiguous Atlantic Area, and to obtain this, the Agreement requests the Contracting Parties to undertake, individually and through collaborations, a series of conservation and management measures and to develop cooperation for research and monitoring to fully implement them. The development of monitoring effort, such as the one represented by the ACCOBAMS Survey Initiative, is clearly designated by the Agreement among the most recommended ways to enhance the knowledge about the biology, ecology, and population dynamics of cetaceans, in order to identify and implement appropriate conservation measures.

4.4 Final remarks

The ACCOBAMS Survey Initiative provided the first synoptic estimate of abundance of eight cetacean species in the Mediterranean Sea, by implementing an unprecedented collaborative aerial survey in the whole region.

Stakeholders, including Governments, scientists, managers, and NGOs, have benefited from the data arising from the ASI survey to address local, national, and regional conservation objectives, including the negotiation of the adoption of the north-western Mediterranean PSSA (Particularly Sensitive Sea Area) by the IMO, conducting Environmental Impact Assessments, and developing Maritime Spatial Planning plans.

One of the ASI concrete outcomes is the recent re-assessment of the conservation status (*sensu* International Union for the Conservation of Nature – IUCN) of several of the Mediterranean sub-population of cetaceans. With no sub-populations listed as Data Deficient nor Extinct, the 77% of the resident Mediterranean subpopulations of cetaceans are considered to be threatened with extinction (IUCN, 2023), specifically listed as Vulnerable or Endangered (ACCOBAMS, 2021a). Ten of the 13 endemic subpopulations of cetaceans that are resident in the Mediterranean Sea are assessed as threatened, three as Critically Endangered, six as Endangered and one as Vulnerable. Two sub-populations previously classified as Data Deficient are now assessed as threatened, the Risso's dolphin and the long-finned pilot whale. Eight of the subpopulations have a declining trend, four have unknown trends, and only striped and bottlenose dolphins have an increasing population trend (IUCN, 2023). This regional assessment sheds light on the conservation status of these sub-

populations and highlights the need for more efforts to prevent the likely declining of the cetacean fauna of the Mediterranean Sea.

In light of the ongoing decline in global biodiversity, there is widespread recognition of the need for monitoring programs that can assess changes in species distributions and abundances over large spatial and temporal scales, in order to predict long-term biological responses to human pressures and global changes. Effective monitoring also supports the implementation of national and regional policies by informing a more comprehensive understanding of populations and environmental status. This, in turn, facilitates long-term conservation targets by addressing the challenges posed by global changes in the context of socioeconomic development. Quantifying the distribution and abundance of species, and changes in these parameters, is inherently complex, particularly when dealing with highly mobile and cryptic species such as cetaceans, whose ranges often span across large areas and are affected by the exposure to anthropogenic activities.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: <https://accobams.org/asi-data-access-request/>.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements.

Author contributions

SP: Conceptualization, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. NP: Data curation, Investigation, Methodology, Writing – original draft, Writing – review & editing. HA: Investigation, Writing – review & editing. LD: Investigation, Writing – review & editing. ND-M: Investigation, Writing – review & editing. GD: Investigation, Methodology, Writing – review & editing, Software. JG: Investigation, Writing – review & editing. DH: Investigation, Writing – review & editing. SL: Data curation, Formal analysis, Methodology, Software, Writing – review & editing. GL: Investigation, Writing – review & editing. R-MP: Investigation, Writing – review & editing. MP: Investigation, Writing – review & editing. DP: Investigation, Writing – review & editing. VR: Conceptualization, Methodology, Resources, Supervision, Writing – review & editing. JV: Investigation, Writing – review & editing. AC: Conceptualization, Data curation, Formal analysis, Methodology, Validation, Writing – original draft, Writing – review & editing.

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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

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https://github.com/NinoPierantonio/Panigada_et_al_ASI_SI_Frontiersin_2023.

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Evaluation of the exposure of the Mediterranean biodiversity to marine litter: the ASI – plastic busters MPAs projects connection

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A number of marine species in the Mediterranean are threatened by the presence of several pressure factors, which include climate change, collisions with vessels, entanglement and ingestion of marine litter, especially plastic. Risk reduction policies can only be conceived starting from an accurate analysis of the exposure to such pressure factors. To estimate spatial abundance of both marine species and plastic litter and to assess the exposure risk, a two-stage analysis approach was applied, using aerial survey data from the ACCOBAMS Survey Initiative (ASI), in synergy with the Plastic Busters MPAs (PB MPAs) project. First, a detection function was fitted to observation data to obtain detection probabilities for individuals, then a Generalized Additive Model (GAM) was employed to estimate the spatial distribution of relative abundance, based on survey observations. A bivariate Local Indicator of Spatial Association (LISA) was then applied to the maps of relative abundance to derive risk maps of exposure of marine species to marine litter. The maps, obtained with a spatial resolution of about 10 km, allow us to identify areas with the highest neighboring abundance of taxa and marine litter, in particular for the MPAs studied by the PB MPAs project, which include the North-Western Mediterranean (Pelagos Sanctuary and Tuscan Archipelago), the Ionian and Aegean Sea (Zakynthos), and the Strait of Sicily (Cabrera Archipelago).

KEYWORDS

marine litter exposure, risk assessment, density surface modelling, marine species distributions, marine protected areas

1 Introduction

The Mediterranean Sea hosts over 17,000 marine species, with approximately 20-30% of them being endemic, resulting in the highest rate of endemism globally (UNEP/MAP and Plan Bleu, 2020). That means that the Mediterranean Sea stands out as a globally significant hotspot and that, relative to its size or geographic extent, it contains a significant number of species that are found exclusively within its waters. This diverse ecosystem faces constant threats due to significant human activities (Micheli et al., 2013; Halpern et al., 2015), particularly in the northern basin where human population growth has stabilized since 1980, contrasting with a more than twofold increase in the south and east during the same period (UN DESA, 2019). Various marine species in the Mediterranean Sea are under threat from human-induced pressures such as fishing, climate change, vessel collisions, and plastic ingestion (Notarbartolo di Sciara, 2016; Panigada et al., 2006; Coll et al., 2012; Fossi et al., 2012, 2017, 2018). Coastal regions and enclosed basins, like the Mediterranean, suffer significantly from marine plastic pollution, posing a growing threat to marine biodiversity (Deudero and Alomar, 2015; Alomar et al., 2020; Darmon et al., 2017; Fossi et al., 2017; Consoli et al., 2019; Fossi et al., 2020). Lambert et al. (2020) provided quantitative assessments of plastic debris in the Mediterranean Sea using aerial survey data, including the ASI dataset. The variable spatial and temporal distribution of marine litter (ML) is influenced by several physical factors, indeed the transport primarily occurs via passive mechanisms, where marine currents, wind, wave dynamics, and Stokes drift exert simultaneous effects. Turbulent momentum transport mechanisms, particularly at mesoscale and submesoscale levels, predominantly drive dispersion (van Sebille et al., 2020). Plastic pieces larger than 30 cm typically exhibit floating behavior and are highly susceptible to atmospheric agents, thereby highlighting the relevance of wind transport effects. Furthermore, the Mediterranean region's significant meteorological and hydrodynamic variability results in diverse regional and seasonal plastic distribution patterns (Robinson et al., 2001; Demirov and Pinardi, 2002; Lolis et al., 2008). Various approaches have been utilized to estimate marine litter exposure based on litter sources, species distributions, and spatial-temporal data. Darmon et al. (2017) assessed sea turtle exposure to marine plastic risks using aerial surveys, while Schuyler et al. (2016) correlated turtle habitat maps with plastic distribution from ocean drifter data. Additionally, initiatives like the Plastic Busters MPAs and the ACCOBAMS Survey Initiative aim to monitor and mitigate the impact of plastic pollution on marine biodiversity, especially in Marine Protected Areas (MPAs) like the Pelagos Sanctuary for Marine Mammals. Data collected by ASI in 2018, shared with Plastic Busters MPAs in 2019, facilitated collaborative efforts to evaluate plastic litter exposure risks to Mediterranean marine biodiversity. Spatial density abundance predictions were derived using Generalized Additive Models (GAM) fitted to ASI data, aiding risk assessments of taxa exposure to marine litter. Models featuring continuous representation of physical and ecosystem variability can effectively address these scale-related aspects

(Guerrini et al., 2019), but they entail inherent uncertainties associated with both physical and biological process modeling, such as the distribution of feeding habitats (Druon et al., 2012).

The aim of this work was to present a method to obtain risk maps of exposure of marine species to ML in the Mediterranean Sea, and to identify potential hotspots, by combining hazard maps of plastic ML distribution with vulnerability maps of different taxa distribution, determined via a Density Surface Modeling (DSM) approach (Hedley and Buckland, 2004; Miller et al., 2013). We used the Agreement Conservation of Cetaceans of the Black Sea, Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) Survey Initiative data, which was performed to obtain robust data on the conservation status of cetacean populations in the Mediterranean ecosystem, in synergy with the Plastic Busters MPAs (PB MPAs) project.

2 The plastic buster MPA project and the ACCOBAMS Survey Initiative (ASI)

Plastic Busters MPAs was a 4-year-long InterregMed-project with the goal of helping to sustain biodiversity and conserve natural ecosystems in pelagic and coastal marine protected areas (MPAs), by defining and implementing a harmonized approach against marine litter. The project entailed actions that addressed the whole management cycle of marine litter, from monitoring and assessment to prevention and mitigation, as well as actions to strengthen networking between and among pelagic and coastal MPAs. This involved evaluating ML presence in both coastal and offshore environments and cross mapping the distribution of litter with the distribution of selected species, mainly top predators. The deployed ML monitoring protocols were identified and elaborated within the study phase of the Plastic Busters MPAs project, and they were tested in those areas where the identification of potential ML distribution patterns was also supported by a lagrangian model implemented in a previous work by LaMMA Consortium (Laboratory of Monitoring and Environmental Modelling for the sustainable development, a joint consortium between Tuscany region and Italian National Council of Research - CNR) (Fossi et al., 2017).

We focus on three types of protected areas studied within the Plastic Buster MPA project:

- a) Large pelagic MPAs (SPAMI - Specially Protected Areas of Mediterranean Importance, EBSA - Ecologically or Biologically Significant Marine Areas): Pelagos Sanctuary SPAMI;
- b) Medium scale MPAs: Tuscan Archipelago National Park (PNAT);
- c) Small scale MPAs: Cabrera Archipelago Marine-terrestrial National Park, National Marine Park of Zakynthos.

The ACCOBAMS Survey Initiative (ASI) aimed to establish an integrated, collaborative, and coordinated monitoring system across the entire ACCOBAMS area to assess the status of cetaceans and

other species of conservation concern, providing robust capacity building, training, and ultimately enhancing conservation efforts and governance throughout the region (ACCOBAMS, 2021). The ASI was conducted from June to August 2018 over most of the Mediterranean Sea. Three types of aircraft were used (Britten-Norman-II, Partenavia, and Cessna 337 G Skymaster), all equipped with bubble windows so that observers could scan the sea surface and sub-surface right below the aircraft on the transect line. Observers were trained to search for all visible species, and mega-debris larger than 30 cm in size present in a 200 m strip on either side of the aircraft, following strip-transect methodology (Buckland et al., 2015). Positions were regularly rotated between data recording, right and left observation. The aircraft flew at a constant speed of 90 knots at a height of 600 feet above sea level. Observation conditions (e.g. sea state, turbidity, cloud cover, glare severity, glare orientation) and a subjective estimation of small cetacean detectability (subjective conditions) were systematically recorded during active survey effort. Flight data and sightings were recorded using VOR v8.6 software during SAMM survey (Suivi Aérien de la Méga-faune Marine en France métropolitaine - winter 2011/12 and summer 2012) and SAMMOA v1.1.2 software during ASI survey (<http://www.observatoire-pelagis.cnrs.fr/publications/les-outils/article/logiciel-sammoa>). The ASI survey design involved delineating various areas (blocks and sub-blocks), with transects following a systematic zig-zag pattern detailed in Panigada et al. (2023) and Cañadas et al. (2023). In this study, data obtained from the ASI survey, which intersects with areas covered by the PB MPAs project, has been used to determine the risk assessment associated with the abundance of taxa and ML.

3 Materials and methods

The data employed were collected in 2018 by the ASI (Panigada et al., 2023), and shared, in 2019, through an agreement with the Plastic Busters MPAs, with the aim of capitalizing the efforts of the two projects in a synergistic way, to evaluate the risk of exposure to plastic litter of the Mediterranean marine biodiversity. To derive a spatial density abundance prediction in each sector, detection functions were fitted to the data (different for each sector and each target). Afterwards, the spatial modeling was accomplished through a Generalized Additive Model technique (GAM), followed by the analysis of the model output and the inference phase. Such a procedure, performed via the *dsm* R-package (Miller et al., 2022), resembles the DSM workflow reported by Miller et al. (2013) and is similar to the one adopted for other analyses of the ACCOBAMS data, as reported in technical reports (i.e. ACCOBAMS, 2021). The risk assessment procedure was carried out by evaluating the exposure of the analyzed taxa to ML. Despite the distinct characteristics driving the movements of analyzed taxa and ML in water, we simplified the analysis by adopting the same approach for both targets, employing respective detectability models. Since observations are not repeated over time across the study areas, the relative abundance distribution of targets obtained can be considered as originating from a series of “snapshots” captured during individual transect surveys. However, given the trade-off

between aforementioned simplification, the demanding nature of this observation campaign, and the importance of having concurrent information about marine species and plastic litter presence, resulting maps serve as valuable indicators of their spatial distribution, in the context of risk assessment procedure design.

3.1 The dataset

The analysis considered 15 distinct ASI survey blocks: (04-08a-08b-09-10-11-12-13-20-21-21b-22a-22b-22c-22d) (Panigada et al., 2023). Additionally, since the dataset wasn't contiguous, it was divided into three parts and analyzed separately: North-West Mediterranean (NWM – 439,300 km²), Strait of Sicily (SOS – 156,600 km²), and Ionian/Aegean Seas (IAS – 268,700 km²) (see Figure 1).

Several steps were identified for the data processing according to the strategy previously proposed by Darmon et al. (2017):

- 1) to define square unit (5-10 nautical miles) for the whole target area;
- 2) to calculate the amount of each variable (ML/cetaceans/sea turtles/fish, etc.) in each square;
- 3) to define the sampling effort in each square (hours of flight/square Km, probability of sampling effort): “maximum” corresponds to the maximum effort or number of hours of flight (1), “zero” is no effort;
- 4) to relate the amounts of each variable/sampling effort in each square (probability of presence): “maximum” (1) corresponds to the maximum amount of each variable (litter/species), “zero” is absence;

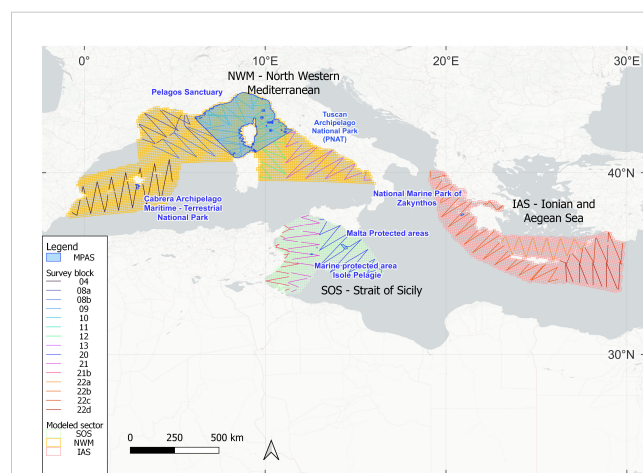


FIGURE 1

View of the transects followed during the ASI aerial surveys (different colors for each survey block as in legend), and also monitored in the PB MPAs project and subdivision in three sectors to conduct the analysis. Each color indicates a differently modeled sector, i.e. orange: North-West Mediterranean (NWM) including part of the Tyrrhenian sea, green: Strait of Sicily (SOS) and red: Ionian/Aegean Seas (IAS). Blue polygons are the main protected areas analyzed in Plastic Buster MPAs Project.

- 5) to map the distributions (standardized by sampling effort): each square as 0 to 1 value;
- 6) to cross-map data of litter and of each variable (cetaceans/sea turtles/fish, etc.). This may be obtained multiplying the indicators from points 3 and 4;
- 7) to analyze the distribution of cross probability (6) through various algorithms such as kernel, kriging, minimum distance, etc.

In ASI surveys, litter observations are marked by a predominance of plastic and various non-plastic items such as wood, oil, fishing debris, and unidentified refuse. For the purpose of ensuring consistency, only data specifically described as “plastic litter” (PL) have been taken into account in this analysis to examine a standardized dataset. Taxonomic groups including several marine species with similar detectability have been modelled. Each taxonomic group mentioned here corresponds to the criteria outlined in the ACCOBAMS protocols (ACCOBAMS, 2021). The work focused on top predators, including whales, dolphins, “small” and “large” fish. For fish, the discrimination was made by size and not by species, because data were retrieved through aerial surveys, and more specific details are seldom present in the available observed dataset.

The selected groups include:

- 1) large Dolphins (LD), including bottlenose and Risso’s dolphins, long-finned pilot whales.
- 2) large Fish (LF), including swordfish (*Xiphias gladius*) and tuna species.
- 3) small Fish (SF), small and unidentified fish categories.
- 4) whales (W), fin whales and unidentified species, excluding minke whales but including sperm whales.

For statistical significance, only areas with a minimum density of 1×10^{-5} per Km^2 observations have been analyzed. In particular, the whales group has been analyzed only in the NWM area (Table 1).

Most of the observations occurred in the NWM sector, which is the largest one and also includes the SPAMI Pelagos Sanctuary. Plastic items observed in the SOS sector are proportionally much higher than in the other sectors. Due to limitations in aerial survey detection, PL can only refer to observations of plastic material larger

than 30 cm (ACCOBAMS, 2021). From now on, PL is only intended as this type of material.

Data spans from 06/06/2018 to 20/08/2018 in IAS, from 12/06/2018 to 29/07/2018 in NWM and from 21/06/2018 to 14/07/2018 in SOS. Hence, the representativeness of derived distributions is limited to the summer period, as the winter conditions are notably much different.

3.2 Fitting the detection function

The distance sampling technique for line transects is a commonly employed method for estimating the spatial density of a biological population (Hedley and Buckland, 2004; Barlow and Forney, 2007; Bilgmann et al., 2019; Di Matteo et al., 2022). The detection function, denoted as $p(x)$, provides an estimation of the ability to detect items as a function of the orthogonal distance x from the surveyed line transect. This approach allows us to relax the assumption that all objects within the analyzed area are detected, thereby estimating the number of missed objects and, consequently, the total number present. Specifically, the probability of detection P is given by:

$$P = \frac{\int_0^w p(x)dx}{w}$$

where w represents the truncation distance, defined as the maximum perpendicular distance from the line transect within which collected observations are considered in the model. Observations of targets that are too distant from the transects hold less significance when fitting the detection function. Furthermore, sparse observations at greater distances, characterized by increased uncertainty and lower frequency, may affect the results, resulting in greater variability in estimates.

In line with other ACCOBAMS reports (e.g., ACCOBAMS, 2021), specific detection functions were derived for each analyzed group and sector. Two distinct functional forms, namely hazard rate (hr) and half normal (hn), were tested as fundamental functions for both PL and taxa groups.

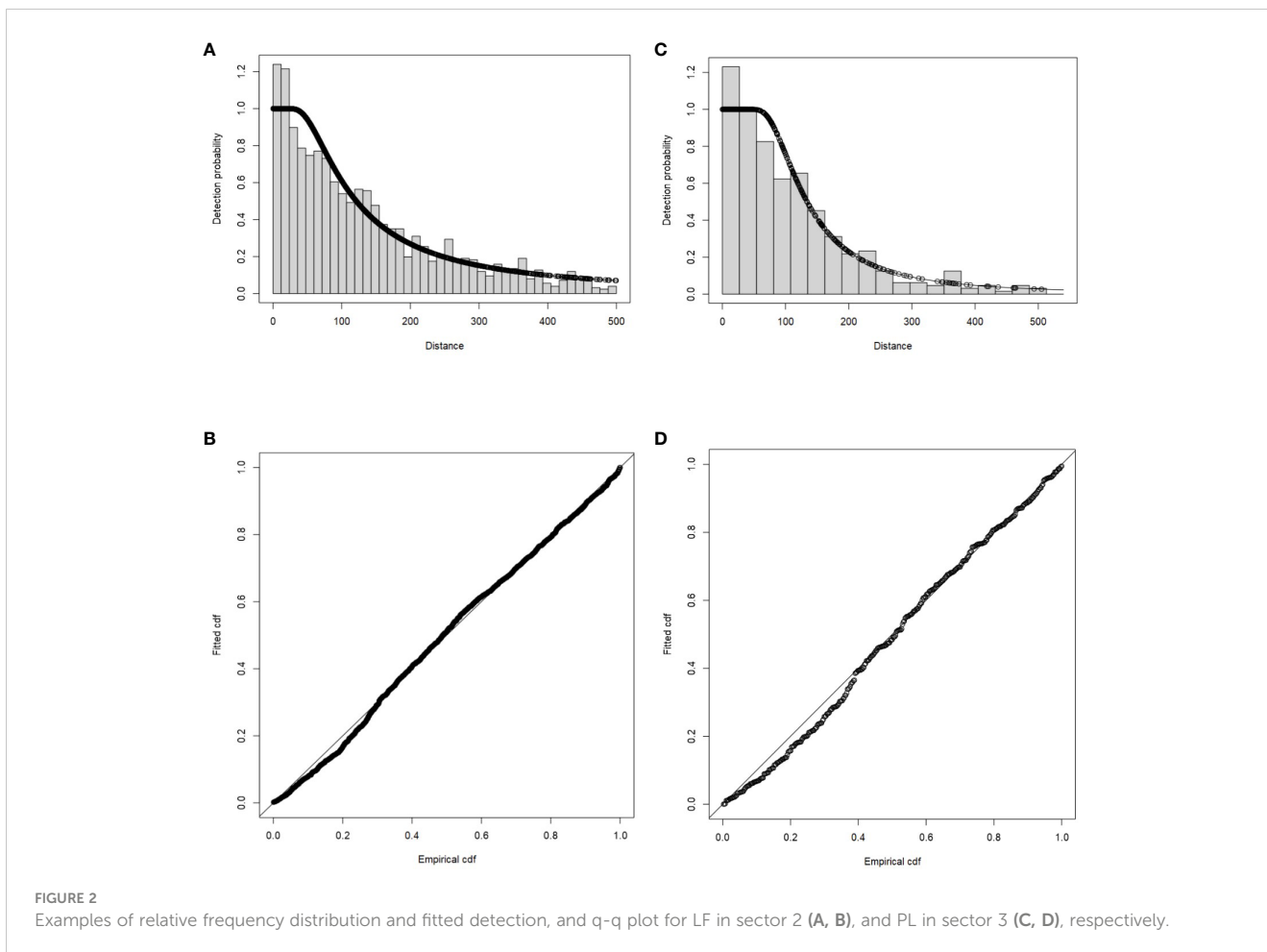
Each detection function, customized for specific sectors and taxonomic groups, was chosen based on rigorous model fitting diagnostics conducted by ACCOBAMS. These diagnostics encompassed evaluations of the Akaike Information Criterion (AIC), goodness-of-fit tests employing Cramer-von Mises statistics, analysis of quantile-quantile plots (Q-Q plots), and examination of fitted function plots (Figure 2).

ACCOBAMS prescribed truncation distances, representing the maximum perpendicular distances allowed for targets, were applied for each analyzed group.

For PL in the North-Western Mediterranean (NWM), three models were tested (refer to Supplementary Information), given that the p -value of the von-Mises test for both hr and hn (with cosine adjustment) fell significantly below 5%. Despite employing a polynomial adjustment to achieve the best fit, it resulted in a higher AIC.

TABLE 1 Number of observations per Km^2 (averaged values) in the 3 analyzed sectors for all the surveyed species (pooling as in ACCOBAMS, 2021); red values corresponds to a number of observations below the threshold assumed to be statistically significant (10^{-5} Obs/ Km^2); so distributions of W in those areas haven’t been modelled.

		IAS	NWM	SOS
Nr. Obs/ Km^2	Large Dolphin (LD)	0.41×10^{-4}	1.32×10^{-4}	1.15×10^{-4}
	Large Fish (LF)	0.37×10^{-4}	9.22×10^{-4}	2.30×10^{-4}
	Small Fish (SF)	1.34×10^{-4}	0.50×10^{-4}	0.83×10^{-4}
	Whales (W)	0.04×10^{-4}	0.84×10^{-4}	0.06×10^{-4}
	Plastic Litter (PL)	34.20×10^{-4}	42.23×10^{-4}	221.39×10^{-4}



3.3 Fitting the DSM and risk assessment procedure

Density surface models were produced by modeling species abundance as a function of spatial distribution of targets. Spatial GAM has been used to estimate the relative abundance of targets (Miller et al., 2013). As this is a well-known procedure also used by ACCOBAMS (2021), we adopted the same method, but to keep the approach as simple as possible, a bivariate smooth of coordinates was used without additional covariates.

Abundance was predicted on a 10x10 kilometers grid. For each cell of the grid, the count (n) of groups_{*i*} in each *i* segment of each cell, was used as the response variable. The general structure of the model is:

$$n_i = A_i \hat{P}_i \exp[\beta_{0,i} + s(x_i, y_i)] + \epsilon_i$$

Where:

- n_i represents the count on each segment in each cell.
- $\beta_{0,i}$ is the intercept and ϵ_i are residuals (i.e., differences between model and observation).
- A_i is the area of each segment, and \hat{P}_i is the detectability (probability of detection) in the segment.

- $s(x_i, y_i)$ is the bivariate smooth of coordinates (longitude and latitude).

The GAM algorithm facilitates the selection of different response distribution types. Here, the negative binomial distribution was chosen to account for overdispersion (Miller et al., 2013; Roberts et al., 2016; Sigourney et al., 2020). A “soap film” smoother algorithm was also employed to account for the complex geometry of the study areas (Wood et al., 2008), particularly for the presence of large islands and narrow channels in sectors 1 and 2, aiding in explaining deviance to a higher degree than other approaches.

The spatial GAM allows predictions over the grid to calculate relative abundance, i.e., the count of targets per square kilometer, normalized in a range between 0 and 1. This facilitates comparison of spatial distributions of examined taxa with plastic trash distribution. Relative abundance maps were checked for autocorrelation to ensure no autocorrelation pattern is present in the residuals. Autocorrelation can lead to misleadingly narrow confidence intervals due to pseudo-replication, where clustered measurements, if not independent, artificially increase sample size without contributing useful information (Hurlbert, 1984).

The risk assessment employed a LISA (Local Indicators of Spatial Association) bivariate approach (Anselin, 1995; Anselin et al., 2002), considering relative spatial distribution between taxa

and plastic litter on a 10x10 km grid. The method correlated, for each cell, the relative abundance of a taxon with the PL relative abundance in the neighboring cells. A threshold was set at the 95th percentile confidence interval for clustering significant values. It must be clear that detected patterns do not give any help in explaining the spatial processes which generated them. Patterns may be due to specific, period-related covariates (not used in this analysis), as well as to not optimal settings of the modeling of the observations under investigation. Moreover, correlation does not imply any actual causation among the phenomena under study.

4 Results

4.1 DSM, relative abundance and uncertainty analysis

The results of the DSM are depicted in Figure 3 for both the taxa and the three sectors: NWM, SOS, and IAS. Generally, while the relative abundance of taxa displays a uniform pattern, the distribution of PL exhibits a patchier aspect. This difference may be due to the fact that, at the analyzed spatial scales, currents tend to cluster passive plastic material (Meacham and Berloff, 2023) following the spatial and temporal variability of winds and surface flow field. Consequently, evolving concentration patterns may emerge, which might be hardly detected through a single survey.

In the NWM, the largest area under study encompassing the Pelagos Sanctuary, it is noteworthy to confirm the substantial presence of the considered cetacean species (fin whales, sperm whales) during the survey period (Figure 3I). Large fish also exhibit high abundance in this area during the same period (Figure 3F), while the presence of dolphins and small fish appears more evenly distributed in the Western Mediterranean (Figures 3E, G).

Regarding the concentration of PL, areas with higher concentrations are notably more evident in the Tyrrhenian than in the Ligurian-Algerian basin (Figure 3H). This behavior, regarding macroplastic concentrations, is not unexpected, as in the summer period, the mixing of waters between the Tyrrhenian and Ligurian basins is interrupted due to thermohaline circulation. Additionally, water transport from the Tyrrhenian to the Ligurian basin, typically directed northwards in winter, is interrupted or even reversed during the summer period.

Uncertainty in the DSM procedure arises from both distance sampling and the determination of the detection function, as well as from the GAM employed. To assess this degree of uncertainty on the estimation of the relative abundance of both taxa and plastic litter across different sectors, the following quantities are determined: the Coefficient of Variation (CV) for the chosen detection function and GAM, and the total coefficient of variation (average sector values reported in Table 2). Furthermore, the algorithm enables calculation of a “per-cell” CV by splitting the data into cells of a grid in each sector. In this case, the obtained CV can be mapped to identify areas with the largest variance. Generally, larger CV values are situated at the borders of the modeled domain

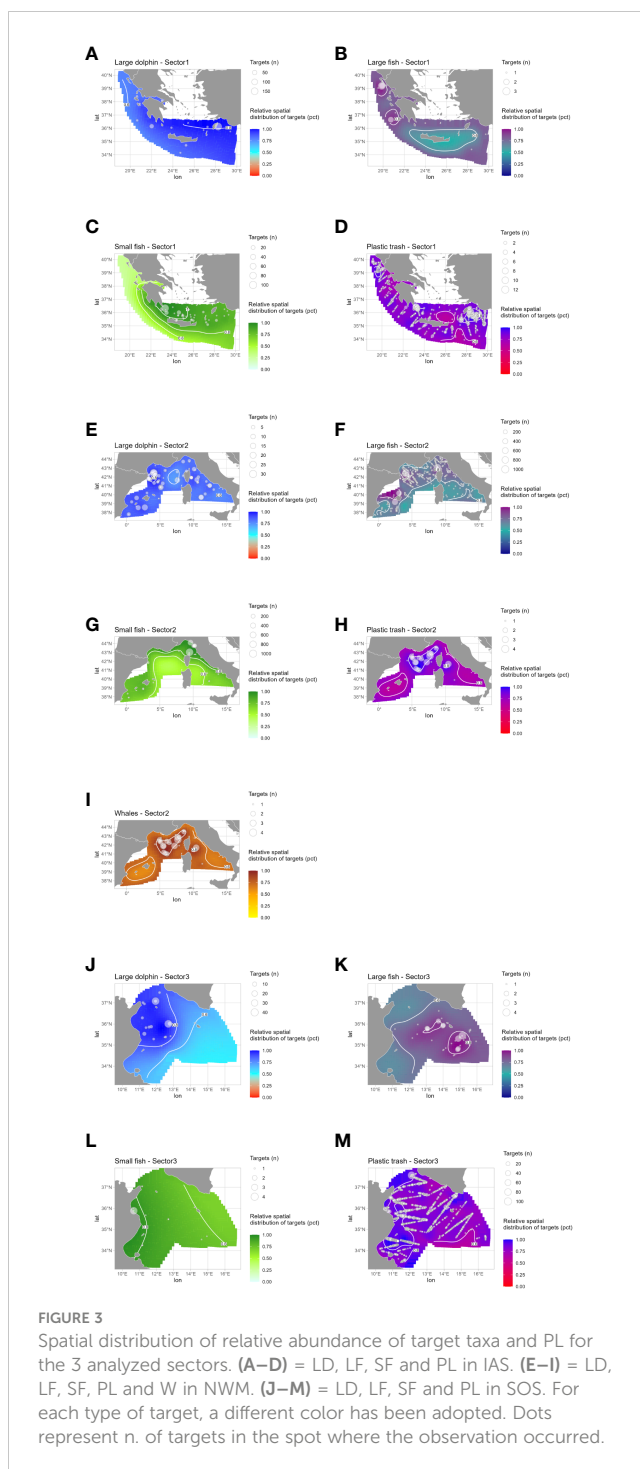


FIGURE 3 Spatial distribution of relative abundance of target taxa and PL for the 3 analyzed sectors. (A–D) = LD, LF, SF and PL in IAS. (E–H) = LD, LF, SF, PL and W in NWM. (J–M) = LD, LF, SF and PL in SOS. For each type of target, a different color has been adopted. Dots represent n. of targets in the spot where the observation occurred.

and within areas characterized by a low density of data (an example of these plots in Figure 4).

4.2 Exposure to PL

The risk assessment regarding the potential exposure of marine fauna to PL was estimated by integrating information from the

TABLE 2 Summary of uncertainty in a density surface model calculated analytically for GAM, with delta method.

Sector	Target	n	CV Detection function	CV GAM	Total CV
IAS	LD	11	0.03559185	0.6915	0.6924
	LF	10	0.03559185	0.5793	0.5804
	SF	36	0.03611962	0.4617	0.4631
	PL	919	0.03559185	0.0518	0.0628
NWM	LD	58	0.01645871	0.233	0.2336
	LF	405	0.01645871	0.5568	0.5571
	SF	22	0.01680367	0.6487	0.6489
	W	37	0.2991768	0.2139	0.3678
	PL	3467	0.01645871	0.0318	0.0318
SOS	LD	18	0.02562528	0.474	0.4747
	LF	36	0.02562528	0.2241	0.2256
	SF	13	0.3068508	0.3963	0.5012
	PL	1855	0.02562528	0.0581	0.0635

- light grey areas: clusters of not significant correlation of group of taxa or PL;
- green areas: clusters of low (about < 1.5 sd) relative abundance of taxa targets in a low relative environment of PL;
- yellow areas: clusters of relatively low relative abundance of taxa targets in high (about > 1.5 sd) relative environment of PL;
- orange areas: clusters of high relative abundance of taxa in areas of low relative environment of PL;
- red areas: clusters of high relative abundance of group targets in high relative environment of PL.

The exposure maps for the different taxa and sectors are reported in Figure 5.

5 Discussion

The exposure maps depicted in Figure 5, while constrained by the limitations inherent in the representativeness of the data, as previously discussed, allow us to clarify specific aspects of each of the investigated areas:

relative abundance of PL and specific taxa using the LISA bivariate approach (Anselin et al., 2002).

This approach involves evaluating, for each cell of the grid, the correlation between taxa and PL abundance in the neighboring cells. In essence, each variable (e.g., taxa) is compared with the spatial distribution of the environmental pressure variable (e.g., PL) in the surrounding cells.

Exposure maps are presented for those taxa with at least 10 targets in each sector. These maps allow us to visualize the potential areas where marine fauna may be at risk of exposure to plastic litter, based on the spatial distribution and abundance of both PL and specific taxa.

To quantify the exposure, we assigned different colors to the analyzed areas, based on the following classes:

1. Within the IAS sector, the assessment reveals that the National Marine Park of Zakynthos MPA exhibits a low risk for most taxa except for Large Fishes (LF), which face relatively higher risks due to their presence in areas characterized by low plastic litter (PL) but elevated LF presence. Notably, the central-eastern areas of this sector pose the highest risk for large dolphins (LD) and small fish (SF), with a pronounced overlap between PL and taxa observed, particularly in the eastern area owing to high PL density.
2. In the NWM sector, characterized by large spatial heterogeneity, the Pelagos Sanctuary emerges as the most significant area of interest. Here, the higher PL presence in the southeastern part poses a notable threat mainly to SF,

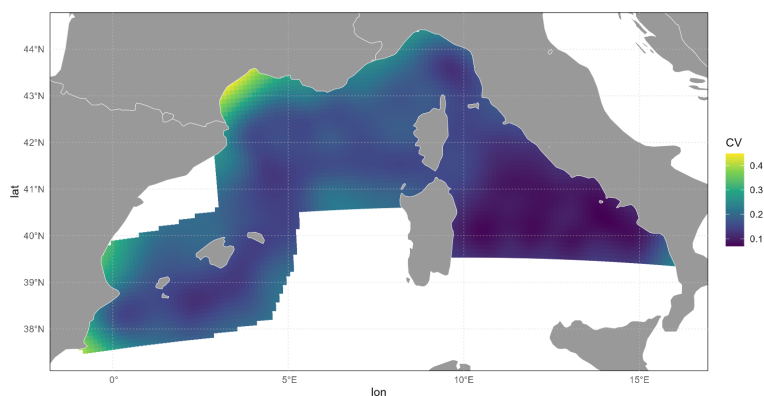


FIGURE 4 Example of CV "per-cell" map of PL GAM in NWM.

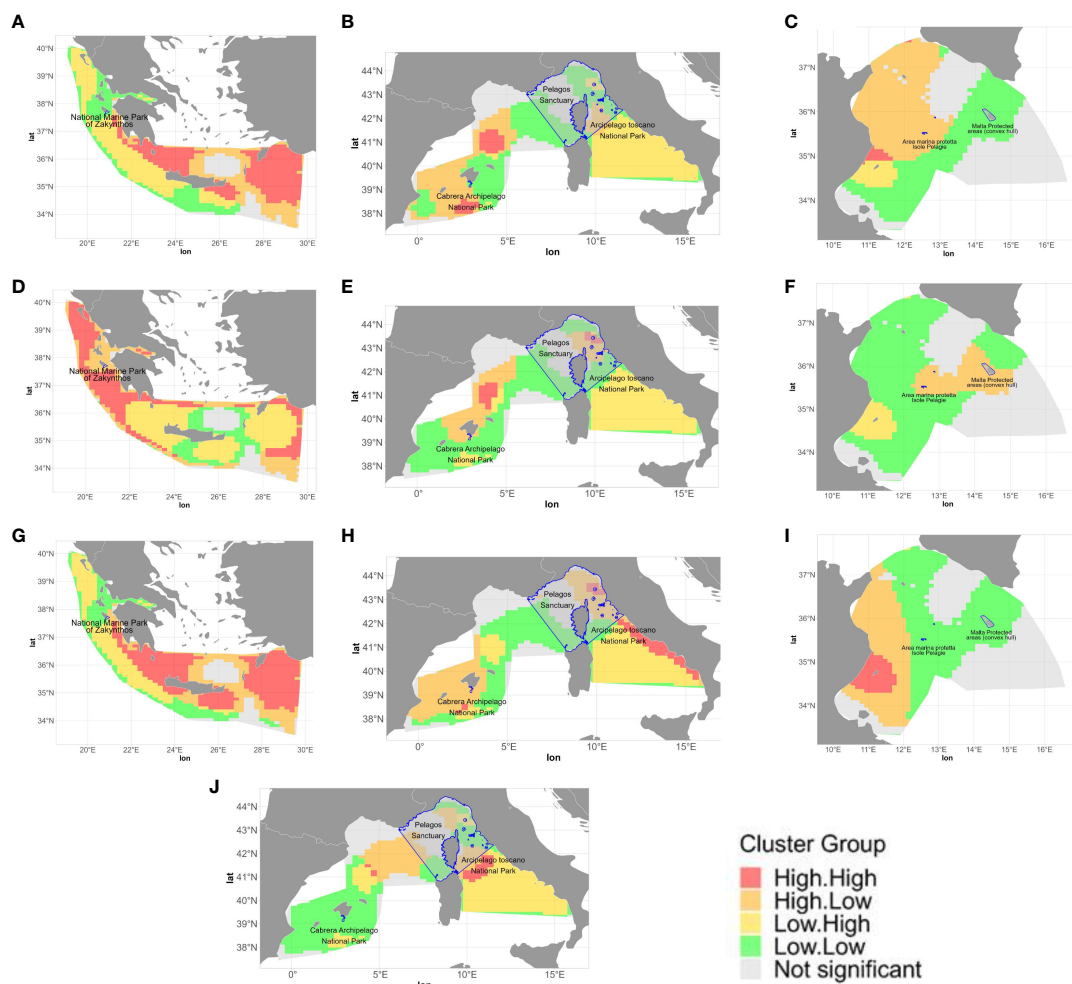


FIGURE 5 Clusters of relative distributions of LD (Large Dolphins) vs PL (Plastic Litter) (Part 1, **A–C**), LF (Large Fish) vs PL (Part 2, **D–F**) and SF (Small Fish) vs. PL (Part 3, **G–I**). In (**J**) panel the W (Whales) group for NWM area. In the legend, the first indication of the cluster name is referred to the taxa examined and the other to the PL.

but the impacts on whales in the northwestern part can also be significant. Additionally, high PL presence is observed in the east-southern area and localized spots around the Balearic Islands and the Gulf of Genoa.

3. Within the SOS sector, PL predominantly accumulates along the eastern side of Tunisian coasts and the west-southern coast of Sicily. The primary threat in this sector pertains to LD and SF, with the “Isole Pelagie” MPA exhibiting higher PL exposure compared to MPAs around Malta. LD and SF concentrate in the western area, where PL is clustered, while LF shows greater clustering in the eastern area, seemingly less impacted by PL.

In the context of the Northwest Mediterranean (NWM), and particularly within the Pelagos Sanctuary area, other studies in the literature have highlighted the significant risk posed to the biodiversity present in this region. For instance, [Guerrini et al. \(2019\)](#), in their decadal assessment of plastic ingestion risk by fin whales within the Pelagos MPA, identified a potential hotspot for fin whales in the Liguro-Provençal basin during the summer feeding season, based on

simulations of plastic debris dispersal and ecological modeling. Similarly, [Darmon et al. \(2017\)](#) in their analysis of aerial surveys, highlighted elevated pollution levels, especially in this Mediterranean area, with potential detrimental effects on biodiversity, notably on sea turtles. [Darmon et al. \(2017\)](#) emphasizes the importance of integrating empirical data with simulation models to understand spatiotemporal variability in sensitive areas and assess factors influencing interactions between species and debris, highlighting the limitations of single-time observations in determining impacts on marine fauna. However, it is noteworthy that the peculiar summer circulation patterns between the Tyrrhenian and Ligurian seas, with limited exchange between the two basins in this period, mitigate the risk of whales’ exposure to macroplastics in the Pelagos Sanctuary during the summer season.

One of the main issues in the current analysis is the sample size used in the spatial model, especially for the IAS area and for LD and LS groups, but also for the SF group in NWM (see [Table 1](#)). This is quite evident in the exposure maps where non-significant correlations areas are commonly low density target areas (both taxa and ML).

As already stated, it is important to remark that the determined patterns of ML ad taxa distributions are related to the moment in

which surveys occurred. Only repeated surveys during the same period of the year might help to map distributions more affordably. Also, in this preliminary study on ASI data, we did not include any covariate in the spatial model (i.e. depth, distance from the coast, SST) which could affect the results, at least for specific groups. Results of spatial distribution hold significance in establishing conservation priorities and are becoming increasingly crucial in decision-making for cost-effective approaches to safeguard biodiversity. Hotspots may exhibit dynamism and fleeting nature, yet this does not detract from their ecological significance.

Regarding the PL hotspots, various authors, utilizing both direct measurements of transport at sea like Lagrangian drifters (Zambianchi et al., 2017) and numerical models of transport and dispersion (e.g., Fossi et al., 2017), have demonstrated the critical contribution of surface currents in determining the surface distribution of plastic litter, even in confined environments such as the Mediterranean basin. In contrast, animal movement ecology operates at a significantly higher level of complexity, encompassing habitat associations, the extent and utilization of geographical ranges, migratory pathways, phenology, and interactions between animals and their environment.

6 Conclusions

The present investigation was aimed at evaluating the risk posed to marine biodiversity in specific Mediterranean areas by exposure to plastic litter (PL), aligning with the objectives of the ASI and Plastic Busters MPAs joint effort. The analysis revealed variability in estimates, particularly in areas with limited data and in coastal and boundary regions, consistent with findings in existing literature.

Furthermore, ecosystem variability, notably in large pelagic marine mammals, exhibits a seasonal behavior influenced by physical and trophic conditions, posing challenges for accurate modeling. Despite limitations in data representativeness, our risk analysis based only on observational data provides a robust foundation for discussing the relative distributions between species and PL.

The study highlights the benefits of cross-mapping the distribution of PL and marine species using observational data for management purposes. Such an approach, although tested at a limited scale due to resource constraints, provides a large-scale assessment to delineate areas at risk and prioritize mitigation measures. The ASI dataset offers a valuable resource, providing a comprehensive dataset of both fauna and PL (macroplastics) acquired simultaneously during monitoring campaigns. It is also important to point out, for the purposes of the conclusions of this analysis, that aerial surveys are effective in identifying larger debris (>30 cm), while smaller debris, including microplastics, are also present and pose risks to marine life (Lambert et al., 2020). Detecting and assessing these smaller debris particles require additional methods and technologies, and needs even greater synergy between different initiatives.

Preservation efforts should focus on continuous monitoring of high-risk areas, such as the Pelagos Sanctuary, to safeguard fragile

habitats and species. Systematic surveys over time, coupled with integrated and harmonized monitoring tools, are crucial for estimating variations in population abundance and PL trends, enhancing our understanding of spatial risk exposure, and facilitating measures to protect vulnerable marine species.

In conclusion, our research emphasizes the critical importance of conducting thorough evaluations of marine debris and its repercussions on biodiversity in the Mediterranean. This has profound implications for devising conservation and management strategies. It is imperative to prioritize further research and monitoring endeavors to tackle the challenges presented by marine debris and to ensure the effective protection of marine ecosystems.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repository and accession number(s) can be found here: <https://accobams.org/asi-dataaccess-request/>.

Ethics statement

Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because this study used observations of animals previously conducted under other research efforts.

Author contributions

MP: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Validation, Visualization, Writing – original draft, Writing – review & editing, Software. CB: Conceptualization, Funding acquisition, Investigation, Methodology, Supervision, Validation, Writing – review & editing. MB: Data curation, Methodology, Validation, Writing – original draft, Writing – review & editing. CL: Validation, Writing – review & editing. FG: Conceptualization, Funding acquisition, Supervision, Writing – review & editing. SP: Conceptualization, Resources, Supervision, Validation, Writing – review & editing. AC: Validation, Writing – review & editing. CP: Validation, Writing – review & editing. MF: Funding acquisition, Supervision, Writing – review & editing.

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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.2024.1352059/full#supplementary-material>

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Density and abundance estimates of cetaceans in the Black Sea through aerial surveys (ASI/CeNoBS)

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Introduction: Population abundance is amongst the most basic and crucial parameters for the assessment of conservation status of any species. Three species of odontocetes, all represented by local subspecies, inhabit the Black Sea: the Black Sea common dolphin *Delphinus delphis ponticus*, the Black Sea bottlenose dolphin *Tursiops truncatus ponticus*, and the Black Sea harbour porpoise *Phocoena phocoena relicta*. Their populations are threatened by multiple factors, including overfishing of their prey, bycatch, pollution and epizootics. Despite this, there are no basinwide estimates for any cetacean species in the Black Sea.

Methods: In 2019, a systematic study was carried out under the EU CeNoBS project. Six strata were designed in the waters of Bulgaria, Georgia, Romania, Türkiye and Ukraine, covering most of territorial and offshore waters, which were surveyed between June 19 and July 4. A line transect distance sampling approach was used, following predefined transects within each stratum, achieving a 5% coverage of the surveyed area. A total of 7,344 kilometres of transects were surveyed recording a total of 1,744 cetacean sightings. Design-based abundance estimates were obtained using a Multiple Covariate Distance Sampling (MCDS) approach. Model-based abundance estimates were also derived using a Generalized Additive Models (GAM) approach, linking species sightings with a number of environmental covariates (e.g., bathymetric features, sea surface temperature, chlorophyll-a) over a grid of 10x10 km.

Results and discussion: The uncorrected (for perception and availability bias) estimates obtained through the model-based analysis were 108,283 (CV=0.07) common dolphins, 22,720 (CV=0.15) bottlenose dolphins and 93,808 (CV=0.06) harbour porpoises. These aerial surveys yielded the first insights on overall

abundance, density and distribution, providing current regional baseline values and density maps for all three cetacean species of the Black Sea during the summer months, to be used for the elaboration of effective conservation measures and to address national and international requirements.

KEYWORDS

Black Sea dolphins, Black Sea porpoises, aerial survey, abundance, distribution, ASI, CeNoBS, MSFD

1 Introduction

The Black Sea is a semi-enclosed sea, connected to the Mediterranean Sea only with the narrow Istanbul Strait, and the world largest meromictic water body with an oxygen-depleted deep layer, known as a *unicum hidrobiologicum* due to its unique physico-chemical properties (Vespremeanu, 2007; Bologa, 2015) and highly vulnerable marine ecosystem (Zaitsev and Mamaev, 1997; Daskalov, 2003). Monitoring and assessing environmental status (based on the ecosystem's biodiversity, functions and services) is crucial to ensure the long-term sustainability of the Black Sea. Cetaceans are apex predators that may be keystone species with a structuring role within ecosystems and the food webs (Power et al., 1996).

Three species of small odontocetes inhabit the Black Sea and adjoining Marmara and Azov Seas (Zalkin, 1938b; Barabash-Nikiforov, 1940; Zalkin, 1940; Kleinenberg, 1956; Tomilin, 1957; Antonescu, 1966; Öztürk and Öztürk, 1996; Gol'din, 2004; Viaud-Martinez et al., 2007; Birkun, 2008b; Notarbartolo di Sciarra and Tonay, 2021; Fontaine et al., 2012; Murariu, 2012; Birkun et al., 2014; Ben Chehida et al., 2020), each represented by an endemic subspecies, the Black Sea common dolphin (*Delphinus delphis ponticus* Barabash, 1935), the Black Sea common bottlenose dolphin (*Tursiops truncatus ponticus* Barabash-Nikiforov, 1940), and the Black Sea harbour porpoise (*Phocoena phocoena relicta* Abel, 1905).

Based mostly on indirect evidence, such as risk assessment and observations of mass mortality or some local surveys, these populations are characterised by IUCN (<https://www.iucnredlist.org/>) as either Endangered or Vulnerable (Birkun, 2008a; Birkun and Frantzis, 2008; Birkun, 2012). All three cetacean species are listed under Annex IV of the EU Habitats Directive 92/43/EEC, while the harbour porpoise and bottlenose dolphins are listed under Annex II as well. It is believed that a number of threats, including past direct takes, habitat degradation, depletion of prey stocks and zoonoses, had already led to drastic declines by the end of 1980s (Birkun, 2002a; Daskalov, 2003). This, plus the ongoing high cetacean bycatch rates (Tonay, 2016; Popov et al., 2020; Aiken et al., 2023; Popov et al., 2023), emphasises the urgent need for new data on the status of Black Sea cetaceans and the threats they face. Also, this means that they require strict protection and their key habitats to be designated

as Special Areas of Conservation (SACs) within the NATURA 2000 network.

A robust estimate of population abundance (or ideally a series of such estimates over time) is one of the fundamental parameters needed for assessing conservation status. In the 1970s and 1980s, abundance estimates were extrapolated from partial surveys to the entire Black Sea (Zemsky and Yablokov, 1974; Çelikkale et al., 1989). More comprehensive historical records had been collected for cetacean distribution by the 1980s (Zalkin, 1938a; Freiman, 1950; Kleinenberg, 1956; Danilevsky et al., 1978; Mikhalev, 2005a; Mikhalev, 2005b; Mikhalev, 2008). Since 2000, research on the distribution and abundance of cetaceans in the Black Sea has been increasing, but covering mostly local areas and the coastal waters (Birkun et al., 2003; Birkun et al., 2004; Birkun et al., 2006; Dede and Tonay, 2010; Birkun et al., 2014; Kopaliani et al., 2015; Panayotova and Todorova, 2015; Gladilina and Gol'din, 2016; Gladilina et al., 2017; Panayotova et al., 2017; Akkaya Baş et al., 2019; Paiu et al., 2019; Uludüz et al., 2020; ANEMONE, 2021; Popov et al., 2021; Paiu et al., 2021a). Prior to the surveys reported here, the latest large scale abundance estimation was made in 2013, when an aerial survey, as well as a ship survey, was coordinated along the NW Black Sea (Birkun et al., 2014) covering Bulgarian, Romanian and Ukrainian (partially) waters.

The EU Marine Strategy Framework Directive (hereinafter MSFD) and the EU Water Framework Directive (WFD) are components of a suite of environmental controls linked to the Directives related to the environment (Borja et al., 2010). The MSFD established a framework for the development of measures designed to achieve the "Good Environmental Status" (GES) in the marine environment, using 11 qualitative descriptors. Descriptor 1 requires that biological diversity is maintained and that the quality and occurrence of habitats and the distribution and abundance of species are in line with prevailing physiographic, geographic, and climatic conditions (Palialexis et al., 2019). The main aim of this paper is to provide a regional assessment of abundance (D1C2) and distribution (D1C4) of cetacean populations, to fill gaps and provide baseline data at the Black Sea regional scale.

In 2019, in cooperation and with support from the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic Area (ACCOBAMS), within the framework of the ACCOBAMS Survey Initiative (ASI, ACCOBAMS, 2021a; ACCOBAMS, 2021b), the "Support MSFD implementation in the

Black Sea through establishing a regional monitoring system of cetaceans (D1) and noise monitoring (D11) for achieving Good Environmental Status” CeNoBS project has implemented an aerial survey to assess cetacean density and abundance in the Black Sea, using robust methods. Standardised protocols were used to facilitate data comparison and to create a baseline of abundance and distribution data which will allow future analyses of trends in space and time. A robust analytical modelling framework was applied to the dataset and is reported in the present article.

2 Materials and methods

Distribution and abundance were assessed using agreed protocols for a regional aerial survey (e.g., Hammond et al., 2002, 2013 and Hammond et al., 2017). Aerial surveys allow large areas to be covered in a short period of time, which is especially important in places, such as the Black Sea, known for rough sea conditions and the capacity of going from 0 (calm sea) to 5 (rough sea) Beaufort wind force scale quickly. The collected data were analysed to estimate abundance, density and assess distribution of the different species, using both model-based and design-based analytical frameworks (see section 2.3 and 2.4).

The aerial survey was conducted using two small twin-engined planes, Cessna 337, with high wings and bubble windows, to allow the observers to view the trackline below the plane. One plane surveyed the waters of Bulgaria, Romania and Ukraine, and the other one surveyed the waters of Georgia and Türkiye. Flights were conducted during daytime in good weather and sea states <4 on the Beaufort wind force scale.

2.1 Study area and timeframe

The survey covered the waters of Bulgaria, Georgia, Romania, Türkiye, and Ukraine, in total 52% of the of Black Sea (Supplementary Figure S1). The survey was conducted between June 19th and July 4th, 2019.

2.2 Survey design and data collection

Distance sampling methods were used following pre-determined transects with a random start, designed to ensure an equal coverage probability and representation of the study area (Buckland, 2001; Buckland, 2004; Thomas et al., 2010; Buckland et al., 2015). This standardised approach has been used in several other regional contexts e.g., Hammond et al. (Hammond et al., 2002, 2013 and 2017) and the ASI project (ACCOBAMS, 2021a; ACCOBAMS, 2021b; Cañadas et al., 2023; Panigada et al., 2024). For each stratum, an equal spaced zigzag design was selected (using the dedicated software Distance 7.3; Thomas et al., 2010) taking into account depth contours and the coastline to ensure sampling across the gradient of expected cetacean density. The strata boundaries represented the best compromise amongst oceanographic zones, bathymetric characteristics and political/jurisdictional constraints. The selected tracks allowed a final coverage of 5% for all the areas.

The target altitude was 183 m (600 feet), as is customary for small cetacean surveys, with a target speed of 100 knots (e.g., see Hammond et al., 2002 and ACCOBAMS, 2021a). The survey was conducted in passing mode (i.e., not diverting from the trackline when sightings were made) unless it was deemed necessary to close with the sighting to obtain reliable estimates of group size or confirm species identity. In such cases, the survey was resumed at the exact point it was left. ‘Secondary sightings’ (i.e., the additional sightings made after leaving the predetermined trackline) were recorded but not used to obtain the abundance and density estimates.

For each sighting, a minimum of species identity, group size, and declination angle to the sighting – when abeam – were recorded. Where possible, group composition (adults and calves), direction of swimming and group behaviour were also recorded. An overall subjective assessment of the detection conditions (i.e., “excellent”, “good”, “moderate” or “poor”, hereafter referred to as “Sightability score”) for each observer was also recorded where, as an instance, “good” conditions corresponded to an observer perceiving the likelihood of spotting a small delphinid within the searching area (within approx. 300 m from the track line) to be good (e.g., Beaufort wind scale ≤ 2 , turbidity < 2 and glare moderate, good or absent). The variables collected were the sea state (Beaufort wind force scale), glare, cloud cover, turbidity and a subjective assessment of overall detectability conditions. Additional information such as the presence of human activities was also recorded.

All data were recorded on a laptop with software (SAMMOA 1.1.2), dedicated to data acquisition on marine megafauna from visual observations during aerial surveys. SAMMOA is connected to a GPS and has a simultaneous audio recording system. The flight plan and tracklines were programmed before take-off, along with observers’ position onboard. The software also allowed data validation based on the voice recordings associated with each observation. The plane’s position, speed and altitude were continuously recorded through a GPS and the angle to each sighting was measured with a clinometer.

2.3 Design-based analysis

Analysis of the data followed standard line transect distance sampling methodology (Buckland, 2001). Density of animals (D) was estimated as:

$$\hat{D} = \frac{1}{2L} \times \sum_{i=1}^n f(0 | z_i) s_i$$

where \hat{D} is the estimated density, n is the number of sightings of groups, s_i is the group size for sighting i , L is the total length of transect searched under acceptable conditions and $f(0 | z_i)$ represents the probability density function of the perpendicular distance [$f(0)$] given the covariates z_i .

Abundance was estimated as:

$$\hat{N} = A \hat{D}$$

where A is the size of the survey area.

The design-based analysis was performed in R (R Core Team, 2017), with an *ad-hoc* script prepared for this dataset, using package Distance (Miller et al., 2019). Segments of tracks and sightings with

sea state 4 (Beaufort wind force scale) or above were excluded from the analysis. Detection functions were fitted to the perpendicular distance data to estimate the effective strip half-width, *esw*. Multi-Covariate Distance Sampling methods were used to allow detection probability to be modelled as a function of other covariates, additional to perpendicular distance from the transect line (Marques et al., 2007). These covariates were defined in the survey design phase and are shown in Supplementary Table S1.

After exploration of the data (histograms of perpendicular distances), it was clear that left truncation was necessary only for Black Sea bottlenose dolphins (40 m) and for Black Sea harbour porpoise (50 m). Given that the planes used bubble windows and there was visibility under the plane, the reduced number of sightings in those first metres from the trackline could be due to observer behaviour (tending to look further away rather than closer to the track line), although this did not happen with common dolphins. The left truncation was treated as a strip transect, where the probability of detecting is considered uniform, at the same level of the chosen left truncation. This means that the detection functions treats the strip as a proper left truncation, but during abundance estimation those observations are taken into account and given the probability of detection at the 40 m distance for Black Sea bottlenose dolphins and 50 m for Black Sea harbour porpoise, including those distances in the effective strip width. It is important to highlight, however, that treating left truncation as a strip transect in a detection function could potentially mean an overestimation of the probability of detection close to the trackline, creating therefore a potential slight underestimation of the abundance estimate.

Different right truncation distances were also tested. A compromise between the comparison of the diagnostics of each of the different truncation distances and the percentage of data lost in each one was considered to decide on the final right truncation. The diagnostics used were the qq-plots and the Cramér-von Mises diagnostics. The final right truncation distances were: 511 m for Black Sea common dolphins, 482 m for Black Sea harbour porpoises and 312 m for Black Sea bottlenose dolphins. Considering both left and right truncation, the number of observations discarded for modelling the detection function were 2 (0.3%) for Black Sea common dolphins, 116 (14.74%) for Black Sea harbour porpoises and 13 (11.93%) for Black Sea bottlenose dolphins. Considering only right truncation, the number of observations discarded for the model-based analysis were 2 (0.3%) for Black Sea common dolphins, 5 (0.64%) for Black Sea harbour porpoise, and 4 (3.67%) for Black Sea bottlenose dolphins.

The best functional form (Hazard Rate model) of the detection function and the covariates retained by the best fitting models were selected based on model fitting diagnostics: AIC, goodness of fit tests, Q-Q plots, and inspection of plots of fitted functions.

2.4 Model-based analysis

Density surface models were produced by modelling species abundance as a function of environmental covariates. These were derived from a large number of data sources and included variables such as water depth (m), distance to the several depth contours (as

proxies for coastal, continental shelf, oceanic habitats, etc.), distance to canyons and seabed slope. As indices of marine hydrology and/or biological activity/primary productivity, variables such as sea surface temperature and levels of chlorophyll-a were examined. For a complete list of variables used, see Supplementary Table S2.

All on-effort transects (i.e., where searching conditions were acceptable) were divided into segments (mean=10.1 km) with homogeneous effort types, and under the assumption that little variability in physical and environmental features occurred. Each segment was associated with the values of the covariates of the specific cell (10 x10 km) in which their centroid fell. As for the design-based method, segments of tracks and sightings with sea state more than 3 (or, for the harbour porpoise, more than 2) were excluded from the analysis, as were sightings beyond the truncation distances for each species. All estimates were produced for each individual strata, as well as for the whole study area (all strata pooled together).

The count of animals in each segment was used as the response variable. The abundance of animals was modelled using a Generalized Additive Model (GAM) with a logarithmic link function, and a Tweedie error distribution. The general structure of the model was:

$$n_i = \exp \left[\ln(a_i) + \theta_0 + \sum_k f_k(z_{ik}) \right]$$

where n_i is the number of animals in the i^{th} segment, the offset a_i is the effective search area for the i^{th} segment (calculated as the length of the segment multiplied by twice the effective strip half-width – *esw*), θ_0 is the intercept, f_k are smoothed functions of the explanatory covariates, and z_{ik} is the value of the k^{th} explanatory covariate in the i^{th} segment. The *esw* was obtained for each species/species group from their detection function, according to the covariates included in it.

An exploration of correlations was performed amongst covariates. As a result, “families” of covariates were created such that only one element of each family could be tested in each model. Supplementary Figure S2 shows the collinearity plots resulting for fixed and dynamic covariates. All correlations equal or above 0.7 were considered as collinear and therefore not used together in the same model. REML (Restricted maximum likelihood) was used to fit the models. Shrinkage smoothers were also used in all models, which reduces the effective degrees of freedom to zero if a covariate explains little variation in the data. A full model (including all covariates) was run. Using REML and shrinkage smoothers, the non-useful covariates were discarded, reducing it only to the covariates to be tested in the final models. The final models were run using all the potential combinations of the “useful” covariates selected by REML and the shrinkage smoothers from the full model, sequentially testing each covariate from each collinear family. The resulting models were judged and ranked automatically by AIC avoiding time consuming and potentially unreliable manual selection.

Two sets of models were run for each species; one allowing for the inclusion of the two-dimension spatial smoothness of the interaction between Longitude and Latitude (“LonLat” from now

on), and another one not allowing it. The rationale behind this is that very often the inclusion of the LonLat improves the fit of the model and the precision of the prediction, being responsible for a large proportion of the deviance explained by the model. This is the most desirable output if your model is predictive (as are ours), and you try to obtain the best possible estimate of density and spatial distribution of the species, as opposed to an explanatory model where the objective is to explain the causes of the observed distribution. However, sometimes it is interesting to explore which environmental covariates would allow a good model, without the help of LonLat.

Abundance of animals in each grid cell was estimated by multiplying the predicted density of animals from the model by the surface area of the grid cell. The total abundance estimates for the whole study area and for each stratum were obtained by summing up the abundance of all the grid cells within the target study area.

Variance of abundance was estimated by a parametric bootstrap procedure (“posterior simulation”). This method generates bootstrap replicates based on resampling the parameters of the best fitting model, instead of resampling the data itself. The delta method was used to combine the CV from the bootstrap with the CV from the detection function and from the model. The 95% CIs were obtained using the final CV and assuming the estimates were lognormally distributed. All modelling was carried out using the statistical software R (R Core Team, 2017) using the mgcv package (Wood, 2011), within an *ad hoc* script created for this dataset.

3 Results

A total of 1,744 cetacean sightings were recorded (Paiu et al., 2021b), with 3,669 individuals from three species (two delphinids and one phocoenid: this order will be kept along the sections) in the study area (Table 1). A total of 12,387.25 kilometers was surveyed by the two planes in the different strata, with 7,324.1 km on effort and 5,063.15 km off effort, as summarised in Table 2. All the analysis and results presented are reflecting the surveyed area (52%) and are not being extrapolated to the rest of 48% of the Black Sea area.

TABLE 1 Total number of sightings and individuals observed during the aerial surveys.

Species	Number of sightings	Number of individuals
Black Sea bottlenose dolphin	117	335
Black Sea common dolphin	715	1762
Delphinid	28	50
Black Sea harbour porpoise	884	1522
Total	1744	3669

3.1 Design-based analysis

The final detection functions chosen for each species and their diagnostics are presented in Supplementary Table S3; Supplementary Figures S3–S5. For common dolphins, the covariates retained in the model were glare under the aircraft, aircraft name and sea state as a factor; for bottlenose dolphins, the only covariate retained was turbidity; for harbour porpoises the final covariates were swell, glint and aircraft name.

The abundance estimates obtained with the design-based analysis for the three species of cetaceans are shown in Tables 3–5. In these tables, “mean group size” is the mean of the observed group sizes, while “expected group size” is the result of dividing the estimated abundance of individuals by the estimated abundance of groups.

3.2 Model-based analysis

The parameters and selected covariates for the density surface modelling for each species are presented in Table 6.

Black Sea common dolphins, which were expected to be the most observed species, were the second most frequently encountered species (Figure 1) with 715 sightings. When it comes to the recorded number of animals, with 1,762 individuals, the species ranks first.

Black Sea bottlenose dolphins were the least frequently observed species during the aerial survey (Figure 2), with 117 sightings, totalling 335 individuals.

Black Sea harbour porpoises were the most frequently observed species, with 884 sightings (1,522 individuals). Based on the recorded sightings, a prediction of abundance maps was created (Figure 3), in which one of the historical hotspots was identified, within the Western Black Sea area, in Bulgarian and Romanian waters (details in the next section).

Tables 7–9 show the results of abundance estimates for the model-based analysis for each species, with and without spatial covariates. Thus, the uncorrected (for perception and availability bias) estimates obtained through the model-based analysis were 108,283 (CV=0.07) (model with LonLat) common dolphins, 22,720 (CV=0.15) (model without LonLat) bottlenose dolphins and 93,808 (CV=0.06) (model without LonLat) harbour porpoises. The best models for common dolphins and bottlenose dolphins were chosen based on the AIC. For harbour porpoises, none of the models with LonLat were significant, except using only LonLat, which caused a strong edge effect, reason for which it was discarded (also gave a higher estimate: 104,567 than all the other models ranging from 88,000 to 93,000). Therefore, the second best model was chosen based on the AIC (Table 6).

4 Discussion

4.1 Methodological considerations

The observations, abundance estimates and data on distribution shown here represent solely the data for the survey area (52% of the whole Black Sea) and are not extrapolated in any way on the whole

TABLE 2 Total number of kilometres covered per strata on-effort and off-effort.

Strata for	Strata no.	Strata area (km ²)	Covered % of the whole TW+EEZ Area	Km on effort	Km off effort	Total Km
Ukraine	1	69785	18.49	767.39	735.7	1503.09
Romania	2	18611	63.84	816.32	548.44	1364.76
Bulgaria	3	32683	95.74	1115.53	159.59	1275.12
Türkiye 1	4	71796	82.15	2211.47	2095.2	4306.67
Türkiye 2	5	69785		2203.03	1405.1	3608.13
Georgia	6	6237	24.31	210.36	119.12	329.48
Total		220169		7,324.1	5,063.15	12,387.25

Black Sea. Also, data from this study characterise a single time period, two weeks during the summer 2019, and are not extrapolated to other seasons or years. The models of animal distribution used by us do not intend to be “explanatory” models, and here we recognise that most physical covariates used by us are proxies for other important processes, such as prey density. However, our models are “predictive”, which means that we use the covariates available to us (including geographic coordinates in some of the models), as proxies in most cases, to identify the areas with higher or lower density.

Moreover, the abundance estimates provided here are underestimates for the survey area, in that they have not yet been corrected for availability bias (animals on the trackline below the surface that could not be seen), or perception bias (animals missed by observers that were available to be seen). It may be possible to collect data in the future that will allow for such correction or use available data from other surveys. In an aerial survey, correction for perception and availability bias occurs simultaneously when using the circle-back or “racetrack” method (Hiby and Lovell, 1998; Hiby, 1999; Hammond et al., 2013, 2017) given that the right amount of time between aircraft passes is enough (around 3 minutes; Hiby and Lovell, 1998). In the cases of an additional two observers behind and isolated from the ‘primary’ two observers (‘double platform’ approach), only perception bias would be accounted for, and availability bias would need to be derived from diving/surfacing times independently.

Correction for such biases can be significant and is essential to obtain the best estimates of absolute abundance. However, trends can be assessed also using uncorrected abundance when data collection methods and estimation models are the same and it can be assumed that the levels of these biases remain constant over time. While the uncorrected estimates can be used as minimum estimates for management, corrected estimates need to reliably assess the impact of human-induced mortality, such as bycatch. For example, using the estimates obtained through the model-based analysis and the correction factors of 0.364 for the harbour porpoise and 0.805 for common dolphins under good sighting conditions in the European Atlantic waters and the North Sea area (SCANS) (Hammond et al., 2017), the abundance estimates for the study area would be around 258,000 harbour porpoises and 135,000 common dolphins on a model-based estimation basis. Although the justification of the use of correction factors from the NE Atlantic to the Black Sea is debatable, given that the Black Sea subspecies might be different surfacing/diving times, such rough numbers suggest the importance of obtaining local data to correct the Black Sea estimates. For example, the corrected estimate for the harbour porpoise helps to provide a conservative estimate of its bycatch (11,826 - 16,200 individuals) in the Black Sea that suggest annual bycatch affects 5–7% of the estimated population (Popov et al., 2023). Non-corrected abundance estimates could be challenged as unreliably high as it will mean the annual bycatch

TABLE 3 Results of the design-based analysis for Black Sea common dolphins.

Stratum	Area km ²	n groups	mean group size*	exp. group size**	CV exp. group size	Effort (km)	Enc. Rate groups (per km)	CV Enc. rate groups	Density (Anim./ km ²)	Abundance	CV	95% Confidence Interval	
Bulgaria	32683	75	2.47	2.48	0.1362	1115.5	0.1658	0.1973	0.3678	12022	0.21	8000	18068
Georgia	6237	7	4.29	4.29	0.2771	180.8	0.1659	0.3986	0.3508	2188	0.40	1002	4776
Romania	18611	27	2.78	2.78	0.3126	816.3	0.0919	0.4016	0.1790	3331	0.40	1533	7242
Türkiye1	71796		2.52	2.52	0.0937	2002.5	0.2881	0.1443	0.5698	40910	0.14	31176	53682
Türkiye2	69785	310	2.31	2.30	0.0661	2146.3	0.3331	0.1219	0.6562	45796	0.12	35851	58498
Ukraine	21057	15	1.87	1.92	0.2515	767.4	0.0365	0.3523	0.0769	1620	0.37	798	3288
Total	220169	663	2.43	2.43	0.0501	7028.8	0.2401	0.0820	0.4808	105867	0.08	90004	124527

Mean group size” is the mean of the observed group sizes, while *“expected group size” is the result of dividing the estimated abundance of individuals by the estimated abundance of groups.

TABLE 4 Results of the design-based analysis for Black Sea bottlenose dolphins.

Stratum	Area km ²	n groups	mean group size*	exp. group size**	CV exp. group size	Effort (km)	Enc. Rate groups (per km)	CV Enc. rate groups	Density (Anim./ km ²)	Abundance	CV	95% Confidence Interval	
Bulgaria	32683	15	3.47	3.38	0.1856	1115.5	0.0134	0.3933	0.1344	4394	0.39	2081	9280
Georgia	6237	0		0	–	180.8	0	0	0	0	0	0	0
Romania	18611	32	2.50	2.34	0.2467	816.3	0.0392	0.2579	0.3912	7281	0.42	3303	16048
Türkiye 1	71796	35	2.94	3.26	0.1696	2002.5	0.0175	0.2097	0.1658	11905	0.32	6417	22087
Türkiye 2	69785	0	0.00	0	–	2146.3	0	0	0	0	0	0	0
Ukraine	21057	14	3.29	3.13	0.2472	767.4	0.0182	0.2812	0.2376	5003	0.41	2305	10857
Total	220169	96	2.93	2.96	0.1079	7028.8	0.0128	0.1368	0.1298	28583	0.23	18403	44393

level is above 10% (14–17%), resulting in extinction of the species in less than 10 years, which obviously is not the case.

4.2 Distribution, habitat preference and factors driving it

Based on the information on the variables available to us (Supplementary Table S2), the proxies of animal density shared by all the cetacean species were the geographic coordinates (when they were allowed in the model). This shows the importance of geographic area for understanding cetacean distribution in the Black Sea during our survey. However, it is clear that prey availability is one of, if not the major factor, determining distribution linked to the migration of fish species (e.g., Kleinenberg, 1956; Bushuev, 2000), and the incompleteness of updated quantitative information on this limits habitat preference modelling considerably. However, recent reviews summarising the distribution of most abundant fish species and status of their stocks are available (STECF, 2010; STECF, 2012; STECF, 2017), and can be considered for partial interpretations of cetaceans’ distribution. Also, there can be at least several other locality-related factors independent of, or weakly correlating environmental covariates assessed here: these include sources of underwater noise

(including marine traffic) and intra- and inter-species biotic interactions. When LonLat was not allowed in the model, amongst the proxies for cetacean occurrence there were the univariate longitude (Lon) and latitude (Lat) measures, distance from the 25, 50 and 1000 m isobaths (Dist25, Dist50, and Dist1000), the current intensity and surface salinity for common dolphins, the mean sea surface temperature (sst) for bottlenose dolphins, and chlorophyll concentration (chl) for harbour porpoises. Also, depth of the mixed layer is an important environmental factor in a meromictic Black Sea, where the water column is stratified and the deep waters are anoxic: not surprisingly, all the three species showed preference for its positive values, since all the prey fish were concentrated only in the oxygen rich layer (Zaitsev and Mamaev, 1997). Equally, the summer sea temperature (and especially the surface temperature) in the Black Sea comes to the higher limit of optimum for the most widespread fish species, such as sprat (*Sprattus sprattus*) and anchovy (*Engraulis encrasicolus*) (Dubinets and Gubanov, 1988), so the negative preference for it by cetaceans following their prey seems obvious. Changes in sea surface height (mean ssh anomaly) affect ocean dynamics, including the upwelling and mixing of nutrient-rich deep waters: negative values enhance upwellings and therefore higher levels of productivity, while positive values tend to suppress such upwellings (Chelton et al., 2011). E.g., common dolphins showed

TABLE 5 Results of the design-based analysis for Black Sea harbour porpoises.

Stratum	Area km ²	n groups	mean group size*	exp. group size**	CV exp. group size	Effort (km)	Enc. Rate groups (per km)	CV Enc. rate groups	Density (Anim./ km ²)	Abundance	CV	95% Confidence Interval	
Bulgaria	32683	316	1.71	1.69	0.0653	1115.5	0.2833	0.0926	1.3413	43838	0.13	33887	56711
Georgia	6237	0		0	–	125.9	0	0	0	0	0	0	0
Romania	18611	46	3.13	3.33	0.6116	795.3	0.0578	0.1975	0.6043	11246	0.66	3409	37098
Türkiye1	71796	177	1.68	1.74	0.0841	1584.3	0.1117	0.1071	0.5294	38008	0.13	29219	49439
Türkiye2	69785	97	1.39	1.36	0.0577	1716.8	0.0565	0.1601	0.2065	14408	0.17	10250	20253
Ukraine	21057	35	2.03	2.09	0.1353	754.7	0.0464	0.2683	0.3389	7137	0.34	3682	13835
Total	220169	671	1.78	1.76	0.0732	6092.6	0.1057	0.0605	0.5207	114637	0.0999	94225	139470

TABLE 6 Density surface modelling results.

Species	Without or with LonLat	Covariates	edf	p	Deviance explained (%)	AIC
B.S. common dolphins	Without LonLat	Dist1000	2.67	0.000089	24.72	2174.84
		Dist50	3.16	0.000000		
		ssc	1.09	0.000000		
		sss	0.97	0.000089		
	With LonLat	Lat,Lon	10.19	0.000001	30.74	2156.12
		DistAbyss	5.51	0.000001		
		chl	0.83	0.023726		
		ssc	1.01	0,000105		
sshn		2.92	0.000001			
B.S. bottlenose dolphins	Without LonLat	Dist50	4.71	0.005347	38.54	727.2944
		Lon	2.36	0.000000		
		sst	0.98	0.000543		
	With LonLat	DistAbyss	0.83	0.016721	39.07	727.9733
		Dist50	4.95	0.004659		
		mld	0.83	0.02013		
		Lat,Lon	2.02	0.000000		
B.S. harbour porpoise	Without LonLat	Dist25	1.18	0.000011	38.49	1855.28
		chl	0.93	0.000127		
		Lat	7.53	0.000001		
		Lon	12.86	0.000000		
	With LonLat	Lat,Lon	34.56	0.000000	47.09	1813.57

The LonLat syntagm was used to underline the two different models, “with LonLat” model allowing for the inclusion of the two-dimension spatial smoothness of the interaction between Longitude and Latitude and the “Without LonLat” does not allow this interaction.

The meaning of the covariates can be consulted in [Supplementary Table S3](#); edf, estimated degrees of freedom; p, significance of the covariate.

preference for negative values of mean ssh anomaly (i.e., higher productivity upwelling waters) during our survey.

4.2.1 Black Sea common dolphins

Common dolphin was the only species for which the model with LonLat was chosen because: a) the AIC was smaller, and b) the deviance explained was larger. The difference with the next model without LonLat was small, but no parameters suggested that we should choose the model without LonLat. The spatial model predicts that common dolphins are most abundant in the southern part of the Black Sea, off the coasts of Türkiye and Bulgaria (Figure 1). The number of sightings increases with a gradient from north to south in the western portion of the Black Sea, with a higher number of sightings starting from the border between exclusive economic zones (EEZ) of Bulgaria and Romania. A relatively small group size was recorded by our study, concurrent with other surveys of the post-industrial dolphin fishery era

(Mikhalev, 2008; Birkun et al., 2014) and possibly showing long-term alterations of the population structure.

Underwater profile seems to be a proxy for the distribution of common dolphins, with higher densities in deeper waters, and lower densities in the shallower northwestern coastal water of the Black Sea. These findings concur with views of earlier authors (e.g. Zalkin, 1938a; Freiman, 1950; Kleinenberg, 1956; Mikhalev, 2008) and more recently (Dede and Tonay, 2010; Birkun et al., 2014; Sánchez-Cabanes et al., 2017). A straightforward explanation for this is their preference for and association with aggregations of the most abundant fish species (e.g. see Kleinenberg, 1956; Dubinets and Gubanov, 1988; Birkun, 2002b; Mikhalev, 2008; Gol'din et al., 2017; Bilgin et al., 2018), most importantly the anchovy (*Engraulis encrasicolus*) and the sprat (*Sprattus sprattus*), which are the primary prey items for common dolphins (Zalkin, 1940; Kleinenberg, 1956). The anchovy is an epipelagic species and the sprat is vertically migrating between the sea bottom and surface

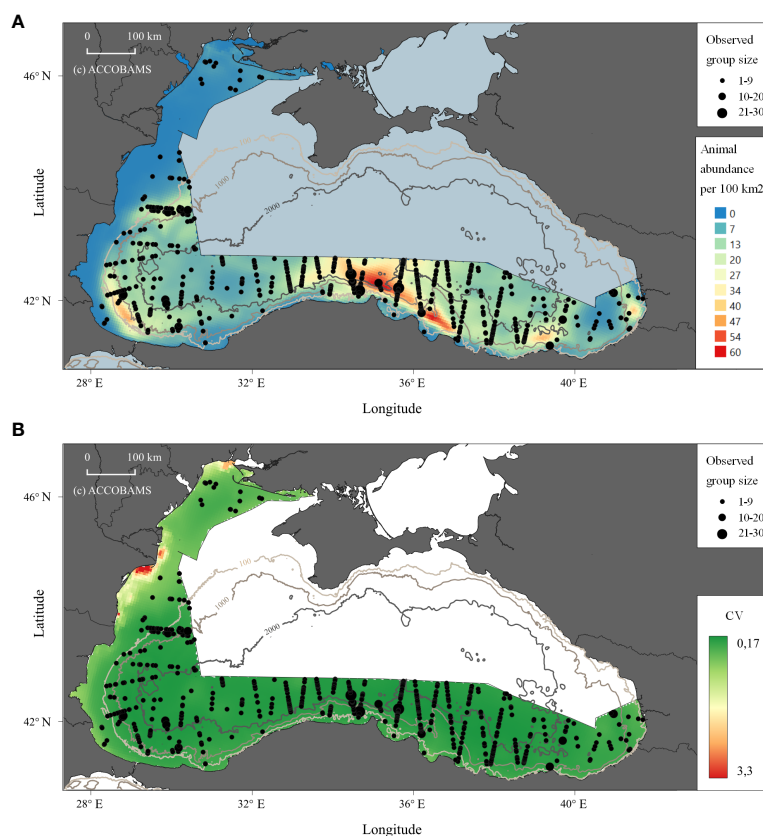


FIGURE 1 Prediction of abundance of animals for Black Sea common dolphins modelling in the study area. (A) Density shown as the animal abundance/100km². (B) CV for the density.

layers. Both species in their summer distribution prefer the shelf areas of 15–110 m deep (sprat) or shelf and sea slope areas of 100–200 m deep (anchovy), and the area of maximum preference by common dolphins (100–500 m deep) fits the area simultaneously occupied by these fishes in summer (STECF, 2010; STECF, 2012; STECF, 2017). Importantly, the sprat and anchovy at present are by far the most abundant (by biomass) fish species in the Black Sea (STECF, 2017) and therefore the common dolphin has the best historically preserved prey resource in comparison to the other cetaceans.

4.2.2 Black Sea bottlenose dolphins

Sightings of Black Sea bottlenose dolphins in all the strata were rarer than those of two other Black Sea cetacean species. The spatial model predictions for the Black Sea bottlenose dolphins (Figure 2) showed a relatively uniform pattern in the waters of Ukraine, Romania and Bulgaria, preference for shallower and coastal waters (not uncommon for this species). This was also true for the coast of Türkiye, in the western part, but with no sightings east of 34° E, including the waters of Georgia. Association with shallow depths in the western Black Sea concurs both with historical evidence (Kleinenberg, 1956) and more recent research (Shpak et al., 2006; Dede and Tonay, 2010; Kopaliani et al., 2015; Panayotova and Todorova, 2015; Gladilina and Gol'din, 2016; Gladilina et al., 2017;

Paiu et al., 2019; Uludüz et al., 2020). Çelikkale et al. (1989) also reported that this species was found in the western and central Turkish Black Sea coast, but rarely seen along the eastern Black Sea coast, with no sighting east of 36°E (Samsun). There were very few sighting records in the eastern Turkish Black Sea and Georgia between March and May 2010 (Sánchez-Cabanes et al., 2017) in Ordu near 38°E in 2021 (Özsandıkçı et al., 2022), and some strandings have been recorded around longitude 35°E (Sinop) by Özsandıkçı et al. (2019). In addition, data from earlier surveys in Georgian waters showed the abundance of bottlenose dolphins was low, 100 to 150 individuals, and they were unevenly distributed (Kopaliani et al., 2015).

Factors underlying occurrence or distribution of Black Sea bottlenose dolphins are less evident than for common dolphins. They are opportunistic feeders with a flexible feeding behaviour (Bushuev, 2000). Amongst their prey items, there are at least 23 fish species, and the pelagic horse mackerel (*Trachurus* spp.) and benthic dwelling, vertically migrating whiting (*Merlangius merlangus*) were found to be the dominants (Gladilina and Gol'din, 2014). However, pelagic sprats and anchovies are equally important for some stocks or seasons, and regular foraging near trawling vessels is observed (Gladilina, 2018). Therefore, these prey preferences alone hardly can explain the patchy distribution of the Black Sea bottlenose dolphins.

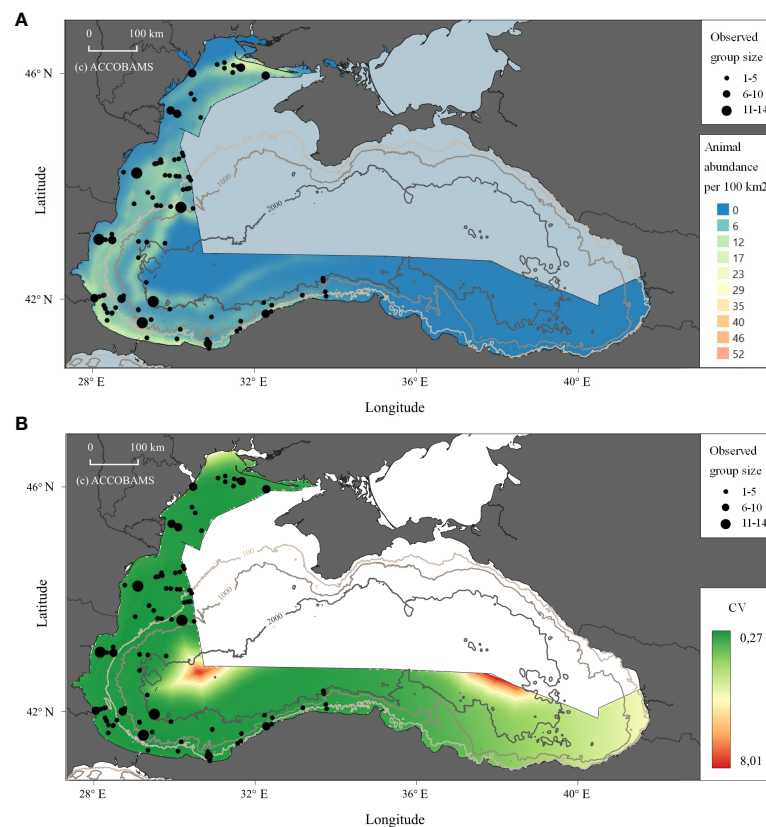


FIGURE 2
Prediction of abundance of animals for Black Sea bottlenose dolphins in the study area. (A) Density shown as the animal abundance/100km². (B) CV for the density.

Black Sea bottlenose dolphins are believed to have a complex sub-population structure, forming a coastal metapopulation, as seen from several recent photo identification studies (Gladilina, 2018; Gladilina et al., 2018). A number of local areas with summer resident or seasonally migrating local groupings have been identified in recent studies (Kopaliani et al., 2015; Gladilina et al., 2017; Gladilina, 2018; Akkaya Baş et al., 2019). It can be suggested that specific cultural traditions of feeding and migration patterns for local stocks, rather than common physical or trophic factors, could explain the overall uneven distribution of bottlenose dolphins in the Black Sea.

4.2.3 Black Sea harbour porpoises

Black Sea harbour porpoises (Figure 3) were the most commonly sighted cetacean species during the survey. Sightings peaked in western waters (Bulgarian and south Romanian EEZ) and decreased in the north and the southeastern Black Sea, with even fewer observations towards the eastern part of the study area, off Türkiye and in Georgia. Similarly, fewer sightings were recorded towards Ukraine, although some individuals occurred in shallower waters in the northern part of the Ukrainian study area.

The distribution found in this survey partly follows previously reported 'preferences' (Kleinenberg, 1956), with sightings close to shelf waters, the highest preference for 50 m deep. Another preference was found for slope habitats which are often situated

at areas 50–200 m deep. Nevertheless, the overall distribution was broader with sightings close to the coast, mainly in the western Black Sea and along the central-east Turkish coasts, as well as offshore, in deep Turkish waters. Historically, such an offshore distribution was pointed out by Mikhalev (2005a).

No sightings were recorded in southeastern (Georgian) waters. The species has been observed there (Kopaliani et al., 2015), with reported seasonal fluctuations in occurrence with the fewest sightings in summer. Combined with our data, this shows seasonality with low summer densities in the eastern Black Sea.

The spatial model predicts highest harbour porpoise density along the border between Bulgaria and Romania. While this concurs with relatively recent evidence from the western Black Sea (Birkun et al., 2014), evidence from the 1980s showed four summer hotspots in the northwest, southwest, northeast and southeast (Mikhalev, 2005a) but only a single southwestern hotspot was confirmed by this survey, despite covering three of the four historical hotspots. Also, interestingly, the spatial model did not predict hotspots in the other areas. It might be only speculated that the environmental proxies for the past distribution of the porpoises could have changed and shifted to a single western area.

The distribution of porpoises, small animals with high energy intake (Yasui and Gaskin, 1986; Rojano-Doñate et al., 2018), would

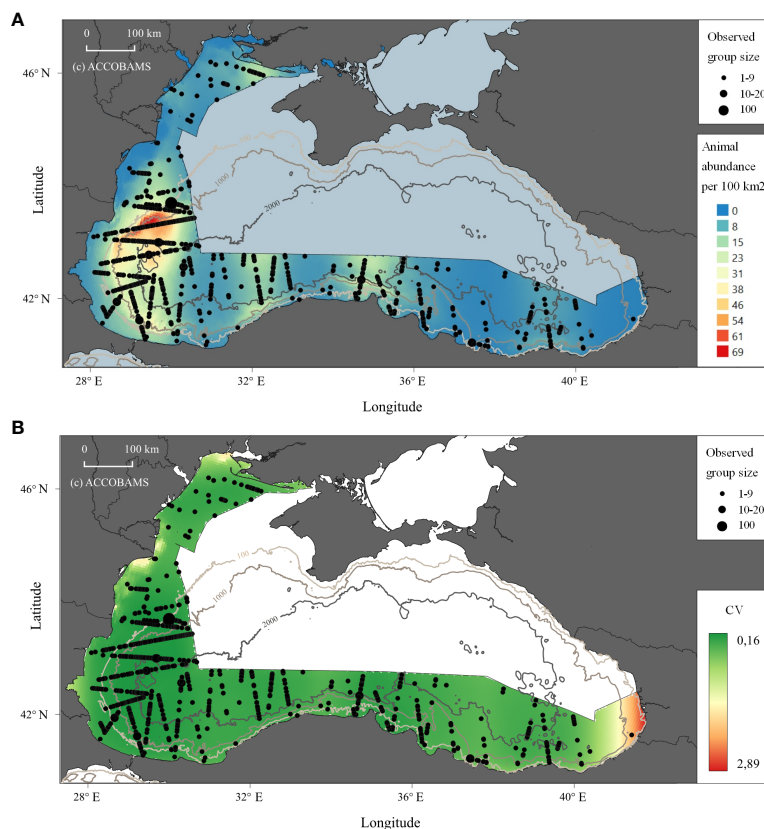


FIGURE 3
Prediction of abundance of animals for Black Sea harbour porpoises in the study area. (A) Density shown as the animal abundance/100km². (B) CV for the density.

be expected to be driven by the distribution of their prey, especially during the summer, which is the nursing season. The main prey species for Black Sea harbour porpoises are sprats and small whittings (Tonay et al., 2007: data for April-June), anchovies, horse mackerels, sand smelts (*Atherina* spp.), and the once dominating and preferred gobies (Zalkin, 1940; Kleinenberg, 1956; Bilgin et al., 2018). Porpoise summer distribution would be expected to be linked to shallow waters if there are gobies or sand smelts in the area, shelf waters for sprats and whittings, and pelagic grounds for anchovies and horse mackerels (Dubinets and Gubanov, 1988; Yakovlev, 1995; STECF, 2010; STECF, 2012; STECF, 2017). Thus, they are mostly observed across the northwestern and northern waters of the Black Sea (Bulgarian and Romanian EEZs) shelf and sea slope zone, for the offshore areas in correlation with the pelagic fish aggregation. The low-density areas recorded in this study are similar to the densities recorded earlier in the northernmost Ukrainian Black Sea (Birkun et al., 2003, 2004).

4.2.4 Considerations for the area not covered by the survey

Clearly, cetaceans inhabit the areas of the Black Sea which were not covered by this survey. There are some recent and many historical data on cetacean occurrence and distribution in the northern and north-eastern Black Sea areas, in the waters of

Crimea and Caucasus. For example, common dolphins were commonly recorded during the summer throughout the Black Sea, including its central part, at depths of 100–1000 m, quite similar to this study (Mikhalev, 2008; Birkun et al., 2014), and it is not unlikely that there is a single basin wide pattern of distribution of this species (Mikhalev, 2008). Bottlenose dolphins form several local aggregations of different size in coastal waters of Crimea and Caucasus including the Kerch Strait and visiting the Azov Sea (Gladilina, 2018; Gladilina et al., 2018); amongst them, particularly large is the aggregation in the waters of the south-eastern Crimea which can number 1500 individuals or even more (Gladilina, 2012; Gladilina and Gol'din, 2016; Gladilina et al., 2018). Other large aggregations can be found near the southern Crimea (Birkun et al., 2014; Gladilina, 2018) and Caucasus (Shpak et al., 2006). However, the details of their overall distribution (especially in offshore areas) or abundance, as well as common environmental predictors, are mostly obscure (Mikhalev, 2005b; Gladilina, 2018), while cultural traditions of prey chasing, or depredation may be important in some areas (Gladilina, 2018). The situation with the distribution of the harbour porpoise is the least clear since it is subject to interannual fluctuations. At least, it is permanently present in Crimean waters, including the Kerch Strait (Gol'din, 2004; Vishnyakova, 2017). Also, the harbour porpoise inhabits the whole Azov Sea at the north-eastern corner of the Black Sea basin during the warm season, and the distinct population in that small

TABLE 7 Results of the model-based analysis for Black Sea common dolphins.

Without or with LonLat	Stratum	Area km ²	mean group size*	CV mean group size	Density (Anim./ km ²)	Abundance	CV	95% Confidence Interval	
Without LonLat	Bulgaria	32683	2.467	0.130	0.397	12962	0.13	10126	16592
	Georgia	6237	4.286	0.133	0.489	3043	0.21	2028	4566
	Romania	18611	2.778	0.322	0.136	2525	0.13	1966	3243
	Türkiye1	71796	2.52	0.082	0.549	39277	0.09	33039	46692
	Türkiye2	69785	2.306	0.062	0.655	45525	0.09	37985	54561
	Ukraine	21057	1.867	0.227	0.090	1800	0.40	850	3813
	Total	220169	2.428	0.047	0.479	105133	0.07	92502	119488
With LonLat	Bulgaria	32683	2.467	0.130	0.395	12885	0.15	9702	17113
	Georgia	6237	4.286	0.133	0.468	2906	0.33	1561	5411
	Romania	18611	2.778	0.322	0.130	2424	0.22	1580	3718
	Türkiye1	71796	2.52	0.082	0.560	40094	0.10	32928	48819
	Türkiye2	69785	2.306	0.062	0.697	48432	0.10	39862	58844
	Ukraine	21057	1.867	0.227	0.074	1542	0.36	773	3075
	Total	220169	2.428	0.047	0.493	108283	0.07	94876	123584

sea is believed to be endangered (Vishnyakova, 2017). Thus, future effort extending the assessment of distribution and abundance of cetaceans onto the whole Black Sea will certainly improve data analysis and modelling frameworks. Moreover, it would be impossible to draw any basin wide extrapolations by simple arithmetical operations or assumptions, since the distribution of cetaceans is uneven and it varies interannually.

4.3 Historical changes in abundance and distribution

Cetacean populations in the Black Sea are believed to have undergone a serious decline during the 20th century. The pristine state of total cetacean population in the Black Sea is unknown, but some estimates exist. Zemsky and Yablokov (1974) and Zemsky

TABLE 8 Results of the model-based analysis for Black Sea bottlenose dolphins.

Without or with LonLat	Stratum	Area km ²	mean group size*	CV mean group size	Density (Anim./ km ²)	Abundance	CV	95% Confidence Interval	
Without LonLat	Bulgaria	32683	3.47	0.234	0.195	6277	0.21	4194	9395
	Georgia	6237	0.00	-	0	0	-	-	-
	Romania	18611	2.50	0.227	0.194	3608	0.19	2515	5177
	Türkiye1	71796	2.94	0.149	0.130	8903	0.16	6489	12215
	Türkiye2	69785	0.00	-	0.001	81	0.76	22	304
	Ukraine	21057	3.29	0.275	0.184	3851	0.27	2293	6469
	Total	220169	2.93	0.104		22720	0.15	16965	30427
With LonLat	Bulgaria	32683	3.47	0.234	0.208	6569	0.20	4484	9623
	Georgia	6237	0.00	-	0	0	-	-	-
	Romania	18611	2.50	0.227	0.179	3328	0.22	2169	5106
	Türkiye1	71796	2.94	0.149	0.123	8549	0.22	5555	13158
	Türkiye2	69785	0.00	-	0.004	257	0.49	103	643
	Ukraine	21057	3.29	0.275	0.238	4963	0.38	2422	10169
	Total	220169	2.93	0.104	0.110	23666	0.16	17438	32118

TABLE 9 Results of the model-based analysis for harbour porpoise.

Without or with LonLat	Stratum	Area km ²	Mean group size*	CV mean group size	Density (Anim./km ²)	Abundance	CV	95% Confidence Interval	
Without LonLat	Bulgaria	32683	1.71	1.022	0.995	32412	0.10	26898	39057
	Georgia	6237			0.004	26	1.14	4	156
	Romania	18611	3.13	4.382	0.337	6258	0.16	4582	8547
	Türkiye1	71796	1.68	1.106	0.524	37162	0.10	30570	45176
	Türkiye2	69785	1.39	0.532	0.168	11592	0.15	8577	15666
	Ukraine	21057	2.03	0.841	0.307	6358	0.28	3744	10797
	Total	220169	1.78	2.319	0.430	93808	0.06	82771	106317
With LonLat	Bulgaria	32683	1.71	1.022	0.977	31887	0.09	26666	38131
	Georgia	6237			0.003	20	1.30	3	140
	Romania	18611	3.13	4.382	0.333	6194	0.14	4674	8209
	Türkiye1	71796	1.68	1.106	0.494	35174	0.10	28715	43085
	Türkiye2	69785	1.39	0.532	0.168	11579	0.16	8457	15853
	Ukraine	21057	2.03	0.841	0.985	19802	0.96	4046	96915
	Total	220169	1.768	2.319	0.482	104657	0.20	71433	153334

(1975) suggested it was between 1,500,000 and 2,000,000. Arseniev et al. (1973) stated that the latter estimate was for common dolphins only, and thus the total population was larger. The animals' distribution was described as ecologically stratified: common dolphins were believed to be mostly pelagic, while bottlenose dolphins and harbour porpoises were primarily or solely coastal.

All the Black Sea cetaceans were extensively hunted between 1931 and 1983 (Kleinenberg, 1956; Smith, 1982; Birkun et al., 2014). The overall catch during this period could exceed 5,000,000 animals (Zemsky, 1996; Birkun, 2002a), and the abundance of common dolphins was thought to have dropped to below 200,000 individuals (Arseniev et al., 1973). Extensive bycatch in bottom gillnets in all the Black Sea added to the direct takes (Pavlov et al., 1996; Birkun et al., 2014). In addition to these removals, the habitat quality of the Black Sea severely deteriorated in 1970–2000 (Daskalov, 2002; Daskalov, 2003), and, despite some improvement during the last decade (Zhang et al., 2020), probably is still well below its pristine state. Changes in river catchment, overfishing, IUU fishing, eutrophication, chemical pollution, degradation of coastal habitats and invasions of alien species eventually led to decline of prey fish stocks (Daskalov, 2003), which have likely become the major factor limiting the restoration of cetacean populations (Bushuev, 2000).

The first direct estimates of cetacean abundance were produced from aerial surveys covering the northern Black Sea, although at that time the methods used were based on extrapolative assumptions and did not include correction on detection probability and confidence intervals (Buckland et al., 1992) (this is not the case here, where the results reflect solely the abundance estimates of the surveyed areas). The 1967–73 average abundance of the common dolphin was estimated at around 155,000 and the bottlenose dolphin as around 85,000 (Zemsky and Yablokov, 1974;

Smith, 1982). Further estimates from 1977–86 showed the average abundance of common dolphins as around 140,000 and bottlenose dolphins as around 50,000 (Bushuev, 2002). Çelikkale et al. (1989), based on vessel surveys of 1987 in the southern Black Sea, estimated cetacean abundance in the entire Black Sea as around 454,000 animals: of which some 53% (237,000) were thought to be harbour porpoises, 33% (149,820) common dolphins and 15% (67,257) bottlenose dolphins.

However, the peak of cetacean population decline is believed to have been reached due to the depletion of fish stocks, particularly after the outbreak of the invasive ctenophore *Mnemiopsis leidyi* in the late 1980s (Vinogradov et al., 1989; Shiganova and Bulgakova, 2000). The subsequent mass mortality of cetaceans in 1989–90, as well as bycatch (Pavlov et al., 1996; Tonay and Öztürk, 2012), primarily affected the harbour porpoise. It has been suggested that the 1988–1997 decade was the time of lowest cetacean abundance in the Black Sea (Birkun, 2002a; Birkun, 2002b), before another alien ctenophore *Beroe ovata* effectively regulated *Mnemiopsis* population and brought some balance to the food webs (Shiganova et al., 2000). Another important change found since the 1970s was the shift in distribution of the harbour porpoises and bottlenose dolphins, which began to frequently occur in pelagic waters (Mikhalev, 2005a; Mikhalev, 2005b). This was interpreted as a response to depletion of once richest benthic fish stocks (Bushuev, 2000).

In 2013, a combined aerial and vessel survey of cetacean abundance using distance sampling methods was conducted in the western Black Sea and included the entire waters of Bulgaria and Romania and, partially, Ukraine (Birkun et al., 2014): the abundance of common dolphins was approximately 60,400 (95% CI = 41316 – 88298), with 26,400 (95% CI = 19586 – 35751)

bottlenose dolphins, and 29,400 (95% CI = 19568 – 44368) harbour porpoises. These more robust estimates are difficult to compare with previous estimates due to *inter alia* differences in the surveyed areas. However, the abundance estimates presented in the current study are generally comparable with those by [Birkun et al. \(2014\)](#), although comparison can be made between data on Romanian and Bulgarian waters, the only area for which a direct comparison. It shows differences for all the species, most probably due to interannual variation in distribution ([Supplementary Table S4](#)). The most significant new feature is the single hotspot of harbour porpoises observed in the western Black Sea in 2019.

Overall, the current distribution patterns in the western and southern parts of the Black Sea are similar to those reported for the 1980s. Recognising that methodological differences render comparisons difficult, the data presented here show that cetacean populations in the Black Sea are still below their pristine state. Their abundance has not reached back to the baseline estimates of the 1930s and no indications for its growth are observed. However, since direct hunting has stopped, at a broad level, it seems that the abundance of common dolphins and possibly harbour porpoises may have returned to the level of 1977–1987, whereas the population of bottlenose dolphins has not.

The Black Sea cetaceans are dependent on small sized pelagic fish known for their fluctuations in abundance ([Galtsoff, 1924](#); [Shulman, 2002](#); [Yankova, 2011](#)). They are also vulnerable to various factors such as underwater noise, bycatch in fishing gears, epidemics, and pollution ([Notarbartolo di Sciara et al., 2002](#); [Birkun, 2008b](#); [Notarbartolo di Sciara and Tonay, 2021](#)) and new potential threats posed by regional conflicts such as wars ([Vishnyakova et al., 2023](#); [Vyshnevskiy et al., 2023](#)). To elaborate long-term effective conservation strategies for these cetaceans, regular surveys covering wider areas and seasons should be realised, as well as appropriate mitigation measures to reduce or eliminate anthropogenic pressures.

5 Conclusions

1. The abundance estimates, obtained by us for 52% of the Black Sea during the 15-day survey at good weather conditions, are less than the hypothetical historical estimates of 1.5–2 million individuals, but they are broadly comparable, with all the precautions and methodological limitations, to those for 1977–87 and more recent ones which were also based on partial sea surveys. This implies that the cetacean populations in the Black Sea have not fully recovered from various human pressures, including direct hunting (continued until 1983) or on-going bycatch in fisheries. Common dolphins and bottlenose dolphins hypothetically passed the lowest point of population decline, as they are under less heavy pressure of bycatch, than harbour porpoises. But new war related threats in most recent years could change this.
2. The distribution of common dolphins and harbour porpoises in the Black Sea can be broadly associated with prey availability in a changing ecosystem, primarily the sprat and anchovy and also whiting, horse mackerel and possibly other fishes. Additional factors may also be important for the bottlenose dolphin. The resources of prey species historically preferred by common dolphins (sprat and anchovy) are in a better condition than those for other cetacean species. Finally, all the species share epipelagic fish as a prey resource and therefore occur in the open sea waters.
3. Shifts in the hotspots of the species distribution were identified from the historic literature, mainly for Black Sea harbour porpoises and Black Sea bottlenose dolphins, together with low or no sightings in areas known to be used by both species. This underlines the need of a broad scale, long-term monitoring programme to be implemented. Considering the seasonal difference in distribution of cetaceans, the temporal and spatial coverage should be increased.
4. These results provide essential information for the assessment of the Criteria under main Descriptor D1, marine biodiversity, in particular for D1C2 (cetaceans populations abundance) and D1C4 (cetacean distributional range), within the framework of European Commission's Marine Strategy Framework Directive. In addition, they could contribute to the other descriptors and should be evaluated in relation to human pressures such as anthropogenic underwater noise and fisheries interactions.
5. This first synoptic, collaborative, and coordinated aerial survey for cetaceans in the Black Sea yielded comprehensive data and the first robust insights on global abundance, distribution and density for all three cetacean species. This systematic effort, complemented with previous data, has provided robust baseline information for identifying Important Marine Mammal Areas (IMMAs) ([IUCN Marine Mammal Protected Areas Task Force, 2021](#); [Tetley et al., 2022](#)), evaluating their current conservation status under the IUCN criteria for Red List Species, assessing their population trends in line with ACCOBAMS provisions and allowing coastal countries to fulfil their commitments under different legal frameworks, such as the EU Habitats Directive, Black Sea Integrated Monitoring and Assessment Programme (Black Sea Commission - BSC).
6. Replication of this large-scale effort should be considered at least every 6 years, following the EU MSFD cycles, to allow the creation of a robust time series, to be used for identification of temporal and spatial trends. The Black Sea Integrated Monitoring and Assessment Programme of BSC as well as Long Term Monitoring Programme of ACCOBAMS (adopted in Malta, during the MOP 8, December 2022, with a specific Resolution) could provide a framework for the basin-wide synergetic cooperation.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: EMODNET Biology (https://ipt.vliz.be/upload/resource?r=marine_mammal_sightings_black_sea_recorded_by_cenobs_aerial_expedition_2019) for the CeNoBS datasets and at by request on the ACCOBAMS website (<https://accobams.org/asi-data-access-request/>). The datasets analysed for this study can be found in the text of this paper and publicly available at <https://cenobs.eu/content/deliverables>.

Ethics statement

Ethical review and approval was not required for the study on animals in accordance with the local legislation and institutional requirements.

Author contributions

RMP, SP, DP, and PG planned the study; RMP, DP, AD, GM, AT, CT, and PG collected data; AC led the analysis; RMP, SP, DP, AO, and PG analysed data with input from GM, AD, CT, NK, DM, and AC. RMP, PG, DP, and SP wrote the initial manuscript draft with input from all the authors. 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.

The handling editor RC declared a past co-authorship with the author AD.

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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.2024.1248950/full#supplementary-material>

SUPPLEMENTARY FIGURE 1

Survey plan used during the sampling over the 6 strata.

SUPPLEMENTARY FIGURE 2

Collinearity plot for fixed and dynamic covariates

SUPPLEMENTARY FIGURE 3

(A) Q-q plot (left) and (B) detection function (right) for B.S. common dolphins. The detection function is scaled to 1.0 at the left-truncated perpendicular distance, and the histograms represent the frequency of the observed sightings at different perpendicular distances. Dots represent individual sightings and the effect of the covariates considered. Kilometre is the distance measurement unit.

SUPPLEMENTARY FIGURE 4

(A) Q-q plot (left) and (B) detection function (right) for B.S. bottlenose dolphins. The detection function is scaled to 1.0 at the left-truncated perpendicular distance, and the histograms represent the frequency of the observed sightings at different perpendicular distances. Dots represent individual sightings and the effect of the covariates considered. Kilometre is the distance measurement unit.

SUPPLEMENTARY FIGURE 5

(A) Q-q plot (left) and (B) detection function (right) for harbour porpoise. The detection function is scaled to 1.0 at the left-truncated perpendicular distance, and the histograms represent the frequency of the observed sightings at different perpendicular distances. Dots represent individual sightings and the effect of the covariates considered. Kilometre is the distance measurement unit.

SUPPLEMENTARY TABLE 1

Covariates tested in the models and their ranges or factor levels.

SUPPLEMENTARY TABLE 2

Covariates tested in the spatial models. The dynamic covariates sst and chl-a were obtained from SeaWiFS and MODIS-Aqua sensors and the sst of MODIS-Terra and MODIS-Aqua. Depth was extracted from ETOPO (a 1 arc-minute global relief model of Earth's surface that integrates land topography and ocean bathymetry, <https://ngdc.noaa.gov/mgg/global/global.html>). Its derivatives were obtained using ArcGIS 10.5.

SUPPLEMENTARY TABLE 3

Parameters and results of the detection functions. Codes: Truncation: L= left truncation (km), R= right truncation (km); n = number groups in detection function; key function HR =hazard-rate; p=probability of detection; CV p = coefficient of variation of the probability of detection; esw = effective half-strip width (km); CvM p = p-value of the Cramér-von Mises goodness of fit.

SUPPLEMENTARY TABLE 4

Comparing the abundance estimates of CeNoBS and Birkun et al. (2014) for Romania and Bulgaria.

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Temporal patterns in dolphin foraging activity in the Mediterranean Sea: insights from vocalisations recorded during the ACCOBAMS Survey Initiative

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Marine organisms continually adapt their physiology and behaviour to temporal variations in their environment, resulting in diurnal rhythmic behaviour, particularly when foraging. In delphinids, these rhythms can be studied by recording echolocation clicks, which can provide indicators of foraging activity. The foraging rhythms of delphinids and their relationship to temporal parameters are poorly documented and most studies so far have used moored passive acoustic systems. The present study provides, for the first time, information on the activity rhythms of delphinids investigated in relation with temporal variables at a basin scale from a moving platform, in the western and central Mediterranean Sea. We used passive acoustic recordings collected by hydrophones towed along transect lines during the ACCOBAMS Survey Initiative in the summer 2018. We extracted variables that may influence daily and monthly rhythms, including time of day, lunar cycle, lunar illumination and sea state and fitted generalised additive models. The nycthemeral and lunar cycles were the two main factors influencing dolphin activity rhythms. Echolocation activity was predominant at night, with a maximum of 0.026 acoustic events per minute at 21:00/22:00 compared to as few as 0.0007 events per minute at 11:00. These events were also more frequent during the third quarter of the moon; 0.033 acoustic events on day 22 of the lunar cycle as opposed to 0.0008 on day 8 of the lunar cycle, corresponding to the first quarter of the moon. Variations in the echolocation activity of delphinids in the Mediterranean Sea could reflect variation in their foraging effort and be related to prey density, composition, accessibility and catchability within dolphin foraging depth range. These results should also improve interpretation of passive acoustic monitoring data.

KEYWORDS

foraging activity rhythms, Generalised Additive Models (GAMs), delphinids, echolocation clicks, Mediterranean Sea

1 Introduction

Marine animals live in habitats that are subject to temporal variations that can be seasonal, lunar or daily and that shape the adaptive strategies of each species (Häfker et al., 2023). Species adapt their physiology and behaviour to these environmental fluctuations and associated rhythms (Pirota et al., 2020). These adaptations result in biological activity being in phase with the main natural cycles, which are governed by the Earth's orbit around the Sun (seasons), the Moon's orbit around the Earth (tides) and the Earth's rotation (nycthemeron). Biological rhythms are controlled by endogenous systems, some of which are light sensitive. Changes in light levels can trigger physiological or behavioural responses (Simonis et al., 2017). Therefore, the nycthemeral cycle can be the source of daily changes and adaptations, although the exact nature of these rhythms also depends on other factors such as habitat type, coordinates, and physicochemical properties (Häfker et al., 2023). The aggregation of marine predators for feeding has been shown to be associated with primary production, bathymetry, and sea surface temperature (Hastie et al., 2004; Prieto et al., 2017; Scales et al., 2016).

The best known example of a biological rhythm in the pelagic environment is the Diel Vertical Migration (DVM) of the Deep Scattering Layer (DSL), composed mainly of planktonic and micronectonic organisms such as a variety of crustaceans (euphausiids, mysids, copepods), fish (e.g., myctophids) or cephalopods (e.g., histioteuthids) which generally migrate towards the surface layer at dusk and return to the depths at dawn (Wang et al., 2019). According to Marohn et al. (2021), this phenomenon would reflect a balance between meeting food requirements and avoiding predators. The most plausible hypothesis is that the vertical migrations of marine organisms at the base of the food web lead to similar rhythms in their mesopelagic and epipelagic predators, such as number of fish, squid and sharks (Häfker et al., 2023) which forage when their prey is most efficiently exploited. Similarly, air-breathing diving top predators such as cetaceans, that feed on these meso- and epipelagic species, would develop a strategy in which the optimal decision about where and when to feed would allow them to maximise their energy intake, especially as these predators must hold their breath during dives to access resources (Miller et al., 2010). The challenge for these marine mammals is to find the best compromise between breathing at the surface and feeding at depth. To achieve this, they are likely to adapt their feeding rhythms to the dynamics, availability, or catchability of their prey (Giorli et al., 2016).

In pelagic ecosystems, cetacean activity tends to be nocturnal, with an increase in activity in the evening, followed by a peak at night and a decrease in the early morning (Linnenschmidt et al., 2013). This pattern likely occurs due to the greater availability of prey closer to the sea surface at night. Furthermore, in demersal ecosystems, cetaceans that prey on bottom-dwelling fish, many of which are not particularly active at night, may exhibit different diel activity patterns (Brandt et al., 2014). In the Mediterranean Sea, where neritic habitats are quite limited, Giorli et al. (2016) highlighted that several typically offshore dwelling cetaceans (Risso's dolphin *Grampus griseus*, long-finned pilot whale

Globicephala melas, Cuvier's beaked whale *Ziphius cavirostris*, and sperm whale *Physeter macrocephalus*) exhibited mostly nocturnal foraging activity. Similarly, Caruso et al. (2017) studied several delphinids (striped dolphin *Stenella coeruleoalba*, bottlenose dolphin *Tursiops truncatus*, common dolphin *Delphinus delphis*, Risso's dolphin and long-finned pilot whale) in the Ionian Sea and found a daily pattern in foraging activity, with greater activity at night. The explanatory hypothesis put forward in these two studies is that the foraging behaviour of odontocetes would be dictated by the presence and dynamics of their prey, which are more active and putatively easier to capture at night.

There are several approaches to study cetacean activity rhythms. Longitudinal approaches (e.g., biologging techniques and focal tracking) have the advantage of following the activity of known individuals over time (Brauer et al., 2022), although they can be very time-consuming to implement and, in the case of visual tracking, are typically dependent on good weather and daylight conditions. Cross-sectional approaches sample activity data across a population as a function of time, for example during acoustic or visual surveys. They provide rapid results that reflect the general characteristics of a population. However, individual variability is not measurable (Brauer et al., 2022). The distribution, dynamics and activity rhythms of vocalising cetaceans can be studied using this second method, thanks to acoustic surveys (Barlow et al., 2021). Here we used a cross-sectional approach to investigate delphinid foraging activity using a large-scale acoustic line-transect survey.

Sound emission is common to all cetaceans, which have developed highly sophisticated sound production systems. Sound plays an important role in their daily lives, being used for communication, socialisation, navigation, and predation (Hildebrand, 2009). Vocalisations are also used to compensate for poor visibility conditions such as night, depth and turbidity, and are a critical component of the evolutionary success of odontocetes in general (Au, 1993). Odontocetes emit different types of acoustic signals, sometimes near the surface, sometimes at depth (Gillespie et al., 2009), which can travel long distances (probably tens of kilometres in some whale species, Bittle and Duncan, 2013). Acoustic signals emitted by odontocetes can be classified into three categories: tonal signals (or whistles), pulsed calls and echolocation clicks (Azzolin et al., 2014). Whistles and certain pulsed calls are long and complex sounds with intermediate frequencies (often below 10 kHz, González-Hernández et al., 2017), modulated and with a narrow bandwidth. They mainly provide information about the identity of a species, population or individual, as well as on the physiological and behavioural state of the emitter. These signals are essential for maintaining organisation and cohesion within social groups (Azzolin et al., 2014). Clicks, emitted in series (known as click trains), and certain pulsed calls, are short, repetitive sounds that cover a wide range of frequencies (from 20 kHz to over 100 kHz, González-Hernández et al., 2017). They are used for foraging, navigating and detection of predators (Au, 2018). Foraging involves all the activities required to reach, locate, detect and capture prey, with searching generally being the most time-consuming activity and this includes echolocation, which is becoming more important for both navigation and prey detection (Madsen and Wahlberg, 2007; Giorli et al., 2016). As clicks allow the

detection of prey in the environment, they are considered to be the most relevant acoustic indicator of foraging activity (Caruso et al., 2017). In this context, we assumed that temporal pattern in foraging activity would strongly affect click production rate. In addition, clicks are the most abundant type of sound emitted, and therefore would provide a larger sample size than the other types of vocalisations. Studying them can therefore help our understanding of how cetacean foraging activity changes over time.

The Mediterranean Sea is home to 7% of the world's marine biodiversity, of which around a fifth is considered endemic (Pace et al., 2015). Eleven cetacean species are known to permanently occur in the Mediterranean Sea and are regularly observed in the basin: the bottlenose dolphin, the striped dolphin, the common dolphin, the Risso's dolphin, the rough-toothed dolphin *Steno bredanensis*, the long-finned pilot whale, the Cuvier's beaked whale, the sperm whale, the fin whale *Balaenoptera physalus*, the orca, *Orcinus orca* and the Black Sea harbour porpoise *Phocoena phocoena relicta*, which is present in the northern Aegean Sea (Cucknell et al., 2016; Notarbartolo di Sciara and Tonay, 2021). The false killer whale *Pseudorca crassidens*, the minke whale *Balaenoptera acutorostrata*, and the humpback whale *Megaptera novaeangliae* are occasional visitors (Notarbartolo di Sciara and Tonay, 2021).

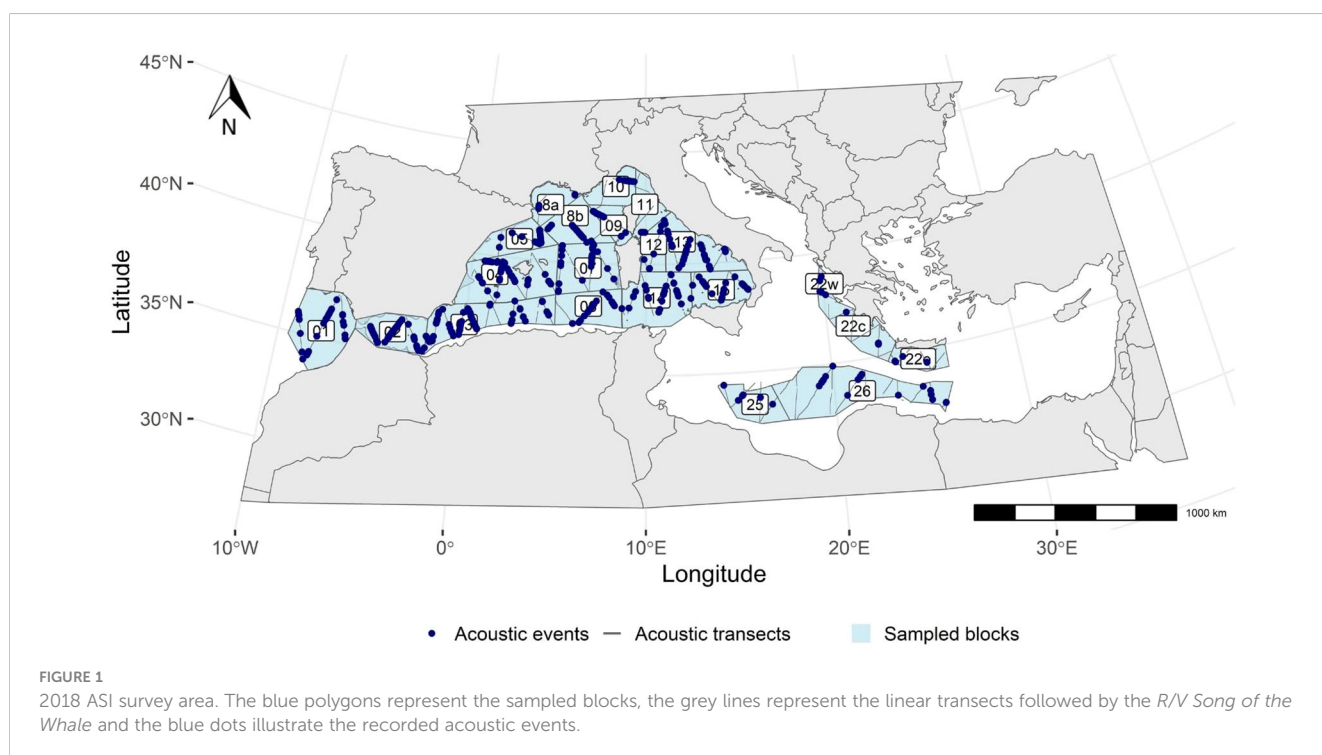
The main objective of this study was to analyse the rhythm of foraging activity of Mediterranean pelagic delphinids of which the striped, common and bottlenose dolphins are the most abundant (Panigada et al., 2024). Most previous studies of delphinid foraging rhythms in the Mediterranean Sea have used fixed acoustic devices within limited geographic areas (Giorli et al., 2016; Cascão et al., 2020). Here we used acoustic recordings provided by the Agreement on the Conservation of Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic area (ACCOBAMS) and collected during the vessel component of the basin-wide 2018 ACCOBAMS Survey Initiative

(ASI), which aimed to assess cetacean abundance and distribution in the Mediterranean Sea. Acoustic recordings were collected day and night using towed hydrophones along predetermined transect lines. Only echolocation clicks were analysed, as they are the most relevant signals to study the foraging activity. Diel rhythms in the number of acoustic events were investigated, as well as their relationship with a selection of temporal parameters. We hypothesised that a strong diel pattern would prevail, with more acoustic events during the night. We first extracted click emissions from acoustic recordings. The number of acoustic events per unit of time was then modelled in a generalised additive model (GAM) framework using a selection of temporal variables thought to be involved in the foraging strategies of delphinids.

2 Materials and methods

2.1 Study area

The study area covered the entire Mediterranean basin, from 34°S to 45°N and from 6°W to 17°E (Figure 1). The Mediterranean Sea is a semi-enclosed mid-latitude sea virtually isolated from the main oceanic systems, stretching east-west over about 3,800 kilometres. The general circulation of the Mediterranean Sea is cyclonic, with Atlantic surface waters flowing along the southern shores of the basin and returning along the northern shores, whose complexity generates many eddies. Winter cooling of the surface waters in the north of the basin is responsible for the formation of rich, deep waters that flow towards the Atlantic Ocean (Robinson et al., 2001; Millot and Taupier-Letage, 2005). Although the Mediterranean Sea is generally considered to range from oligotrophic to ultra-oligotrophic, it is characterised by significant spatial variability in primary productivity, with decreasing



productivity observed from west to east (Lazzari et al., 2012). Geomorphological features such as canyons, seamounts and deep trenches also provide a variety of unique habitats (Aissi et al., 2015) resulting in highly diverse and rich ecosystems.

2.2 Data collection

The acoustic data used in this study were collected from May to September 2018 during the ASI survey, during which two types of platforms were used to collect cetacean data; aircraft conducted visual detection only, and vessels supplemented the visual observations collected by the aerial component with acoustic data, particularly for deep-diving sperm whales and Cuvier's beaked whales (Boisseau et al., 2024; Panigada et al., 2024). In this study, we focused only on acoustic data from delphinids collected aboard the *R/V Song of the Whale*. Overall, the visual data collected from the boat indicated that delphinids were represented by three main species: the striped, the common and the bottlenose dolphins. Risso's dolphin, long-finned pilot whale and rough-toothed dolphin were also present, although less frequently.

The acoustic survey was conducted continuously (24 hours per day) wherever permitted by the riparian states, except where weather conditions, water depth (minimum 50 m) or technical constraints did not allow it. It focused on the Atlantic region near the Strait of Gibraltar, the western Mediterranean basin from the Alborán Sea to the Tyrrhenian Sea, and parts of the central Mediterranean basin (the Ionian waters and the Hellenic Trench; Figure 1). No survey effort was allocated in the Aegean Sea. The study area was divided into 21 blocks, designed to estimate cetacean abundance (ACCOBAMS, 2021).

Sampling was carried out along linear transects, and the hydrophone array was towed 400 m behind the vessel. This sampling method was used to maximise the coverage of the study area and to provide a robust estimate of species density and abundance (Buckland et al., 2004). Transects were defined using the Distance software (version 7.3, ACCOBAMS, 2021) and a zig-zag pattern was used to achieve equal probability coverage. A total of 17,271 km of transects were travelled, representing approximately 74,000 minutes of effort. This total sampling effort was divided into one-minute duration segments. The vessel speed was maintained between 5 and 8 knots to minimise bias due to species movement. The hydrophone array was housed in an oil-filled tube and consisted of two pairs of hydrophones in a linear configuration. The pair of broadband hydrophones, spaced 0.25 m apart, recorded sounds from 1 to 100 kHz with a sensitivity of -204 dB re 1V/μPa, in the frequency band in which most odontocete vocalisations are produced. The outputs of the broadband hydrophones were digitised at a sampling rate of 192 kHz. The audio recordings were stored in 16-bit wav files.

2.3 Acoustic data analysis

Field recordings were compressed into binary storage files using PAMGuard (version 2.2.7.0, Gillespie et al., 2008). These files were manually analysed using the 'click detector' module, which allows

the user to identify acoustic events, following the methodology of Ollier et al. (2023). Only click series, defined as sequences of clicks produced by the same animal or group of animals that exhibited a consistent change in bearing (*i.e.*, from the bow to the stern of the vessel) were examined. Sequences of clicks that did not show a consistent bearing trajectory or did not cross the 90° line were not considered as acoustic events. To ensure that the series of echolocation clicks identified were indeed from delphinids, the characteristics of the clicks making up the series were examined. The waveform of a click had to cross the horizontal axis several times (known as "zero crossing"). The spectrum of each click should ideally have a frequency peak between 20 kHz and 40 kHz. If this frequency peak was below 10 kHz, it could be a sound signal emitted by a boat (ACCOBAMS, 2021).

2.4 Statistical analysis

2.4.1 Variables

We selected variables relevant to temporal scales ranging from hourly to monthly time resolution such as time of day, lunar cycle, lunar illumination and sea state (Table 1). The time of day of the acoustic events was considered as a circular variable to account for the temporal cyclicity (so that the statistical models considered that 00:00 = 24:00). This improved the accuracy of the model and captured the large cyclical variations in the data. As the study area was relatively large, and to avoid any time zone bias, the times at which these acoustic events were detected, given in Coordinated Universal Time (UTC), were converted to solar time (Waugh, 1973) by using the equation:

$$\text{Solar Time} = 4(\text{longitude}) + \text{Coordinated Universal Time}$$

The longitude in the equation is expressed in decimal degrees. The value 4 represents minutes and comes from the fact that the Earth rotates in 24 hours (= 1440 minutes), *i.e.* one degree of rotation every 4 minutes. Depending on whether the longitude is positive or negative (whether the acoustic event was detected east or west of the Greenwich meridian), the duration in minutes obtained is added to or subtracted from Coordinated Universal Time to obtain Solar Time (Waugh, 1973).

Sea state is expected to affect foraging efficiency on epipelagic prey. It was recorded during the ASI survey according to the Beaufort scale. The lunar cycle and lunar illumination are expected to modulate the extent and intensity of the diel vertical migration of planktonic and micronectonic organisms. The lunar cycle lasts 29.53 days, and during this cycle the moon goes through different phases (Wang et al., 2015): new moon (day 1 of the lunar cycle), first quarter (day 8 of the lunar cycle), full moon (day 15 of the lunar cycle), and last quarter (day 22 of the lunar cycle). The lunar cycle is also a circular variable as the lunar phases change from day to day, completing a cycle at the end of a lunation. The phase of the lunar cycle and the lunar illumination of each day were obtained using the 'lunar' R-package (Lazaridis 2022). To obtain the day of the lunar cycle, the lunar phase was divided by 0.212769 (the lunar phase increases by this value per day for a complete cycle).

TABLE 1 Temporal and spatial variables used in the GAMs that potentially influence the cetacean rhythm activity.

Variable	Description/Relevance to the topic	Source	Variable type	Range of values/Units	Resolution
Time of day	Time of acoustic events	PAMGuard analysis	Circular	Hour [0-23]	Temporal
Geographical coordinates (Longitude, Latitude)	Environmental parameter - indicator of habitat use	ASI survey	Continue	Degrees	Spatial
Sea state	Environmental parameter - indicator of habitat use	ASI survey	Category	Beaufort scale [0-6]	Temporal and spatial
Lunar cycle	Influences the vertical migration of organisms (Simonis et al., 2017)	Lunar R-package (Lazaridis, 2022)	Circular	Days [1-29] 1: New moon 15 th : Full moon	Temporal
Lunar illumination	Influences the vertical migration of organisms (Simonis et al., 2017)	Lunar R-package (Lazaridis, 2022)	Continue	[0-1] 0: New moon 15 th : Full moon	Temporal

Beside nocturnal illumination, the lunar cycle also determines tidal range. Although the tide is limited in the Mediterranean Sea it does exist with an amplitude from 0 around the Balearic Islands to 60 cm in the Gulf of Gabès, Tunisia. Tidal range is greater at full and new moon and is minimal at first and last quarters, following a cyclical pattern with a period of approximately 15 days. Recognising the importance of spatial distribution of delphinids in the Mediterranean Sea (Cañadas et al., 2023), we included longitude and latitude as spatial factors rather than as explanatory variables to focus on temporal patterns.

2.4.2 Generalised additive models

GAMs (Wood, 2006) were fitted to determine how the number of acoustic events could be explained by these different variables. GAMs were chosen because unlike linear models, they offer great flexibility in describing non-linear relationships between predictors and a response variable. They have the advantage that the data

dictate how the shape of the response variable is affected by each covariate by fitting non-parametric models (Marian et al., 2021). The explained variable was the number of acoustic events per minute. Correlations between variables were assessed using a correlation matrix. If the Pearson coefficient between two variables was greater than 0.5, then the variables could not be included together in any model selection. Due to the very large number of null values and the overdispersion of the data, a Tweedie distribution was used for the analysis (Foster and Bravington, 2013). For the time of day and lunar cycle variables, the degrees of freedom (k) were set to a maximum of 15 and 7 respectively, to allow the relationships to have multiple inflection points (non-linear responses expected for these two variables). To account for the circularity of these two variables, we have included circular smoothing terms (splines, bs = 'cc'). This approach allowed the model to better capture temporal variations and effectively model the cyclicity of the data. For the other variables, we set the degrees

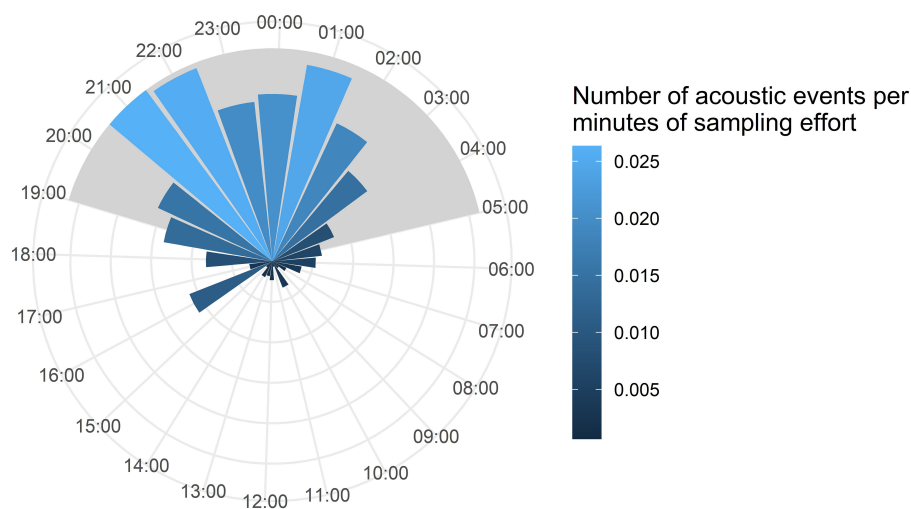


FIGURE 2

Temporal distribution of acoustic events corrected by sampling effort over the nycthemeral cycle. The shaded area represents the nocturnal phase. The largest radius has a value of 0.026 acoustic events per minute of effort at 21:00.

of freedom to a maximum of 4, as the expected relationships were less complex, and used thin plate regression splines ($bs = 'tp'$) as these are considered optimal (Wood, 2017). GAMs were fitted using the 'gam' function in the 'mgcv' R-package (Wood, 2017). Model selection was performed to determine the best model from all the models tested (combinations of 1 to 6 variables). The best model was selected based on the Akaike information criterion (AIC; Akaike, 1998), the AIC weight ('akaike.weight' function in the 'qpcR' R-package, Spiess, 2018), and the explained deviance. The importance of each variable was determined by summing the Akaike weights of the models in which the variable was selected (Symonds and Moussalli, 2011). The variables were then ranked according to their individual contribution to the models. All analyses were performed using the R software (version 4.1.2, R Core Team, 2022).

3 Results

Approximately 12,000 km were covered by the *R/V Song of the Whale* between May and September 2018, which represents a total of 74,000 minutes of sampling effort. Almost 40,000 minutes were sampled during the day while 34,000 were sampled at night. We identified a total of 741 acoustic events in PAMGuard during this sampling period. 41 events were recorded in May, 288 in June, 290 in July, 64 in August and 58 in September. Acoustic events were detected in all blocks sampled (Figure 1). More acoustic events were recorded at night (shaded area, Figure 2) with two peaks, reaching around 0.026 acoustic events per minute at 21:00/22:00 (solar time) and then around 01:00. After 01:00, the number of events decreased considerably; down to 0.0007 events per minute at 11:00 (Figure 2).

No correlations were found between the variables in the correlation matrix (Figure 3). Therefore, all the variables could be selected simultaneously in the models tested (Supplementary Table 1). The variables that contributed most to explaining the number of events were the time of day and the sea state, with Akaike weights of 99-100% (Table 2). They were followed by the lunar cycle

with an AIC weight of 80%. In contrast, lunar illumination, had a relatively low AIC weight of 60% (Table 2). The best model explained 14.3% of the deviance and included all the variables tested, although lunar illumination was not significant ($p = 0.39$, Supplementary Table 1). The second model also explained 14.3% of the deviance and did not include lunar illumination. As the top two models were very similar ($\Delta AIC < 2$), the second model was selected as the final model because lunar illumination was not significant (Supplementary Table 1). The final model included the spatial effect and the variables time of day, lunar cycle and sea state.

The number of acoustic events increased as night approached, peaking around 21:00/22:00 and at 01:00, with only a slight decrease between these two periods (Figure 4A). The number of acoustic events then decreased to a diurnal minimum around 11:00 (Figure 4A). For the lunar cycle variable, the number of events decreased from day 0, corresponding to the new moon, to a minimum on day 8, corresponding to the first quarter of the moon (Figure 4B). The response variable then increased, reaching a maximum during the third quarter of the moon (day 22 of the lunar cycle) and decreasing again until the end of the lunar cycle (day 29, Figure 4B). The relationship with sea state was unimodal from 1 to 4 and became positive at higher sea states (above 4, Figure 4C). Longitudinal and latitudinal gradients were observed, with more frequent acoustic events in the northern part of the western and central Mediterranean sub-regions (Figure 4D).

4 Discussion

4.1 General considerations

To date, knowledge of the dynamics of delphinid foraging activity throughout the Mediterranean basin has been relatively patchy. Most studies analysing delphinid foraging rhythms have been carried out using fixed passive acoustics and have focused on specific Mediterranean subregions or even local sites (e.g. Sicily, Caruso et al., 2017; Ligurian Sea, Giorli et al., 2016). These studies

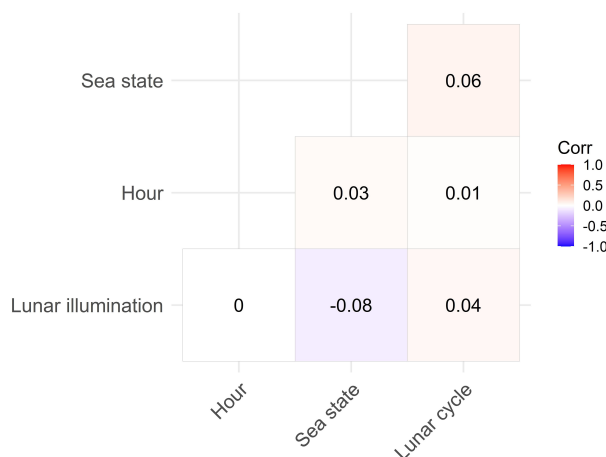


FIGURE 3

Correlation matrix including all variables tested in model selection. The numbers represent the coefficients of Pearson.

showed a diel foraging activity pattern with a peak at night. However, in these settings, it can be challenging to discriminate true patterns in echolocation activity around the recording stations from local movements in and out of the detection radius of these stations. The present study provides, for the first time, information on the activity rhythms of delphinids investigated at a basin scale from a moving platform, in the western and central Mediterranean Sea. At such a large scale, we can assume that the abundance of dolphins in the study area does not change during a diel cycle. Therefore, any change in the detection rate of acoustic events on the hydrophone array is assumed to be primarily due to changes in the dolphin vocal behaviour in relation to their foraging activity rather than changes in their occurrence. This is an inherent benefit of using large spatial datasets to identify activity rhythms. The GAM results suggested that delphinids in the Mediterranean Sea exhibit a strong diel foraging pattern, as evidenced by a nocturnal predominance of acoustic events. The distribution of effort between day and night condition was fairly well balanced and cannot be a source of bias in the number of acoustic events per unit time. The lunar cycle and also had a clear influence on the number of acoustic events, with more events being detected during the third quarter of the moon.

Carrying out this study at the scale of the western and central Mediterranean provided an overall picture of delphinid activity in the area but required spatial variability and time zone to be considered. To do this, we transformed all UTC times into solar time so that the time zone did not bias the results (Carlucci et al., 2016) and we included a spatial effect in the model to smooth out the effect of the distribution of animals in the model. A very clear signal of the influence of selected temporal variables on the number of acoustic events was found as the uncertainties associated with the relationships obtained were very low (narrow confidence intervals, Figure 4), suggesting that the highlighted patterns were quite reliable.

However, the selected model only explained 14.3% of the deviance suggesting that processes other than those considered in this study could also contribute to explain the variation in the number of acoustic events. For example, tidal height or sea surface temperature were considered by Gauger et al. (2022) and found to influence the number of acoustic events. Benoit-Bird and Au (2003) analysed and compared the relative abundance and density of spinner dolphins (*Stenella longirostris*) and the mesopelagic community off three Hawaiian islands using a modified echo-

sounder and showed that the dolphins followed the vertical dynamics of their prey. In this study, we were limited to using temporal variables that were available at all locations in a large study area, *i.e.* through available remote sensing datasets or oceanographic model outputs. This lack of direct data on prey behaviour throughout the study area necessitated the use of other parameters such as time of day or lunar cycle, thought to influence prey density and catchability at dolphin foraging depths. More detailed information on local delphinid prey activity and their catchability may have allowed for a more precise explanation of delphinid activity rhythms.

Grouping species which have distinct ecological and behavioural requirements (Cipriano et al., 2022) may also contribute to the relatively low deviance explained by the model. The exclusive use of echolocation clicks provides a solid description of delphinids foraging activity but clicks do not allow the precise identification of the species recorded. Here, delphinids were mainly represented by three species, the striped, bottlenose and common dolphins, which may have different diel activity patterns, with other species like the long-finned pilot whale, the Risso's dolphin and the rough-toothed dolphin being extremely marginal in the data set (ACCOBAMS, 2021; Ollier et al., 2023). Striped dolphins, for example, typically live in productive waters deeper than 350 m off the continental shelf (Carlucci et al., 2016). They exploit mesopelagic fish, cephalopods, and planktonic crustaceans (Würtz and Marrale, 1993) and follow their vertical distribution and dynamic movements. The bottlenose dolphin is quite flexible in its requirements, with populations living in extreme coastal, estuarine or even lagoon habitats, while others live offshore in oceanic waters (Wursig and Wursig, 1979). Its diet is quite eclectic, with combinations of benthic, demersal and pelagic shelf species versus oceanic assemblages according to the habitats where it is found (e.g., Bearzi et al., 2009; Blanco et al., 2001; Neri et al., 2023; Queiros et al., 2018). The common dolphin occurs in both pelagic and neritic environments, often sharing the former with striped dolphins and the latter with bottlenose dolphins (Notarbartolo di Sciara and Tonay, 2021). The temporal patterns presented here result from a combination of the foraging strategies of these three species in approximate proportions of 80% striped dolphins, 10% bottlenose dolphins and 10% common dolphins (proportions derived from table 4 in Panigada et al., 2024). Hence, the present results might be considered as a fair image of striped dolphin behaviour, slightly blurred by limited inputs from the other two species. However, as we were not able to distinguish species from the click trains alone in this study, any assumptions regarding species identity should be treated with caution.

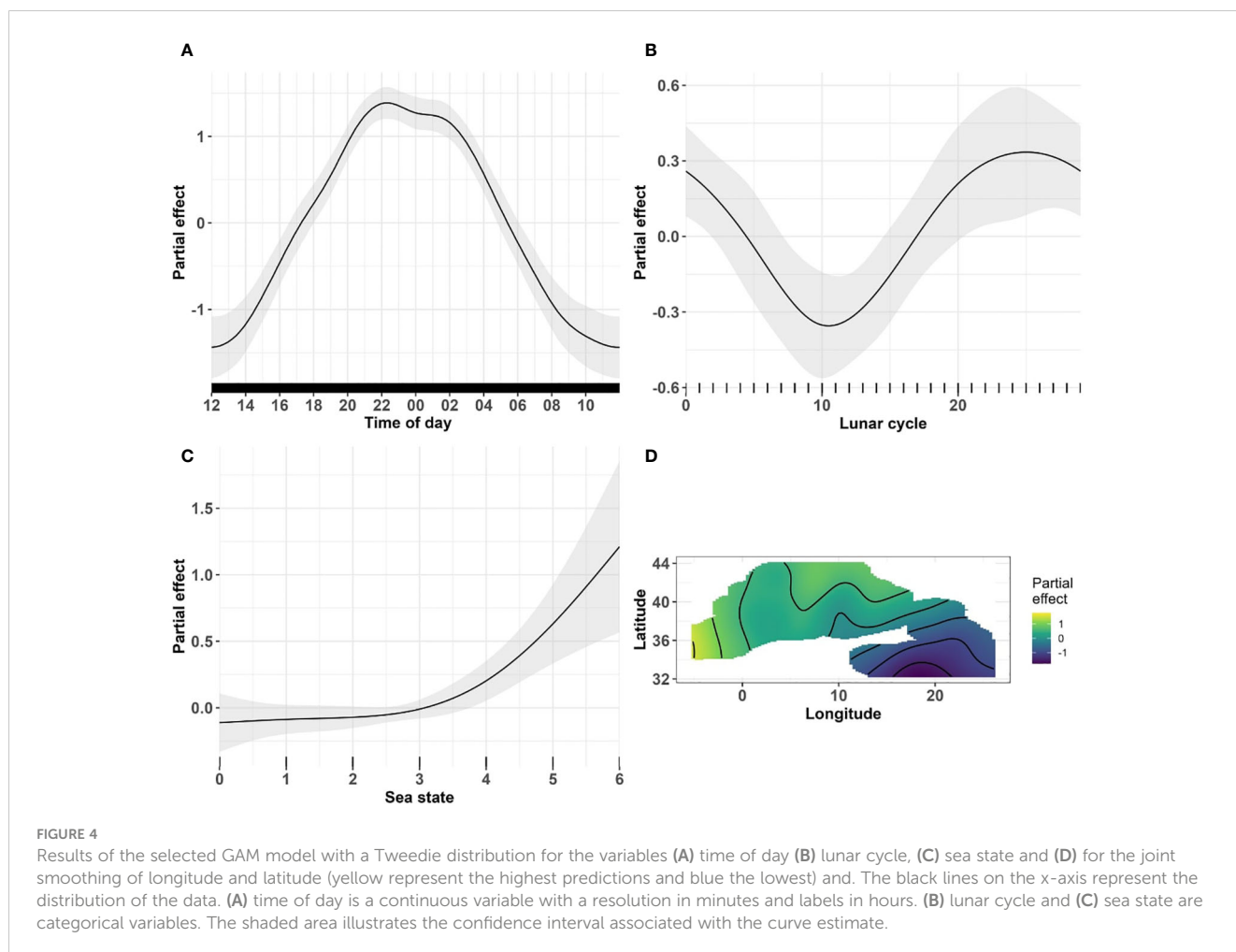
4.2 Activity rhythms of delphinids in the Mediterranean Sea

The results of this study, showing that Mediterranean delphinids have a very distinct diel rhythm of acoustic event production, mainly displayed processes related to the striped dolphins in offshore habitats (Panigada et al., 2024). The other species present did not contribute much to the overall picture and

TABLE 2 Ranking of variables according to their AIC weight.

Variable	Count	Percent	Akaike weight
Time of day	8	50	100
Sea state	8	50	99
Lunar cycle	8	50	80
Lunar illumination	8	50	60

The Count column corresponds to the number of times the variable appears in all the models tested, the Percent column expresses the associated percentage, and the Akaike weight corresponds to the relative contribution of the variable in the model.



were likely merely contributing to the uncertainty around the observed general patterns. These variations in the number of acoustic events illustrated the activity or foraging strategy of the dolphins, which would vary according to the availability and type of prey (Benoit-Bird and Au, 2003).

The variable that contributed most to our model was the time of day of the nycthemeral cycle. Here, the variation in the number of acoustic events expresses a change in foraging activity, based on the availability of prey. The latter is shaped by depth distribution and density of prey. Dolphins assess the accessibility of prey, the potential encounter rate during foraging dives, and the associated costs in order to maximise their foraging success (MacArthur and Pianka, 1966). When prey is scarce or too deep, dolphins would stop foraging because it would not be profitable (Cascão et al., 2020), hence few acoustic events would be recorded. When prey is more abundant and accessible, dolphins would forage actively (MacArthur and Pianka, 1966). In the latter situation, it is also possible to imagine that when prey is most abundant less foraging effort would be necessary to meet calorific requirements, whereas when there is slightly less prey but still enough for foraging to be profitable, then foraging would be greater, hence acoustic events more numerous.

In our study, dolphins exhibited a higher number of acoustic events emitted during the night, suggesting a nocturnal peak in foraging activity. This is thought to occur in response to nocturnal changes in the dynamics and vertical distribution of cetacean prey (Thompson and Miller, 1990). The latter migrate vertically between the surface to feed at night and deeper layers to escape from predators during the day (Marohn et al., 2021). During the ascent of the DSL to the surface, the defence mechanisms of organisms, particularly small pelagic fish, are thought to be reduced; they would be present in less aggregated schools and would have a reduced individual swimming speeds (Zein et al., 2019). Furthermore, as night-time can lead to a loss of visual information and an increase in backscatter (Mass and Supin, 2018), the nocturnal increase in prey concentration near the surface would be advantageous for nocturnal air-breathing predators; this is the case for odontocetes, whose highly developed sonar allows them to detect prey regardless of light conditions.

To maximise foraging success while minimising dive duration, pelagic delphinids might be expected to concentrate their foraging activity at night, when the DSL get closer to the surface (Giorli et al., 2016). Maximum amounts of prey would then be available, easy to capture and accessible near the surface. Dolphins consume more

oxygen per unit time than deep diving odontocetes which may consume less oxygen due to evolutionary adaptations (Tyack et al., 2006). As a result, delphinids deplete their oxygen reserves more quickly before reaching their aerobic diving limit. To maximise hunting time relative to vertical transit time, they would ideally limit their foraging depth and thus foraging at night may be more favourable to them. Simonis et al. (2017) have highlighted a similar diurnal pattern in certain delphinids, stating that foraging activity was mainly nocturnal, and that social behaviours tended to take place during the day.

The number of acoustic events were found to be higher in the north and west of the western and central Mediterranean subregions. According to Goffredo and Dubinsky (2013), the different Mediterranean subregions are characterised by different micro phytoplanktonic compositions, with diatoms dominating in the west, and coccolithophorids and dinoflagellates in the east. The Mediterranean basin would then be characterised by significant spatial variability in terms of primary production, with decreasing levels of primary productivity from west to east, and to a lesser extent from north to south. This gradient could be a cause for a lower availability of cetacean prey, resulting in a lower densities of cetaceans (see Cañadas et al., 2023 for an analysis of gradient in cetacean distribution observed during the ASI) and therefore fewer acoustic events in the eastern and southern parts of the basin.

Other parameters may influence the increase in acoustic events, but more indirectly, as they could affect the migration dynamics of the DSL. This is the case for the lunar cycle. The number of acoustic events was minimal on day 8, corresponding to the first quarter of the moon, and maximal during the third quarter (22st day of the lunar cycle). According to Ochoa and collaborators (2013), the extent of these vertical migrations would depend on the intensity of nocturnal versus diurnal illumination and would only be possible if light intensity fluctuates sufficiently during the diel cycle. During full moon nights, when nocturnal illumination is the highest, vertical migrations would be delayed or attenuated (Ochoa et al., 2013) and fish would therefore be less available near the surface. As a result, small cetaceans may reduce their use of acoustic foraging behaviour or switch to visual predation, resulting in fewer acoustic events. However, the results of this study are inconsistent with previous research that have observed minimal acoustic foraging activity during the full moon. Simonis et al. (2017) found that common dolphin echolocation activity in the Southern California Bight was the lowest during the full moon and the highest during the third quarter phase. They suggested that the brightness of the full moon delayed or shortened vertical migrations to avoid predators, causing predators to reduce foraging or switch to visual predation. During the third quarter, a prolonged period of darkness after sunset allows mesopelagic prey to migrate to the surface before the moon rises, making them more vulnerable and available to predators. In the Mediterranean Sea, Caruso et al. (2017) found no evidence of a relationship between echolocation activity and the lunar cycle in a pelagic area. These mixed results

suggest that the response of small cetaceans to the lunar cycle is more complex than previously thought (Benoit-Bird et al., 2009; Caruso et al., 2017; Simonis et al., 2017; Shaff and Baird, 2021; Cohen et al., 2023). Therefore, in our study, lunar illumination alone cannot explain the variation in the rhythm of delphinid activity as maximum acoustic activity was found close to the third quarter of the moon and not at full moon. The mesopelagic community would be affected by the lunar cycle in a more complex way than just a direct effect of nocturnal light that would limit the DSL vertical migration and in cascade reduce nocturnal prey density in the surface layer at full moon. Indeed, detailed analyses of the DSL composition at night suggest that the main phyla constituting the DSL, i.e. fish, squids and crustaceans, and probably the main taxa constituting those, might display more complex variation in relation to the lunar cycle, drastically affecting prey availability for top predators (e.g. Battaglia et al., 2020). For example, blue-finned tuna (*Thunnus thynnus*) in the Strait of Messina show extensive changes to their prey composition during the lunar cycle, with mesopelagic fish being predominant during first quarter and full moon, mesopelagic squid after first and third quarters and other invertebrates at third quarter and new moon. If different prey types are available at different times of the lunar cycle, dolphins may use different foraging strategies, including variable echolocation rates.

Sea state was also selected in the best model. This variable may have an influence on cetacean sound activity. The number of acoustic events increased from Beaufort 4. However, it is difficult to interpret this trend due to the limited data coverage in these conditions. Changes could appear with significant ambient noise, whether natural or anthropogenic, as an adaptation strategy (Isojunno et al., 2022). For example, cetaceans may compensate for higher ambient noise by increasing the amplitude or shifting the frequency or temporal pattern of their acoustic signals (Isojunno et al., 2022). Elevated ambient noise may also affect the detection range of a hydrophone array. An independent measure of ambient noise may be useful in future surveys to explore whether dolphins adjust their sound activity to ambient noise.

4.3 Monitoring and conservation implications

Our results have important implications for the monitoring of small delphinid populations using passive towed acoustics. Highlighting the strong signal in the intensity of delphinid acoustic activity as a function of time may have implications for cetacean abundance estimation programmes. A uniform acoustic detection probability over the nycthemeral cycle cannot be used because the relationship between the number of acoustic events and the number of individuals would change with the activity of those individuals. Therefore, a variable cue rate should be included when estimating dolphin abundance in order to duly consider variation in acoustic detection probability with the time of day. This would apply to the analysis of data from towed passive acoustic surveys

following a linear transect strategy. In contrast to stationary passive acoustic surveys using moored hydrophones, changes in dolphin activity on the one hand and small scale movements affecting local dolphin density within the detection range of the hydrophone on the other hand, determine together the number of acoustic detection per unit time and are hard to disentangle.

The existence of these activity rhythms may also have conservation implications. While fishing may occur day or night (Levy et al., 2015), depending on the diversity of fishing practices, increased foraging activity at night could make dolphins more vulnerable to fisheries bycatch as they would be more focused on foraging and less alert to environmental hazard (Todd et al., 2020). Anthropogenic sound sources in the same frequency bands as dolphins can have a masking effect on these signals (Marian et al., 2021) which can affect foraging success and, indirectly, the survival of the species.

5 Conclusion

This study is the first of its kind to be carried out across a large part of the Mediterranean Sea. It was conducted to explore the activity rhythms of Mediterranean delphinids, mostly represented by the striped dolphin in offshore habitats. A nycthemeral rhythm of echolocation activity was demonstrated. These dolphins showed a predominantly nocturnal foraging pattern, illustrated by much higher echolocation clicks at night. This rhythmicity of foraging activity was shown to be linked to temporal variables such as time of day, sea state and lunar cycle. This result is consistent with available knowledge on the DSL vertical migration. In the context of foraging ecology, the use of echolocation clicks by towed passive acoustics has proved to be highly relevant for identifying diel patterns in delphinid foraging activity.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Author contributions

MS: Data curation, Methodology, Formal analysis, Writing – original draft. CO: Data curation, Methodology, Formal analysis, Conceptualization, Writing – review & editing. OB: Writing, review & editing. VR: Conceptualization, Methodology, Writing – review & editing. AV: Conceptualization, Methodology, Formal analysis, Writing – review & editing.

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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.2024.1378524/full#supplementary-material>

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