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Risks, threats, and conservation status of cetaceans in the Mediterranean and Black Seas

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Risks, threats, and conservation status of cetaceans in the Mediterranean and Black Seas

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Editorial: Risks, threats, and conservation status of cetaceans in the Mediterranean and Black Seas

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Editorial on the Research Topic
Risks, threats, and conservation status of cetaceans in the Mediterranean and Black Seas

Together, the Mediterranean Sea and the Black Sea cover less than 1% of the oceans' surface, but constitutes one of the most important hotspots of biodiversity at a global scale. Unfortunately, they are also amongst the most impacted ecoregions as a result of anthropogenic pressures on coastal and offshore ecosystems that threaten many species (Coll et al., 2010; Avila et al., 2018). Conservation of cetaceans in these basins is a critical issue, as these top-order animals structure ecosystems through trophic cascades, and aid the maintenance of marine food web stability (Schwarzmüller et al., 2015). However, current information about the conservation status of cetaceans is fragmented and requires assessments at geographical scales sufficiently large enough to encompass the movement patterns of these mobile species (ACCOBAMS, 2021). The Research Topic "*Risks, threats, and conservation status of cetaceans in the Mediterranean and Black Seas*" addresses this issue by presenting 14 studies on subjects including distribution and ecology, genetic structure of populations and major threats including maritime traffic, interaction with fisheries, contaminants and diseases. Together, this research contributes to a better understanding of the relatedness, status and impact of human activities on populations of cetaceans within the region.

Knowledge of keyhabitats where cetaceans exhibit essential activities is central to the optimization of conservation strategies. Five papers in this Topic identified these habitats by exploring the long-term stability or changes in the spatiotemporal distribution of several species over the last decade. Chicote et al. found that the submarine canyons of northern Catalonia (Spain) in the north-western Mediterranean Sea were critical habitats for Risso's dolphins (*Grampus griseus*), whereas Pace et al. found that the coastal areas off the Tuscany and Lazio regions of Italy were important for the common bottlenose dolphin (*Tursiops truncatus*). Within the eastern basin, the southern coastal waters of Israel were found to be important for common dolphins (*Delphinus delphis*) by Mevorach et al. and the eastern Rhodes and Finike basins hosted two priority areas for deep diving cetaceans and a large area for delphinids off Türkiye (Awbery et al.). A statistical analysis using a spatial log–Gaussian Cox Process was used by Pace et al. to highlight the persistent presence of bottlenose dolphins in the same coastal areas both in winter and summer. Similarly, Mevorach et al. employed photo-identification to show the long-term site fidelity and residency of a critically endangered population of common dolphins, with a recent decline in abundance of this species. In contrast, a statistical analysis using generalized additive models by Chicote et al. showed a displacement of Risso's dolphins to more pelagic waters off Catalonia in recent years. Finally, Arcangeli et al. tested four potential indicators (the Observed Distributional Range: ODR, the Ecological Potential Range: EPR, the Range Pattern and the proportion of ODR over the EPR) to assess the range and short-term changes in habitat of three species of deep diving cetaceans that occur at low densities in trans-border fixed-transects. Changes in range were found for all three species. This combination of complementary indicators proved to be the most valuable approach to evaluate the significance of changes for highly mobile species such as cetaceans. The concurrent analysis of species with similar ecologies was found critical to determine whether the detected changes were species-specific or representative of broader trends. Combined, these five papers contribute to a better understanding the ecological preferences of cetacean species, recognize priority areas to be protected and underline the importance of monitoring changes over space and time for a better definition of conservation strategies.

Antonacci et al. explored genetic diversity in a local population of striped dolphins (*Stenella coeruleoalba*) in the northern Ionian Sea (central-eastern Mediterranean Sea) using two mtDNA markers. Their results suggested a population now in rapid expansion after a period of reduction in size and diversity, together with at least two lineages, the former shared with the overall Mediterranean population and the latter specific to the local region. They also found a potential problem of hybridization between striped and common dolphins, which needs to be further investigated, as presence of admixed individuals and human disturbances that cause hybridization can led to genomic extinction of already threatened species or population or conservation units.

Other manuscripts in the Research Topic investigated human pressures that can affect cetacean populations locally or on a wider scale. Interaction with fisheries is one of the most predominant threats for cetaceans (Read, 2008; Avila et al., 2018; ACCOBAMS, 2021) and was the focus of four manuscripts. Two of these characterized cetacean interactions with the pelagic longline fishery for albacore tuna (*Thunnus alalunga*) based on commercial logbooks and interviews, onboard observations and stranding data, from the waters around Cyprus in the eastern Mediterranean (Papageorgiou et al.) and in all the active fisheries operating in the region off Valencia, Spain NW Mediterranean Sea (Izquierdo-Serrano et al.). In both studies, common bottlenose dolphins and striped dolphins were the main species involved in depredation and/or bycatch, with differences between fishing gears and areas. By quantifying the economic consequences of depredation, estimating dolphin bycatch and identifying dolphin-fisheries conflict areas, they provide information to support the implementation of mitigation strategies to minimize interactions between dolphins and fisheries. The third manuscript approached the interaction of bottlenose dolphins with bottom trawlers near the River Tiber estuary (Italy, NW Mediterranean Sea) from the perspective of social dynamics of individuals of this species of dolphin Pace et al. This study showed that in common bottlenose dolphin sex-specific social dynamics and interactions with anthropogenic activities may affect grouping and induce changes in relationships between individuals. Indeed, females show stronger association compared to any other individuals while individuals interacting with fisheries (only rarely females with calves) showed weaker and short-term associations. This suggested that social and ecological drivers that can influence individual pattern of association were essential aspects affecting animals' responses to both human-related pressures and conservation strategies. Finally, Carlucci et al. applied a multi-species bio-economic approach to estimate the characteristics of the Otter Trawl Bottom fishery in potential cetacean conservation areas (CCAs) and fishing grounds in the northern Ionian Sea, adopting the quantitative model SMART (Spatial Management of demersal Resources for Trawl fisheries). The results showed that spatial fishing restrictions due to the establishment of CCAs would have no or negligible effects on trawlers, highlighting the importance of considering spatially integrated information during the designation of CCAs, following the principles of ecosystem-based management.

Maritime traffic and ship strike, another major threat for cetaceans (Pennino et al., 2017; David et al., 2022), was studied in two important cetacean habitats. Castro et al. estimated the monthly risk of ship strikes for fin whales (*Balaenoptera physalus*) in a seasonal feeding aggregation area off the Catalan coast, Spain (NW Mediterranean Sea) that overlaps with major shipping lanes. On the other side of the basin, Awbery et al. delineated potential risk areas in the eastern Mediterranean Sea off Türkiye, where high cetacean encounter rates and dense maritime traffic overlap. In both cases, mitigation of collision risk will require active management actions including rerouting of shipping outside the habitat and/or reducing vessel speed.

Finally, three manuscripts in the Research Topic addressed the widespread threat contaminants (Van Bressem et al., 2009) and their connection with diseases. Giovani et al. brought new insights about the toxic potential of the chemical additives released by plastic debris, by exposing dolphin cell cultures to increasing doses of one of the most used plasticizer in plastic production, the di(2-ethylhexyl)phthalate DEHP. The results indicate that potential chromosome loss could constitute a threat for marine mammals that are constantly exposed to plastic marine litter. Romani-Cremaschi et al. suggested that the chronic parasitic infestation and the immunosuppressive effects of organochlorine contaminants were likely to have compromised the health of a Cuvier's beaked whale (*Ziphius cavirostris*). They argued this, predisposed the whale to an opportunistic bacterial infection, leading to the first described case of bacterial septicemia with central nervous system involvement in a wild cetacean due to infection by *Morganella morganii*. Infections of this bacteria also occur in humans. Similarly, Morick et al. reported infection by the bacteria *Streptococcus agalactiae* in a stranded common dolphin in Israel. This is the first published report of an infection by this pathogen in a common dolphin, with the potential for dispersion throughout the Mediterranean subpopulation, which is already endangered.

With an overall number of 14 manuscripts, this Research Topic brings together a wide range of research spread throughout the Mediterranean Sea.

Author contributions

LD: Writing – original draft, Writing – review & editing. AAk: Writing – review & editing. AAr: Writing – review & editing. PG: Writing – review & editing. SM: Writing – review & editing. MV: Writing – review & editing. RC: Writing – review & editing.

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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References

ACCOBAMS. (2021). *Conserving whales, dolphins and Porpoises, in the Mediterranean Sea, Black Sea and adjacent area: an ACCOBAMS status report*. Eds. N. di Sciara and A. M. Tonay (Monaco: ACCOBAMS).

Avila, I. C., Kaschner, K., and Dormann, C. F. (2018). Current global risks to marine mammals: Taking stock of the threats. *Biol. Conserv.* 221, 44–58. doi: 10.1016/j.biocon.2018.02.021

Coll, M., Piroddi, C., Steenbeek, J., Kaschner, K., Ben Rais Lasram, F., Aguzzi, J., et al. (2010). The biodiversity of the mediterranean sea: estimates, patterns, and threats. *PloS One* 5 (8), e11842. doi: 10.1371/journal.pone.0011842

David, L., Arcangeli, A., Tepsich, P., Di-Meglio, N., Roul, M., Campana, I., et al. (2022). Computing ship strikes and near miss events of fin whales along the main ferry routes in the Pelagos Sanctuary and adjacent west area. *Aqua. Conserv.* 32, 442–456. doi: 10.1002/aqc.3781

Pennino, M. G., Arcangeli, A., Prado Fonseca, V., Campana, I., Pierce, G. J., Rotta, A., et al. (2017). A spatially explicit risk assessment approach: Cetaceans and marine traffic in the Pelagos Sanctuary (Mediterranean Sea). *PloS One* 12, e0179686. doi: 10.1371/journal.pone.0179686

Read, A. J. (2008). The looming crisis: interactions between marine mammals and fisheries. *J. Mammalogy* 89 (3), 541–548. doi: 10.1644/07-MAMM-S-315R1.1

Schwarzmüller, F., Eisenhauer, N., and Brose, U. (2015). 'Trophic whales' as biotic buffers: weak interactions stabilize ecosystems against nutrient enrichment. *J. Anim. Ecol.* 84, 680–691. doi: 10.1111/1365-2656.12324

Van Bressem, M., Raga, J. A., Di Guardo, G., Jepson, P. D., Duignan, P. J., Siebert, U., et al. (2009). Emerging infectious diseases in cetaceans worldwide and the possible role of environmental stressors. *Dis. Aquat. organisms* 86 (2), 143–157. doi: 10.3354/dao02101

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First Isolation and Characterization of *Streptococcus agalactiae* From a Stranded Wild Common Dolphin (*Delphinus delphis*)

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Streptococcosis is an infectious bacterial disease of both homeotherms and poikilotherms. Among the *Streptococcus* species that infect marine animals, *Streptococcus agalactiae* has the broadest host spectrum, including different aquatic organisms in freshwater and marine environments. The common dolphin (*Delphinus delphis*) is categorized as Endangered in the Mediterranean Sea. There are few reports of a streptococcal infection of *D. delphis*, caused by *Streptococcus phocae* and *Streptococcus iniae*. Here we report the isolation and identification of *S. agalactiae* in a stranded, wild male common dolphin that was found dead in September 2020 on the seashore next to the city of Bat-Yam, Israel. The carcass was fresh with a moderate nutritional status and with no apparent fishing gear or other anthropogenic-related signs. A *post-mortem* examination did not reveal an apparent cause of death, but further laboratory analysis demonstrated a *S. agalactiae* bacterial presence in urine, lungs and pericardial fluid that was characterized as type Ia-ST7 by whole genome sequencing. Interestingly, this isolate was found to be almost identical to another isolate recently recovered from a wild sandbar shark (*Carcharhinus plumbeus*) in the same area in Israel, the eastern Mediterranean Sea.

Keywords: *Delphinus delphis*, common dolphin, *Streptococcus agalactiae*, streptococcosis, type Ia-ST7, whole-genome sequencing (WGS), phylogeny

INTRODUCTION

Bacterial infections are considered to be a significant threat to marine mammals, causing significant morbidity and mortality. Members of the genus *Streptococcus* are among the most commonly reported pathogens in marine mammals, including cetaceans (Dunn et al., 2001; Numberger et al., 2021). Although these bacteria have been isolated from apparently healthy individuals, they have also been associated with significant pathological changes and zoonotic potential (Díaz-Delgado et al., 2017). Streptococcosis is a septicemic disease that affects freshwater, brackish and marine animals in wild and farmed populations. Ten streptococcal species have been isolated from marine mammals, including *Streptococcus agalactiae* (Group B *Streptococcus*, GBS) and other closely related streptococci like *S. phocae* and *S. iniae* (Numberger et al., 2021). In the area of the eastern Mediterranean Sea, there are very limited data regarding the presence and importance of streptococcal infection in the marine environment and *Streptococcus* spp. are reported in wild marine fish (Zlotkin et al., 1998; Corloni et al., 2002; Berzak et al., 2019). The common dolphin (*Delphinus delphis)* is a small cetacean species with a wide distribution. *Delphinus delphis* was once abundant in the Mediterranean Sea, but from the 1960s the species declined dramatically in the region and the Mediterranean subpopulation contains fewer than 2,500 mature individuals (Notarbartolo di Sciara and Tonay, 2021). It is estimated to continue and decline of at least 20% in two generations, and a reduction of 66% is suspected in the past three generations (Bearzi et al., 2003; Del Mar Otero and Conigliaro, 2012; Notarbartolo di Sciara and Tonay, 2021). At a global level, the species is classified as "Least Concern" (Braulik et al., 2021). However, due to its dramatic decrease, the Mediterranean subpopulation was listed as "Endangered" in the International Union for Conservation of Nature (IUCN) Red List (Bearzi, 2012). In the Mediterranean, *D. delphis* are found in both pelagic and neritic environments, forming mixed groups along with Striped (*Stenella coeruleoalba*) and Risso's Dolphins (*Grampus griseus*) (Frantzis and Herzing, 2002). Isolated groups are also recorded, with about 50–70 individuals (Bearzi et al., 2003). Threats to the species in the region include competition with commercial fisheries (Bearzi et al., 2003), PCB pollution (Borrell et al., 2001), bycatch and climate change (Bearzi, 2012). As with other Mediterranean marine mammals, data on health status is limited, and few publications described *Erysipelothrix rhusiopathiae*, *Toxoplasma gondii* and dolphin morbillivirus as the primary infectious agents, affecting the common dolphin in the Mediterranean Sea area (Vella et al., 2021). Here we report the first isolation and identification of *S. agalactiae* type Ia-ST7 in a common dolphin from the Mediterranean subpopulation, and the first report of this infection for the species worldwide.

METHODS

In September 2020, a male common dolphin was found stranded nearby Bat-Yam, Israel. The carcass was collected for necropsy at the Morris Kahn Marine Research Station in Ashdod, Israel. A post-mortem examination was conducted, based on the procedure described by Kuiken and García Hartmann (1993) and Ijsseldijk et al. (2019), and tissue samples were aseptically collected to avoid cross contamination. An exhaustive pathological examination was not performed, as other research groups were involved in the necropsy and removed the brain and gastrointestinal tract for functional MRI and microplastic analysis, respectively. Due to several ongoing studies described above, the sampling protocol was also performed with limitations. Samples of the penis, spleen, testicle, liver and several sections of the lung and kidney were fixed in 10%-buffered formalin for 48 h. Subsequently, the fixed samples were reduced in size, trimmed, dehydrated, embedded in Paraplast®, and stained with Hematoxyline & Eosin and Gram stain for routine histological evaluation. Liver, spleen, kidney and lungs were sampled by sterile swabs, and pericardial fluid and urine (from the urinary bladder) were collected aseptically *via* sterile needle and syringe during the necropsy. Before swabbing, the surface of the organ was seared with a hot blade, then incised with a sterile scalpel and, finally, a sterile swab was inserted into the incision. Two aliquots, one for PCR (stored at -20°C) and one for microbiological investigations (stored at 4°C), were collected. These samples were sent to the department of bacteriology at the Kimron Veterinary Institute were the pericardial fluid and urine were aseptically swabbed and further used for the following tests. All the mentioned above swabs were screened by molecular tools for canine distemper virus (CDV) following Elia et al. (2006). For bacteriology, all swabs were inoculated onto tryptic soy agar (TSA), blood agar (5% sheep blood enriched TSA), and MacConkey agar, and incubated for 24-48 h at 37°C. The samples were inoculated onto Brucella agar as previously described (Markey et al., 2013) for 10 days and onto Mycoplasma broth followed by Mycoplasma agar incubated at 37°C for up to 10 days in CO_2 enriched atmosphere as described before (Blum et al., 2010). Confirmation of bacteria species was initially done by Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry (MALDI-TOF MS), according to the manufacturing protocol (Autoflex, Bruker). Antimicrobial sensitivity test was performed to this strain by *in vitro* susceptibility testing by standard disk diffusion method and inhibition zones were measured as previously described (Elad et al., 2018). DNA was extracted from bacterial colonies isolated from the urine using a Wizard SV Genomic System (Promega, WI, USA) by genomic DNA purification protocol following the manufacturer's instructions for tissue lysates. The quantity and purity of the DNA were estimated using NanoDrop One (Thermo Scientific, Rockford, CA, USA). The genomic DNA obtained was stored at −20°C until use. The quantity and purity of the DNA were estimated using NanoDrop One (Thermo Scientific, Rockford, CA, USA). The isolates were further serotyped at the Public Health National Reference Laboratory (Ministry of Health, Israel) using a molecular serotyping method by multiplex-PCR for species confirmation and direct identification of capsular type (Poyart et al., 2007). Additionally, whole-genome sequencing (WGS) was performed. The DNA for

WGS was extracted using the QIAsymphony® SP system and the QIAsymphony® DNA mini kit (Qiagen) according to the manufacturers' recommendations. A DNA library was prepared using the Nextera XT library preparation kit (Illumina, CA, USA), followed by WGS using the Illumina MiSeq system with the read length of 250 bp paired-end. Reads were assembled using SPAdes by the BioNumerics 8.0 platform.

RESULTS

The dolphin weighed 85.5 kg, had a girth measuring 107 cm, a total length of 211 cm and was classified as mature according to Murphy and Rogan (2006); we assumed that the animal was not of old age base on mild erosion of the teeth. At necropsy, no external signs of interaction with fishing gear were observed. The decomposition state of the carcass was classed as 'fresh' (condition code 2; DCC2), as defined by Ijsseldijk et al. (2019), with a moderate nutritional status. Little gross changes were evident, including an assumed papilloma at the end of the penis. Emphysema was found in the cranial parts of both lungs along with a white foam material in the bronchioles. Five ml of mucopurulent discharge was observed in the urinary bladder (**Figure 1**). Histological examination of lungs revealed numerous bacterial colonies in subpleural alveoli and numerous bacterial colonies in pulmonary blood vessels (**Figure 2A**). The splenic architecture appeared within normal ranges with no evidence of lymphoid depletion. The liver was partially autolyzed. However, extracellular bacteria were present inside hepatic blood vessels but no lesions were observed in the parenchyma (**Figure 2B**). Bacterial colonies were also observed in the capillaries of the kidneys (**Figure 2C**). Aerobic culture yielded pure bacterial colonies of spherical or ovoid cocci, 1-2 μm in diameter, grayish-white, smooth, glossy and translucent, with a narrow zone of β hemolysis. Colonies, consistent with the genus *Streptococcus*, appeared on the blood agar plates 48 h post-inoculation from the mucopurulent discharge from the urine, lungs and pericardial fluid. Gram staining confirmed the presence of Gram-positive bacteria (**Figures 2D, E**). The bacteria were confirmed as *S. agalactiae* by MALDI-TOF MS, and further by the mentioned above molecular methods. No other bacteria were isolated from the tested samples. The isolate was resistant to Gentamicin with intermediate susceptible to Amoxicillin/clavulanic acid, Ampicillin, Fluoroquinolones and Tetracyclines and susceptible to Erythromycin, Sulfamethoxazole/Trimethoprim, Florfenicol, Penicillin, Penicillinase resistant penicillins, First gen. cephalosporins and Clindamycin. The assembled genome was submitted to the PubMLST *S. agalactiae* database as ICLGBS002 (ST35685). The strain was identified as ST-7 strain by wgMLST comparison analysis of ST-7 strains. Using the Genome Comparator (GC) tool, allelic profiles of the 2,207 loci were retrieved and imported to BioNumerics 8.0 (Applied Maths) in order to generate a phylogenetic tree (**Figure 3**). The present isolate is almost identical with a two-SNP difference in comparison of 1,969,033 bases (96.7% genome size), to an isolate described by Morick et al. (2020), collected from a moribund sandbar shark (*Carcharhinus plumbeus*) found on Netanya's shoreline (Israel – east Mediterranean coast) in

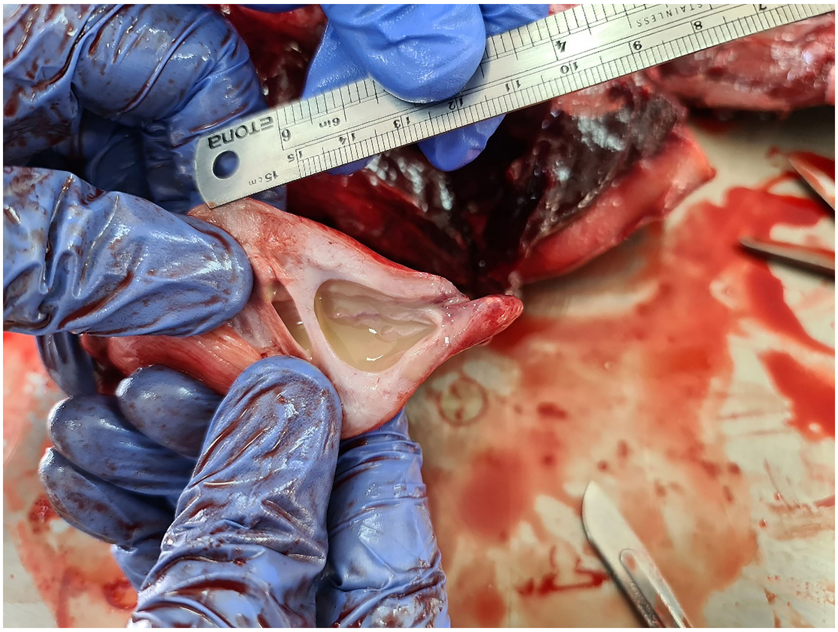

FIGURE 1 | Gross pathology of the urinary bladder of a common dolphin (*Delphinus delphis*). About five ml of mucopurulent discharge was observed in the urinary bladder.

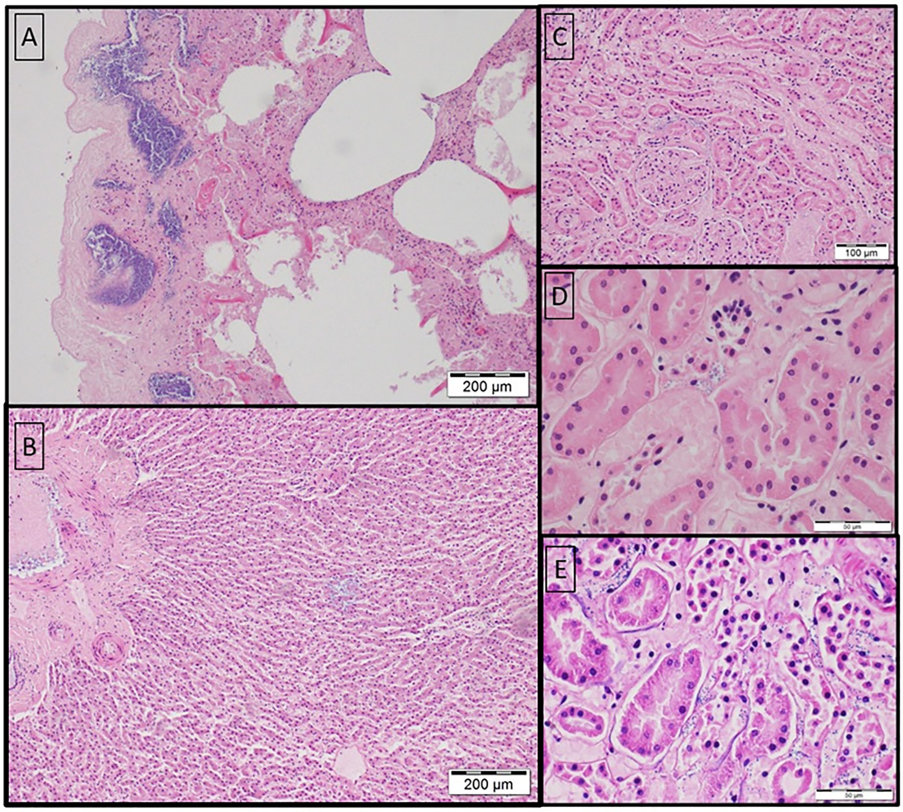

FIGURE 2 | *Streptococcus agalactiae* in lungs, liver, and kidney of a common dolphin. **(A)** Colonies of spherical or ovoid cocci, 1-2 µm in diameter, detected in subpleural alveoli and lung blood vessels (H&E stain). **(B)** Colonies identified inside hepatic blood vessels. No lesions or pathology was observed in the parenchyma (H&E stain). **(C)** Colonies and detached bacteria detected in the capillaries of the kidneys (H&E stain). Higher magnification of the kidney **(D)** and Gram-positive cocci confirmation in kidney samples **(E)**; (Gram stain).

2018. Other tests that were performed and described in the methods section produced negative results.

DISCUSSION

Although rapid population declines have been reported for the species in the Mediterranean, the causes are still a matter of speculation. Studies indicate that synergistic environmental and anthropogenic causes contributed to the reduction reported in the region (Vella et al., 2021). Overexploitation of their prey (Bearzi et al., 2008) and niche competition with striped dolphins (Giménez et al., 2018) are also potential causes. Nevertheless, quantitative data on abundance and decline rates are not available for this subpopulation, so a reduction in population size was inferred at more than 50% over a three-generation period (30 to 45 years) (Bearzi, 2012). This subpopulation was listed as “Endangered” based not only on population declines but also on its extent of occurrence, as well as a deterioration of habitat quality (Bearzi et al., 2003). One of the most commonly used proxies to assess a population (and its habitat) status is the systemic health of individuals. Environmental alterations can affect the immune response of cetaceans, making them more susceptible to infectious diseases and associated mortality (Romano et al., 2002). Concerning diseases affecting this subpopulation (both infectious and non-infectious), neoplasms with metastatic potential (Di Guardo et al., 2005; Días-Delgado et al., 2012), *Erysipelothrix rhusiopathiae* infection (Fernández-Maldonado, 2016), toxoplasmosis (Sobrino et al., 2007) and intestinal parasites (Quiñones et al., 2013) have already been described for *D. delphis* Mediterranean subpopulation. There is also evidence that shared habitat and foraging resources between common and stripped dolphins may lead to disease outbreaks, as morbillivirus epizootics are reported for both species since the 1990s (Birkun et al., 1999; Raga et al., 2008). The immunosuppressive effect of morbillivirus in wild populations can make common dolphins predisposed to other infections that can seriously compromise population health and recovery from declines caused by other stressors.

Streptococcus agalactiae is a Gram-positive coccus, and *S. agalactiae* ST-261 was first reported in 1988 from farmed Nile tilapia (*Oreochromis niloticus*) leading to outbreaks in Israel (Eldar et al., 1994). *Streptococcus* outbreaks were also reported in farmed cows in the region (and worldwide), but due to a

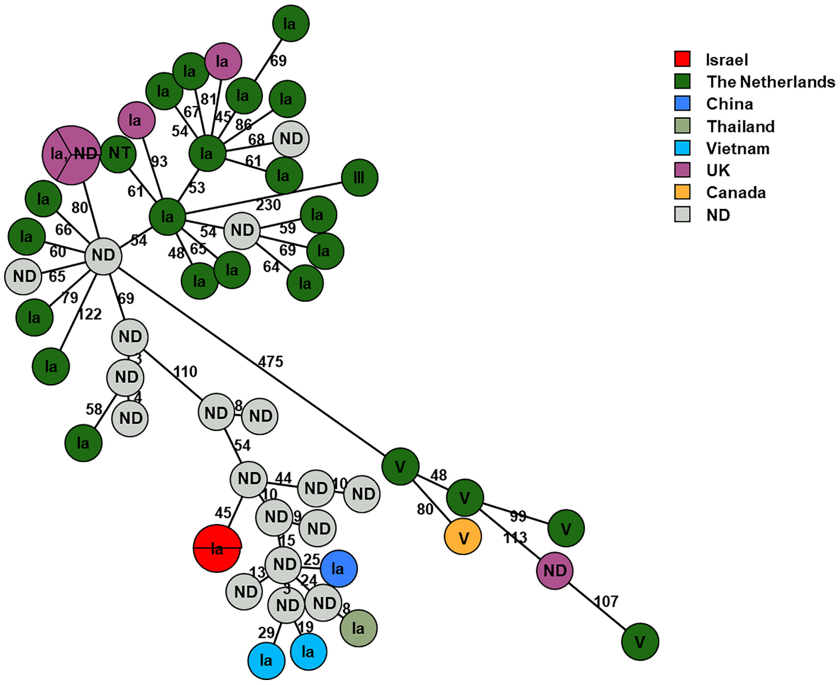

FIGURE 3 | Whole genome MLST phylogenetic analysis of 55 publicly available global ST-7 sequences. Minimum spanning tree was based on wgMLST scheme (2,207 genes). The numbers on branches indicate allelic differences between strains. Group B *Streptococcus* (GBS) serotype is shown on top of the nodes. Color-coding is by country of origin ND = no data available regarding the serotype of the isolate.

successful eradication program, it is no longer a sanitary issue in Israel (Lavon et al., 2019). In the late 1980s, it had also been described in association with marine mammal infections and cause of death, when it was isolated from wounds and tissue lesions of grey seals in Scotland (*Halichoerus grypus*) and Antarctic fur seals (*Arctocephalus gazella*) on Bird Island (Baker, 1988; Baker and McCann, 1989). The first cetacean isolations were in the early 2000s, as a fatal fasciitis and myositis in a captive common bottlenose dolphin (*Tursiops truncatus*), then a wild animal with no associated pathology (Zappulli et al., 2005; Evans et al., 2006). *Streptococcus agalactiae* has also been described as part of the nasal flora of healthy captive Hawaiian monk seals (*Monachus schauinslandi*) in Hawaii (Kissel et al., 2011). Consumption of diseased fish was a means of bacterial transmission to marine mammals, as *S. agalactiae* is a significant piscine pathogen (Numberger et al., 2021). The results of the antibacterial drug sensitivity test showed susceptibility of this isolate to the drugs that are most frequently used in human and veterinary medicine in this region. The resistance to gentamicin is reasonable and was described before for *S. agalactiae* isolated from a captive common bottlenose dolphin (*Tursiops truncatus*) (Zappulli et al., 2005). Additional studies should be conducted to increase the knowledge of GBS sensitivity profile to antimicrobials in marine animals. *Streptococcus agalactiae* was isolated from mucopurulent discharge from the urinary bladder, lungs and pericardial fluid, suggesting possible antemortem peracute septicemia. There was no significant gross nor microscopical findings available that could be associated with streptococcal infection. Unfortunately, the cause of death or primary causes for disease in this common dolphin remains undefined, but the dissemination of *S. agalactiae* may have contributed to stranding and death. The current isolate was assigned to ST7, which is a human pathogenic lineage, associated with septicemia and meningitis of immunocompromised people (Harris et al., 2011). *Streptococcus agalactiae*, previously reported in association with marine mammals (Zappulli et al., 2005), but also associated with outbreaks of fish disease, suggesting links between human, fish, and cetacean cases (Evans et al., 2006; Evans et al., 2008; Delannoy et al., 2013).

This is the first published report of a *Streptococcus agalactiae* infection in a common dolphin, with the potential to dispersion throughout the already endangered Mediterranean subpopulation. To date, conservation measures for the species are recommendations from the Convention on Biological Diversity, the Barcelona Convention (trough the Protection of the Marine Environment and Coastal Region of the Mediterranean), the Bern Convention (trough the Convention on the Conservation of European Wildlife and Natural Habitat), the Bonn Convention (trough the Convention on Migratory Species), legal protection trough the Legal Notice 203 (2003, Malta), the Convention on International Trade in Endangered Species of Wild of Flora and Fauna (CITES, Appendix II), and the Habitats Directive (92/43/EEC) (Vella et al., 2021). Marine

Protected Areas are cited as a promising strategy to protect common dolphin populations, especially from human-induced disturbances (Giménez et al., 2021). Although such measure is extremely relevant for the species, infectious agents with zoonotic potential adhere to statutory delineations, thus confounding the management of diseases that might compromise species conservation. Long-term monitoring should be done, and funds should be constantly allocated to better understand the demographic and evolutionary effects of infectious diseases (Vella et al., 2021), as well as the genetic basis of susceptibility. This pathogen has been diagnosed in other marine fish (Morick et al., 2020) and now in a marine mammal in the eastern Mediterranean Sea. Possible transmission routes of this pathogen into marine mammals can include infected prey, sea bird feces and human and terrestrial animals wastes *via* sewage and rivers (Numberger et al., 2021). Further inquiry into the importance of *S. agalactiae* in wild marine animals in this area and its zoonotic potential is needed.

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://pubmlst.org/bigsdb?db=pubmlst_sagalactiae_isolates&page=profiles, ICLGBS002 (ST35685).

ETHICS STATEMENT

This animal study was authorized with permits issued by Israel Nature and Parks Authority (n. 42548).

AUTHOR CONTRIBUTIONS

DM, ND, EB, and AS contributed to field collections, necropsy procedure, and sample processing. ZZ-S, TH, SL, DT, NW, and ES contributed to data processing, pathological interpretation, and writing of the manuscript. MR, AR, SB, and MF perform bacterial isolation and molecular characterization. All authors participated in drafting the manuscript, contributed to writing the article, and approved the submitted version.

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REFERENCES

Baker, J. R. (1988). Further Studies on Grey Seal (*Halichoerus Grypus*) Pup Mortality on North Rona. *Br. Vet. J.* 144 (5), 497–506. doi: 10.1016/0007-1935(88)90090-5

Baker, J. R., and McCann, T. S. (1989). Pathology and Bacteriology of Adult Male Antarctic Fur Seals, *Arctocephalus Gazella*, Dying at Bird Island, South Georgia. *Br. Vet. J.* 145 (3), 263–275. doi: 10.1016/0007-1935(89)90079-1

Bearzi, G. (2012). "Delphinus Delphis," in *The IUCN Red List of Threatened Species 2012*. Downloaded on 28 November 2021. doi: 10.2305/IUCN.UK.2012-1.RLTS.T134817215A195829089

Bearzi, G., Agazzi, S., Gonzalvo, J., Costa, M., Bonizzoni, S., Politi, E., et al. (2008). Overfishing and the Disappearance of Short-Beaked Common Dolphins From Western Greece. *Endanger. Species Res.* 5, 1–12. doi: 10.3354/esr00103

Bearzi, G., Reeves, R. R., Notarbartolo Di Sciara, G., Politi, E., Canadas, A., and Mussi, B. (2003). Ecology, Status and Conservation of Short-Beaked Common Dolphins *Delphinus Delphis* in the Mediterranean Sea. *Mamm. Rev.* 33, 224–252. doi: 10.1046/j.1365-2907.2003.00032.x

Berzak, R., Scheinin, A., Davidovich, N., Regev, Y., Diga, R., Tchernov, D., et al. (2019). Prevalence of Nervous Necrosis Virus (NNV) and *Streptococcus* Species in Wild Marine Fish and Crustaceans From the Levantine Basin, Mediterranean Sea. *Dis. Aquat. Org.* 133 (1), 7–17. doi: 10.3354/dao03339

Birkun, A.Jr., Kuiken, T., Krivokhizhin, S., Haines, D. M., Osterhaus, A. D. M. E., Van de Bildt, M. W. G., et al. (1999). Epizootic of Morbilliviral Disease in Common Dolphins (*Delphinus Delphis Ponticus*) From the Black Sea. *Vet. Rec.* 144, 85–92. doi: 10.1136/vr.144.4.85

Blum, S., Elad, D., Zukin, N., Lysnyansky, I., Weisblith, L., Perl, S., et al. (2010). Outbreak of *Streptococcus Equi Subsp. Zooepidemicus* Infections in Cats. *Vet. Microbiol.* 144 (1-2), 236–239. doi: 10.1016/j.vetmic.2009.12.040

Borrell, A., Cantos, G., Pastor, T., and Aguilar, A. (2001). Organochlorine Compounds in Common Dolphins (*Delphinus Delphis*) From the Atlantic and Mediterranean Waters of Spain. *Environ. Pollut.* 114 (2), 265–274. doi: 10.1016/s0269-7491(00)00213-x

Braulik, G., Jefferson, T. A., and Bearzi, G. (2021). "Delphinus Delphis (Amended Version of 2021 Assessment)," in *The IUCN Red List of Threatened Species 2021*. Downloaded on 28 November 2021. doi: 10.2305/IUCN.UK.2021-2.RLTS.T134817215A199893039

Corlorni, A., Diamant, A., Eldar, A., Kvitt, H., and Zlotkin, A. (2002). Streptococcus iniae Infections in Red Sea Cage-Cultured and Wild Fishes. *Dis. Aquat. Org.* 49 (3), 165–170. doi: 10.3354/dao049165

Delannoy, C. M., Crumlish, M., Fontaine, M. C., Pollock, J., Foster, G., Dagleish, M. P., et al. (2013). Human Streptococcus Agalactiae Strains in Aquatic Mammals and Fish. *BMC Microbiol.* 13 (1), 1–9. doi: 10.1186/1471-2180-13-41

Del Mar Otero, M., and Conigliaro, M. (2012). *Marine Mammals and Sea Turtles of the Mediterranean and Black Seas* Vol. 32 (Gland: IUCN), ISBN: .

Díaz-Delgado, J., de los Monteros, A. E., Fernández-Maldonado, C., Arbelo, M., Quesada-Canales, O., Andrada, M., et al. (2012). Mixed Testicular Neoplasia in a Short Beaked Common Dolphin *Delphinus Delphis*. *Dis. Aquat. Org.* 101, 257–260. doi: 10.3354/dao02525

Díaz-Delgado, J., Sierra, E., Vela, A. I., Arbelo, M., Zucca, D., Groch, K. R., et al. (2017). Coinfection by Streptococcus Phocae and Cetacean Morbillivirus in a Short-Beaked Common Dolphin *Delphinus Delphis*. *Dis. Aquat. Org.* 124 (3), 247–252. doi: 10.3354/dao03124

Di Guardo, G., Marruchella, G., Affronte, M., Zappulli, V., and Benazzi, C. (2005). Heterotopic Kidney Tissue in the Lung of a Free-Living Common Dolphin (*Delphinus Delphis*). *Vet. Pathol.* 42, 213–214. doi: 10.1354/vp.42-2-213

Dunn, L. J., Buck, J. D., and Robeck, T. R. (2001). "Bacterial Diseases of Cetaceans and Pinnipeds," in *Handbook of Marine Mammal Medicine: Health, Disease, and Rehabilitation*. Eds. L. A. Dierauf and F. M. D. Gulland Boca Raton, Florida: (CRC Press), 309–336.

Elad, D., Blum, S. E., Perreten, V., Fleker, M., Avni, Z., and Weisbelith, L. (2018). Emergence and Prevalence Decline of a Phenotypically Multidrug-Resistant

Staphylococcus Pseudintermedius in Israel. *Israel J. Vet. Med.* 73 (3), 14–18. doi: 10.7892/boris.121224

Eldar, A., Bejerano, Y., and Bercovier, H. (1994). *Streptococcus Shiloi* and *Streptococcus Difficile*: Two New Streptococcal Species Causing a Meningoencephalitis in Fish. *Curr. Microbiol.* 28 (3), 139–143. doi: 10.1007/BF01571054

Elia, G., Decaro, N., Martella, V., Cirone, F., Lucente, M. S., Lorusso, E., et al. (2006). Detection of Canine Distemper Virus in Dogs by Real-Time RT-PCR. *J. Virol. Methods* 136 (1-2), 171–176. doi: 10.1016/j.jviromet.2006.05.004

Evans, J. J., Bohnsack, J. F., Klesius, P. H., Whiting, A. A., Garcia, J. C., Shoemaker, C. A., et al. (2008). Phylogenetic Relationships Among *Streptococcus Agalactiae* Isolated From Piscine, Dolphin, Bovine and Human Sources: A Dolphin and Piscine Lineage Associated With a Fish Epidemic in Kuwait is Also Associated With Human Neonatal Infections in Japan. *J. Med. Microbiol.* 57 (11), 1369–1376. doi: 10.1099/jmm.0.47815-0

Evans, J. J., Pasnik, D. J., Klesius, P. H., and Al-Ablani, S. (2006). First Report of *Streptococcus Agalactiae* and *Lactococcus Garvieae* From a Wild Bottlenose Dolphin (*Tursiops Truncatus*). *J. Wildl. Dis.* 42 (3), 561–569. doi: 10.7589/0090-3558-42.3.561

Fernández-Maldonado, C. (2016). Patologías Y Causas De La Muerte De Los Cetáceos Varados En Andalucí–2014) (PhD Thesis) (Universidad de las Palmas de Gran Canaria, Las Palmas, Spain).

Frantzis, A., and Herzing, D. L. (2002). Mixed Species Associations of Striped Dolphin (*Stenella Coeruleoalba*), Short-Beaked Common Dolphin (*Delphinus Delphis*) and Risso's Dolphin (*Grampus Griseus*), in the Gulf of Corinth (Greece, Mediterranean Sea). *Aquat. Mamm.* 28 (2), 188–197.

Giménez, J., Cañadas, A., de Stephanis, R., and Ramírez, F. (2021). Expanding Protected Areas to Encompass the Conservation of the Endangered Common Dolphin (*Delphinus Delphis*) in the Alboran Sea. *Mar. Environ. Res.* 168, 105305. doi: 10.1016/j.marenvres.2021.105305

Giménez, J., Cañadas, A., Ramírez, F., Afán, I., García-Tiscar, S., Fernández-Maldonado, C., et al. (2018). Living Apart Together: Niche Partitioning Among Alboran Sea Cetaceans. *Ecol. Indic.* 95, 32–40. doi: 10.1016/j.ecolind.2018.07.020

Harris, P., Siew, D. A., Proud, M., Buettner, P., and Norton, R. (2011). Bacteraemia Caused by Beta-Haemolytic Streptococci in North Queensland: Changing Trends Over a 14-Year Period. *Clin. Microbiol. Infect.* 17 (8), 1216–1222. doi: 10.1111/j.1469-0691.2010.03427.x

Ijsseldijk, L. L., Brownlow, A. C., and Mazzariol, S. (2019). "Best Practice on Cetacean Post-Mortem Investigation and Tissue Sampling," in *Joint ACCOBAMS and ASCOBANS Document*.

Kissel, L. N., Bankowski, M. J., Koyamatsu, T. L., Nagai, R. Y., Seifried, S. E., and Crow, G. L. (2011). Aerobic Microorganisms Identified Over a Fourteen-Month Period From the Upper Respiratory Tract of Captive Hawaiian Monk Seals (*Monachus Schauinslandi*). *Aquat. Mamm.* 37 (3), 377–385. doi: 10.1578/AM.37.3.2011.377

Kuiken, T., and García Hartmann, M. (1993). Cetacean Pathology: Dissection Techniques and Tissue Sampling. *ECS Newslett.* 17, Special Issue, 1–43.

Lavon, Y., Leitner, G., Kressel, Y., Ezra, E., and Wolfenson, D. (2019). Comparing Effects of Bovine Streptococcus and Escherichia Coli Mastitis on Impaired Reproductive Performance. *J. Dairy Sci.* 102 (11), 10587–10598. doi: 10.3168/jds.2019-16673

Markey, B., Leonard, F., Archambault, M., Cullinane, A., and Maguire, D. (2013). *Clinical Veterinary Microbiology E-Book* (Amsterdam: Elsevier Health Sciences), 857.

Morick, D., Davidovich, N., Bigal, E., Rosenbluth, E., Bouznach, A., Rokney, A., et al. (2020). Fatal Infection in a Wild Sandbar Shark (*Carcharhinus Plumbeus*), Caused by *Streptococcus Agalactiae*, Type Ia-St7. *Animals* 10 (2), 284. doi: 10.3390/ani10020284

Murphy, S., and Rogan, E. (2006). External Morphology of the Short-Beaked Common Dolphin, *Delphinus Delphis*: Growth, Allometric Relationships and Sexual Dimorphism. *Acta Zool.* 87 (4), 315–329. doi: 10.1111/j.1463-6395.2006.00245.x

Notarbartolo di Sciara, G., and Tonay, A. M. (2021). *Conserving Whales, Dolphins and Porpoises in the Mediterranean Sea, Black Sea and Adjacent Areas: An ACCOBAMS Status Report, (2021)* (Monaco: ACCOBAMS), 160.

Numberger, D., Siebert, U., Fulde, M., and Valentin-Weigand, P. (2021). Streptococcal Infections in Marine Mammals. *Microorganisms* 9 (2), 350. doi: 10.3390/microorganisms9020350

Poyart, C., Tazi, A., Réglier-Poupet, H., Billoët, A., Tavares, N., Raymond, J., et al. (2007). Multiplex PCR Assay for Rapid and Accurate Capsular Typing of Group B Streptococci. *J. Clin. Microbiol.* 45 (6), 1985–1988. doi: 10.1128/JCM.00159-07

Quiñones, R., Giovannini, A., Raga, J. A., and Fernández, M. (2013). Intestinal Helminth Fauna of Bottlenose Dolphin, *Tursiops Truncatus*, and Common Dolphin, *Delphinus Delphis*, From the Western Mediterranean. *J. Parasitol.* 99, 576–579. doi: 10.1645/GE-3165.1

Raga, J. A., Banyard, A., Domingo, M., Corteyn, M., Van Bressem, M.-F., Fernández, M., et al. (2008). Dolphin Morbillivirus Epizootic Resurgence, Mediterranean Sea. *Emerg. Infect. Dis.* 14, 471–473. doi: 10.3201/eid1403.071230

Romano, T. A., Olschowka, J. A., Felten, S. Y., Quaranta, V., Ridgway, S. H., and Felten, D. L. (2002). "Immune Response, Stress, and Environment: Implications for Cetaceans," in *Cell and Molecular Biology of Marine Mammals*. Ed. C. J. Pfeiffer (Malabar, Florida: Krieger Publishing Co., Inc).

Sobrino, R., Cabezón, O., Millán, J., Pabón, M., Arnal, M. C., Luco, D. F., et al. (2007). Seroprevalence of *Toxoplasma Gondii* Antibodies in Wild Carnivores From Spain. *Vet. Parasitol.* 148, 187–192. doi: 10.1016/j.vetpar.2007.06.038

Vella, A., Murphy, S., Giménez, J., de Stephanis, R., Mussi, B., Vella, J. G., et al. (2021). The Conservation of the Endangered Mediterranean Common Dolphin (*Delphinus Delphis*): Current Knowledge and Research Priorities. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 31, 110–136. doi: 10.1002/aqc.3538

Zappulli, V., Mazzariol, S., Cavicchioli, L., Petterino, C., Bargelloni, L., and Castagnaro, M. (2005). Fatal Necrotizing Fasciitis and Myositis in a Captive Common Bottlenose Dolphin (Tursiops Truncatus) Associated With Streptococcus Agalactiae. *J. Vet. Diagn. Invest.* 17 (6), 617–622. doi: 10.1177/104063870501700620

Zlotkin, A., Hershko, H., and Eldar, A. (1998). Possible Transmission of *Streptococcus Iniae* From Wild Fish to Cultured Marine Fish. *Appl. Environ. Microbiol.* 64 (10), 4065–4067. doi: 10.1128/AEM.64.10.4065-4067.1998

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ORIGINAL RESEARCH
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Ship Strike Risk for Fin Whales (*Balaenoptera physalus*) Off the Garraf coast, Northwest Mediterranean Sea

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Ship strikes are a widespread conservation issue for many cetacean species globally. Population level impacts depend on the occurrence and severity of collisions, which may lead to life altering injuries or fatalities. Such impacts are a major concern for large, long-lived, and reproductively slow species like the fin whale. Since 2014, a seasonal feeding aggregation of fin whales has been monitored from February to June off the Catalan coast (Spain), in the northwest Mediterranean Sea. Oceanographical factors influence the occurrence and high density of krill within submarine canyons along the continental shelf, resulting in high whale abundance within a small spatial area. The study area extends 37 km offshore across a 1,944 km^2 marine strip situated between the towns of Torredembarra and Castelldefels. This fin whale feeding ground is exposed to high density marine vessel traffic, given its location between the northern Mediterranean shipping lane, which links Barcelona and Tarragona Ports to the Atlantic Ocean and wider Mediterranean Basin. Ship strikes represent the greatest conservation threat for fin whales in the Mediterranean Sea. At least four fin whales have been found dead in Barcelona Port since 1986 due to ship strikes and seven live whales have been documented with injuries in the study area since 2018. Fin whale distribution was mapped with known high-risk marine vessels' (cargo, tanker and passenger vessels) shipping lanes. Vessel density and shipping lanes characterised by speed were considered. Collision risk was estimated monthly based on the predicted fin whale occurrence and traffic density. Several shipping lanes crossed the fin whale feeding habitat every month with an average speed of 15 kn. Cargo vessels displayed the highest ship-strike risk during April, overlapping with the peak of fin whale sightings in the critical feeding area. Slower vessel speeds (8 kn) in waters <200 m depth or along the continental shelf should be implemented along the Catalan coast, during the whale season. These suggestions should be applied into the Barcelona Port transport separation scheme. Ship strike risk for this species will persist unless active management plans are adapted in the region to mitigate its risk.

Keywords: fin whale, ship strike, collision, marine traffic, injury, feeding, Catalonia, Mediterranean Sea$_8$

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1. INTRODUCTION

The fin whale (*Balaenoptera physalus*) (Linnaeus, 1758) is the largest cetacean species present in the Mediterranean Sea and the only baleen whale species regularly found within the Mediterranean basin year-round (Notarbartolo di Sciara et al., 2016; Aguilar and García-Vernet, 2018). Two populations have been identified within the Mediterranean Sea: the Northeast Atlantic Ocean (NENA) population and the Mediterranean subpopulation (Notarbartolo di Sciara et al., 2016). Both populations cohabit annually in the Balearic Sea off southern Spain and along the Catalan coast (Castellote et al., 2008; Castellote et al., 2012a; Gauffier et al., 2020). The NENA population moves through the Strait of Gibraltar into the Mediterranean basin between November and April and leaves between May and October (Notarbartolo di Sciara et al., 2016; Gauffier et al., 2018; Gauffier et al., 2020). On the other hand, the Mediterranean subpopulation is found in the Ligurian Sea in summer from July to September and in the Eastern Mediterranean in winter, where they have been observed feeding around Lampedusa Island in February. However, there is some evidence for the species presence in Ligurian between October and December (Canese et al., 2006; Panigada et al., 2006; Pintore et al., 2021).

Fin whales primarily feed on northern krill (*Meganyctiphanes norvegica*) (M. Sars, 1857) in the Mediterranean Sea (Notarbartolo di Sciara et al., 2016; Borrell et al., 2021). *M. norvegica* requires high levels of primary productivity that are linked to oceanic fronts (Druon et al., 2012). These oceanic fronts occur seasonally in patches across the Mediterranean and are linked to fin whale movements (Druon et al., 2012; Panigada et al., 2017; Lydersen et al., 2020). Over the past decade, during the spring, a new feeding ground has been discovered along the continental shelf off the Catalan coast, between Torredembarra and Castelldefels. Additionally, this feeding aggregation was discovered around the Cunit and Foix submarine canyons (EDMAKTUB, 2021, in press). These underwater geographic formations are particularly important due to the upwelling effect present in the area, which triggers elevated krill densities during spring (EDMAKTUB, 2021, in press; Sabates et al., 1989; Puig et al., 2000).

Distinct behavioural patterns in fin whales have been documented on the Garraf Coast. Surface feeding behaviour was observed approximately within the first 10 m of the water column, by EDMAKTUB (2018). While feeding, fin whales exhibit irregular movements in a zig-zag pattern or circular motion (Tort et al., 2017). Breathing patterns were observed to oscillate on average between two to six min between surface intervals (Tort et al., 2017). Moreover, travelling, and resting behaviour have been identified in this area. While travelling, fin whales conduct a linear path with a constant speed. Both resting and travelling have been described with a regular breathing pattern (Tort et al., 2017). Resting behaviour is defined when a whale remains stationary at the surface, or at a shallow depth just below the surface.

The fin whale is threatened by a variety of natural diseases, such as the cetacean morbillivirus (CeMV), and by anthropogenic effects including persistent pollutants, noise pollution, fishing gear entanglement, climate change and ship strikes within the Mediterranean Sea (Panigada et al., 2006; Mazzariol et al., 2016; Notarbartolo di Sciara et al., 2016). Moreover, it is a long-lived species with a high mortality rate in the first stage of life (77%), which decreases with maturity (Arrigoni et al., 2011). Female fin whales become reproductively active between 22.8 and 36.8 years, making the species highly vulnerable to natural and anthropogenic pressures. This leads to the high mortality rate recorded in the Mediterranean populations (Panigada et al., 2006; Arrigoni et al., 2011).

Fin whales are exposed to several anthropogenic threats (Panigada et al., 2006; Notarbartolo di Sciara et al., 2016). The Mediterranean Sea is one of the most heavily populated and anthropogenically impacted marine habitats globally, despite comprising only 0.8% of the global marine surface area (Micheli et al., 2013; Vaes and Druon, 2013). In this area, shipping has grown exponentially since 1992, reaching up to 30% of the global maritime traffic annually (Vaes and Druon, 2013). 220,000 ships >100 gross tons operate within it, accessing 300 ports, while following shipping lanes along the north of African coast or the southern coast of Europe (Panigada et al., 2010; Vaes and Druon, 2013).

The International Union for Conservation of Nature (IUCN) red list assessment recently classified the conservation status of the Mediterranean fin whale population as endangered (Panigada et al., 2021) and the status of the NENA population as vulnerable (Cooke, 2018). The species is protected across its range by the European Union Habitats Directive as an Annex IV species, the Marine Strategy Framework Directive (MSFD) and the Agreement on the Conservation of Cetaceans in the Black Sea, the Mediterranean Sea and Contiguous Atlantic Area (ACCOBAMS) (Vaes and Druon, 2013; Notarbartolo di Sciara et al., 2016). Within Spanish waters, cetaceans are protected from disturbance and harm with exclusion zones applied around animals under the Royal Decree 1727/2007, on December 21st. Additionally, fin whales are included in the list of wild species under special protection regime and in the Spanish catalogue of threatened species, being catalogued as vulnerable by Royal Decree 139/2011, of September 2021.

To protect migratory and resident cetacean species, the Mediterranean Cetacean Migration Corridor (CMC) declared the area as a Specially Protected Area of Mediterranean Importance (SPAMI) at the COP21, which was adopted by the Barcelona Convention in 2019 (OceanCare, 2021). The CMC was established by the Royal Decree 699/2018 in June 2018. The CMC comprises 46,385 km^2 between Catalonia and Valencia within the Balearic archipelago in the Balearic Sea (OceanCare, 2021). Likewise, the Pelagos Sanctuary was designated within the Ligurian Sea between France and Italy in 1999 (Notarbartolo di Sciara et al., 2016). This designation was in response to the region's importance for the species in the summer, as it supports the highest density of foraging fin whales in the Mediterranean basin (Notarbartolo di Sciara et al., 2016; Panigada et al., 2017). An Important Marine Mammal Area (IMMA) was also implemented across the North-West Mediterranean Sea, slope and canyon system, between the Pelagos Sanctuary and the Balearic Sea (IUCN, 2017; Torreblanca et al., 2019). This

IMMA acts as a guide for the designation of marine protected areas (MPA's) for important habitats for species warranting formal protection measures. Furthermore, the Coast of Garraf is included in the Natura 2000 Network area, which establishes a robust policy framework to ensure ecosystem sustainability and contributes to international efforts in marine conservation (Natura 2000, European Commission), **Figure 1**.

Ship strikes represent the current greatest threat to large whales in the Mediterranean Sea (Laist et al., 2001; Panigada et al., 2006; Vaes and Druon, 2013; Di-Meglio et al., 2018; Frantzis et al., 2019; Schoeman et al., 2020; Winkler et al., 2020). Fin whales are the cetacean species most often killed by ship strikes globally (Laist et al., 2001; Panigada et al., 2006; Winkler et al., 2020). When collided with, fin whales can be pinned onto the bow of large vessels or become dislodged from the bow and float due to decomposition gases (Laist et al., 2001; Moore et al., 2020). Injuries resulting from ship strikes vary in severity depending on the speed and size of the vessel involved in the incident (Laist et al., 2001; Panigada et al., 2006). They typically occur along the lateral and dorsal sides of whales and vary from lacerations caused by propellor movements, blunt force trauma causing broken bones and scarring to deformations and amputations of body parts (Laist et al., 2001; Panigada et al., 2006; de Reuver et al., 2021). Collisions occur most often over continental shelf waters (Laist et al., 2001). The possibility for large vessels to spot and successfully evade hitting surfacing whales is inversely proportional to the size and speed of the vessel (Laist et al., 2001). Once vessel speed reaches ≥14 kn (25.9 km/h) the likelihood of severe injuries or a fatal interaction to occur between a whale and a ship (≥ 80 m) increases greatly (Laist et al., 2001; Panigada et al., 2006; Keen et al., 2019). Damage to vessels and injuries to the crew onboard have also been reported on some occasions (Schoeman et al., 2020; Sèbe et al., 2020; Laist et al., 2001). Collision risk areas are dynamic regions where environmental factors, prey density, whale occurrence and marine traffic combine to threaten whales (Ham et al., 2021). Within the Mediterranean between 1972 and 2001, 46 (16%) of 287 stranded fin whale carcasses examined were killed by ship strikes primarily within or adjacent to the Pelagos Sanctuary, where near miss events were also noted from 2008 to 2019 (Panigada et al., 2006; David et al., 2022). The risk posed by ship strikes to fin whales has been extensively studied within the Ligurian Sea in the North-Western Mediterranean (Panigada et al., 2006; Vaes and Druon, 2013; Ham et al., 2021; David et al., 2022). In this area, an overlap of important shipping lanes and satellite tagged fin whales exhibiting foraging behaviour, demonstrated that the species uses seasonal foraging habitats in high-risk areas (Panigada et al., 2017). However, there is a lack of knowledge about the risk posed by ship strikes in the Balearic Sea.

The aim of this study was to identify critical areas where marine traffic and fin whale presence overlapped off the Catalan coast of Garraf, an area between the large ports of Barcelona and Tarragona. This study was structured in different steps. Firstly, fin whale behaviour was evaluated, and fin whale distribution was estimated in the study area. Secondly, photographed fin whales displaying collision marks and historical strandings data were accessed and evaluated. Finally, marine traffic maps to detect ship route concentrations were created considering types of vessels with known high ship strike risk. Vessel speed by vessel type and length was evaluated while route density maps were created which was used to identify potential fin whale ship strike risk areas within the study area. This study will contribute to risk mitigation and protection measures in order to promote conservation, help to make management decisions and reduce the risk of collisions occurring while fin whales are seasonally present.

2 THE METHODOLOGY

2.1 Study Area

The study area comprises a 1,944 km^2 region, centred around the city of Vilanova i la Geltrú and extending 37 km offshore. The area stretches between Torredembarra and Castelldefels out to

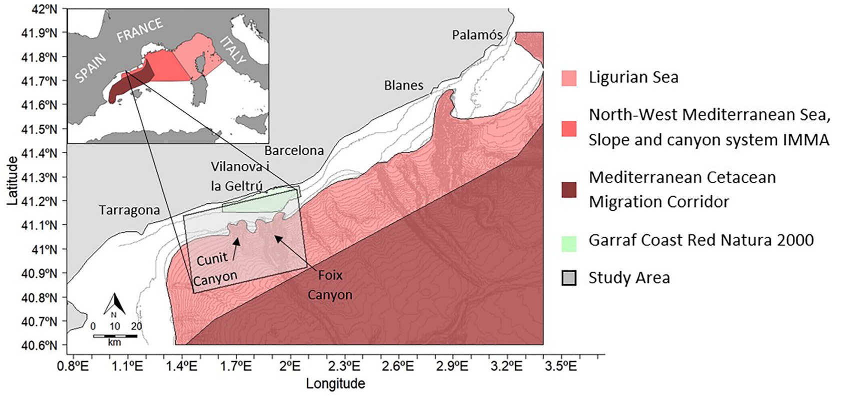

FIGURE 1 | Fin whale project study area along the Garraf coast. The Foix Canyon (largest system) is located on the right side of the study area and the Cunit canyon is situated on the left side. The bathymetry lines have an interval of 50 meters. In the top left it can be seen an image of the W- Mediterranean in which there are represented the SPAMI areas;.In dark red the cetacean corridor, in light pink the Pelagos Sanctuary, in red the North-West Mediterranean Sea, Slope and canyon system IMMA a.nd in light green the Garraf Coast Red Natura 2000.

40°81.38’ N, 1°46.50’ E and 40°92.43’ N, 2°09.21’ E (**Figure 1**). The Cunit and Foix Canyons are situated within the middle of the survey area along the continental shelf edge. The bathymetry rapidly deepens from the continental shelf (100 m) to 2,000 m in the Foix Canyon (Puig et al., 2000). These canyon systems and the Liguro-Provençal-Catalan current create an upwelling effect which promotes primary production during spring months resulting in high densities of krill along the shelf edge (Puig et al., 2000).

The survey fieldwork used for this study was carried out in 2021, between Mach and May. A 14.3 m catamaran *RV MAKTUB* (model catana 471) was the research platform where dedicated visual surveying took place, on a near daily basis during the fieldwork season. The area was surveyed under engine power and by sail during suitable weather conditions (< Beaufort Sea State 4 and < 2 m swell) and with a sustained speed of five to six knots. The area being surveyed did not follow a systematic approach. The daily survey effort depended on where whale activity was detected over previous days, reported by fisherman, or determined by the weather conditions in the study area (sea state and wind strength/direction). The continental shelf edge and submarine canyon systems, where the species regularly forages, were targeted for surveying.

2.2 Fin Whale Data Collection

Fin whale sightings were obtained from dedicated research surveys. Visual effort began and finished at the 30 m in depth mark. Four observers continuously scanned the sea for whales from all 4 sides of the catamaran to have 360° coverage. Fin whales were detected either by visually spotting surfacing animals and their tall columnar blow with the naked eye or with the use of 8 x 42 binoculars. One of the observers on a break was responsible for data recording during sightings. Positions were rotated every 30 min to avoid fatigue and to minimise observer bias. These observers were trained at the beginning of the season and remained throughout it to ensure consistency in the data collection.

Once a fin whale was visually detected, survey effort to detect whales was ended and the following protocol began. Sightings occurred when the individual(s) were first seen and ended when identification data and/or biological samples were obtained. To minimise duplicating sightings of the same individuals, new sightings were recorded in three circumstances: (1) if more animals were sighted in a period over 45 min and identified as different through photo-identification, (2) if animals surfaced >3 km away from the last whale dive position and were observed in less than 10 mins from the last observation, (3) if they were sighted >2 hrs after the initial sighting. In each sighting the GPS position, species (if other cetacean species were encountered), the number of animals, the start/end time, the angle, and the estimated distance of the whale when sighted for the first time were recorded. Distance was estimated using a compass and judgement from the skipper. Environmental conditions at the beginning of the sighting were documented. During the sighting, additional data was recorded, such as photo-identification pictures, drone videos and behaviour of the animal(s).

2.3 Evidence of Ship Strikes on Live and Dead Fin Whales

Live fin whales were photographed and/or filmed with DSLR cameras (Nikon D7100 with a 150 - 400 mm telephoto lens and Canon 90D with a 70 – 200 mm telephoto lens) and a drone (DJI Mavic Pro 2/Phantom 3 Pro) during the field season for photo-identification purposes. Whales with lesions and damage consistent with ship strikes were compiled to gauge the prevalence of non-lethal ship strikes in the study area.

Strandings of fin whales were assessed from along the Garraf coast using the literature and newspaper reports to identify whales with evidence of ship strike damage such as acute abrasion and hematoma bruising on the dorsal side of the body (Laist et al., 2001; Panigada et al., 2006). Fin whales calve standings were also investigated within the study area and along the Catalan coast. In addition, records of fin whale bones recovered by bottom trawling fishermen in the study area between the ports of Ametlla de Marand Palamós were also compiled.

2.4 Fin Whale Distribution

The statistical analysis was conducted using R software version 4.1.1 (R Core Team, 2020). QGis version 3.4.3 – Madeira (QGIS Development Team, 2018) was used to map the fin whale presence and the traffic data.

Fin whale sightings recorded by dedicated research surveys and accounting for observer effort were used to determine the presence and absence of fin whales across the study area. The observer effort was defined as the time where observers were actively surveying. Only on effort transects were considered for analysis. The 2021 season was used to perform the distribution model. The aim of the model was to compute the collision risk map. The complete traffic database available was the EMODnet database (www.emodnet-humanactivities.eu) which starts in 2019 (see section 2.5). 2019 was an unusual year with few sightings and in 2020 the COVID-19 pandemic stopped the season, so data collection was not possible. Therefore, only 2021 data was used for the purposes of this study.

The data collected was assessed per month (from March to May). The data was transferred to a 3x3 km grid. To define the sampled area, only the vessel tracks, in which observers were on effort were considered. A buffer of 3.7 km perpendicular distance from the vessel track was generated, according to the maximum distance where whales were sighted during surveys. The probability of fin whale detection depended on their distance from the vessel, so a probability of detection was assigned to the buffer area. It was 1 for the first 1.8 km, where it was assumed that all fin whales would definitely be detected if present, according to the sightings recorded and as followed by other authors such as Ham et al. (2021). The detection probability then decreased with increasing distance ranges: 0.75 from 1.8 to 2.4 km, 0.5 from 2.4 to 2.7 km and 0.25 from 2.7 to 3.7 km. The buffer threshold was established according to the percentages of sightings for each distance range, being 0.85, 0.95 and 0.97 respectively. These buffers were then overlaid on the 3 km grid. A grid cell was only considered sampled if ≥50% of its area was covered by the buffer with a detection probability greater than 0.5, otherwise, it was excluded. For each sampled grid cell, a unit effort associated with the buffer was defined.

The unit effort was defined as the weighted average of the probability of detection by coverture on each pixel. A grid cell may be covered by multiple buffers during a month (e.g., a vessel track might pass more than once by the same grid cells in a month). Therefore, all unit efforts within each cell were then monthly summed. This grid sampled effort (hereafter called effort) was included into the fin whale distribution model as a correction variable.

Fin whale presence and absence within the sampled area were associated with each grid cell. When there was a sighting, it was assigned the value according to the number of animals observed. The total observations within each grid cell were then monthly summed to calculate the total number of sightings recorded in that particular cell. A zero was assigned when there were no sightings in a sampled effort cell.

Four oceanographic variables were considered as explanatory oceanographic variables to describe the fin whale distribution over the study area: chlorophyll a concentration (Chla in mg/m3), sea surface temperature (SST in K), mean depth (m) and mean slope angle (°) of the grid cell. The Chla and the SST were obtained from E.U. Copernicus Marine Services Information, both with a daily resolution of 1x1 km and 0.01°x0.01° raster respectively in a L3 processing level. Bathymetry data were derived from a fine resolution 115x115 m raster provided by the European Marine Observation and Data Network (EMODnet) (www.emodnet-humanactivities.eu). The slope angle of the seafloor was also computed through EMODnet using the slope function in QGis.

The oceanographic factors were summarised by month (from March to May) using the 75 quantile for the Chla and the mean for the SST. Then, all oceanographic values were gridded into the 3 km cells by computing the mean value with the Zonal Statistics function in QGis. Having a resultant grid with a mean Chla, SST, depth, and slope value per month.

The fin whale distribution for 2021 was modelled using Generalised Additive Models (GAMs) with Poisson distribution family and log link function and were computed using the mgcv package (Wood, 2011). All the available oceanographic variables were considered in a preliminary analysis to select the most relevant ones. A parametric linear term for each variable was checked against a smooth alternative and interactions. The variable selection was based on two criteria; (1) to determine which covariates have the strongest effects on the number of sightings, enhancing model interpretability, and (2) improving its prediction accuracy, aiming for a balance between fit and parsimony. For all the possible combinations, the prediction error criteria General Additive Cross-Validation (GACV) was used to compare models. Environmental variables that were not interpretable were discarded even if they improved the model fit.

The relationship between fin whale distribution and the recurring spring phytoplankton bloom that primarily occurs in coastal waters, continental shelf breaks and around the submarine canyons was investigated by these models. Krill does not move far from the continental shelf into shallower waters, therefore, fin whales are typically found in waters >50 m in depth, where krill aggregations can be found. Hence, a coastal correction factor (CC) was used to mask coastal areas and exclude high Chla values caused by river runoff that might affect results interpretation.

2.5 Marine Traffic Data

In this study, three complementary marine traffic databases were used for different aims. Firstly, commercial port data was acquired from the Barcelona Port authority and from the literature (OceanCare, 2021; Barcelona Port, 2022). The annual usage of Barcelona Port by cargo ships (Ro-Ro ships, Lo-Lo ships, bulk carrier and container ships), tanker vessels (oil tankers) and passenger vessels (cruise ships and ferries) were analysed from 2016 to 2021 with the aim of characterizing the marine traffic in the area. Cargo boats were defined as 70 to >200 m in length and ≤ 15 kt in speed, tankers were >80 m in length and 15 kt in speed and passenger ships were >80 m in length and <24 kt in speed. Secondly, two sources of Automated Identification System (AIS) data were analysed: (1) from the antenna located at the Laboratory of Applied Bioacoustics (LAB) of the Technical University of Catalonia, BarcelonaTech (UPC); and (2) from EMODnet. The aim, in this case, was to map and characterize the traffic corridors in the study area. The EMODnet database was used to compensate for the constraints from the LAB database, as explained below.

The AIS data was used to identify the position of vessels, their course and speed, as required by the International Maritime Organisation (IMO). The IMO requires AIS use by all vessels >500 gross tons, for any vessel >300 gross tons that is engaged on international voyages and for all passenger vessels irrespective of size (IMO, 2002). However, AIS transceivers are commercially available and are also used on vessels that do not meet the requirements by law.

High-risk ship strike vessels were selected based on criteria published in the literature considering overall length and speed (Laist et al., 2001; Panigada et al., 2006; Winkler et al., 2020; Stepanuk et al., 2021). Following Stepanuk et al. (2021), three types of vessels (cargo, tanker and passenger ships) were selected using their IMO, a unique identification number. These vessel types were previously identified as high risk in Panigada et al. (2006). The occurrence of these vessel types, seasonality and movement patterns were then assessed within the study area.

The LAB AIS data was accessible from 2011 to 2020. It was stored through the data stream of the shallow water OBSEA platform in the Mediterranean Sea. OBSEA (www.obsea.es) is a cabled seafloor underwater observatory located 4 km (41°10.92' N, 1°45.14' E) off the Vilanova i la Geltru coast and placed within a fishing protected area. In the AIS data from the LAB, there were gaps and much variability mainly due to two factors. Firstly, the AIS data was saved through OBSEA and therefore it was only stored when OBSEA was active. Secondly, the range of the antenna receiver decreased over time, resulting in a lack of data in recent years (e.g., in 2020, 90% of the data was below an 8 km range and less than 1% of vessels were detected at more than 15 km from OBSEA).

A two-step quality control check was implemented. Vessels detected on land or with erroneous values were deleted and then, only data from days that registered ships >42 km from the LAB, in Vilanova i la Geltrú were selected for analysis. Thus, only days with a detection range which covered the whole study area were considered for analysis, ensuring that biassed AIS data (with a partial detection range over the study area) were excluded. Given the goal of this study was to identify the principal marine traffic lines through the survey area (which remain consistent annually), all the years of data from 2011 to 2020 were pooled by month.

Shipping lanes and vessel speed within the study area from February to June were identified. Cargo, tanker, and passenger vessel shipping lanes were represented by monthly maps, characterised by three speed ranges: low (up to 8 kn), moderate (8 to 16 kn) and high (>16 kn). Vessel traffic speed through the study area was also analysed. Vessel speed distributions were compared between years and within years to evaluate the reliability of pooling all the AIS data. The normality assumption was checked to use more statistically powerful parametric tests. However, since the normality assumption failed, three non-parametric tests were computed. The Kolmogorov-Smirnoff test, a test of the equality of continuous, one-dimensional probability distributions; the Mann-Whitney-U test (also called Wilcoxon rank-sum test) which consider as null hypothesis equal medians; and the Kruskal-Wallis test by ranks, the non-parametric equivalent of the ANOVA but comparing that the medians of all groups are equal. The speed distribution by vessel type and length were also compared. We classified AIS transmissions into vessel length classes as in Panigada et al. (2006): 50–100, 100–200, 200–300, and 300–400 m. Records that contained no information on vessel classification were not included in the analyses.

Due to the constraints with the LAB data quality, a vessel density map per month and year was unfeasible but needed to model the ship strike risk assessment in 2021. Therefore, a parallel analysis using EMODnet data was implemented. Marine traffic data between March and May 2021 was acquired. Route density maps produced and provided to EMODnet by the European Maritime Safety Agency (EMSA) were obtained for cargo, tanker, and passenger vessels. The route density of vessels in the study area was then calculated by computing the mean of the number of routes per month in the same 3x3 km grid squares for each vessel type in 2021, using EMODnet dataset.

2.6 Ship Strike Risk Assessment

The critical fin whale habitat assessment was adapted from Tregenza et al. (2000). The risk of vessel strike (Ri) was defined as the probability of lethality to fin whales, from 0 (low) to 1 (high). Ri was described by this equation:

$$R_i = (W_i + 0.64L) * 10^{-3} * Dcell * T * NAD * VD$$

Where Wi is the mean hull width in metres according to David et al. (2011), L is the length of a whale in metres, Dcell is the mean distance travelled per pixel, T is the percentage of time near the surface, NAD is the density of fin whales according to the distribution model and VD is the density of vessels computed from the EMODnet database (see section 2.5).

Fin whale size (L) in the study area was estimated to be approximately 17 m, by using drone measures and according to (Szegedi et al., 2019). The percentage of time near the surface was set to 30, which was extracted from David et al. (2011). To convert the number of fin whales into a probability density, the predicted number of animals by grid cell was divided by the total number of fin whales estimated (NAD) in the grid for the whole season.

These variables were computed monthly in a 3x3 km^2 grid, the same as in the distribution model. The Dcell was estimated following the formula from Druon (2014):

$$Dcell = 0.5 * Dmax$$

Where Dmax is the maximum distance which can be travelled by a vessel in a grid cell.

3 RESULTS

3.1 Fin Whale Sightings

Between March and May in 2021, 149 fin whale sightings were recorded. These sightings consisted of 187 individuals from across the study area (**Figure 2**). Generally, single whales were recorded in each sighting (n=116), except for some groups consisting of between two to five whales (n=33), which were mostly observed in May. Sightings varied each month from a minimum of 32 in March to a maximum of 77 in April. The number of fin whale individuals sighted per month ranged from 42 to 87. April had the highest number of fin whales recorded (**Figure 2**). Fin whales were also photographed off Barcelona and Tarragona Ports in the presence of vessels on eight occasions during the season from March to May (**Supplementary Figure S1**).

The percentage of fin whales observed that were engaged in surface feeding behaviour was 71% of observations, whilst 9% was travelling behaviour and 4% resting behaviour. For the remaining 16% of the sightings, the behaviour of the whale was undefined. In the first 10 m of the water column, 17 whales were filmed feeding in the area by the drone. Most feeding behaviours (55%) were observed in the evening. Resting behaviour was observed on six occasions.

3.2 Fin Whale Distribution

The selected monthly model was a GAM model using Poisson family distribution and log link function, fitted to investigate the relationship between the logarithm of the number of sightings (response variable NS) and the oceanographic variables mentioned above. This model included a multidimensional isotropic smooth function of the Chla, plus the smooth interaction between Chla and SST and the interaction between Chla and slope. The linear effect of depth and coastal correction factor, adjusted by the effort in a logarithmic scale was added. This is the resulting model (R code can be found in

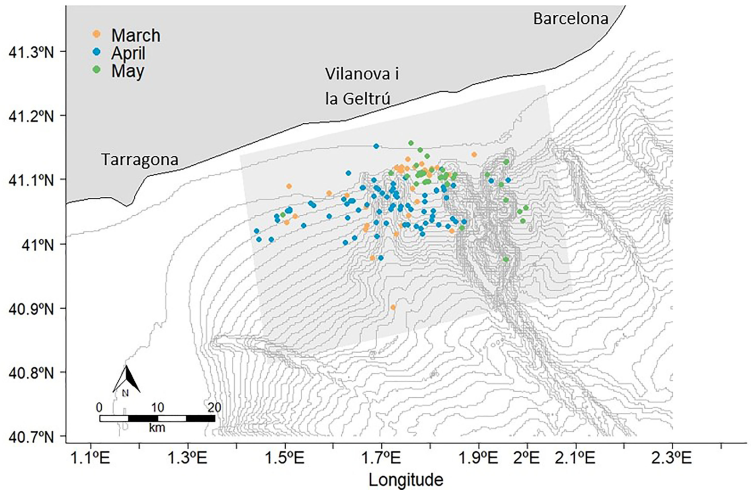

FIGURE 2 | Fin whale sightings between March-May 2021. The bathymetry lines have an interval of 50 meters.

Supplementary Figure S2):

$$E[log(NS)\ ] \sim s(Chla, bs = cs) + s(Chla, SST) + s(Chla, slope)$$

$$+ depth + CC - \log(Effort) + \epsilon_i$$

$$\epsilon_i \sim Poisson$$

All the tested parameters were significant, except for the coastal correction (CC). The model had a 57.9% deviance explained. The dataset presented a zero-inflation problem which was observed in the residual plots, but it did not invalidate the model (**Supplementary Figure S3**). The explanatory variables plots can be found in the **Supplementary Figure S4, S5**. The main standard deviation of the distribution maps was 0.27, 0.37 and 0.38 for each month. The distribution maps with the standard deviation per grid cell can be found in the supplementary material (**Supplementary Figure S6**).

Bathymetry was found to be a strong predictor of fin whale habitat preference. The fin whale distribution in March and April was very consistent with a high concentration of sightings on the continental shelf break and between Cunit and Foix canyons as displayed in the distribution model (**Figure 3**). In May, the distribution started to decrease at the shelf break and moved more offshore to deeper waters (1,000 - 2,000 m) inside the Foix Canyon.

3.3 Fin Whale Ship Strike Evidence

Seven whales displaying ship strike injuries were observed. Scars were the most prevalent around the dorsal fin, both in the anterior (n = 2), and in the posterior (n = 1) sides of the fin. On one individual, a lateral scar was observed along its right flank. In addition, a deep cut with scar tissue on the tailstock of one whale was noted. However, the most severe injury recorded was a whale with a dorsal fin collapsed to its right side. Due to this injury, an 'idiopathic' scoliosis posterior to the dorsal fin on its right side was developed (**Figure 4**) (Alves et al., 2017; de Reuver et al., 2021). This injury compromised its swimming speed and diving abilities, causing it to lift the right side of its tail fluke out of the water while diving. It was in a very poor nutritional condition with its vertebral column visible (Castrillon and Bengtson Nash, 2020) (**Supplementary Figures S7-S10**). In the study area, only one other whale was recorded across all seasons, exhibiting injuries in April 2018. This whale also displayed 'idiopathic' scoliosis at its dorsal fin, where the vertebral column curved to the right. It was not re-sighted in any other season. Fin whales were seen on six occasions in the presence of cargo and tanker vessels during the 2021 season (**Supplementary Figure S11**). Two individuals were seen outside Barcelona Port on 19 May and one whale was seen off Tarragona Port on 10 May (**Supplementary Figure S11**).

Four fin whales have been recorded as ship strike fatalities in Barcelona Port since 1986 (**Table 1**). In 1986, two whales were found on the bow of vessels entering the port. One male whale was brought in by storm conditions on 9th of May 2002, displaying abraded skin on its central left flank posterior to the pectoral fin. Moreover, a male individual was located floating in the port in 2016. The skin along the juvenile male whale's left flank had been removed in an acute area, with a depression and signs of haemorrhaging (**Figure 5**). The haemorrhaging on the body was used to indicate that the whale circulation system was still active meaning that the whale was alive when hitting. The location of the collision marks on the body also indicates that they were alive. Orange coloured faeces were found in the gastrointestinal tract indicating that the whale had recently fed on krill prior to being struck.

3.4 Marine Traffic

Barcelona port data was available for cargo, tanker, and passenger vessels from 2014 to 2021. Between 4,468 and 3,587 cargo ships, 852 and 1042 tanker ships and 3869 and 2156 passenger ships visit Barcelona Port annually. Most cargo ships using the port were from foreign cargo vessels (Barcelona Port, 2022). The marine traffic was evaluated during the fin whale season between 2016 and 2021 (**Table 2**).

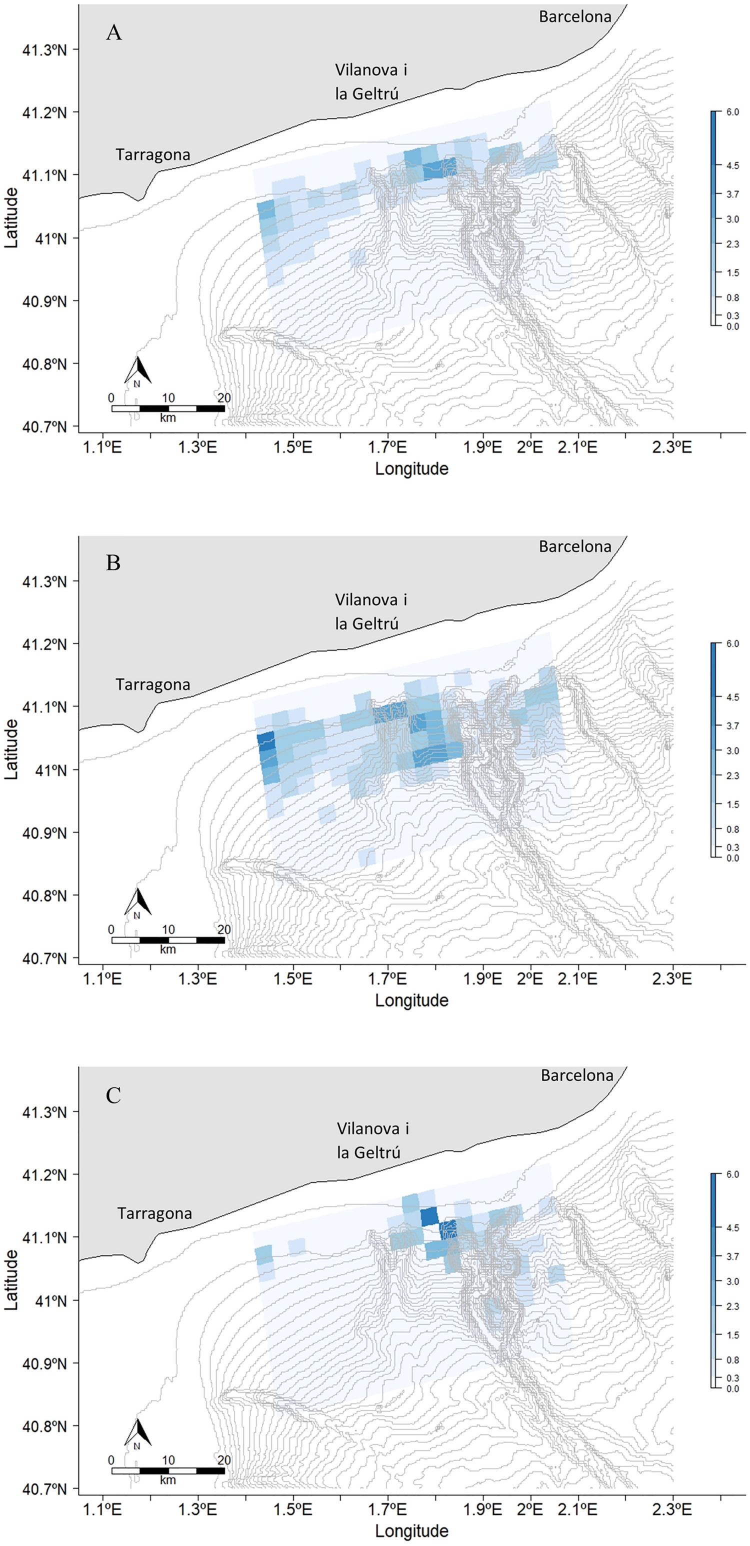

FIGURE 3 | Fin whale distribution in **(A)** March, **(B)** April and **(C)** May 2021 within the study area. The bathymetric lines are drawn every 50 m depth. The Number of animals sighted per pixel varied between 0-6.

FIGURE 4 | A fin whale displaying severe damage to its vertebral column and dorsal fin on 4 April 2021. **(A)** lateral and **(B)** overhead view.Photographs by Dr. Eduard Dellogada.

Well established shipping lanes were identified transiting the study area. Cargo ships were the most prevalent vessel type across the study area, especially in the northwest section, due to the proximity to the Barcelona Port. The shipping lane splits into three routes that diagonally cross the study area. Three shipping lanes to Tarragona Port in the west of the study area also transit through it (**Supplementary Figures S12-S15**).

Tanker vessels use both Barcelona and Tarragona Ports. One main shipping lane emerges from each port and diagonally crosses the study area from the northeast and northwest. Cargo and tanker vessels also moved parallel to the coast along the continental shelf edge (**Figure 6** and **Supplementary Figures S16-S19**). Passenger vessels transit through the study area in its southeast corner (**Figure 6** and **Supplementary Figures S20-S23**). This lane moves southwards towards Ibiza in the Balearic Islands (OceanCare, 2021).

3.5 Shipping Speed

The vessel speed distribution and the comparison of medians between years were significantly different due to the reception variability of the LAB AIS antenna. The p-values of the Kolmogorov-Smirnoff, the Mann-Whitney-U and the Kruskal-Wallis test, were $p < 0.001$. Within each year, there was also a significant difference ($p < 0.001$) in the vessel speed between months (**Figure 7** and **Supplementary Figure S24**). In years when the antenna range was stable, there was a consistent pattern where the vessel speed increased from February to June. However, vessel speed was consistent across months for the different types of vessel or sizes (**Supplementary Figure S25**). The marine traffic lanes with the highest speed vessels that exited from or arrived at Barcelona Port were cargo and passenger vessels (**Figures 6**, **7** and **Supplementary Figures S20-S23**, **S26-S29**). Both vessel speed patterns, by vessel type and by vessel length, were consistent for all the months (**Figure 7** and **Supplementary Figures S26-S29**). Cargo vessels were the most numerous vessel types that moved through the Garraf coast from February to June. The same number of tanker and passenger vessels were recorded during this five-month period. Despite the lowered numbers present, passenger vessels exhibited the highest speeds through the study area, followed by cargo ships and tankers. Approximately 80% of vessels that exceeded 20 kn were

TABLE 1 | Confirmed fin whale ship strikes at Barcelona Port since 1986.

Date	Sex	Length (m)	Weight (t)	Location	Source
23/01/1986	Female	12.5	N/A	On bow	Raga et al., 1991, Panigada et al., 2006
10/08/1986	N/A	12	N/A	On bow	Raga et al., 1991, Panigada et al., 2006
09/05/2002	Male	N/A	N/A	Floating in port	REUTERS
14/04/2016	Male	13.5	14.5	Floating port	Degollada pers. obs.

passenger vessels, travelling through the southeast corner of the study area (**Figure 6**). In addition, vessels between 200 and 400 m in length exhibited the highest speeds, exceeding a speed median threshold of 15 kn most frequently (**Figure 7** and **Supplementary Figures S26-S29**).

3.6 Shipping Density

Cargo vessels displayed the highest densities through the study area in all months and peaked in May. The vessel route density went from 9.75 to 10.97 with a maximum of 50.56 in April. For tanker vessels the mean route went from 3.99 to 4.26 with a

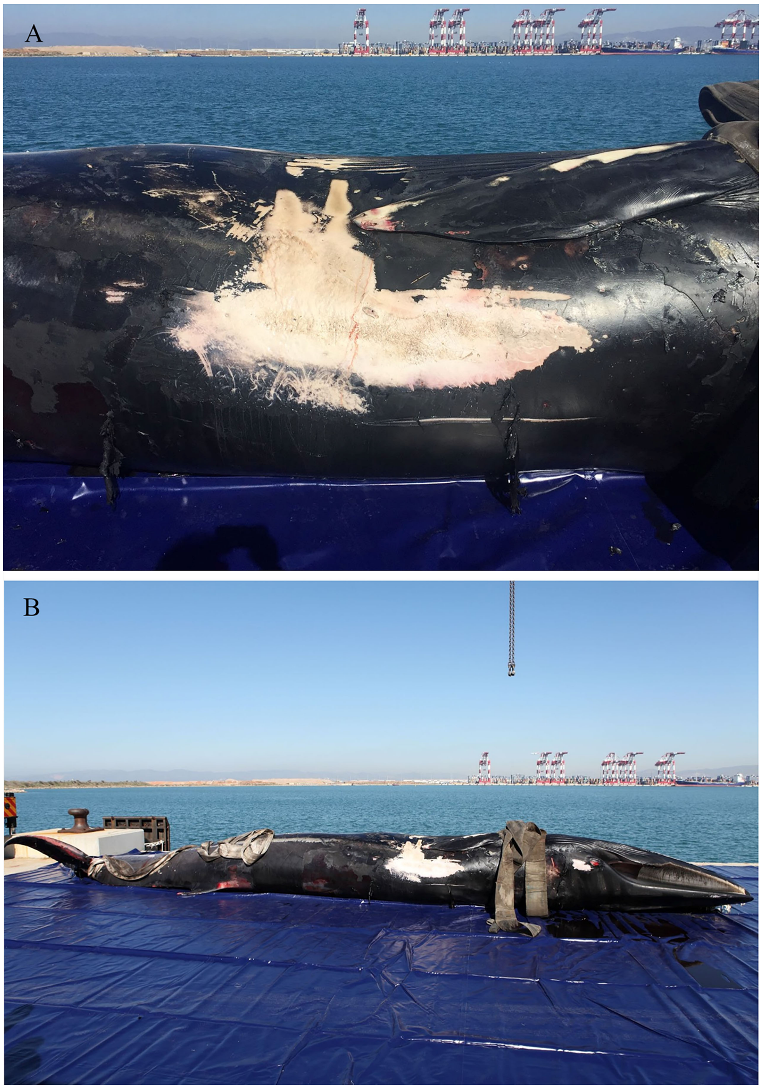

FIGURE 5 | Fin whale in Barcelona Port with evidence of ship strike on its leftright flank on the 14 April 2016. **(A)** A closeup view of the trauma and **(B)** an overall view of the whale. Photographs by Dr. Eduard Dellogada.

TABLE 2 | Number of vessels that use Barcelona port annually during the fin whale season (from March to May) between 2016 and 2021. The vessel numbers for 2020 are lower due to the COVID-19 pandemic.

Vessel type	2016	2017	2018	2019	2020	2021
Cargo	1,190	1,102	1,141	1,036	827	972
Tanker	238	247	243	270	248	431
Passenger	782	911	957	934	430	421

maximum value of 15.89 in March. Passenger ships were the ones with the lowest density in the study area with a mean route between 0.12 and 0.19. In this case the maximum number of vessel routes per pixel was 1.9 in May (**Figure 8** and **Supplementary Figures S30-S32**).

3.7 Ship Strike Assessment

The collision risk maps performed (**Figure 9** and **Supplementary Figures S33-S35**) indicate that April is the month with highest risk of vessel collision at a mean risk of 0.066. On the opposite, May had the lowest risk recorded with a mean value of 0.031 in the study area. The area with the highest collision risk is the left side of the Foix Canyon, because of the proximity to Barcelona port.

4 DISCUSSION

4.1 Fin Whale Ship Strike and Fatalities

The whales photographed in the study area generally exhibited ship strike injuries high in the dorsal side, which is the most raised body part when the animal dives. This was especially noted when the whale dove quickly (Laist et al., 2001). The injuries were similar to those reported elsewhere in the Mediterranean and Strait of Gibraltar (de Stephanis and Urquiola, 2006; Panigada et al., 2006; Gauffier et al., 2018). The aforementioned two cases of severe vertebral column deformity and likely idiopathic scoliosis in 2018 and 2021, were determined as severe welfare cases. In these, the whales were unable to swim correctly or at typical speeds, which reduced their capacity to feed efficiently (Goldbogen et al., 2006; Gough et al., 2019; de Reuver et al., 2021). Neither animal was subsequently resighted during the following seasons (2018 whale) or the remaining 2021 season (2 months).

Nine fin whales (out of 383) were photo-identified with ship strike injury evidence in the Ligurian Sea from 1990 to 2001 (Panigada et al., 2006). Only one fin whale was photographed with ship strike evidence off the Garraf coast prior to 2021, when 6 individuals were documented. This sudden increase in visual marks from ship strikes may indicate that the risk posed from this threat has increased. The four confirmed ship strike fatalities in Barcelona Port displayed typical ship strike characteristics for the species with dorsal side collision marks. Two fatal ship strikes occurred during the fin whale season (April and May) from the data available in 2002 and 2016. These whales were still young given their length range was between 12 and 14.5 m. These results are limited and there have likely been more whale strandings associated with ship strikes in proximity to Barcelona Port as indicated by the concentration of strandings on Mediterranean Database of Cetacean Strandings (MEDACES). Further work is required in evaluating the strandings from this location to assess past ship strike occurrence in the area.

Fin whales traveling in groups in the Bay of Biscay displayed lowered attentiveness rates in proximity to ferries, which may increase the risk of ship strikes (Aniceto et al., 2016). This “group effect”, the slower traveling speed of mother-calf pairs (6.6 km/hr in one study) and increased time at the surface, may increase the susceptibility of these whales to ship strikes in the study area and in the vicinity of Barcelona Port (Aniceto et al., 2016; Smultea et al., 2017). The low survival rate of calves in the Mediterranean Sea and that additional loss of sexually mature females with calves poses a high threat to the species (Panigada et al., 2006; Arrigoni et al., 2011). Poor nutritional condition and maternal separation was attributed to one live stranded calf that was euthanised and necropsied at Tarragona on 13th November 2015 after appearing in Barcelona Port on 10th/11th November (Cuvertoret-Sanz et al., 2020). Only four mother-calf pairs have been recorded present in the study area since the project began in March 2014 (EDMAKTUB unpublished data).

4.2 Fin Whale Ship Strike Risk on the Garraf Coast

Ship strike risk appears to be higher in April when the number of fin whale sightings is highest. May was the month with less risk despite it having the highest marine traffic levels. The lower risk may be due to the offshore movements of fin whales in May. The study area´s lower risk cannot be extrapolated to other close-by zones, given the proximity of Barcelona Port, the surrounding traffic routes, and the changes in oceanographic conditions along the Catalan Coast, as reported by the fisherman along seasons. Future studies are required to evaluate the whole Catalan Coast and the Balearic Sea with fin whale data in order to assess the risk of collision in other areas.

Fin whales follow a complex migratory pattern subject to food availability in the Mediterranean Sea (Geijer et al., 2016; Notarbartolo di Sciara et al., 2016; Panigada et al., 2017). The relationship between their presence and oceanographic variables (which act as indicators of productivity and consequently to food abundance) do not follow simple linear regressions. For this reason, one of the most used models for cetacean distribution is the GAM model. It offers a flexible and robust approach for the exploration and characterization of complex, non-linear relationships among variables (Panigada et al., 2008; Zerbini et al., 2015). The oceanographic model used to fit this model used Chla in order to get the primary production indicator and the

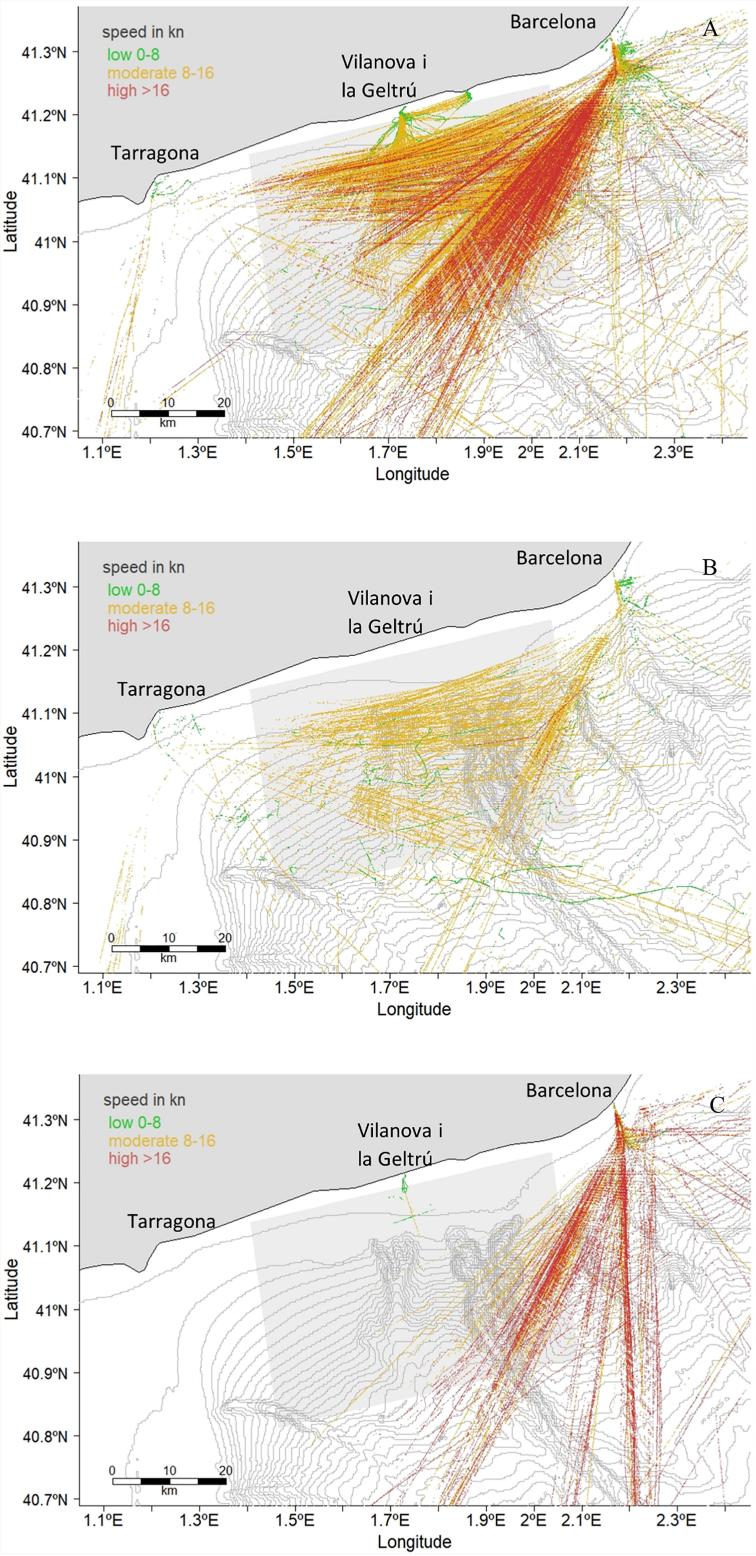

FIGURE 6 | Vessel positions for Cargo **(A)**, Tanker **(B)** and Passenger **(C)** vessels in April from pooled AIS data between 2011 and 2020. The bathymetry is represented by isobaths every 50 m and the study area is shaded.

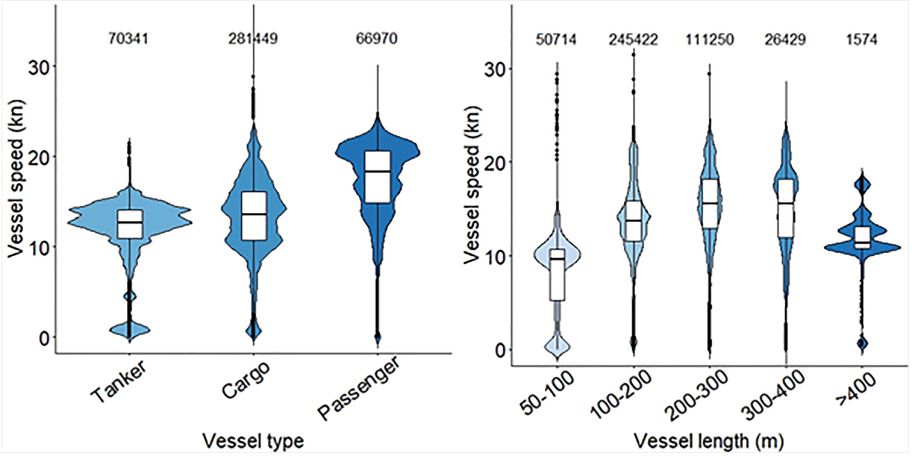

FIGURE 7 | The distribution of vessel speeds in April (from pooled AID data between 2011 and 2020) by vessel type and vessel length in the study area, where fin whales were observed surface feeding. The number of unique vessels in each category is indicated at the top of each violin plot.

SST as the indicator for column mixing which implies an enrichment of nutrients in superficial waters which implies more productivity (Croll et al., 2005; Baines and Reichelt, 2014).

The bathymetry, depth and continental shelf slope factors appear to be an important factor to correlate with chlorophyll a and sea surface temperature, as they determine where krill occurs. Northern krill is a mesopelagic species which perform a diurnal-vertical migration to feed on phytoplankton at night (Panigada et al., 1999; Kaartvedt, 2010). Fin whale surface feeding behaviours, especially lunge feeding, usually occur at dusk for this reason which was recorded often off the Garraf coast. Fin whales also remain at the surface for extended periods of time, swim slower and shallower at night (Calambokidis et al., 2019; Keen et al., 2019). These behaviours may reduce their reaction times while the crew of vessels cannot visually see animals in the dark, which substantially increases the risk of collision (Calambokidis et al., 2019; Keen et al., 2019; Guazzo et al., 2021).

In the Ligurian Sea, fin whales primarily feed in habitats reaching 2,000 m in depth and can dive to reach ~470 m to feed (Panigada et al., 1999; Panigada et al., 2005). The deep foraging dives performed in the Ligurian Sea, reduce the time that fin whales spend at the surface. 82% of the Mediterranean Sea's fatal ship strikes were recorded between 1972 and 2001 in the Ligurian Sea area (Panigada et al., 2006). However, the shallower surface feeding behaviours exhibited by fin whales along the Catalan coast increases their presence at the surface exponentially. Surface lunge feeding in the Mediterranean has been documented off Lampedusa Island (Canese et al., 2006). This fact may expose the whales in this region to a higher risk of collision, given the whales are closer to the surface for extended periods of time, while both feeding and resting.

The intensity and visibility of fin whale blows appears to vary depending on their behaviour and the environmental conditions (Stone et al., 1992; Kopelman and Sadove, 1995; Goldbogen et al., 2006; Horton et al., 2019; Martins et al., 2020). The blow intensity may also be affected by the time of the day within the study area (Stone et al., 1992). This variation in blow visibility would influence the detection rate of whales by the crew of marine vessels.

The persistent acoustic noise from vessels has been recorded altering fin whale behaviour (Nieukirk et al., 2011; Castellote et al., 2012b). Acoustic calls to avoid masking caused by shipping noise, have also resulted in displacement behaviours while foraging in the Mediterranean Sea (Castellote et al., 2012b; Notarbartolo di Sciara et al., 2016). Adaptation to the increased noise levels within the Mediterranean Sea may make the species more susceptible to ship strikes (Castellote et al., 2012b; Notarbartolo di Sciara et al., 2016).

Of the 43 near-miss events recorded in the Ligurian Sea between 2008 and 2019, 63.4% of whales surfaced in front of ferries <50 m away (David et al., 2022). No behavioural changes were observed when whales were travelling or resting in proximity to ferries, indicating that the acoustic output of marine traffic is not suitable for the species as an avoidance signal for marine vessels (David et al., 2022). The animals observed in the study area did not show any behavioural changes that could be attributed to the presence of vessels. The species spatial awareness alters depending on their behaviour and group size which may make them more susceptible to being hit while engaging in behaviours such a surface feeding and resting (Friedlaender et al., 2015; Aniceto et al., 2016; Panigada et al., 2017; Calambokidis et al., 2019; Irvine et al., 2019). The near surface feeding behaviours recorded in the Garaff study area during the evening time, when krill migrate higher in the water column, have the potential to increase the risk of collision. During the hours of darkness whales would not be detected by visual observation unless by thermal imaging (Zitterbart et al., 2020).

Cargo vessels pose the highest ship strike risk to fin whales on the Garraf coast. They transit through important foraging areas at speeds between 10 to 20 kn, reaching a probability of having a

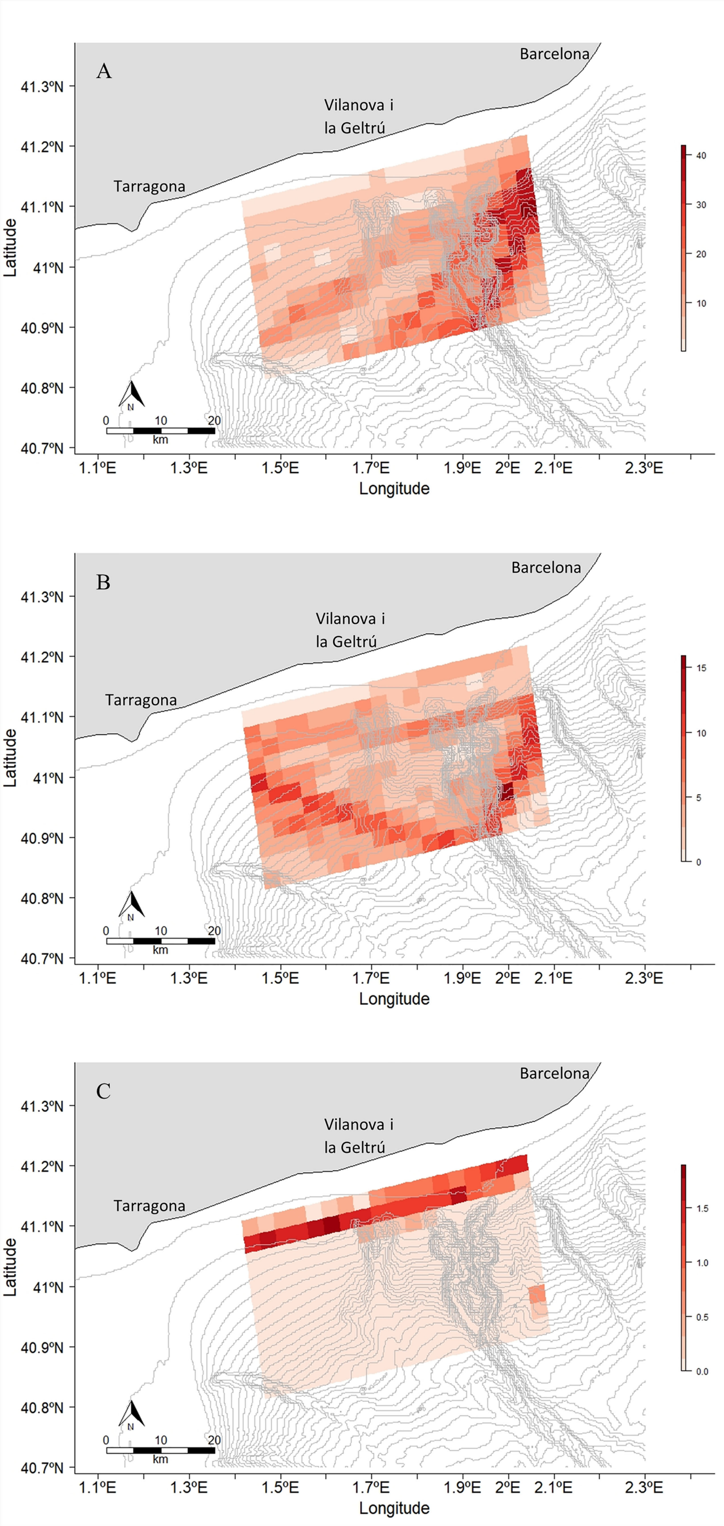

FIGURE 8 | Vessel route density maps within the study area in 2021. The first map **(A)** represents the cargo ship route density in May, the second **(B)** represents the tanker ship route density in March and the third **(C)** one represents the passenger ship route density in May. These maps represent the months with highest marine traffic for each type of vessel. The maps of the months left can be found in the **Supplementary Figures S29-S31**.

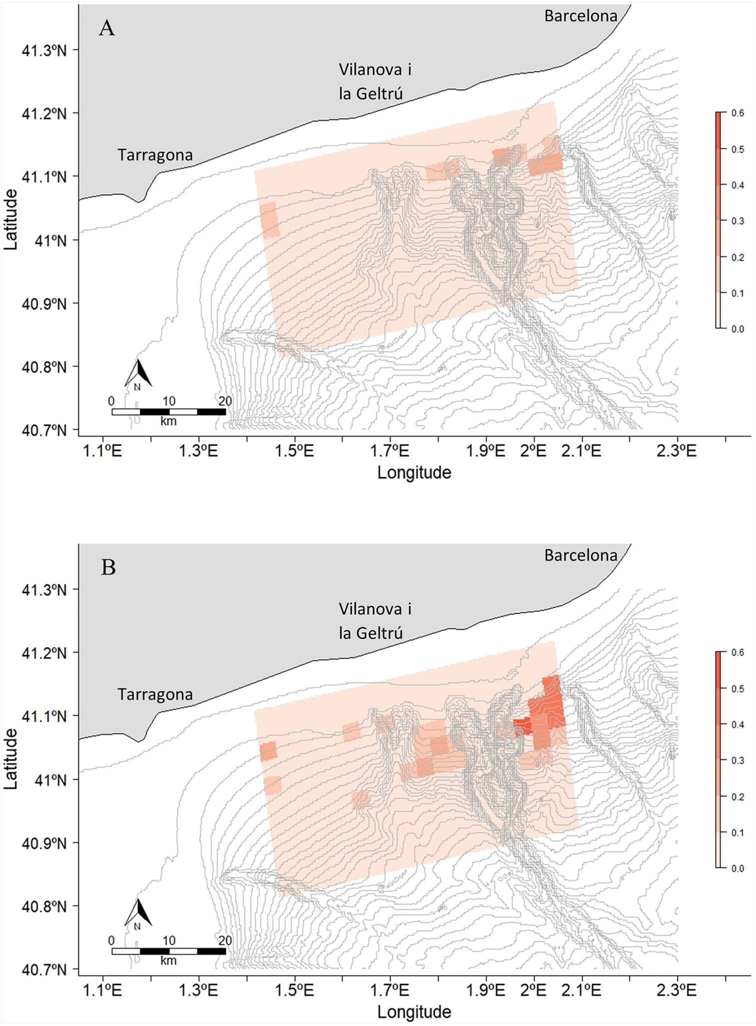

FIGURE 9 | Risk of collision between a fin whale and a cargo ship in **(A)** March and **(B)** April. The risk range is between 0 and 0.6. April was the month with the highest risk. The other maps with the risk of collision with cargo ships in May, with tanker ships and with passenger ships can be found in the **Supplementary Figures S32-S34**.

fatal strike to around 79% (Vanderlaan and Taggart, 2007; Conn and Silber, 2013). Half of global ship strikes occur at moderate vessel speeds (below 15 kn) (Winkler et al., 2020). Death is possible even at low speeds (Kelley et al., 2021). Tanker ships recorded the slowest mean speed at around 10 kn. They crossed the study area from both sides, due to the presence of Tarragona and Barcelona Ports. In this case, even though the traffic density was low, the collision risk was high for this type of vessel, due to its prevalence in crossing the study area.

While passenger's vessels did not display a high risk in the study area (because they transit through a small section of it), they do represent a major threat towards the species along the Catalan coast and in the Balearic Sea. They move at very fast speeds, averaging 20 kn regularly between Barcelona and the Balearic Islands, France, and Italy. In the Ligurian Sea, ship strike risk increases during the summer "high tourist season" months, when ferries move between the French and Italian mainland to Corsica and Sardinia, while fin whales forage in the area (Grossi et al., 2021; Ham et al., 2021). Pleasure boats operate in coastal waters of the study area and may move at fast speeds (>20 kn), however, it is predominantly sailing boats that occur over the Cunit and Foix Canyons due to the distance from shore. Nonetheless, sailing boats may pose a reduced risk due to manoeuvrability limitations when the sails are raised in some environmental conditions (Ritter, 2012). Further study of tourism season threat and impact on the fin whales is required off the Catalan coast for ferries and pleasure boats such as yachts (Carreño and Lloret, 2021).

4.3 Mitigation Measures to Reduce Ship Strike Risks

Ship strikes are a huge conservation concern for cetacean species globally, especially for large whales (Laist et al., 2001; Sèbe et al., 2019; Winkler et al., 2020). For this reason, a variety of mitigation measures have been implemented to minimise the risk of ship strikes worldwide (Panigada et al., 2006; Winkler et al., 2020). The most common measures used are speed reductions and the implementation of traffic separation schemes (TSS) (Guzman et al., 2013; Bezamat et al., 2015; Guzman et al., 2020; OceanCare, 2021). For example, the chances of lethal injury from ship strikes drops to 50% at 8.8 kn speed (Vanderlaan and Taggart, 2007; Conn and Silber, 2013). Thus, a speed limitation to 8 kn, along the entire Catalan coast under 200 m depth and in the areas closest to the continental shelf edge and submarine canyons between February and June, would greatly protect fin whale habitats over a wide spatial area. Moreover, slower vessel speeds would aid climate action efforts in reducing the amount of CO2 emissions being released by marine traffic (Leaper, 2019).

In order to prevent collisions with the critically endangered north Atlantic right whale (*Eubalaena glacialis*) (Müller, 1776) in the Gulf of Maine, the IMO subcommittee on ship strikes implemented a measure forcing ships larger than 300 gross tons to report their position, speed and destination while entering key right whale habitats: New England (foraging area) and coastal Georgia and Florida (calving area) (Conn and Silber, 2013; Cooke, 2020). Reporting ships receive an automatically generated message that provides information about right whale sightings, their vulnerability, and actions to avoid ship strikes. In addition, in the eastern Mediterranean Sea, re-routing of shipping lanes by 20 km in the Hellenic Trench, Greece was predicted to decrease the ship strike risk to sperm whales (*Physeter macrocephalus*) (Linneaus 1758) by 70%, with no additional cost to shipping companies transiting the area (Frantzis et al., 2019).

The use of fin whale distribution models influenced by oceanographic conditions would be useful to implement flexible regulation measures, considering that fin whale presence and behaviour is affected by foraging conditions along the Garraf coast. Marine traffic has been seasonally diverted away from areas where ship strike risk increased greatly during certain months in other countries; it has been introduced at New Scotia and Gulf of Maine, Canada coast and the Gulf of Panama (Vanderlaan and Taggart, 2007; Guzman et al., 2020). Other suggested measures include vessels moving offshore to avoid crossing known foraging habitats once they departed port. The continental shelf edge should be crossed during daylight hours, while the vessel's master or a member of the crew actively watch for fin whales and evade any sighted animals at a far distance. This would increase the chances of the ship to avoid ship strike or collision (Gende et al., 2019). This mitigation measure could be implemented in the proposed TSS of Barcelona and Tarragona Ports.

In other areas out of the Mediterranean Sea, Marine Mammal Observers (MMO) accompany the vessels during the spring season, to help locate fin whales in the path of the vessel, and therefore, helping to avoid ship strikes (Flynn and Calambokidis, 2019; Zitterbart et al., 2020). The use of Infrared cameras may alternatively be used to detect whale blows and their body heat signature while at the surface (Zitterbart et al., 2020). Zitterbart et al. (2020) reported a 70% detection rate of whales within 2 km of vessels in Australia. Detections were also observed at night while the whales feed or rest near the surface (Calambokidis et al., 2019; Keen et al., 2019). This technology would be very useful in the Garraf study area given the area's dusk, and the night-time fin whale foraging behaviour. The efficiency of this technique for fin whales should be evaluated in this region.

Funding should be provided to survey the Balearic Sea, particularly within the Mediterranean cetacean Migration Corridor and in the new Specially Protected Areas of Mediterranean Importance (SPAMI) area. In order to provide conservation managers with more robust data and aid in future studies about the ship strike risk for fin whales species, additional population and abundance data on fin whales within the Balearic Sea during springtime is required. Previous studies in the Balearic Sea using dedicated aerial and opportunistic boat-based surveys indicate the presence of fin whales elsewhere in the Balearic Sea. Further research is required to understand their seasonality and movement patterns (Torreblanca et al., 2019; ACCOBAMS, 2021). Consideration must also be made for the use of acoustic monitoring techniques along the Catalan coast and in the SPAMI area, in order to evaluate the noise pollution under the Marine Strategy Framework Directive descriptor 11. Evaluating this anthropogenic noise would provide opportunities to detect the cetacean presence and seasonality (including sperm whales and Cuvier's beaked whales (*Ziphius cavirostris*) (Cuvier, 1823) and to monitor this region's cetacean communities further (OceanCare, 2021).

In light of the high abundance of fin whales seasonally feeding off the Garraf coast of Catalonia and the high volume of marine traffic transiting the area, careful consideration is required to reduce the ship strike risk to this species. The endangered status of the Mediterranean fin whale population warrants an urgent need to minimise the loss of individuals from the population through anthropogenic impacts such as ship strikes. The Royal Decree protecting fin whales in Spanish waters should engage with and enforce conservation measures for this species from ship strike risk along the Mediterranean coast. The Barcelona and Tarragona Port TSS provides a unique opportunity to develop a plan with stakeholder engagement, to implement the recommendations made in this study and limit the loss of fin whales.

DATA AVAILABILITY STATEMENT

The marine traffic data used in this publication was made available by the EMODnet Human Activities project, www.emodnet-humanactivities.eu, funded by the European Commission Directorate General for Maritime Affairs and Fisheries. The marine traffic AIS data used in this publication was made available by the Laboratorio de Aplicaciones Bioacústicas of the Polytechnic University of Catalonia. This data is not available to download. The bathymetric data used in this publication was made available by the EMODnet Bathymetry project, https://www.emodnet-bathymetry.eu, funded by the European Union under Regulation (EU) No 508/2014 of the European Parliament and of the Council of 15 May 2014 on the European Maritime and Fisheries Fund. The oceanographic data used in this publication was made available by the Marine Copernicus, (https://marine.copernicus.eu) funded by the European Commission (EC) and implemented by Mercator Ocean International.

ETHICS STATEMENT

The animal study was reviewed and approved by the Ministry of Ecological Transition Spain, under the ministry authorization: DGBBD/BDM/AUTSPP/17/2021.

AUTHOR CONTRIBUTIONS

EDB, BC and SO'C assisted with the data collection. SO'C, BC and RG drafted the manuscript. BC conducted the main analysis. RG assisted and analysed the LAB AIS data. SO'C compiled present and historical collision evidence. All authors contributed perspectives and assisted with manuscript review.

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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.2022.867287/full#supplementary-material

REFERENCES

ACCOBAMS (2021). *Estimates of Abundance and Distribution of Cetaceans, Marine Mega-Fauna and Marine Litter in the Mediterranean Sea From 2018-2019 Surveys*. Eds. S. Panigada, O. Boisseau, A. Canadas, C. Lambert, S. Laran, R. McLanaghan and A. Moscrop (Monaco: ACCOBAMS - ACCOBAMS Survey Initiative Project), 177 pp.

Aguilar, A., and García-Vernet, R. (2018). "Fin Whale Balaenoptra Physalus," in *Encyclopaedia of Marine Mammals 3rd Edition*. Eds. B. Würsig, J. G. M. Thewissen and K. Kovacs (Royal Society). Available at: 10.1098/rspb.2009.1135.

Alves, F., Towers, J. R., Baird, R. W., Bearzi, G., Bonizzoni, S., Ferreira, R., et al. (2017). The Incidence of Bent Dorsal Fins in Free-Ranging Cetaceans. *J. Anat.* 232, 263–269. doi: 10.1111/joa.12729

Aniceto, A. S., Carroll, J. L., Tetley, M. J., and van Oosterhout, C. (2016). Position, Swimming Direction and Group Size of Fin Whales (Balaenoptera Physalus) in the Presence of a Fast-Ferry in the Bay of Biscay. *Oceanologia* 57, 235–240. doi: 10.1016/j.oceano.2016.02.002

Arrigoni, M., Manfredi, P., Panigada, S., Bramanti, L., and Santangelo, G. (2011). Life-History Tables of the Mediterranean Fin Whale From Stranding Data. *Marine Ecol.* 32, 1–9. doi: 10.1111/j.1439-0485.2011.00437.x

Baines, M. E., and Reichelt, M. (2014). Upwellings, Canyons and Whales: An Important Winter Habitat for Balaenopterid Whales Off Mauritania, Northwest Africa. *J. Cetacean Res. Manage.* 14, 57–67.

Barcelona Port (2022) *Port Authority Statistics [WWW Document]*. Available at: https://www.portdebarcelona.cat/es_ES/web/autoritat-portuaria/estadisticas.

Bezamat, C., Wedekin, L. L., and Simões-Lopes, P. C. (2015). Potential Ship Strikes and Density of Humpback Whales in the Abrolhos Bank Breeding Ground, Brazil. *Aquat. Conserv.: Marine Freshwater Ecosyst.* 25, 573–586. doi: 10.1002/aqc.2523

Borrell, A., Gazo, M., Aguilar, A., Raga, J. A., Degollada, E., Gozalbes, P., et al. (2021). Niche Partitioning Amongst Northwestern Mediterranean Cetaceans Using Stable Isotopes. *Prog. Oceanogr.* 193, 102559. doi: 10.1016/j.pocean.2021.102559

Calambokidis, J., Fahlbusch, J. A., Szesciorka, A. R., Southall, B. L., Cade, D. E., Friedlaender, A. S., et al. (2019). Differential Vulnerability to Ship Strikes Between Day and Night for Blue, Fin, and Humpback Whales Based on Dive and Movement Data From Medium Duration Archival Tags. *Front. Marine Sci.* 6. doi: 10.3389/fmars.2019.00543

Canese, S., Cardinali, A., Fortuna, C. M., Giusti, M., Lauriano, G., Salvati, E., et al. (2006). The First Identified Winter Feeding Ground of Fin Whales (Balaenoptera Physalus) in the Mediterranean Sea. *J. Marine Biol. Ass.* 86, 903–907. doi: 10.1017/S0025315406013853

Carreño, A., and Lloret, J. (2021). Environmental Impacts of Increasing Leisure Boating Activity in Mediterranean Coastal Waters. *Ocean Coastal Manage.* 209, 105693. doi: 10.1016/j.ocecoaman.2021.105693

Castellote, M., Clark, C. W., and Lammers, M. O. (2012a). Fin Whale (Balaenoptera Physalus) Population Identity in the Western Mediterranean Sea. *Marine Mammal Sci.* 28, 325–344. doi: 10.1111/j.1748-7692.2011.00491.x

Castellote, M., Clark, C. W., and Lammers, M. O. (2012b). Acoustic and Behavioural Changes by Fin Whales (Balaenoptera Physalus) in Response to Shipping and Airgun Noise. *Biol. Conserv.* 147, 115–122. doi: 10.1016/j.biocon.2011.12.021

Castellote, M., Esteban, J. A., and Clark, C. W. (2008). Fin Whale (Balaenoptera Physalus) Movements Along the Spanish Mediterranean Coast. *J. Acoustical Soc. America* 123, 3775. doi: 10.1121/1.2935401

Castrillon, J., and Bengtson Nash, S. (2020). Evaluating Cetacean Body Condition; a Review of Traditional Approaches and New Developments. *Ecol. Evol.* 10, 6144–6162. doi: 10.1002/ece3.6301

Conn, P. B., and Silber, G. K. (2013). Vessel Speed Restrictions Reduce Risk of Collision-Related Mortality for North Atlantic Right Whales. *Ecosphere* 4(4): 43. doi: 10.1890/ES13-00004.1

Cooke, J. G. (2018). Balaenoptera Physalus, Fin Whale. The IUCN Red List of Threatened Species. e.T2478A50349982. doi: 10.2305/IUCN.UK.2018-2.RLTS.T2478A50349982.en

Cooke, J. G. (2020). Eubalaena Glacialis, North Atlantic Right Whale. The IUCN Red List of Threated Species. e.T41712A178589687. doi: 10.2305/IUCN.UK.2018-1.RLTS.T41711A50380694.en.

Croll, D. A., Marinovic, B., Benson, S., Chavez, F. P., Black, N., Ternullo, R., et al. (2005). From Wind to Whales: Trophic Links in a Coastal Upwelling System. *Marine Ecol. Prog. Ser.* 289, 117–130. doi: 10.3354/meps289117

Cuvertoret-Sanz, M., Lόpez-Figueroa, C., O'Byrne, A., Canturri, A., Martí-Garcia, B., Pintado, E., et al. (2020). Causes of Cetacean Stranding and Death on the Catalonian Coast (Western Mediterranean Sea), 2012-2019. *Dis. Aquat. Org.* 142, 239–253. doi: 10.3354/DAO03550

David, L., Alleaume, S., and Guinet, C. (2011). Evaluation of the Potential of Collision Between Fin Whales and Maritime Traffic in the North-Western Mediterranean Sea in Summer, and Mitigation Solutions. *JMATE* 4, 17–28.

David, L., Arcangeli, A., Tepsich, P., Di-Meglio, N., Roul, M., Campana, I., et al. (2022). Computing Ship Strikes and Near Miss Events of Fin Whales Along the Main Ferry Routes in the Pelagos Sanctuary and Adjacent West Area, in Summer. *Aquat. Conserv*. 32 (3), 442–456. doi: 10.1002/aqc.3781

de Reuver, S., IJsseldijk, L. L., Homans, J. F., Willems, D. S., Veraa, S., van Stralen, M., et al. (2021). What a Stranded Whale With Scoliosis Can Teach Us About Human Idiopathic Scoliosis. *Sci. Rep.* 11, 7218. doi: 10.1038/s41598-021-86709-x de Stephanis, R., and Urquiola, E. (2006). "Collisions Between Ships and Cetaceans in Spain," in *Document Presented to the Scientific Committee of the International Whaling Commission SC/58/BC5*.

Di-Meglio, N., David, L., and Monestiez, P. (2018). Sperm Whale Ship Strikes in the Pelagos Sanctuary and Adjacent Waters: Assessing and Mapping Collision Risks in Summer. *J. Cetacean Res. Manage* 18, 135–147.

Druon, J.-N. (2014). *Monitoring Pelagic Habitats to Support Future EU Policie) - Strategic View on How the Daily Monitoring of Marine Water-Column Habitats Will Support the Future EU Integrated Maritime Policy (MSP, CFP, MSFD) to Stimulate Ecosystem Health and Blue Growth*. (Luxembourg: Publications Office of the European Union, 2014)

Druon, J. N., Panigada, S., David, L., Gannier, A., Mayol, P., Arcangeli, A., et al. (2012). Potential Feeding Habitat of Fin Whales in the Western Mediterranean Sea: An Environmental Niche Model. *Marine Ecol. Prog. Ser.* 464, 289–306. doi: 10.3354/meps09810

EDMAKTUB (2021) *Edmaktub. Fin Whale Project Results 2021*. Available at: https://www.edmaktub.org/en/results/.

EDMAKTUB (2018) *Edmaktub. Fin Whale Project Results 2018*. Available at: https://www.edmaktub.org/results/.

E.U. Copernicus Marine Service Information. Mediterranean Sea - High Resolution and Ultra High Resolution L3S Sea Surface Temperature Model. doi: 10.48670/moi-00171

E.U. Copernicus Marine Service Information. Mediterranean Sea - Reprocessed Surface Chlorophyll Concentration Adn Phytoplankton Functional Types From Multi Satellite Observations Model. doi: 10.48670/moi-00112

European Commission *Natura 2000 Network in the Marine Environment*. Available at: https://ec.europa.eu/environment/nature/natura2000/marine/index_en.htm.

Flynn, K. R., and Calambokidis, J. (2019). Lessons From Placing an Observer on Commercial Cargo Ships Off the U.s. West Coast: Utility as an Observation Platform and Insight Into Ship Strike Vulnerability. *Front. Marine Sci.* 6. doi: 10.3389/fmars.2019.00501

Frantzis, A., Leaper, R., Alexiadou, P., Prospathopoulos, A., and Lekkas, D. (2019). Shipping Routes Through Core Habitat of Endangered Sperm Whales Along the Hellenic Trench, Greece: Can We Reduce Collision Risks? *PLoS ONE* 14(2): e0212016. doi: 10.1371/journal.pone.0212016

Friedlaender, A. S., Goldbogen, J. A., Hazen, E. L., Calambokidis, J., and Southall, B. L. (2015). Feeding Performance by Sympatric Blue and Fin Whales Exploiting a Common Prey Resource. *Marine Mammal Sci.* 31, 345–354. doi: 10.1111/mms.12134

Gauffier, P., Borrell, A., Silva, M. A., Víkingsson, G. A., López, A., Giménez, J., et al. (2020). Wait Your Turn, North Atlantic Fin Whales Share a Common Feeding Ground Sequentially. *Marine Environ. Res.* 155. doi: 10.1016/j.marenvres.2020.104884

Gauffier, P., Verborgh, P., Giménez, J., Esteban, R., Sierra, J. M. S., and de Stephanis, R. (2018). Contemporary Migration of Fin Whales Through the Strait of Gibraltar. *Marine Ecol. Prog. Ser.* 588, 215–228. doi: 10.3354/meps12449

Geijer, C. K. A., Notarbartolo di Sciara, G., and Panigada, S. (2016). Mysticete Migration Revisited: Are Mediterranean Fin Whales an Anomaly? *Mammal Rev*. 46, 284–296 doi: 10.1111/mam.12069

Gende, S. M., Vose, L., Baken, J., Gabriele, C. M., Preston, R., and Noble Hendrix, A. (2019). Active Whale Avoidance by Large Ships: Components and Constraints of a Complementary Approach to Reducing Ship Strike Risk. *Front. Marine Sci.* 6. doi: 10.3389/fmars.2019.00592

Goldbogen, J. A., Calambokidis, J., Shadwick, R. E., Oleson, E. M., McDonald, M. A., and Hildebrand, J. A. (2006). Kinematics of Foraging Dives and Lunge-Feeding in Fin Whales. *J. Exp. Biol.* 209, 1231–1244. doi: 10.1242/jeb.02135

Gough, W. T., Segre, P. S., C.Bierlich, K. K., Cade, D. E., Potvin, J., Fish, F. E., et al. (2019). Scaling of Swimming Performance in Baleen Whales. *J. Exp. Biol.* 222 (20), jeb204172. doi: 10.1242/jeb.204172

Grossi, F., Lahaye, E., Moulins, A., Borroni, A., Rosso, M., and Tepsich, P. (2021). Locating Ship Strike Risk Hotspots for Fin Whale (Balaenoptera Physalus) and Sperm Whale (Physeter Macrocephalus) Along Main Shipping Lanes in the North-Western Mediterranean Sea. *Ocean Coastal Manage.* 212, 105820. doi: 10.1016/j.ocecoaman.2021.105820

Guazzo, R. A., Durbach, I. N., Helble, T. A., Alongi, G. C., Martin, C. R., Martin, S. W., et al. (2021). Singing Fin Whale Swimming Behavior in the Central North Pacific. *Front. Marine Sci.* 8. doi: 10.3389/fmars.2021.696002

Guzman, H. M., Capella, J. J., Valladares, C., Gibbons, J., and Condit, R. (2020). Humpback Whale Movements in a Narrow and Heavily-Used Shipping Passage, Chile. *Marine Policy* 118, 103990. doi: 10.1016/j.marpol.2020.103990

Guzman, H. M., Gomez, C. G., Guevara, C. A., and Kleivane, L. (2013). Potential Vessel Collisions With Southern Hemisphere Humpback Whales Wintering Off Pacific Panama. *Marine Mammal Sci.* 29, 629–642. doi: 10.1111/j.1748-7692.2012.00605.x

Ham, G. S., Lahaye, E., Rosso, M., Moulins, A., Hines, E., and Tepsich, P. (2021). Predicting Summer Fin Whale Distribution in the Pelagos Sanctuary (North-Western Mediterranean Sea) to Identify Dynamic Whale–Vessel Collision Risk Areas. *Aquat. Conserv.: Marine Freshwater Ecosyst* 31, 2257–2277. doi: 10.1002/aqc.3614

Horton, T. W., Hauser, N., Cassel, S., Klaus, K. F., de Oliveira, T. F., and Key, N. (2019). Doctor Drone: Non-Invasive Measurement of Humpback Whale Vital Signs Using Unoccupied Aerial System Infrared Thermography. *Front. Marine Sci.* 6. doi: 10.3389/fmars.2019.00466

International Maritime Organization (IMO) (2002) *Guidelines for the Onboard Operational Use of Shipborne Automatic Identification Sysems (AIS)*. Available at: http://www.navcen.uscg.gov/pdf/AIS/IMO_A_917(22)_AIS_OPS_Guidelines.pdf.

Irvine, L. M., Palacios, D. M., Lagerquist, B. A., and Mate, B. R. (2019). Scales of Blue and Fin Whale Feeding Behavior Off California, USA, With Implications for Prey Patchiness. *Front. Ecol. Evol.* 7. doi: 10.3389/fevo.2019.00338

IUCN (2017). *North West Mediterranean Sea, Slope and Canyon System. Important Marine Mammal Area Factsheet* (IUCN Joint SSC/WCPA Marine Mammal Protected Areas Task Force).

Kaartvedt, S. (2010). Diel Vertical Migration Behaviour of the Northern Krill (Meganyctiphanes Norvegica Sars). *Adv Mar Biol*. 57, 255–275. doi: 10.1016/S0065-2881(10)57009-3

Keen, E. M., Scales, K. L., Rone, B. K., Hazen, E. L., Falcone, E. A., and Schorr, G. S. (2019). Night and Day: Diel Differences in Ship Strike Risk for Fin Whales (Balaenoptera Physalus) in the California Current System. *Front. Marine Sci.* 6. doi: 10.3389/fmars.2019.00730

Kelley, D. E., Vlasic, J. P., and Brillant, S. W. (2021). Assessing the Lethality of Ship Strikes on Whales Using Simple Biophysical Models. *Marine Mammal Sci.* 37, 251–267. doi: 10.1111/mms.12745

Kopelman, A. H., and Sadove, S. S. (1995). Ventilatory Rate Differences Between Surface-Feeding and Non-Surface Feeding Fin Whales (Valaenoptera Physalus) in the Waters Off Eastern Long Island, New York, U.s.a-1987. *Marine Mammal Sci.* 11, 200–208. doi: 10.1111/j.1748-7692.1995.tb00518.x

Laist, D. W., Knowlton, A. M. R., Mead, J. G., Collet, A. S., and Podesta, M. (2001). Collisions Between Ships and Whales. *Marine Mammal Sci.* 17, 35–75. doi: 10.1111/j.1748-7692.2001.tb00980.x

Lavanguardia (2002) *Una Ballena De 12 Metros Aparece Muerta En La Bahía Del Port De La Selva*. Available at: https://www.lavanguardia.com/vida/20020619/51262756893/una-ballena-de-12-metros-aparece-muerta-en-la-bahia-del-port-de-la-selva.html.

Leaper, R. (2019). The Role of Slower Vessel Speeds in Reducing Greenhouse Gas Emissions, Underwater Noise and Collision Risk to Whales. *Front. Marine Sci.* 6. doi: 10.3389/fmars.2019.00505

Lydersen, C., Vacquié-Garcia, J., Heide-Jørgensen, M. P., Øien, N., Guinet, C., and Kovacs, K. M. (2020). Autumn Movements of Fin Whales (Balaenoptera Physalus) From Svalbard, Norway, Revealed by Satellite Tracking. *Sci. Rep.* 10. doi: 10.1038/s41598-020-73996-z

Martins, M. C. I., Miller, C., Hamilton, P., Robbins, J., Zitterbart, D. P., and Moore, M. (2020). Respiration Cycle Duration and Seawater Flux Through Open Blowholes of Humpback (Megaptera Novaeangliae) and North Atlantic Right (Eubalaena Glacialis) Whales. *Marine Mammal Sci.* 36, 1160–1179. doi: 10.1111/mms.12703

Mazzariol, S., Centelleghe, C., Beffagna, G., Povinelli, M., Terracciano, G., Cocumelli, C., et al. (2016). Mediterranean Fin Whales (Balaenoptera Physalus) Threatened by Dolphin Morbillivirus. *Emerging Infect. Dis.* 22, 302–305. doi: 10.3201/eid2202.150882

Micheli, F., Halpern, B. S., Walbridge, S., Ciriaco, S., Ferretti, F., Fraschetti, S., et al. (2013). Cumulative Human Impacts on Mediterranean and Black Sea Marine Ecosystems: Assessing Current Pressures and Opportunities. *PLOS ONE* 8(12): e79889. doi: 10.1371/journal.pone.0079889

Moore, M. J., Mitchell, G. H., Rowles, T. K., and Early, G. (2020). Dead Cetacean? Beach, Bloat, Float, Sink. *Front. Marine Sci*. 7. doi: 10.3389/fmars.2020.00333

Nieukirk, S. L., Mellinger, D. K., Moore, S. E., Klinck, K., Dziak, R. P., and Goslin, J. (2011). Sounds From Airguns and Fin Whales Recorded in the Mid-Atlantic Ocea–2009. *J. Acoustical Soc. America* 131, 1102–1112. doi: 10.1121/1.3672648

Notarbartolo di Sciara, G., Castellote, M., Druon, J. N., and Panigada, S. (2016). Fin Whales, Balaenoptera Physalus: At Home in a Changing Mediterranean Sea? *Adv. Marine Biol.* 75, 75–101. doi: 10.1016/bs.amb.2016.08.002

OceanCare (2021). *Quiet Waters for Whales and Dolphins: The One-Time Opportunity to Avoid, Reduce and Mitigate Noise-Generating Activities in the Mediterranean Cetacean Migration Corridor*. (Switzerland: Ocean Care).

Panigada, S., Donovan, G. P., Druon, J. N., Lauriano, G., Pierantonio, N., Pirotta, E., et al. (2017). Satellite Tagging of Mediterranean Fin Whales: Working Towards the Identification of Critical Habitats and the Focussing of Mitigation Measures. *Sci. Rep.* 7, 3365 . doi: 10.1038/s41598-017-03560-9

Panigada, S., Gauffier, P., and Notarbartolo di Sciara, G. (2021). Fin Whale the IUCN Red List of Threatened Species: Balaenoptera Physalus Mediterranean Subpopulation. e.T16208224A50387979. doi: 10.2305/IUCN.UK.2021-3.RLTS.T16208224A50387979.en.

Panigada, S., Leaper, R., and Tejedor Arceredillo, A. (2010). Ship Strikes in the Mediterranean Sea: Assessment and Identification of Conservation and Mitigation Measures. *J. Cetacean Res.*, 1–5. MANAGE.

Panigada, S., Notarbartolo di Sciara, G., Zanardelli Panigada, M., Airoldi, S., Borsani, J. F., and Jahoda, M. (2005). Fin Whales (Balaenoptera Physalus) Summering in the Ligurian Sea: Distribution, Encounter Rate, Mean Group Size and Relation to Physiographic Variables. *J. Cetacean Res. Manage.* 7, 137–145.

Panigada, S., Pavan, G., Borg, J. A., Galil, B. S., and Vallini, C. (2008). "Biodiversity Impacts of Ship Movement, Noise, Grounding and Anchoring," in *Maritime Traffic Effects on Biodiversity in the Mediterranean Sea.* Chapter 1. (Malaga, Spain: IUCN Centre for Mediterranean Cooperation).

Panigada, S., Pesante, G., Zanardelli, M., Capoulade, F., Gannier, A., and Weinrich, M. T. (2006). Mediterranean Fin Whales at Risk From Fatal Ship Strikes. *Marine Pollut. Bull.* 52, 1287–1298. doi: 10.1016/j.marpolbul.2006.03.014

Panigada, S., Zanardelli, M., Canese, S., and Jahoda, M. (1999). How Deep Can Baleen Whales Dive? *Mar Ecol. Prog. Ser.* 187, 309–311. doi: 10.3354/meps187309

Pintore, L., Sciacca, V., Viola, S., Giacoma, C., Papale, E., and Giorli, G. (2021). Fin Whale (Balaenoptera Physalus) in the Ligurian Sea: Preliminary Study on Acoustics Demonstrates Their Regular Occurrence in Autumn. *J. Marine Sci. Eng.* 9, 966. doi: 10.3390/jmse9090966

Puig, P., Palanques, A., Guillén, J., and García-Ladona, E. (2000). Deep Slope Currents and Suspended Particle Fluxes in and Around the Foix Submarine Canyon (NW Mediterranean). *Deep-Sea Res. Part I* 47, 343–366. doi: 10.1016/S0967-0637(99)00062-X

QGIS Development Team (2018). *Qgis Geographic Information System* (Open Source Geospatial Foundation Project). Available at: http://qgis.osgeo.org.

Quadern el Pais (2016) *Un Pescador Troba Una Balena De 12 Metres Al Delta De l'ebre*. Available at: https://cat.elpais.com/cat/2016/01/28/catalunya/1453983052_549792.html.

R Core Team (2020). *R: A Language and Environment for Statistical Computing* (Vienna, Austria: R Foundation for Statistical Computing). Available at: https://www.R-project.org/.

Ritter, F. (2012). Collisions of Sailing Vessels With Cetaceans Worldwide: First Insights Into a Seemingly Growing Problem. *J. Cetacean Res. Manage*. 12 (2).

Raga, J. A., Raduán, A, Balbuena, J. A., Aguilar, A, Grau, E, and Borell, A. (1991). Varamientos De Cetáceos En Las Costas Españolas Del Mediterráneo Durante El Período 1982–1988. *Miscellania Zoologica* 15, 215–226.

Sabatés, A., Gili, J. M., and Pagés, E. (1989). Relationship Between Zooplankton Distribution, Geographic Characteristics and Hydrographic Patterns Off the Catalan Coast (Western Mediterranean). *Marine Biol.* 103, 153–159. doi: 10.1007/BF00543342

Schoeman, R. P., Patterson-Abrolat, C., and Plön, S. (2020). A Global Review of Vessel Collisions With Marine Animals. *Front. Marine Sci*. 7. doi: 10.3389/fmars.2020.00292

Sèbe, M., Kontovas, C. A., and Pendleton, L. (2019). A Decision-Making Framework to Reduce the Risk of Collisions Between Ships and Whales. *Marine Policy* 109. doi: 10.1016/j.marpol.2019.103697

Sèbe, M., Kontovas, C. A., and Pendleton, L. (2020). Reducing Whale-Ship Collisions by Better Estimating Damages to Ships. *Sci. Total Environ.* 713, 136643. doi: 10.1016/j.scitotenv.2020.136643

Smultea, M., Fertl, D., Bacon, C., Moore, M., James, V., and Wursig, B. (2017). Cetacean Mother-Calf Behavior Observed From a Small Aircraft Off Southern California. *Anim. Behav. Cogn.* 4, 1–23. doi: 10.12966/abc.01.02.2017

Stepanuk, J. E., Heywood, E. I., Lopez, J. F., DiGiovanni, R. A.Jr., and Thorne, L. H. (2021). Age-Specific Behavior and Habitat Use in Humpback Whales: Implications for Vessel Strike. *Marine Ecol. Prog. Ser.* 663, 209–222. doi: 10.3354/meps13638

Stone, G. S., Katona, S. K., Mainwaring, A., Allen, J. M., and Corbett, H. D. (1992). Respiration and Surfacing Rates of Fin Whales (Balaenoptera Physalus) Observed From a Lighthouse Tower. *Report of the International Whaling Commission*. 42, 739–745.

Szegedi, A., Tort, B., Amigó, N., and Degollada, E. (2019). "Drone Videos: A New Method for Photogrammetry of Fin Whales (Balaenoptera Physalus)?," in *World Marine Mammal Conference* (Barcelona: WMMC).

Torreblanca, E., Caminãs, J. A., MacIás, D., Garciá-Barcelona, S., Real, R., and Baéz, J. C. (2019). Using Opportunistic Sightings to Infer Differential Spatio-Temporal Use of Western Mediterranean Waters by the Fin Whale. *PeerJ.* 7, e6673. doi: 10.7717/peerj.6673

Tort, B., Serrallonga, S., Amigó, N., and Degollada, E. (2017). *Comportamiento Del Rorcual Común (Balaenoptera Physalus) En El Mar Balear Como Zona De Alimentación* (Valencia: SEC).

Tregenza, N., Aguilar, N., Carrillo, M., Delgado, I., Díaz, F., Brito, A., et al. (2000). Potential Impact of Fast Ferries on Whale Populations a Simple Model With Examples From the Canary Islands. *Eur. Res. Cetaceans* 14, 195–197.

Vaes, T., and Druon, J.-N. (2013). Mapping of Potential Risk of Ship Strike With Fin Whales in the Western Mediterranean Sea. A Scientific and Technical Review Using the Potential Habitat of Fin Whales and the Effective Vessel Density. *JRC Sci. Policy Rep*. 28 pp. doi: 10.2788/8520. European Commission.

Vanderlaan, A. S. M., and Taggart, C. T. (2007). Vessel Collisions With Whales: The Probability of Lethal Injury Based on Vessel Speed. *Marine Mammal Sci.* 23, 144–156. doi: 10.1111/j.1748-7692.2006.00098.x

Winkler, C., Panigada, S., Murphy, S., and Ritter, F. (2020). Global Numbers of Ship Strikes: An Assessment of Collisions Between Vessels and Cetaceans Using Available Data in the IWC Ship Strike Database. *Report to the International Whaling Commission, IWC/68B/SC HIM09*. 33pp.

Wood, S. N. (2011). Fast Stable Restricted Maximum Likelihood and Marginal Likelihood Estimation of Semiparametric Generalized Linear Models. *R. Stat. Soc. (B)* 73, 3–36. doi: 10.1111/j.1467-9868.2010.00749.x

Zerbini, A. N., Mendez, M., Rosenbaum, H. C., Sucunza, F., Andriolo, A., Harris, G., et al. (2015). *Tracking Southern Right Whales Through the Southwest Atlantic: New Insights Into Migratory Routes and Feeding Grounds* (San Diego, USA: IWC Scientific Committee).

Zitterbart, D. P., Smith, H. R., Flau, M., Richter, S., Burkhardt, E., Beland, J., et al. (2020). Scaling the Laws of Thermal Imaging-Based Whale Detection. *Journal of Atmospheric and Oceanic Technology*. doi: 10.1175/JTECH-D-19

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Understanding the Interactions Between Cetaceans and Other Megafauna With the Albacore Tuna Fishery: A Case Study From the Cyprus' Pelagic Longline Fishery

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Depredation by cetaceans on fisheries is a major issue globally, both in terms of conservation and fisheries economics. The present study conducted in Cyprus, Eastern Mediterranean Sea, aimed to understand the extent, level, and type of cetacean depredation on the albacore tuna pelagic longline fishery, and in particular to quantify and evaluate the economic consequences of depredation and identify potential dolphin-longline conflict areas and mitigation practices for management. The data were obtained from fisher's logbooks, interviews and onboard observations between June and August 2018. A novel and simple approach was applied to estimate the depredation rate and economic loss by using simple calculations including the number and weight of depredated fish, landings and fishing effort. The results revealed that there is an estimated economic loss per fishing trip of 313.07± 486.19 EUR and an estimated annual economic loss for the entire fleet of 259,272 EUR from depredation caused by cetaceans. The study also estimated that 16,639 albacore tunas were depredated in 2018 and the depredation rate ranged between 0% to 100% with a mean depredation rate of 17% per fishing trip. Depredation by the common bottlenose dolphin and striped dolphin was reported in more than 50% of their fishing trips. Other species that were found to be involved in depredation were the neon flying squid, the shortfin mako shark and the Risso's dolphin. This is the first official record worldwide of depredation from the common bottlenose dolphin, the striped dolphin and the neon flying squid on the pelagic longline albacore tuna fishery. A total bycatch of 62 individuals of common bottlenose dolphins and one individual of stripped dolphin were reported in interviews as a result of depredation on bait and catch. The study also identified depredation hotspots and possible depredation mitigation measures. Such information could support the development of management action plans and measures to minimise interactions between cetaceans and pelagic longlines.

Keywords: dolphin-fisheries interactions, depredation, economic loss, bycatch, mitigation measures, common bottlenose dolphin, striped dolphin, eastern Mediterranean Sea

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1 INTRODUCTION

Fisheries interactions with cetaceans have been well documented in almost all existing fishing gears (Northridge and Hofman, 1999; Dalla Rosa and Secchi, 2007; Forney et al., 2011; Guinet et al., 2015) with different targeted species (Hamer et al., 2012) and at different geographical areas (Lauriano, 2004; Díaz López, 2006; Brotons et al., 2008; Maccarrone et al., 2014; Gonzalvo et al., 2015). These interactions are associated with negative economic and conservation consequences (Hall and Donovan, 2002; Lauriano, 2006; Zollet and Read, 2006; Brotons et al., 2008), which may lead to controversial practises like culling of cetaceans to avoid depredation (Bearzi et al., 2004). The interactions between cetaceans and fisheries, can be biological or operational. Biological (indirect) interaction refers to the competition for the same biological resource at the population level (Northridge and Hofman, 1999), whereas operational (direct) interaction refers to the physical interaction of cetaceans with fishing gear and catch by removing the bait and/or catch, usually with negative consequences like bycatch (Harwood, 1992). Depredation is a form of operational interaction, which refers to the damage or removal of captured fish or bait from fishing gear by marine predators (Gilman et al., 2006). These interactions often result in significant damage to the fishing gear and catch and to the bycatch of cetaceans with consequences that may lead to dolphins' injury, death from drowning, and sometimes to the direct killing by angry fishers as a retaliatory measure (Zollett and Read, 2006).

Cetaceans are well known for their advanced learning abilities and the fast knowledge transfer within populations, enabling them to quickly discover new foraging grounds and opportunities (Whitehead et al., 2004). An example is their ability to develop familiarity with the sound produced by fishing vessels, including the sound produced by the engine, fishing gear and electric equipment facilitating the cetaceans to follow vessels or identify fishing grounds to take advantage of the catch (Gilman et al., 2006; Hernandez-Milian et al., 2008). Chilvers and Corkeron (2001) studied bottlenose dolphin (*Tursiops aduncus*) populations in Australia and found that some communities could become fully dependent on fisheries as an easily accessible feeding source. Many studies dealing with depredation and cetacean-fisheries interactions showed that this is a common strategy among cetaceans and that depredation on fishing gear is a practice that is taught within populations (Pennino et al., 2013). Consequently, the practise of depredation seems to be increasing compared to previous decades and is, therefore, more frequently reported in the literature (Hamer et al., 2012).

Interactions with cetaceans and longlines have been reported since 1952 when the global pelagic tuna longline fishing began in the Indian, Atlantic and Pacific Oceans (Sivasubramaniam, 1964). Among other fishing methods, longline is the most impacted from depredation worldwide (Northridge and Hofman 1999; Gilman et al., 2006; Garrison, 2007; Hamer et al., 2012) with more than 31 odontocete species, six mysticete species, 15 pinniped species and two sirenian species been reported to interact with longline fisheries (Werner et al., 2015). From the fishermen perspective, depredation on longlines is known to cause significant damage on fishing gear and catch and is also related with increased fishing effort to avoid competition with cetaceans and reach quota levels and annual profits (Peterson et al., 2014; Tixier et al., 2015; Werner et al., 2015). Depredation on longlines provides an easy access to prey resource that could modify the energy balance of local ecosystem dynamics due to the changes in the natural predator-prey interactions (Northridge and Hofman 1999; Morissette et al., 2012). Depredation could also lead to overexploitation and biased stock assessments, if losses due to depredation are not accounted for in fish stock assessments (Roche and Guinet, 2007). As most Mediterranean (Vasilakopoulos et al., 2014; Froese et al., 2018) and global (Zeller and Pauly, 2005; FAO, 2020) fish and invertebrate stocks are declining, prey availability for megafauna is also declining (Bearzi et al., 2006). This could explain, to some extent, the increased frequency of conflicts between fisheries and dolphins (Bearzi, 2002).

To the best of our knowledge, information on the interactions between cetaceans and the pelagic longline fishery has never been published in the Mediterranean Sea. In Cypriot waters, depredation by dolphins has already been reported in the literature for set-nets (Snape et al., 2018); however, in the pelagic longline fishery, though known for many years, it has not been described or quantified/estimated prior to this study. Previous personal author's communication with the pelagic longline fishers revealed that cetaceans are the main species impacting their fishing operations and secondarily impacted by other taxa like elasmobranchs and cephalopods. Hence, the main objective of this study was to describe the interactions and impact primarily caused by cetaceans and secondarily by other megafauna on the pelagic longline fishery targeting albacore tuna (*Thunnus alalunga*) in Cyprus. This study, specifically aimed to 1) identify megafauna species that interact with the pelagic longline fishery, 2) evaluate and quantify the interactions between cetaceans and the albacore tuna longline fishery and 3) identify potential dolphin-longline conflict areas and mitigation practices employed by longline fishers in response to cetacean depredation.

2 MATERIALS AND METHODS

2.1 Study Area and Description of the Cypriot Longline Fishery

The study took place within the Exclusive Economic Zone of the Republic of Cyprus, in the marine areas off Larnaca Bay and Paphos – Limassol (southeastern and western coasts of Cyprus, respectively to a maximum distance of 40 nautical miles from the nearest shore. The total fishing effort of the entire fleet targeting albacore tuna in 2018 was 600 days. The albacore tuna fishing period lasts approximately three months, from late May to August. The pelagic longline fleet consisted of 30 vessels of lengths between 12 and 18 meters capable of using various gears (polyvalent vessels) permitted to adjust fishing activities according to the season and the presence and movement of various species of fish. The main fishing gear used is the drifting longline and secondary gears are trammel nets, gillnets, bottom longlines, and traps. The longline fishery is active within small distances from the shore, targeting demersal and mesopelagic species using bottom longline and nets, and away from the shore and outside the territorial waters, using drifting longlines, mainly targeting swordfish and albacore tuna, and other pelagic species (Department of Fisheries and Marine Research, 2019). The swordfish fishing period is between September to December whereas demersal species are targeted between December to May. Sardine is always used as a bait for fishing albacore tuna, and mackerel and squid is always used for swordfish fishing.

2.2 Data Collection

2.2.1 Sampling Scheme

The sampling scheme for data collection was fishery-dependent and the data were derived using three methodologies: self-reporting in logbooks, onboard observations, and interviews. Fishers who may participated in one or more of the three different methodologies provided different information according to aims of each method and therefore there was no effect on the data gathered.

2.2.2 Logbooks

Four professional longline fishers (out of 30 licensed vessels) were provided with logbooks for data collection during 71 fishing trips (days) that took place between June to August 2018. A fishing trip could last between one to three days maximum and each different day in the sea was counted as one fishing trip. Every day is a new setting and hauling for the pelagic longline and only one longline was set per day. For every fishing trip/day, fishers reported the fishing gear characteristics including the number of hooks set on the pelagic longline, length on longline, type of bait, soak time, the depth of fishing, bottom depth, the coordinates of the position of longline and if they used or not acoustic deterrent devices. In all cases, they were targeting albacore tuna. Information was also recorded about catch and depredation, including the caught species, the number of individuals caught, the total weight of landed fish, the number of individuals depredated, and the species involved in depredation events. Depredation was only recorded when the predators were visible and identified otherwise the data were excluded from the analysis. The length of each longline per trip was approximately 50 km with 4,000 hooks set and a soak time ranging between six to 12 hours. No acoustic deterrent devices were used during the fishing trips recorded in logbooks.

Fishers who participated in the study were all trained in self-reporting methods and on the identification of cetaceans and elasmobranchs species that are encountered and often incidentally caught on pelagic longlines through the 'Cyprus Bycatch Project' (Papageorgiou et al., 2020). This was done to ensure the validity of the data recorded on logbooks. In addition to the logbooks, fishers were provided with an identification guide for vulnerable species, including cetaceans, elasmobranchs, marine turtles and seabirds. Only fishers that committed to completing and returning the logbooks and showed great interest in the study during the training sessions were selected to participate in the study. Fishers were regularly visited at ports and monitored, and the logbooks were checked to ensure that were filled correctly. Fishers were also asked to provide photographic evidence, when possible, that was recorded from their cell phones as a confirmation material of depredation.

2.2.3 Onboard Observations

A small number of onboard observations (N = 9) were conducted as part of the 'Cyprus Bycatch Project' survey (Papageorgiou et al., 2020). This was used as an opportunity to train fishers on self-reporting and dolphin identification and also check the validity of the data recorded by fishers on logbooks. During this time, the observer confirmed that depredation on albacore tuna was caused by the common bottlenose dolphin and the striped dolphin as well as other species and photographic and video evidence was collected.

2.2.4 Interviews

Interviews is a very useful qualitative data collection tool for collecting narrative data that allow investigating people's knowledge, experiences, perspectives, and views in great depth (Kvale and Brinkmann, 2009). The value of this method is to help build the complete picture about a subject, to report detailed views of the people in the sample and to enable participants to tell their own story and express their feelings, thoughts and perspectives (Berg, 2004).

Structured interviews were conducted to 20 pelagic longline fishers (who are also the owners of vessels) in September 2020. The sample size (20 vessels) represented 63% of the entire fleet. Interviews seek to gain a better understanding of fishers' knowledge relating to depredation and dolphin behaviour. In the beginning, fishers were introduced to the study and were ensured that all data would be anonymous. This was an important step to gain trust among fishers and to confirm that this study had no associations with regulatory and fishing authorities. Also, the fact that many fishers already knew the interviewer from previous collaborations (e.g., Giannakis et al., 2020; Papageorgiou et al., 2020) played a significant role in the clarity and consistency of their responses. Based on this pre-existing relationship and trust with the fishers, the interviewer was recommended to other fellow fishers. The snowball sampling method was used to interview the participants of the study (Goodman, 1961). This technique is used to interview people in the sample that are referred from the person being interviewed based on their knowledge and experience of the topic, in this case, dolphin depredation. This method has been previously used in numerous fisheries science studies (e.g., Braga and Schiavetti, 2013; Dmitrieva et al., 2013; Peterson and Carothers, 2013; Zappes et al., 2016). A pilot survey among four fishers was conducted to test the questionnaire prior to the study. The structure of the interview was composed of three main parts: 1) fisher's personal information; 2) information on depredation, dolphin population size, fish stocks and identification of dolphin depredation hotspots, and 3) information on depredation mitigation measures. An open-ended interview (unstructured) was followed after the end of the structured interview where fishers' empirical knowledge on longline-dolphins interactions was obtained as well.The interviewer (the leading author, native speaker of Greek) followed standardized interview methodologies to maximise clarity and consistency in the way the questions were asked and recorded. The interviewer appeared neutral during interviews to avoid influencing fishers' responses and to assure fishers that there was no risk in participating in the study and therefore minimise concerns on reporting bycatch. The interviewer recorded any strange responses during interviews and assessed the reliability of the information provided for each fisher. The interviewer is an experienced onboard observer in small-scale and pelagic longline fisheries and has extensive knowledge of the fishing sector in Cyprus.

2.3 Data Analysis

The analysis of the data was conducted in four phases. First, the rates and estimates for depredation and economic loss were calculated. Second, correlations and comparisons between parameters and variables were conducted. The third step aimed to qualitatively and quantitatively understand depredation, bycatch and mitigation practices based on fishers' knowledge using descriptive statistics. Fourth, a non-parametric spatial analysis method was used to identify possible fisheries-dolphin conflict areas.

All the statistical analyses were performed using the R software (R Core Team, 2020). Statistical significance set at $a<0.01$ was used for all statistical tests to account for the increased probability of type I error multiple testing. The standard deviation (SD) for all averages is given unless specified otherwise.

2.3.1 Calculations

Only fish that were landed and sold to retailers were included in the Landings Per Unit of Effort (LPUE), and only the fish that were depredated and discarded were included in the Depredated Per Unit of Effort (DPUE).

The Landings Per Unit of Effort (LPUE) was calculated as:

$$\mathbf{LPUE} = \text{Total weight of fish landed (kg)} \times (\text{1000 hooks (standardised)} / \text{No. of hooks set per fishing trip})$$

The Depredated Per Unit of Effort (DPUE) was calculated as:

$$\mathbf{DPUE} = (\text{No. of individuals depredated} \times \text{average weight for a tuna per fishing trip (kg)}) \times (\text{1000 hooks (standardised)} / \text{No. of hooks set per fishing trip})$$

Then, Catch Per Unit of Effort (CPUE) was calculated as:

$$\mathbf{CPUE} = LPUE + DPUE + other\ \ discrads$$

whereas *other discards* refer to any other species that were caught and discarded. No other discards were recorded in the current study except the depredated tunas.

To estimate the economic loss and depredation rate, the calculations from (FAO 2019a; 2019b) manuals that were developed to estimated bycatch and discard rates and were modified and used accordingly for the purpose of this study. To the best of our knowledge, this is the first time such an approach was used to estimate the depredation rate and economic loss.

The percentage depredation rate (D%) was calculated for each fishing trip and it was defined as the percentage of the total depredated fish per fishing trip. The following equation was used:

$$\mathbf{Percentage\ Depredation\ rate\ (D\%)} = (Summed\ \ depredated\ \ individuals\ / (Summed\ \ depredated\ \ individuals + summed\ \ landed\ \ individuals)) \times 100$$

The depredation rate (R) estimates the rate of depredation on the targeted species based on the number of sampled fishing trips. Knowing the number of depredated fish and the number of sampled fishing trips (71), it was possible to calculate the depredation rate for each depredated species using the following equation:

$$\mathbf{Depredation\ \ rate\ (R)} = Summed\ \ depredated\ \ individuals\ / Number\ \ of\ \ sampled\ \ fishing\ \ trips$$

Then, the estimated annual depredation rate (EDR) of each species was calculated as follows:

$$\mathbf{Estimated\ \ annual\ depredation\ rate\ (EDR)} = Depredation\ \ rate\ (R) \times Total\ \ number\ \ of\ \ fishing\ \ trips\ \ during\ \ reference\ \ year$$

The second step of the calculations concerns the estimation of the economic loss (EL) per fishing trip, which is essential to evaluate the impact of depredation on the catch. For the calculations of the economic loss, only the damaged catch was considered and not the damage on bait or fishing gear. The economic loss was calculated for each fishing trip by knowing the total average weight of individuals depredated, which was obtained by multiplying the average weight of tunas landed per fishing trip with the total number of individuals depredated of the specific fishing trip. The average weight of tunas landed was estimated by calculating the average weight of all individuals which landed from each specific fishing trip recorded in logbooks. Following up, the total average weight of individuals depredated was multiplied by the price per kilo of each species as of 2018. The average price sold from the fisher to the retailer was 2.30 EUR per kilo. In 2018, the price was stable with only 0.10EUR variation within the three months. The equation to estimate the economic loss per fishing trip is as follows:

$$\textbf{Economic Loss (EL)} = (No.\ of\ individuals\ depredated\ per\ fishing\ trip \times Average\ weight\ of\ tuna\ in\ the\ specific\ fishing\ trip) \times price\ per\ kilo$$

Then, the economic loss rate (ER) was calculated as follows:

$$\textbf{Economic loss rate (ER)} = EL\ /\ Number\ of\ sampled\ fishing\ trips$$

Therefore, the annual economic loss rate (EER) was calculated as:

$$\textbf{Estimated annual economic loss rate (EER)} = Economic\ loss\ rate\ (\mathrm{ER}) \times \text{Total number of fishing trips carried out during reference year}$$

2.3.2 Correlation of Parameters and Mean Comparisons

A Shapiro-Wilk's normality test, frequency density and q-q plots were used to test for normality prior to any other statistical analysis tests. The Mann-Witney test was used to compare the LPUE at the presence and absence of dolphins and also to compare the fishing area with D%, DPUE and LPUE. To compare means of LPUE, DPUE, CPUE, D% and EL with the month of fishing, the non-parametric Kruskal-Wallis test was conducted.

2.3.3 Depredation, Bycatch and Mitigation Practices

Descriptive statistics were conducted to show the results of the interviews including information on fish stocks, interactions, depredation, bycatch and mitigation practices. A Spearman rank correlation test was conducted to explore the relationship between the total bycatch of dolphins and years in the profession as well as between the fishing effort (days at sea) and the total number of days with depredation in 2019.

2.3.4 Spatial Analysis for Dolphin's Depredation Hotspots

Based on fishers' knowledge, a heat map was created to show dolphin depredation hotspots based on the number of times specific areas have been reported with dolphin depredation. For the heat-map generation, the Kernel Density Estimation (KDE) has been used, as it is one of the most classic spatial statistical algorithms to capture spatial point patterns that obeys Tobler's First Law of Geography by introducing kernel function and attenuation effect (Yuan et al., 2019). The algorithm behind the tool fits smoothly curved surface over each point. The surface value is highest at the point location and diminishes as the distance from the point increases. It becomes zero at the search radius (bandwidth) distance from the point. Bandwidth selection is a critical step while applying KDE-based heat maps (Lampe and Hauser, 2011; Li et al., 2014). The bandwidths can either be invariant (fixed KDE) or spatially variant (adaptive KDE) across sample points. In order to be able to tell where any clusters in our data exist, several kernel bandwidths have been examined and analyzed, choosing as more suitable for our case and for visualization purposes, the search radius of 10km (based on the linear unit of the projection of the output spatial reference). The output cell size has been set to 20x20m and the output density value (is dolphins count divided by area) on the map has been set to HIGH and LOW. The analysis was performed using ArcGIS$^{\mathrm{TM}}$ (Esri Inc, 2013).

3 RESULTS

3.1 Logbooks and Onboard Observations

3.1.1 Characterisation of Depredation

Four different species were identified depredating on the albacore tuna from the board observations by an onboard observer (leading author). These were: the common bottlenose dolphin (*Tursiops truncatus*), the striped dolphin (*Stenella coeruleoalba)*, the neon flying squid (*Ommastrephes bartrami*) and the shortfin mako shark (*Isurus oxyrinchus*). There are distinctive differences of depredation between the different species that can be identified from the photos taken onboard (**Figure 1**). Fishers were able to identify if depredation was caused by dolphins or the other predators by the damage caused on the catch, even without the visual observation of predators. Confirmation of the species producing the damage to the catch comes from direct observation or bycatch of the depredator (**Figure 2**).

Depredation by cetaceans is most often caused by the common bottlenose dolphin and less often by the striped dolphin. However, there are cases where the two species are found in the same pod and simultaneously depredate on catch or bait. This observation has been confirmed by the onboard observer and by the fishers when asked to explain which cetacean species are involved in depredation. For this reason, depredation recorded in the logbooks that was caused by cetaceans was not differentiated between the two species to avoid misidentification, misinformation and bias on results. Hereafter, cetacean depredation refers to the depredation that was caused by the common bottlenose dolphin or the striped dolphin or both.

3.1.2 Estimates of Interaction, Depredation and Economic Loss

The total fishing effort in June, July and August was 15, 39 and 44 days, respectively. The total number of days with depredation was 10 in June and July, and 5 in August. According to the fishing effort of each month, dolphin depredation was higher in June followed by July and August (**Figure 3**). The mean LPUE was compared in the presence and absence of depredation, and the results were 89.81 ± 92.65 and 117.71 ± 121.82, respectively.

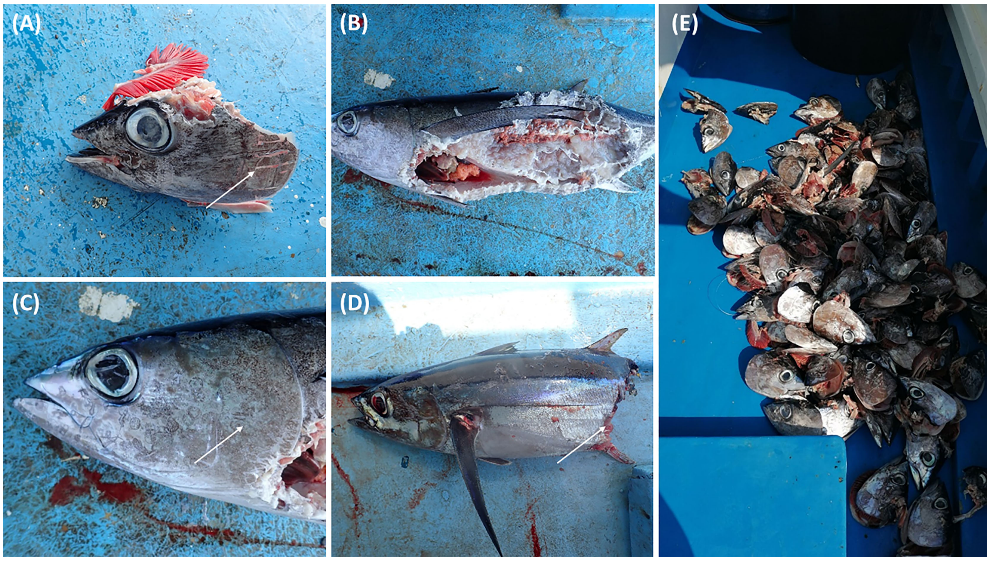

FIGURE 1 | Examples of depredated albacore tuna (*Thunnus alalunga*) by common bottlenose dolphin (*Tursiops truncatus*) (**A**; arrow shows marks odontocete tooth lacerations on head), as opposed to neon flying squid (*Ommastrephes bartrami*) (**B, C**; arrow shows marks of the squid suckers) and shortfin mako shark (*Isurus oxyrinchus*) (**D**; arrow indicates the sharp cut) during pelagic longline fishing in July 2018. Depredated tunas (>200 heads) by the common bottlenose dolphin on a single fishing trip in July 2018 **(E)**.

Results from the Mann-Whitney test revealed no significant difference (W = 1002.5, p-value = 0.47) between the mean LPUE at presence and absence of dolphins. Days with depredation were classified as days with the presence of dolphins whereas days with no depredation were classified as days with the absence of dolphins. The total DPUE, LPUE and CPUE were 3334.85, 10838.2 and 14173, respectively. This indicates that 23.53% of the total catch (CPUE) was depredated (DPUE).

Cetacean depredation on albacore tuna occurred between June – August 2018 with a higher number of depredated fish during June and July 2018, where the catchability and presence of albacore tuna are higher. Depredated albacore tunas ranged from 0 to 120 individuals with a mean of 28.04 ± 33.7 per fishing trip (N=71).

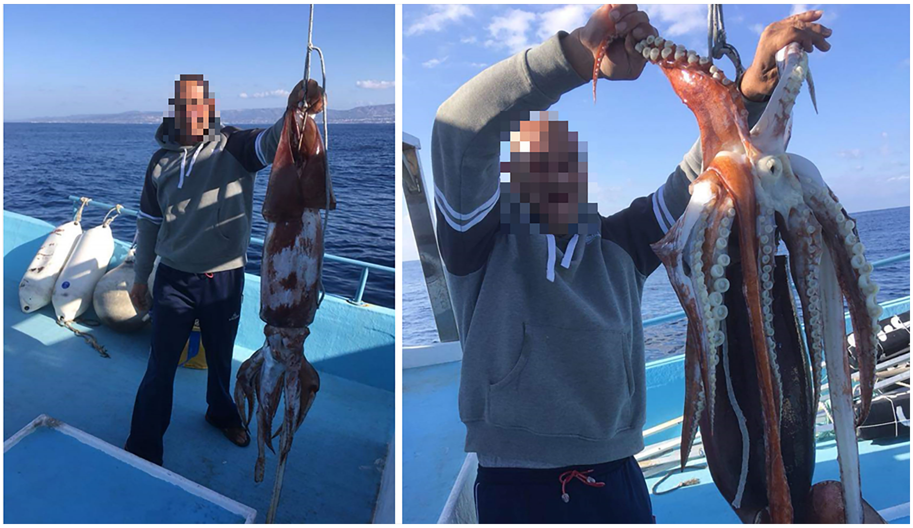

FIGURE 2 | A neon flying squid that was incidentally caught on the pelagic longline fishery in May 2019 when attempting to depredate the albacore tuna.

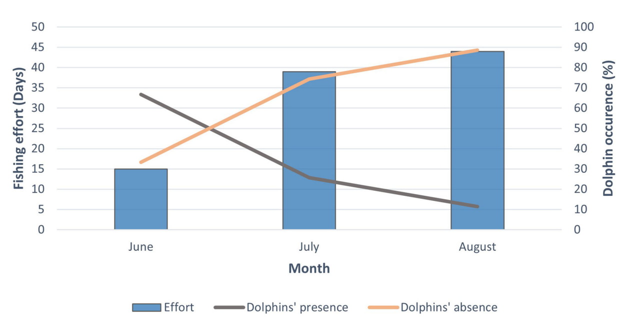

FIGURE 3 | Relationship between fishing effort (days) and days with and without dolphin depredation (%) for each month.

The D% varied from 0% to 100% with a mean of 16.9 ± 23.01%. The estimated R was 27.8 and the estimated EDR was 16,639, meaning that each year this amount of albacore tunas is estimated to be depredated in the entire fleet. The EL ranged from 0 to 1,800.00 EUR with a total EL of 30,680.60 EUR and a mean of 313.07 ± 486.19 EUR per fishing trip. The estimated ER was 423.12 EUR and the estimated EER was 259,272.68 EUR for the entire fleet in 2018. The Kruskal-Wallis test revealed no significant differences between the month of fishing with D% (χ^2 = 50.66, df = 47, df = 47, p-value = 0.33) and EL (χ^2 = 50.66, df = 47, p-value = 0.33) (**Figure 4**).

The mean D% in June, July and August was 29.2 ± 25.6, 20.8 ± 24.7 and 9.3 ± 17.8, respectively. The mean EL in June, July and August was 524.6 ± 653.5, 398.7 ± 542.3 and 165.0 ± 299.3, respectively. The mean D% of the three different months of the albacore tuna fishing period in relation to the mean ER is shown in **Figure 5**.

No significant trend in DPUE, LPUE and CPUE was detected between the months of fishing, range and mean values are shown in **Table 1**. The Kruskal-Wallis test showed no significant differences between month of fishing with DPUE (χ^2 = 50.66, df = 47, p-value = 0.33), LPUE (χ^2 = 78.39, df = 70, p-value = 0.23) and CPUE (χ^2 = 86.56, df =80, p-value = 0.29) (**Figure 6**).

3.1.3 Spatial Distribution of Fishing Trips, Depredation Rate, DPUE and LPUE

Fishing grounds were situated in two broad geographical areas and grouped as Larnaca and Paphos-Limassol. In total, 36 and 35 fishing trips were conducted at Larnaca and Paphos-Limassol, respectively. The days with depredation and the D% were slightly higher in Larnaca than in Paphos-Limassol area. Days with depredation were 24 (67% out of total fishing trips) and 22 (63%) at Larnaca and Paphos-Limassol, respectively. At Larnaca and Paphos-Limassol the mean D% was 26.9 ± 24.8 and 15.2 ± 22.4, the mean DPUE was 62.3 ± 64.8 and 30.8 ± 43.5, and the mean LPUE was 153 ± 122.1 and 148.6 ± 101.6, respectively. The results from the Mann-Whitney test revealed a significant difference between D% and fishing area (W = 794, p = 0.05) but no significant difference between fishing area and

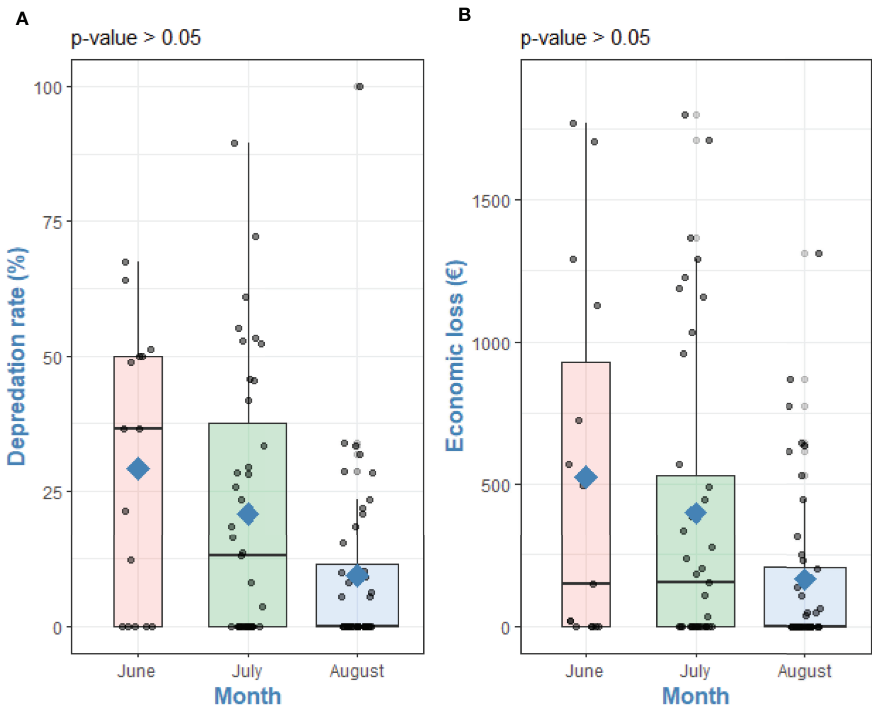

FIGURE 4 | Differences between **(A)** Depredation rate (%) and **(B)** Economic loss (€) and with month of fishing. Kruskal-Wallis tests *p*-values. Points are raw survey data. Rhombuses represent the mean of each group.

DPUE (W = 785, p-value = 0.07) and LPUE (W = 622, p-value = 0.93). The fishing trips with information regarding depredation events and D% are shown in **Figure 7**; DPUE and LPUE per fishing trip are shown in **Figure 8**.

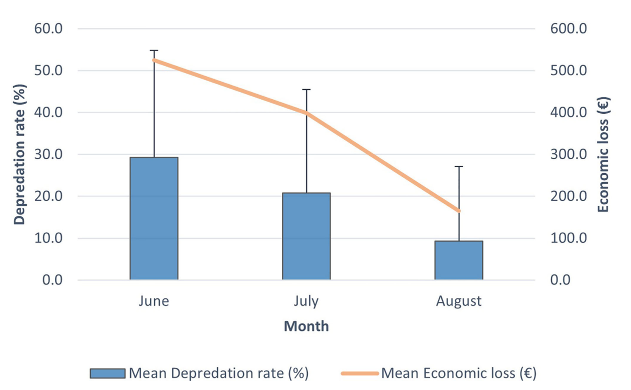

FIGURE 5 | Mean depredation rate (%) and mean economic loss (€) for each of the three fishing months in 2018. Error bars indicate standard deviation.

3.2 Interviews

3.2.1 Species Involved in Depredation

The mean age of pelagic longline fishers interviewed was 51 ± 8.2 years old with 19 ± 12.6 years in the profession. Without any exception, all the fishers interviewed reported that they had experienced depredation of their catch at some point in their career. The most common species reported in the interviews to depredate the catch was the common bottlenose dolphin (100%) followed by the striped dolphin (85%), the neon flying squid (80%), the shortfin mako shark (75%) and the Risso's dolphin (35%) (**Figure 9**).

TABLE 1 | Range and monthly means (± SD) of Landings Per Unit of Effort (LPUE), Depredation Per Unit of Effort (DPUE), and Catch Per Unit of Effort (CPUE) during the 3-month survey in 2018.

	Range (Mean ± SD)	June	July	August
LPUE	0-625 (110.6 ± 115.3)	119.8 ± 115.9	112.8 ± 84.4	105.5 ± 138.7
DPUE	0-195.6 (34.0 ± 52.8)	57.0 ± 71.0	43.3 ± 58.9	17.9 ± 32.5
CPUE	2.5-659.3 (144.6 ± 136.6)	176.8 ± 150.9	156.2 ± 98.9	123.4 ± 158.4

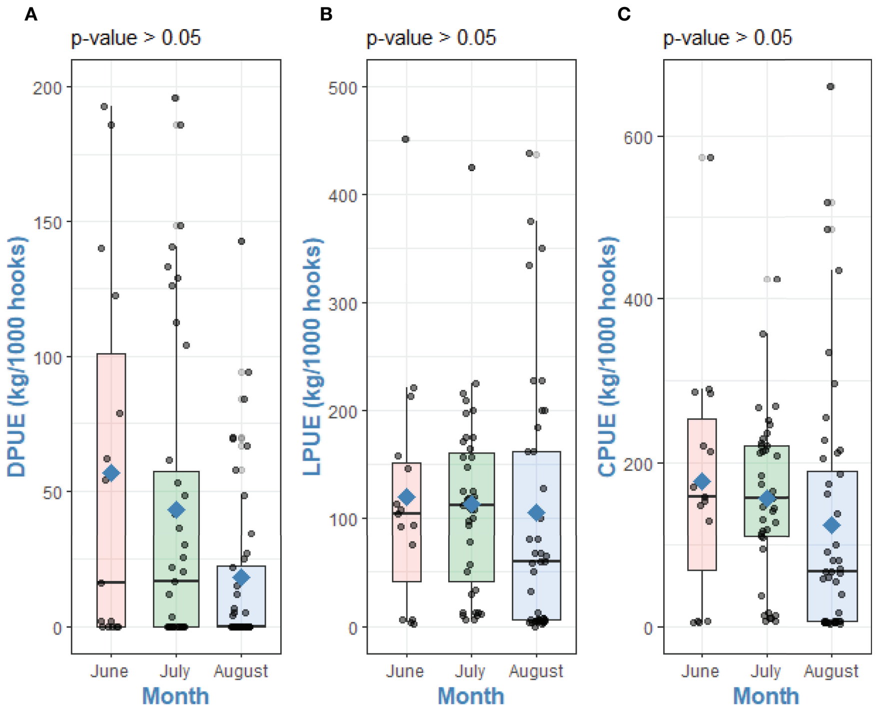

FIGURE 6 | Differences between **(A)** DPUE, **(B)** LPUE and **(C)** CPUE with month of fishing. Kruskal-Wallis tests *p*-values. Points are raw survey data. Rhombuses represent the mean of each group.

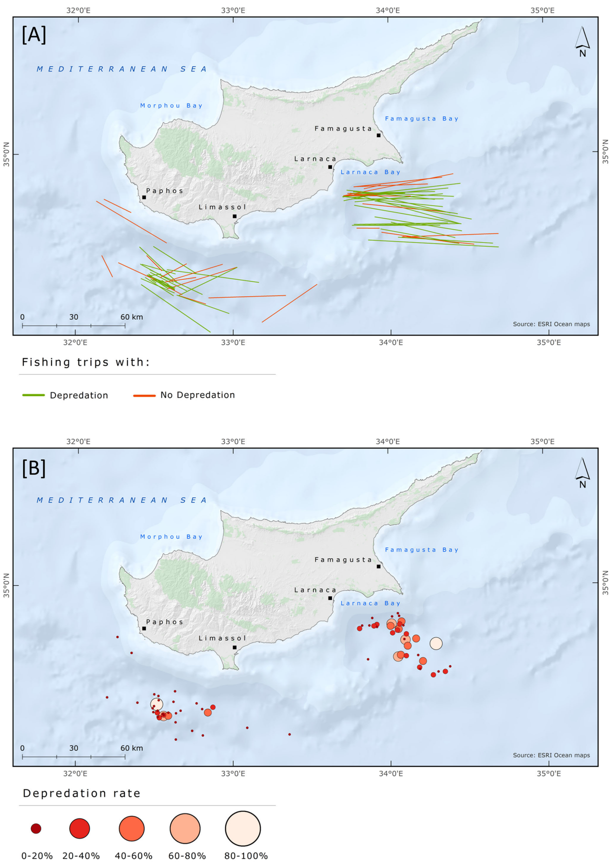

FIGURE 7 | Spatial distribution of pelagic longline fishing trips targeting albacore tuna between June and August 2018 with information regarding **(A)** depredation events and **(B)** Depredation rate (%).

3.2.2 Characteristics of Depredation and Perception on Stocks

All of the respondents (100%) reported that the most common species depredating the catch is *T. truncatus* and the species mostly depredated is *T. alalunga*. All respondents reported that the interactions between dolphins and fisheries have increased in the past 10 years. These interactions mostly occur between May and August during the tuna fishing period (100% of responses). Ninety per cent of the respondents reported that depredation events with dolphins happen very often during their fishing trips targeting albacore tuna whilst only ten per cent reported that depredation happens often. Sixty per cent of the respondents reported that over the past 10 years the tuna stocks have decreased while 40% said that they remained the same. Respondents reported that over the past ten years the swordfish stocks have decreased (70% of respondents), remained the same (20% of respondents) and ‘didn’t know’ (10% of respondents). All respondents reported that all dolphins cause significant damage to the gear when depredation events occur and that depredation causes serious economic losses on their business.

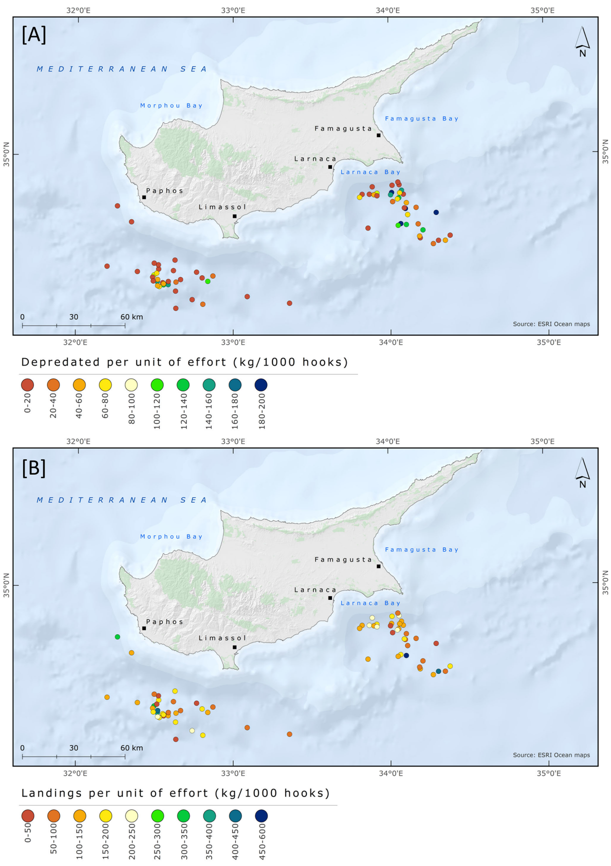

FIGURE 8 | Spatial distribution of pelagic longline fishing trips targeting albacore tuna between June and August 2018 with information regarding **(A)** Discards Per Unit of Effort and **(B)** Landings Per Unit of Effort.

3.2.3 Cetacean Bycatch

Results from the Spearman rank correlation coefficients test revealed a quasi-significant positive relationship (rho = 0.53, p-value = 0.017) between dolphin bycatch and years in professional fishing. Out of the total 20 interviews conducted, 15 fishers (75%) reported bycatch of the common bottlenose dolphin, five (25%) reported no dolphin bycatch and one (5%) reported bycatch of the striped dolphin. The mean fishing effort of responders in

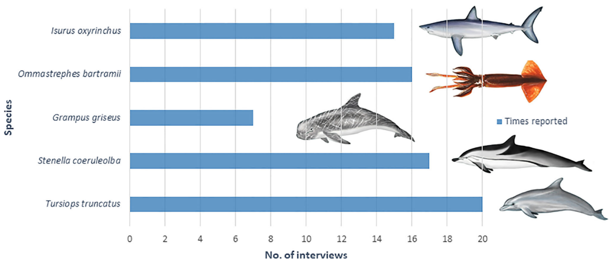

FIGURE 9 | Number of times in which each species was reported to depredate the catch during the interviews (N = 20).

2019 was 74 ± 34.4 days and the mean number of days that had experienced depredation in 2019 was 37 ± 16, essentially 50% of their fishing trips. Results from the Spearman rank correlation coefficients test revealed that there is a significant positive relationship (rho = 0.69, p-value < 0.001) between the fishing effort (days at sea) and the total number of days with depredation in 2019. Fishers reported a total of 62 individuals of *T. truncatus* and one individual of *S. coerueoalba* bycaught throughout their fishing careers. Five fishers reported no bycatch of dolphins and one fisher reported the bycatch of about 20 individuals of *T. truncatus* throughout his career. A juvenile common bottlenose dolphin was incidentally caught on the pelagic longline during the albacore tuna fishing period in June 2019. The incident was video recorded and can be found in the **Supplementary Video (S1)**. The video clearly shows other two dolphins (perhaps family members) that did not leave the area until the juvenile dolphin was released. The fisher reported that the incident happened during the attempt of the juvenile dolphin to depredate the catch and he also reported high dolphin depredation on that day. Recently, a juvenile *S. coerueoalba* was bycaught on pelagic longlines targeting swordfish in September 2021 during the attempt to depredate the bait and drowned (**Figure 10**).

FIGURE 10 | Bycatch of a juvenile *Stenella coerueoalba* on pelagic longlines targeting swordfish in September 2021. Photograph taken onboard by the vessel's captain.

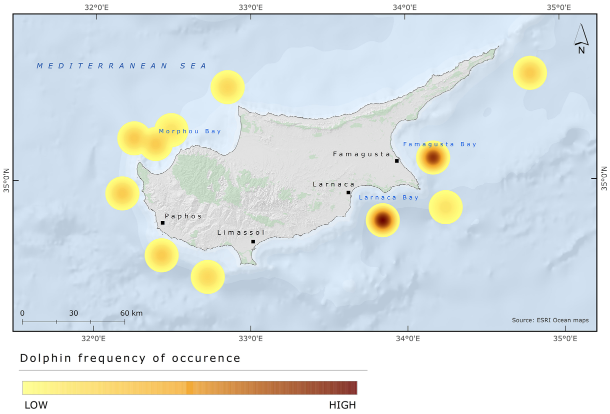

FIGURE 11 | Heat map of dolphins' frequency of occurrence from interviews (N = 20) of fishers.

3.2.4 Depredation Hotspots

Based on the information provided by fishers about areas where dolphins (*T. truncatus* and *S. coeruleoabla*) were most commonly encountered, a heat map was created to show the distribution of dolphin depredation hotspots based on the number of records in each area (**Figure 11**). The areas of Larnaca (Southeast) and Protaras (East) were found to have the highest probability of dolphin occurrence followed by the areas in the Northwest (Chrysochou Bay). There were also records of dolphin depredation in the areas of Akamas, Pegeia, Paphos, Pissouri, north of Morphou Bay and east of Apostolos Andreas.

3.2.5 Mitigation Measures

All fishers interviewed (N=20) were aware of dolphin anti-depredation devices (pingers) and all of them were willing to explore and test possible mitigation measures. Fishers were then asked to report if they take any depredation mitigation measures. Fifteen fishers (75%) reported that they were not taking any measures to avoid dolphin depredation and only five (25%) reported they were using dolphin anti-depredation devices (**Table 2**). Another depredation measure reported which is a common practice among longline fishers was the avoidance of areas with high cetacean abundance and fishers moving to other fishing grounds.

3.2.6 Fishers' Experience and Empirical Knowledge

There is a common belief among fishers that since the beginning of the swordfish longline fishing in Cyprus in 1973, depredation levels have gradually increased over the years. They accept as true that the practice of depredation is taught and passed on to the new generations of dolphins. Fishers reported that in just two years after the beginning of the albacore tuna fishing in Cyprus in 2004, dolphins identified their fishing grounds and learned to depredate the catch. Six fishers reported that only in the last four years (since 2018) dolphins have learned to depredate the bait, whereas in the previous years' depredation was only happening on the catch.

All fishers who participated in the interviews and have reported cetacean bycatch have also reported that in all cases the individuals bycaught were juveniles and, in most cases, they were drowned, indicating a high chance of mortality when bycaught. Some fishers have also reported that the pod did not leave the area until the bycaught dolphin was disentangled and released back to the sea. During these events, fishers have noticed sounds described as mourning coming from dolphins from the pod. One fisher has reported a rare event where other dolphins from the pod were observed carrying the dead young dolphin on their backs outside the water, presumably to allow it to breathe. In most cases of a dead dolphin, fishers reported a "vengeful" behaviour by the pod, where dolphins frantically removed caught fish from longlines and caused substantial damage to the gear.

Another common observation by fishers was that when the pod had consumed enough tunas caught on the longline, the pod displayed a playful behaviour, often swimming and "porpoising"

TABLE 2 | Depredation mitigation measure (dolphin anti-depredation pinger) used by longline fishers and their effectiveness on mitigating dolphin interactions.

Respondent	Pinger Brand	Model	Effectiveness	Effective on	Not effective on
1	STM industrial Electronics	DDD03U/DiD 01	Very effective	*T. truncatus;* *S. coeruleoalba*	Don't know
2	STM Industrial Electronics	DDD03U/DiD 01	Very effective	*T. truncatus;* *S. coeruleoalba*	Don't know
3	STM Industrial Electronics	DDD03U/DiD 01	Very effective	*T. truncatus*	*S. coeruleoalba*
4	Fishtek Marine	Dolphin Anti-Depredation Pinger (40kHz)	Moderate effective	*T. truncatus*	Don't know
5	Fishtek Marine	Dolphin Anti-Depredation Pinger (40kHz)	Slightly effective	*T. truncatus*	Don't know

around the boat and in some cases playing with the depredated tunas by throwing them outside the water and up in the air. In other reports, dolphin pods follow the boat during setting to depredate on the bait (sardine) and during hauling to depredate on the catch. Four fishers alleged that depredation events are more frequent during the full moon.

Two fishers have reported rare depredation events by the species *G. griseus*, mentioning that the species is very shy, not "showing off" and that its behaviour is not aggressive like *T. truncatus* and *S. coeruleoalba*. Fishers also mentioned that the latter has a more aggressive behaviour than the *T. truncatus* and even if depredation events are less frequent with this species, the damage on the catch and gear is higher when it happens.

4 DISCUSSION

By examining the results of the self-reporting, the onboard observations and the interviews, the study confirmed for the first time the depredation by the common bottlenose dolphin, the striped dolphin and the neon flying squid on the albacore tuna pelagic longline fishery in Cyprus. To the best of our knowledge, this is also the first time that the two cetacean species and the cephalopod species have been reported to depredate on pelagic longlines worldwide. The study shows that depredation has been responsible for causing serious socioeconomic and ecological problems, often leading to catch and gear loss, cetacean bycatch and in some extreme cases, death. Most worrying is the rise on the frequency of depredation events and the increased negative impacts on both, fishers, and dolphins. The very few available estimates on depredation rates and economic loss found in the literature, followed different approaches, thus precluding any possible direct comparison with the current study.

4.1 Self-Reporting: Estimations of Depredation Rate and Economic Loss

The findings of this study have shown that the species that are most frequently involve in depredation and cause the highest impact on fishing operations are the common bottlenose dolphin and the striped dolphin. The common bottlenose dolphin is the most frequently reported species to interact with small-scale fishing activities in the Mediterranean Sea (Lauriano, 2004; Díaz López, 2006; Brotons et al., 2008; Rocklin et al., 2009; Blasi et al., 2015; Pennino et al., 2015; Snape et al., 2018; Pardalou and Tsikliras, 2020), probably due to its opportunistic feeding habits and wide spatial distribution (Barros and Odell, 1990). This species has a flexible and cosmopolitan diet (Shane et al., 1986; Barros and Odell, 1990; Connor et al., 2000) and its distribution is often related to prey distribution, which in many cases overlaps with the distribution of species targeted by a fishery (Barros et al., 2000), and thus, inevitably leading to conflicts between dolphins and fisheries. There are several reports worldwide of the common bottlenose dolphin interacting with fishing gear involving hook and line (Werner et al., 2015). Interactions between the striped dolphin *Stenella coeruleoalba* and fisheries have been also documented in the Mediterranean with small-scale and purse seine fisheries (Magnaghi and Podestà, 1987; Di Natale and Notarbartolo di Sciara, 1994; Bearzi, 2002; Northridge et al., 2013; Crosti et al., 2017; Bruno et al., 2021).

The results revealed that there is an estimated mean economic loss per day of about 313 EUR and an estimated annual economic loss for the entire fleet of about 260,000 EUR from depredation. In other regions, the economic loss by cetacean depredation on set demersal longlines targeting halibut (*Reinhardtius hippoglossoides)*, arrowtooth flounder (*Atheresthes stomias)* and Patagonian toothfish (*Dissostichus eleginoides)* has been estimated to range between 1,034 USD to 8,449 USD per day (Roche and Guinet, 2007; Tixier et al., 2010; Hamer et al., 2012 and references therein), which shows substantially higher economic loss per day from the findings of the current study. Regarding the percentage of depredation rate (D%), we found that it ranges from 0 to 100 (mean D% = 17%), which is similar to the depredation rate (17.7%) found by Tixier et al. (2010) regarding the longline depredation on Patagonian toothfish, while other studies which included depredation on species of tuna, provided a broad range of depredation rates (0.5-100%) (Secchi and Vaske, 1998; Purves et al., 2004; Williams et al., 2009; Hamer et al., 2012) It has to be addressed that, the actual cost and rate of depredation from the current study, may be underestimated, as fish completely removed from the hooks were not possible to quantify. The rate of depredation may be affected either by the number of fish available or by the size of the group or from both factors. Both, D% and LPUE decrease from June to August, whilst the fishing effort increases. This can be explained by the albacore tuna migration at the end of the fishing season in August which corresponds to less catch for fishers and LPUE, and subsequently less available depredation resources for dolphins and D%.

Currently, pelagic longline fishers in Cyprus are being compensated annually by the government 300 EUR per fisher, for the damage caused on their fishing gear due to dolphin depredation (Ministry of Agriculture, Rural Development and the Environment, Department of Fisheries and Marine Research, 2020). This is considered by the fishers to be very low compared to the actual damage they are experiencing. Our findings support their arguments since we estimated the mean economic loss, during the fishing seasons, to be 313EUR daily per fisher. Personal author's communication with fishers, unveiled that many fishers expressed aggression towards dolphins, often considering harming them, especially during fishing trips on days with high depredation. However, this was not always the case as a minority of fishers consider themselves as "outsiders", meaning that they did not naturally belong in the dolphin's environment and that they should respect and protect them.

4.2 Onboard Observations: Identification Between Depredating Species

The photographic evidence collected from onboard observations confirmed the depredation by the common bottlenose dolphin and striped dolphin, the shortfin mako shark and the neon flying squid on albacore tuna. Depredation by the cetacean species was distinguishable from a shark- and squid-damaged fish. Odontocetes removed the entire torso and left only the head of the fish up to the gills and in some cases up to the jaw. In other cases, dolphins tore the body of the fish, leaving bites with ragged borders and with the head of the fish left on the hook. Also, in all cases, marks from their pencil-like teeth were visible on the wounded flesh (**Figure 1A**). Apart from that, depredation from dolphins is in almost all cases verified by the presence of dolphins close to the vessel.

The depredation caused by squid was distinguished from dolphins and sharks, by the small beak bites throughout the torso of the caught fish. In most cases, squids attack the belly and middle body of the caught fish, possibly aiming for the highly nutritious eggs (**Figure 1B**). Another distinguishable feature was the visible marks from the squid suckers on the body and head of the caught fish (**Figure 1C**). The squid was also reported by fishers to depredate on the bait and often get caught on the hook. Apart from the photographic evidence showing an individual neon flying squid that was bycaught during depredation (**Figure 2**), the fishers who participated in the interviews were asked to confirm the species by showing them pictures of the Mediterranean squids and they all identified it as *O. bartrami*. Fishers also reported catches of the neon flying squid of individuals weighing between 15 - 20 kg. Taking this information into consideration as well as the specimen photographed in **Figure 2**, no other known squid species in the Mediterranean can reach this body weight.

Shark depredation was distinguishable by the clean cuts, and bite-shaped portionsof flesh from the torso of the caught fish, leaving the surrounding body of the fish undamaged (**Figure 1D**). The species of the shark was identified by the observer since the depredation event happened exactly at the time of towing the longline. The shark arrived at the surface to depredate on the caught fish while towing the longline and dove back in the water. Similar results on the identification of odontocetes and shark depredation on the depredated catch with almost identical marks have been reported in the literature from other parts of the world (Dalla Rosa and Secchi, 2007; Gilman et al., 2007; Hamer et al., 2012).

Pelagic shark and squid species have been also reported in the literature to depredate on the catch on different fisheries and fishing gears. Shark depredation on the longline fishery targeting mainly different tuna species, swordfish and toothfish, has been previously documented (Gilman et al., 2008; Mandelman et al., 2008; Hamer et al., 2012; Rabearisoa et al., 2018; Tixier et al., 2021) however, such information was none existed in the Mediterranean Sea until very recently. Malara et al. (2021) reported for the first time shark depredation in the Mediterranean Sea on the swordfish harpoon fishery. The shark species reported in the study that potentially depredated on swordfish were the blue shark (*Prionace glauca*), the shortfin mako (*I. oxyrinchus*) and great white shark (*Carcharodon carcharias*) (Malara et al., 2021). The blue shark, the shortfin mako and the bigeye thresher (*Alopias superciliosus*) are the most commonly bycaught shark species in the Cypriot pelagic longline fishery (Department of Fisheries and Marine Research 2016 - 2021). The very limited published information on squid depredation mentioned the depredation by the colossal squid (*Mesonychoteuthis hamiltoni*) on longlines targeting the Antarctic toothfish in the Southern Ocean (Remeslo et al., 2015) and by the purpleback flying squid (*Sthenoteuthis oualaniensis*) on gillnets targeting tuna in the Northern Arabian Sea (Moazzam, 2019). However, squid depredation has never been reported before in the Mediterranean fisheries.

4.3 Interviews: Depredating Species, Bycatch and Potential Ecological Effects

The results from the interviews showed that the species causing depredation on the albacore tuna were the *T. truncatus*, followed by *S. coeruleoalba*, *O. bartrami, I. oxyrinchus and G. griseus*. The results from the interviews confirmed the findings from the logbooks and onboard observations. The interviews also revealed another species that is involved in depredation for which little is known about as it appears scarcer in the Levantine basin, the Risso's dolphin (Boisseau et al., 2017). Even the though the depredation by this species is considered less frequent and rare as described by some fishers, there are a few known areas that this species is known to inhabit of which the fishers of the area know and try avoiding fishing there. The depredation by the neon flying squid is becoming a great concern for longline fishers as this species depredates on bait and on the albacore tuna as well as on swordfish (*Xiphias gladius*). However, the species is edible, not prohibited to catch and sell, and according to fishers, taste-wise it is highly valued. This could be an opportunity for fishers to target the species in the future and support their income, in case of available market niche.

The results also showed the high probability of dolphin bycatch during depredation on both bait and catch. Fishers reported that in most cases the bycaught dolphins were juveniles that often drowned. The entanglement and hooking of cetaceans on longlines have been previously reported as an occasional event often leading to serious injuries and mortality (e.g. Forney and Kobayashi, 2007; Garrison, 2007). Even if the fishers cut the branchline to release the bycaught dolphin, it is very likely that the hook and the line will be ingested, injure vital organs and hence, the animal will die as a result (Wells et al., 2008). Considering that fishers have reported a decrease in tuna and swordfish stocks and significant damage on gear, catch and bait from depredation, it is very likely that they resort to extreme measures, using harmful methods to prevent depredation and sustain their operational costs. Practices to prevent depredation, such as direct shooting or using explosives have been reported previously in the literature (e.g. Northridge and Hofman, 1999; Gilman et al., 2006; Zollett and Read, 2006). This is a major concern for cetacean conservation because mortality and injury resulting from those actions may have significant consequences on a population level, especially on small, isolated populations associated, with islands such as Cyprus. However, there are no official records of cetacean harassment in Cyprus.

Although both dolphin species are responsible for depredation, it is unclear from the data which species has the greater impact. Fishers participated in the study reported that depredation by the common bottlenose dolphin is more frequent than the striped dolphin, however, they mentioned that in many cases have observed both species in the same group. Therefore, it is particularly important to understand the precise depredation rate by each species as well as their population dynamics. This will require intense sampling of direct observations by trained and experienced observers.

4.4 Possible Mitigation Strategies

The study also aimed to understand fishing practices and strategies that are employed by fishers which seek to minimize interactions, depredation and bycatch of the odontocetes with the pelagic longline fishery. It was possible to identify several applicable mitigation measures that if applied alone or in combination could potentially decrease longline-dolphin interactions. However, cetaceans are well known for their adaptive capabilities and a single, universal solution is unlikely to exist.

The 'move-on' rule is another practical method to avoid longline-dolphin interactions that is applied among fishers and require collaborative behaviour on their part. When a triggered event occurs between dolphins and fisheries, all fishers of the area are informed and are expected to avoid this specific fishing ground for a specific period (Gilman et al., 2006; Dunn et al., 2014). This method requires good communication and coordination by the fishing industry and fishery associations (Gilman et al., 2006). The method is already practised by fishers in Cyprus as they retain good collaboration among them but with low effectiveness on avoiding dolphin interactions. This is mainly because the tuna fishing grounds are restricted to specific areas and the options for the tuna longline fishery are limited. Additionally, when a pod of dolphins identifies a vessel or a fishing activity it can follow the vessel for miles away.

Moreover, the cause of acoustic discomfort to dolphins with the use of acoustic deterrent devices is a promising mitigation technique, although scientific evidence of their effectiveness is contradicting (Northridge et al., 2003; Brotons et al., 2008; Gazo et al., 2008; Buscaino et al., 2009) and habituation to the acoustic harassment device may occur (Tixier et al., 2015). The current study identified deterrent devices that are currently used by longline fishers in Cyprus and their effectiveness in mitigating depredation (**Table 2**). Although no scientific experiment was conducted in the current study to prove their effectiveness, the feedback from fishers is that only one type of high-intensity acoustic deterrent devices (DDDs/DiD from STM industrial Electronics) seem to be effective in mitigating interaction with the common bottlenose dolphin and striped dolphin, even after a long period of usage (more than two years). Other studies have tested the DDDs devices and showed positive results on cetacean bycatch and depredation (Buscaino et al., 2009; Northridge et al., 2011). Based on fishers' feedback, dolphin acoustic deterrent devices are considered for the moment as the only practical solution to mitigate interactions and depredation by dolphins. It is important to mention that in 2020 the government of the Republic of Cyprus announced a call for grant proposals for the purchase of repellent equipment (specifically dolphin anti-depredation pingers) for the protection of marine protected species. The percentage of co-funding to the beneficiaries (fishers) ranged from 30% to 80%, depending on the case and the remaining percentage was private participation (Thalassa 2014-2020, 2020). However, the potential ecological adverse effects of using high-intensity acoustic deterrent devices have not been assessed in the current study and the current knowledge on their short- and long-term effects is limited. It is likely that using such devices could alter the cetacean foraging strategies and distribution and in some cases damage hearing (Gilman et al., 2006).

Another alternative option, less invasive than deterrent devices, is the predictive forecasting; a method that identifies areas with fishing-dolphin conflicts to avoid these areas in the future through habitat modelling (Peterson and Carothers, 2013; Passadore et al., 2015). However, this method requires a high effort of data collection and analysis over a long period and the quality of such models is variable. In this study, a map (**Figure 11**) was developed to show areas of highly probable longline-dolphin interactions and the probability of dolphins' occurrence in these areas based on fishers' experience. This approach for data collection and analysis was low-cost and could be easily applied to other regions and fishing gears; these results could be used to inform fishers about conflict-prone regions and could also be applied for management measures, such as temporally spatial closures. It is also important to mention that areas identified as longline-dolphin conflict areas are also important fishing grounds with high catchability rates making any management recommendations of spatial closures difficult. It is strongly suggested that any decisions taken by policy-makers should ideally result in following consultations with stakeholders, in this case, the fishers.

4.5 Cetaceans' Foraging Strategy and the 'Dinner-Bell' Effect

The current study showed that feeding on the albacore tuna caught on pelagic longlines represents an alternative foraging strategy for dolphins. The albacore tuna is not included in the diet neither of the common bottlenose dolphin (Blanco et al., 2001; Nowacek, 2002; Bearzi et al., 2005; Díaz López, 2006; Bearzi et al., 2009; Pardalou and Tsikliras, 2020) nor of the striped dolphin (Würtz and Marrale, 1993; Blanco et al., 1995; Spitz et al., 2006; Perrin et al., 2008; Matsuda et al., 2020), however, both species are known to exhibit high dietary plasticity. Other studies from the Mediterranean Sea and around the world have shown similar foraging strategies between cetaceans and static nets (Lauriano, 2004; Díaz López, 2006; Brotons et al., 2008; Bearzi et al., 2011; Maccarrone et al., 2014; Snape et al., 2018), trawling (Zollett and Read, 2006) and longlines (Dalla Rosa and Secchi, 2007; Ramos-Cartelle and Mejuto, 2008; Rabearisoa et al., 2015). Such findings support the optimal foraging theory where an animal makes foraging decisions according to prey type and availability and based on its individual fitness benefits with the aim to increase the net energy intake per unit of time spent foraging (MacArthur and Pianka, 1996). Considering that the literature on the diet of the common bottlenose dolphin and the striped dolphin the albacore tuna is not included; depredation as a foraging strategy intends to increase foraging efficiency while decreasing foraging energy costs.

All fishers interviewed in the study strongly believed that dolphins were able to identify their fishing ground by hearing the engine sound and even follow their vessels from hours to days. Fishers who had previously used low-intensity acoustic deterrent devices (from Fishtek Marine) reported that they were effective only at the beginning while after some time of usage they became ineffective which even acted as a signal for food for dolphins that were previously exposed and had learned the sound. Similar findings were reported by Pardalou and Tsikliras (2018). The behaviour of dolphins to relate acoustic signals from the vessel engines and acoustic deterrent devices to the presence of prey is clear evidence of the 'dinner-bell' effect (e.g., Visser, 2000; Cox et al., 2004; Carretta and Barlow, 2011; Wargo Rub and Sandford, 2020).

The gradual increase of depredation incidents could be the result of foraging decisions and due to cetaceans advanced learning abilities and knowledge transfer within populations (Whitehead et al., 2004; Pennino et al., 2013). However, depredation incidents may have also become more frequent due to the increased fishing effort as a result of the increasing demand for seafood worldwide (FAO, 2020). The increased incidents of cetacean depredation have also motivated researchers worldwide to study cetacean depredation and since the 2000s, the number of studies published on the topic has significantly increased (Hamer et al., 2012).

4.6 Study Limitations

The main limitation of the study is the data collection methodology. Certainly, logbooks cannot replace the quality and accuracy of the data that can be collected from onboard observations by experienced researchers. To achieve optimal coverage of onboard observations and collect sufficient data that are representative of the entire fleet, it is often very expensive, especially when fishers require compensation for their services. On the other hand, self-reporting (logbooks) and interviews are relatively inexpensive, but the data gathered can be inaccurate and biased. However, this approach is widely used and has proven to be an important tool of research if its adequately implemented and with clear protocols for the surveys (e.g. Azzurro et al., 2011; Azzurro et al., 2019; Lopes et al., 2019). To limit the factors that could lead to inaccurate and biased data, the participants in our study were carefully selected and under certain criteria (see Methods section). In addition, the fishers who participated in the self-reporting were previously actively participating and collaborating through the actions of the Cyprus Bycatch Project (2018 – 2022) and other projects (in previous years) and this was also an important criterion during the selection process. The existing trust and respect that was built between the authors of this study and the selected fishers also played a significant role in the quality of the data recorded in logbooks. We rule out any bias in the data arising from the professional relationship with the interviewees (i.e., reporting what is expected to conform to the norm); the onboard surveys confirmed the veracity of the data. This was also another reason why only four out of the 30 licensed vessels/fishers were selected for the self-reporting and anonymity was assured. Definitely, the more the participants the better the extrapolations and representation of the fleet. Nevertheless, the selected participants are among the most active pelagic longline fishers in Cyprus.

Even though the identification of the different depredators from the fish carcasses is in most cases obvious as shown in **Figure 1**, there might be cases which are not. For this reason, a possible misidentification might occur when fishers reported dolphin depredation in their logbooks. To limit this bias, fishers were asked to send photographic evidence of the depredated tunas when possible.

Another limiting factor is that the studies undertaken to assess the economic costs from cetacean depredation on pelagic longlines are very limited and for the Mediterranean do not exist yet. In addition to that, the lack of a standardized methodology that assesses depredation rate and costs is preventing any direct comparison with other studies.

5 CONCLUSION

The combination of the different data collection methodologies makes the study particularly dynamic and have strengthen the efforts made to understand the issue of depredation. In data deficient research topics and difficult-to-reach populations, it is important and efficient to combine a variety of data collection methods to address an issue. The results from the logbooks and interviews as well as personal communication from the leading author with fishers showed that depredation by the common bottlenose dolphin and the striped dolphin on pelagic longlines during the albacore tuna longline fishery is a frequent event in Cyprus. The findings of this study show for the first time the detrimental effects of species depredation on longline fisheries in Cyprus and vice versa, mainly from the cetaceans but also from other taxa including a shark and a cephalopod species. Considering the increasing competition between cetaceans and fisheries, mitigation of this problem should become a high priority by researchers and authorities whilst more effort should be placed by government bodies to support fishers and their fishing operations to mitigate interactions with dolphins, minimize economic loss and avoidance of dolphin bycatch on longlines. Alternative fishing methods and acoustic deterrent devices could support this action. The results could be used as a reference for future work on the taxa causing depredation on the catch to help the correct calculation of the damage caused from different depredating taxa and apply the suitable mitigation measures.

Beyond the economic consequences from depredation and bycatch, the evaluation methodology for depredation and the results from the current study can have important implications on the conservation and management of the albacore tuna stocks as losses due to depredation are not counted for in quota allocation processes and fish stock assessments. The results and the method developed here could also support the development of a standardised data collection methodology and methods for depredation assessment and quantification. Additionally, the data presented in this study could support national management action plans and set the foundations for future research on depredation and bycatch mitigation practices as well as to develop fishery-specific assessments on species interactions.

The empirical knowledge from longline fishers to reduce cetacean-longline interactions as well as to address other future industry problems should always be taken into consideration. Fishers have considerable ecological and empirical knowledge (i.e., traditional knowledge) that could significantly contribute to any scientific experiment and the development of potential management actions. We are certain that fishers should be active participants in fisheries research and collaboratively participate in the development of any management activities and best fishing practices to ensure the long-term effectiveness of these actions. However, this approach should never, in any case, replace scientific research by experienced researchers in the field but rather be used as a tool that supplements fisheries research and to build trust, cooperation and a sense of responsibility among fishery stakeholders.

Depredation from cetaceans is an evolving practice that seems to change from time to time. Research that aims to understand and quantify depredation must be continued. Future research that will aim to understand and quantify bait depredation and its associated costs as well as other indirect costs including gear loss, fuel, salaries, etc. is especially important. Most importantly, future research should aim to mitigate this global issue by testing new mitigation measures and technologies.

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 study on human participants in accordance with the local legislation and institutional requirements. The patients/participants provided their written informed consent to participate in this study. Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

AUTHOR CONTRIBUTIONS

MP conceptualized, administrated, and designed the study, developed methodology, cured data, performed the statistical analysis and visualized results, and wrote the original draft. CJ and AP supervised and reviewed the manuscript and approved the submitted version. AG performed the maps. LH acquired project funding, reviewed the manuscript and approved the submitted version. 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.2022.868464/full#supplementary-material

REFERENCES

Azzurro, E., Moschella, P., and Maynou, F. (2011). Tracking Signals of Change in Mediterranean Fish Diversity Based on Local Ecological Knowledge. *PloS One* 6 (9), e24885. doi: 10.1371/journal.pone.0024885

Azzurro, E., Sbragaglia, V., Cerri, J., Bariche, M., Bolognini, L., Ben Souissi, J., et al. (2019). Climate Change, Biological Invasions, and the Shifting Distribution of Mediterranean Fishes: A Large-Scale Survey Based on Local Ecological Knowledge. *Global Change Biol.* 25, 2779– 2792. doi: 10.1111/gcb.14670

Barros, N. B., and Odell, D. K. (1990). “Food Habits of Bottlenose Dolphins in the Southeastern United States.” in *The Bottlenose Dolphin*. Eds. S. Leatherwood and R. R. Reeves (San Diego, CA: Academic Press), 309–328, 653pp.

Barros, N. B., Parsons, E. C. M., and Jefferson, T. A. (2000). Prey of Offshore Bottlenose Dolphins From the South China Sea. *Aquat. Mamm.* 26 (1), 2–6.

Bearzi, G. (2002). “Interactions Between Cetacean and Fisheries in the Mediterranean Sea.” in *Cetaceans of the Mediterranean and Black Seas: State of Knowledge and Conservation Strategies*. Ed. G. Notarbartolo Di Sciara (Monaco: A report to the ACCOBAMS Secretariat), 20 pages.

Bearzi, G., Bonizzoni, S., and Gonzalvo, J. (2011). Dolphins and Coastal Fisheries Within a Marine Protected Area: Mismatch Between Dolphin Occurrence and Reported Depredation. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 21 (3), 261–267. doi: 10.1002/aqc.1179

Bearzi, G., Fortuna, C., and Reeves, R. (2009). Ecology and Conservation of Common Bottlenose Dolphins Tursiops Truncatus in the Mediterranean Sea. *Mamm. Rev.* 39 (2), 92. doi: 10.1111/j.1365-2907.2008.00133.x

Bearzi, G., Holcer, D., and Notarbartolo di Sciara, G. (2004). The Role of Historical Dolphin Takes and Habitat Degradation in Shaping the Present Status of Northern Adriatic Cetaceans. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 14 (4), 363. doi: 10.1002/aqc.626

Bearzi, G., Politi, E., Agazzi, S., and Azzellino, A. (2006). Prey Depletion Caused by Overfishing and the Decline of Marine Megafauna in Eastern Ionian Sea Coastal Waters (Central Mediterranean). *Biol. Conserv.* 127 (4), 373–382. doi: 10.1016/j.biocon.2005.08.017

Bearzi, G., Politi, E., Agazzi, S., Bruno, S., Costa, M., and Bonizzoni, S. (2005). Occurrence and Present Status of Coastal Dolphins (Delphinus Delphis and Tursiops Truncatus) in the Eastern Ionian Sea. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 15 (3), 243–257. doi: 10.1002/aqc.667

Berg, B. L. (2004). “Methods for the Social Sciences,” in *Qualitative Research Methods for the Social Sciences* (Boston: Pearson Education).

Blanco, C., Aznar, J., and Raga, J. A. (1995). Cephalopods in the Diet of the Striped Dolphin Stenella Coeruleoalba From the Western Mediterranean During an Epizootic in 1990. *J. Zool.* 237 (1), 151–158. doi: 10.1111/j.1469-7998.1995.tb02753.x

Blanco, C., Salomón, O., and Raga, J. A. (2001). Diet of the Bottlenose Dolphin (Tursiops Truncatus) in the Western Mediterranean Sea. *Mar. Biol. Assoc. Unit. Kingd.* 81 (6), 1053. doi: 10.1017/S0025315401005057

Blasi, M. F., Giuliani, A., and Boitani, L. (2015). Influence of Trammel Nets on the Behaviour and Spatial Distribution of Bottlenose Dolphins (Tursiops Truncatus) in the Aeolian Archipelago, Southern Italy. *Aquat. Mamm.* 41 (3), 295–310. doi: 10.1578/AM.41.3.2015.295

Boisseau, O., Frantzis, A., Petrou, A., Van Geel, N., McLanaghan, R., Alexiadou, P., et al. (2017). “Visual and Passive Acoustic Survey Report,” in *Report Submitted to the Department of Fisheries and Marine Research by the AP Marine Environmental Consultancy Consortium*, 49. Nicosia: AP Marine Environmental Consultancy.

Braga, H. D. O., and Schiavetti, A. (2013). Attitudes and Local Ecological Knowledge of Experts Fishermen in Relation to Conservation and Bycatch of Sea Turtles (Reptilia: Testudines), Southern Bahia, Brazil. *J. Ethnobiol. Ethnomed.* 9 (1), 1–13. doi: 10.1186/1746-4269-9-15

Brotons, J. M., Grau, A. M., and Rendell, L. (2008). Estimating the Impact of Interactions Between Bottlenose Dolphins and Artisanal Fisheries Around the Balearic Islands. *Mar. Mamm. Sci.* 24 (1), 112–127. doi: 10.1111/j.1748-7692.2007.00164.x

Bruno, C. A., Caserta, V., Salzeri, P., Ferraro, G. B., Pecoraro, F., Lucchetti, A., et al. (2021). Acoustic Deterrent Devices as Mitigation Tool to Prevent Dolphin-Fishery Interactions in the Aeolian Archipelago (Southern Tyrrhenian Sea, Italy). *Mediter. Mar. Sci.* 22 (2), 408–421. doi: 10.12681/mms.23129

Buscaino, G., Buffa, G., Sara, G., Bellante, A., Tonello, A. J., Hardt, F. A. S., et al. (2009). Pinger Affects Fish Catch Efficiency and Damage to Bottom Gill Nets Related to Bottlenose Dolphins. *Fish. Sci.* 75 (3), 537–544. doi: 10.1007/s12562-009-0059-3

Carretta, J. V., and Barlow, J. (2011). Long-Term Effectiveness, Failure Rates, and “Dinner Bell” Properties of Acoustic Pingers in a Gillnet Fishery. *Mar. Technol. Soc. J.* 45 (5), 7–19. doi: 10.4031/MTSJ.45.5.3

Chilvers, L. B., and Corkeron, P. J. (2001). Trawling and Bottlenose Dolphins' Social Structure. *Proc. R. Soc. Lond. Ser. B.: Biol. Sci.* 268 (1479), 1901–1905. doi: 10.1098/rspb.2001.1732

Connor, R. C., Wells, R. S., Mann, J., and Read, A. J. (2000). “The Bottlenose Dolphin: Social Relationships in a Fission-Fusion Society.” in *Cetacean Societies: Field Studies of Dolphins and Whales*. Eds. J. Mann, R. C. Connor, P. L. Tyack and H. Whitehead (Chicago, IL: Chicago University Press), 91–126.

Cox, T. M., Read, A. J., Swanner, D., Urian, K., and Waples, D. (2004). Behavioral Responses of Bottlenose Dolphins, Tursiops Truncatus, to Gillnets and Acoustic Alarms. *Biol. Conserv.* 115 (2), 203–212. doi: 10.1016/S0006-3207(03)00108-3

Crosti, R., Arcangeli, A., Romeo, T., and Andaloro, F. (2017). Assessing the Relationship Between Cetacean Strandings (Tursiops Truncatus and Stenella Coeruleoalba) and Fishery Pressure Indicators in Sicily (Mediterranean Sea) Within the Framework of the EU Habitats Directive. *Eur. J. Wildl. Res.* 63 (3), 1–13. doi: 10.1007/s10344-017-1111-8

Dalla Rosa, L., and Secchi, E. R. (2007). Killer Whale (Orcinus Orca) Interactions With the Tuna and Swordfish Longline Fishery Off Southern and South-Eastern Brazil: A Comparison With Shark Interactions. *J. Mar. Biol. Assoc. Unit. Kingd.* 87 (1), 135–140. doi: 10.1017/S0025315407054306

Department of Fisheries and Marine Research (2016 - 2021). *Cyprus Annual Report for Data Collection in the Fisheries and Aquaculture Sectors* (Nicosia: Ministry of Agriculture, Rural Development and Natural Resources). Available at: https://datacollection.jrc.ec.europa.eu/ars.

Department of Fisheries and Marine Research (2019). *Annual Report 2018* (Nicosia: Department of Fisheries and Marine Research).

Díaz López, B. (2006). Interactions Between Mediterranean Bottlenose Dolphins (Tursiops Truncatus) and Gillnets Off Sardinia, Italy. *ICES J. Mar. Sci.* 63 (5), 946–951. doi: 10.1016/j.icesjms.2005.06.012

Di Natale, A., and Notarbartolo di Sciara, G. (1994). A Review of the Passive Fishing Nets and Trap Fisheries in the Mediterranean Sea and of Cetacean Bycatch. *Rep. Int. Whal. Comm.* Special Issue 15), 189–202.

Dmitrieva, L., Kondakov, A. A., Oleynikov, E., Kydyrmanov, A., Karamendin, K., Kasimbekov, Y., et al. (2013). Assessment of Caspian Seal by-Catch in an Illegal Fishery Using an Interview-Based Approach. *PloS One* 8 (6), e67074. doi: 10.1371/journal.pone.0067074

Dunn, D. C., Boustany, A. M., Roberts, J. J., Brazer, E., Sanderson, M., Gardner, B., et al. (2014). Empirical Move-on Rules to Inform Fishing Strategies: A N Ew E Ngland Case Study. *Fish. Fish.* 15 (3), 359–375. doi: 10.1111/faf.12019

Esri Inc (2013). *ArcGIS Desktop (Version 10.1)* (Esri Inc). Available at: https://www.esri.com/en-us/arcgis/products/arcgis-desktop/overview.

FAO (2019a). “Monitoring Discards in Mediterranean and Black Sea Fisheries: Methodology for Data Collection,” in *FAO Fisheries and Aquaculture Technical Paper No. 639* (Rome: FAO).

FAO (2019b). “Monitoring the Incidental Catch of Vulnerable Species in Mediterranean and Black Sea Fisheries: Methodology for Data Collection,” in *FAO Fisheries and Aquaculture Technical Paper No. 640* (Rome: FAO).

FAO (2020). *The State of World Fisheries and Aquaculture 2020. Sustainability in Action* (Rome: FAO). doi: 10.4060/ca9229en

Forney, K. A., and Kobayashi, D. R. (2007) Updated Estimates of Mortality and Injury of Cetaceans in the Hawaii-Based Longline Fishery, 1994-2005*, Report, November 2007; United States* (University of North Texas Libraries: Libraries Government Documents Department). Available at: https://digital.library.unt.edu/ark:/67531/metadc1050058https://digital.library.unt.edu (Accessed July 5, 2021)).

Forney, K. A., Kobayashi, D. R., Johnston, D. W., Marchetti, J. A., and Marsik, M. G. (2011). What’s the Catch? Patterns of Cetacean Bycatch and Depredation in Hawaii-Based Pelagic Longline Fisheries. *Mar. Ecol.* 32 (3), 380–391. doi: 10.1111/j.1439-0485.2011.00454.x

Froese, R., Winker, H., Coro, G., Demirel, N., Tsikliras, A. C., Dimarchopoulou, D., et al. (2018). Status and Rebuilding of European Fisheries. *Mar. Policy* 93, 159–170. doi: 10.1016/j.marpol.2018.04.018

Garrison, L. P. (2007). Interactions Between Marine Mammals and Pelagic Longline Fishing Gear in the US Atlantic Ocean Between 1992 and 2004. *Fish. Bull.* 105 (3), 408–417. doi: hdl.handle.net/1834/25527

Gazo, M., Gonzalvo, J., and Aguilar, A. (2008). Pingers as Deterrents of Bottlenose Dolphins Interacting With Trammel Nets. *Fish. Res.* 92 (1), 70–75. doi: 10.1016/j.fishres.2007.12.016

Giannakis, E., Hadjioannou, L., Jimenez, C., Papageorgiou, M., Karonias, A., and Petrou, A. (2020). Economic Consequences of Coronavirus Disease (COVID-19) on Fisheries in the Eastern Mediterranean (Cyprus). *Sustainability* 12 (22), p.9406. doi: 10.3390/su12229406

Gilman, E., Brothers, N., McPherson, G., and Dalzell, P. (2006). A Review of Cetacean Interactions With Longline Gear. *J. Cetac. Res. Manage.* 8 (2), 215.

Gilman, E., Clarke, S., Brothers, N., Alfaro-Shigueto, J., Mandelman, J., Mangel, J., et al. (2007). “Shark Depredation and Unwanted Bycatch in Pelagic Longline

Fisheries: Industry Practices and Attitudes, and Shark Avoidance Strategies," Honolulu: Western Pacific Regional Fishery Management Council.

Gilman, E., Clarke, S., Brothers, N., Alfaro-Shigueto, J., Mandelman, J., Mangel, J., et al. (2008). Shark Interactions in Pelagic Longline Fisheries. *Mar. Policy* 32 (1), 1–18. doi: 10.1016/j.marpol.2007.05.001

Gonzalvo, J., Giovos, I., and Moutopoulos, D. K. (2015). Fishermen's Perception on the Sustainability of Small-Scale Fisheries and Dolphin–Fisheries Interactions in Two Increasingly Fragile Coastal Ecosystems in Western Greece. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 25 (1), 91–106. doi: 10.1002/aqc.2444

Goodman, L. A. (1961). Snowball Sampling. *Ann. Math. Stat*, 32 (1), 148–170. doi: 10.1214/aoms/1177705148

Guinet, C., Tixier, P., Gasco, N., and Duhamel, G. (2015). Long-Term Studies of Crozet Island Killer Whales are Fundamental to Understanding the Economic and Demographic Consequences of Their Depredation Behaviour on the Patagonian Toothfish Fishery. *ICES J. Mar. Sci.* 72 (5), 1587–1597. doi: 10.1093/icesjms/fsu221

Hall, M. A., and Donovan, G. P. (2002). Environmentalists, Fishermen, Cetaceans and Fish: Is There a Balance and Can Science Help to Find It? *Mar. Mamm.*, 491–521 (Boston, MA: Springer). doi: 10.1007/978-1-4615-0529-7_14

Hamer, D. J., Childerhouse, S. J., and Gales, N. J. (2012). Odontocete Bycatch and Depredation in Longline Fisheries: A Review of Available Literature and of Potential Solutions. *Mar. Mamm. Sci.* 28 (4), E345–E374. doi: 10.1111/j.1748-7692.2011.00544.x

Harwood, J. (1992). Assessing the Competitive Effects of Marine Mammal Predation on Commercial Fisheries. *South Afr. J. Mar. Sci.* 12 (1), 689–693. doi: 10.2989/02577619209504733

Hernandez-Milian, G., Goetz, S., Varela-Dopico, C., Rodriguez-Gutierrez, J., Romón-Olea, J., Fuertes-Gamundi, J. R., et al. (2008). "Results of a Short Study of Interactions of Cetaceans and Longline Fisheries in Atlantic Waters: Environmental Correlates of Catches and Depredation Events," in *Essential Fish Habitat Mapping in the Mediterranean* (Dordrecht: Springer), pp. 251–268. doi: 10.1007/978-1-4020-9141-4_19

Kvale, S., and Brinkmann, S. (2009). *Interviews: Learning the Craft of Qualitative Research Interviewing* (Thousand Oaks, CA: Sage Publications).

Lampe, O. D., and Hauser, H. (2011). "Interactive Visualization of Streaming Data With Kernel Density Estimation," in *2011 IEEE Pacific Visualization Symposium* (IEEE: Hong Kong), (pp. 171–178). doi: 10.1109/PACIFICVIS.2011.5742387

Lauriano, G. (2004). Interactions Between Bottlenose Dolphins (Tursiops Truncatus) and the Artisanal Fishery in Asinara Island National Park (Sardinia): Assessment of Catch Damage and Economic Loss. *J. Cetac. Res. Manage.* 6 (2), 165–173.

Li, C., Baciu, G., and Han, Y. (2014). "Interactive Visualization of High Density Streaming Points With Heat-Map," in *2014 International Conference on Smart Computing* (IEEE: Hong Kong), (pp. 145–149). doi: 10.1109/SMARTCOMP.2014.7043852

Lopes, P. F., Verba, J. T., Begossi, A., and Pennino, M. G. (2019). Predicting Species Distribution From Fishers' Local Ecological Knowledge: A New Alternative for Data-Poor Management. *Can. J. Fish. Aquat. Sci.* 76 (8), 1423–1431. doi: 10.1139/cjfas-2018-0148

MacArthur, R. H., and Pianka, E. R. (1966). On Optimal Use of a Patchy Environment. *Am. Nat.* 100 (916), 603–609. doi: 10.1086/282454

Maccarrone, V., Buffa, G., Di Stefano, V., Filiciotto, F., Mazzola, S., and Buscaino, G. (2014). Economic Assessment of Dolphin Depredation Damages and Pinger Use in Artisanal Fisheries in the Archipelago of Egadi Islands (Sicily). *Turk. J. Fish. Aquat. Sci.* 14 (1), 173–181. doi: 10.4194/1303-2712-v14_1_19

Magnaghi, L., and Podestà, M. (1987). "An Accidental Catch of 8 Striped Dolphins, Stenella Coeruleoalba (Meyen 1833), in the Ligurian Sea (Cetacea Delphinidae)," in *Atti Società Italiana Di Scienze Naturali, Museo Civico Di Soria Naturale, Milano*, vol. 128, 235–239.

Malara, D., Battaglia, P., Consoli, P., Arcadi, E., Longo, F., Stipa, M. G., et al. (2021). When Opportunistic Predators Interact With Swordfish Harpoon Fishing Activities: Shark Depredation Over Catches in the Strait of Messina (Central Mediterranean Sea). *Eur. Zool. J.* 88 (1), 226–236. doi: 10.1080/24750263.2021.1879284

Mandelman, J. W., Cooper, P. W., Werner, T. B., and Lagueux, K. M. (2008). Shark Bycatch and Depredation in the US Atlantic Pelagic Longline Fishery. *Rev. Fish. Biol. Fish.* 18 (4), 427–442. doi: 10.1007/s11160-008-9084-z

Matsuda, A., Yamada, T. K., Tajima, Y., Kunisue, T., Amano, M., and Matsuishi, T. F. (2020). Diet of Mass-Stranded Striped Dolphins (Stenella Coeruleoalba) in Southern Japan (East China Sea). *Mamm. Stud.* 46 (1), 1–8. doi: 10.3106/ms2020-0005

Ministry of Agriculture, Rural Development and the Environment and Department of Fisheries and Marine Research (2020). "ΠΡΟΤΕΡΑΙΟΤΗΤΑ ΤΗΣ ΕΝΩΣΗΣ 1," in *Προώθηση Της ΠϵριΒαλλοντικά Βιώσιμης, Αποδοτικής Ως Προς Τους Πόρους, Καινοτόμου, Ανταγωνιστικής Και Βασιζόμϵνης Στη Γνώση Αλιϵίας. Μέτρο 1.19: Προστασία Και Αποκατάσταση Της Θαλάσσιας Βιοποικιλότητας - Συστήματα Για Την Αποζημίωση Των Ζημιών Που Προκαλούνται Στα Αλιϵύματα Από Προστατϵυόμϵνα Θηλαστικά Και Πτηνά – ΑΡΘΡΟ 40.1Η «Σχέδιο Για Την Αποζημίωση Των Ζημιών Που Προκαλούνται Στα Αλιϵύματα Από Προστατϵυόμϵνα Θηλαστικά»* (Nicosia: Department of Fisheries and Marine Research).

Moazzam, M. (2019). "Depredation of Purpleback Flying Squid (Sthenoteuthis Oualaniensis) on Tuna Caught by Gillnet Fisheries in the Northern Arabian Sea: A Major Cause of Concern for Fishermen," in *IOTC Working Party on Ecosystem and Bycatch*, vol. 3-7. (France September: WPEB15 La Reunion), 11p. 2019. IOTC-2019-WPEB15-13.

Morissette, L., Christensen, V., and Pauly, D. (2012). Marine Mammal Impacts in Exploited Ecosystems: Would Large Scale Culling Benefit Fisheries? *PloS One* 7 (9), e43966. doi: 10.1371/journal.pone.0043966

Northridge, S., and Hofman, R. J. (1999). "Marine Mammal-Fishery Interactions," in *Conservation and Management of Marine Mammals*. Eds. J. R. Twiss and R. R. Reeves (Washington, DC: Smithsonian Institution Press), 99–119.

Northridge, S., Kingston, A., Mackay, A., and Lonergan, M. (2011). "Bycatch of Vulnerable Species: Understanding the Process and Mitigating the Impacts," in *Final Report to Defra Marine and Fisheries Science Unit, Project No MF1003* (Defra, London: University of St Andrews), 99pp.

Northridge, S., Vernicos, D., and Raitsos-Exarchopolous, D. (2003). *Net Depredation by Bottlenose Dolphins in the Aegean: First Attempts to Quantify and to Minimise the Problem. IWC Sc/55/Sm25* (Cambridge: International Whaling Commission).

Northridge, S., Waples, D., and Read, A. J. (2013). To Ping or Not to Ping: The Use of Active Acoustic Devices in Mitigating Interactions Between Small Cetaceans and Gillnet Fisheries. *Endanger. Spe. Res.* 19 (3), pp.201–pp.221. doi: 10.3354/esr00464

Nowacek, D. (2002). "Sequential Foraging Behaviour of Bottlenose Dolphins, Tursiops Truncatus,", vol. 139. (Sarasota Bay: FL. Behaviour) 139 (9), pp.1125–1145. doi: 10.1163/15685390260437290

Papageorgiou, M., Papadopoulou, A., and Hadjioannou, L. (2020). "Cyprus Bycatch Project "Understanding Multi-Taxa 'Bycatch' of Vulnerable Species and Testing Mitigation a Collaborative Approach in Cyprus"," in *Technical Report: Results of Phase 1 (2018-20019) of the Bycatch Monitoring Programme in Cyprus* (Nicosia: BirdLife Cyprus and Enalia Physis Environmental Research Centre), Pp32.

Pardalou, A., and Tsikliras, A. C. (2018). Anecdotal Information on Dolphin-Fisheries Interactions Based on Empirical Knowledge of Fishers in the Northeastern Mediterranean Sea. *Ethic. Sci. Environ. Polit.* 18, pp.1–pp.8. doi: 10.3354/esep00179

Pardalou, A., and Tsikliras, A. C. (2020). Factors Influencing Dolphin Depredation in Coastal Fisheries of the Northern Aegean Sea: Implications on Defining Mitigation Measures. *Mar. Mamm. Sci.* 36 (4), 1126–1149. doi: 10.1111/mms.12702

Passadore, C., Domingo, A., and Secchi, E. R. (2015). Analysis of Marine Mammal Bycatch in the Uruguayan Pelagic Longline Fishery Operating in the Southwestern Atlantic Ocean. *ICES J. Mar. Sci.* 72 (5), 1637–1652. doi: 10.1093/icesjms/fsu250

Pennino, M. G., Mendoza, M., Pira, A., Floris, A., and Rotta, A. (2013). Assessing Foraging Tradition in Wild Bottlenose Dolphins (Tursiops Truncatus). *Aquat. Mamm.* 39 (3), 282. doi: 10.1578/AM.39.3.2013.282

Pennino, M. G., Rotta, A., Pierce, G. J., and Bellido, J. M. (2015). Interaction Between Bottlenose Dolphin (Tursiops Truncatus) and Trammel Nets in the Archipelago De La Maddalena, Italy. *Hydrobiologia* 747 (1), 69–82. doi: 10.1007/s10750-014-2127-7

Perrin, W. F., Robertson, K. M., and Walker, W. A. (2008). “Diet of the Striped Dolphin, Stenella Coeruleoalba,” in *The Eastern Tropical Pacific Ocean* (Agencies and Staff of the U.S. Department of Commerce). Available at: http://digitalcommons.unl.edu/usdeptcommercepub/23.

Peterson, M. J., and Carothers, C. (2013). Whale Interactions With Alaskan Sablefish and Pacific Halibut Fisheries: Surveying Fishermen Perception, Changing Fishing Practices and Mitigation. *Mar. Policy* 42, 315–324. doi: 10.1016/j.marpol.2013.04.001

Peterson, M.J., Mueter, F., Criddle, K., and Haynie, A.C. (2014). Killer Whale Depredation and Associated Costs to Alaskan Sablefish, Pacific Halibut and Greenland Turbot Longliners. *PLoS One* 9(2), p.e88906. doi: org/10.1371/journal.pone.0088906

Purves, M. G., Agnew, D. J., Balguerias, E., Moreno, C. A., and Watkins, B. (2004). Killer Whale (Orcinus Orca) and Sperm Whale (Physeter Macrocephalus) Interactions With Longline Vessels in the Patagonian Toothfish Fishery at South Georgia, South Atlantic. *CCAMLR. Sci.* 11, 111–26.

Rabearisoa, N., Bach, P., and Marsac, F. (2015). Assessing Interactions Between Dolphins and Small Pelagic Fish on Branchline to Design a Depredation Mitigation Device in Pelagic Longline Fisheries. *ICES J. Mar. Sci.* 72 (5), 1682–1690. doi: 10.1093/icesjms/fsu252

Rabearisoa, N., Sabarros, P. S., Romanov, E. V., Lucas, V., and Bach, P. (2018). Toothed Whale and Shark Depredation Indicators: A Case Study From the Reunion Island and Seychelles Pelagic Longline Fisheries. *PloS One* 13 (8), 1–26 doi: 10.1371/journal.pone.0202037

Ramos-Cartelle, A., and Mejuto, J. (2008). “Interaction of the False Killer Whale (Pseudorca Crassidens) and Depredation on the Swordfish Catches of the Spanish Surface Longline Fleet in the Atlantic, Indian and Pacific Oceans,” in *Report, International Commission for the Conservation of Atlantic Tunas (ICCAT), Collective Volume of Scientific Papers (SCRS/2007/025)*, vol. 62. , 1721–1783.

R Core Team (2020). “R: A Language and Environment for Statistical Computing,” (Vienna, Austria: R Foundation for Statistical Computing). Available at: https://www.R-project.org/.

Remeslo, A. V., Yakushev, M. R., and Laptikhovsky, V. (2015). Alien vs. Predator: Interactions Between the Colossal Squid (Mesonychoteuthis Hamiltoni) and the Antarctic Toothfish (Dissostichus Mawsoni). *J. Natural Histor.* 49 (41-42), pp.2483–2491. doi: 10.1080/00222933.2015.1040477

Roche, C., and Guinet, C. (2007). Marine Mammals and Demersal Longline Fishery Interactions in Crozet and Kerguelen Exclusive Economic Zones: An Assessment of Depredation Levels. *CCAMLR. Sci.* 14, pp.67–pp.82.

Rocklin, D., Santoni, M. C., Culioli, J. M., Tomasini, J. A., Pelletier, D., and Mouillot, D. (2009). Changes in the Catch Composition of Artisanal Fisheries Attributable to Dolphin Depredation in a Mediterranean Marine Reserve. *ICES J. Mar. Sci.* 66 (4), 699–707. doi: 10.1093/icesjms/fsp036

Rub, A. M. W., and Sandford, B. P. (2020). Evidence of a ‘Dinner Bell’effect From Acoustic Transmitters in Adult Chinook Salmon. *Mar. Ecol. Prog. Ser.* 641, pp.1–pp11. doi: 10.3354/meps13323

Secchi, E. R., and Vaske, T. J. (1998). Killer Whale (Orcinus Orca) Sightings and Depredation on Tuna and Swordfish Longline Catches in Southern Brazil. *Aquat. Mamm.* 24, 117–122.

Shane, S. H., Wells, R. S., and Würsig, B. (1986). Ecology, Behavior and Social Organization of the Bottlenose Dolphin: A Review. *Mar. Mamm. Sci.* 2 (1), 34–63. doi: 10.1111/j.1748-7692.1986.tb00026.x

Sivasubramaniam, K. (1964). Predation of Tuna Longline Catches in the Indian Ocean, by Killer-Whales and Sharks. *Bull. Fish. Res. Stat. Ceyl.* 17 (2), 221–236. doi: hdl.handle.net/1834/32494

Snape, R. T. E., Broderick, A. C., Çiçek, B. A., Fuller, W. J., Tregenza, N., Witt, M. J., et al. (2018). Conflict Between Dolphins and a Data-Scarce Fishery of the European Union. *Hum. Ecol.* 46 (3), 423–433. doi: 10.1007/s10745-018-9989-7

Spitz, J., Richard, E., Meynier, L., Pusineri, C., and Ridoux, V. (2006). Dietary Plasticity of the Oceanic Striped Dolphin, Stenella Coeruleoalba, in the Neritic Waters of the Bay of Biscay. *J. Sea. Res.* 55 (4), 309–320. doi: 10.1016/j.seares.2006.02.001

Thalassa 2014-2020 (2020) *Σχέδιο Χορηγιών Για Αγορά Απωθητικού Εξοπλισμού Για Προςταςία Των Θαλάςςιων Προςτατευόμϵνων Ειδών - 1Η Πρόςκληςη ΥποΒολής Προτάςϵων - 05/05/2020*. Available at: http://www.moa.gov.cy/moa/opf/opf2014.nsf/All/98ECCEEADB3C0335C225855F00513070?OpenDocument.

Tixier, P., Gasco, N., Duhamel, G., and Guinet, C. (2015). Habituation to an Acoustic Harassment Device (AHD) by Killer Whales Depredating Demersal Longlines. *ICES J. Mar. Sci.* 72 (5), 1673–1681. doi: 10.1093/icesjms/fsu166

Tixier, P., Gasco, N., Duhamel, G., Viviant, M., Authier, M., and Guinet, C. (2010). Interactions of Patagonian Toothfish Fisheries With Killer and Sperm Whales in the Crozet Islands Exclusive Economic Zone: An Assessment of Depredation Levels and Insights on Possible Mitigation Strategies. *CCAMLR. Sci.* 17, pp.179–pp.195.

Tixier, P., Lea, M. A., Hindell, M. A., Welsford, D., Mazé, C., Gourguet, S., et al. (2021). When Large Marine Predators Feed on Fisheries Catches: Global Patterns of the Depredation Conflict and Directions for Coexistence. *Fish. Fish.* 22 (1), pp.31–pp.53. doi: 10.1111/faf.12504

Vasilakopoulos, P., Maravelias, C. D., and Tserpes, G. (2014). The Alarming Decline of Mediterranean Fish Stocks. *Curr. Biol.* 24 (14), 1643–1648. doi: 10.1016/j.cub.2014.05.070

Visser, I. N. (2000). Killer Whale (Orcinus Orca) Interactions With Longline Fisheries in New Zealand Waters. *Aquat. Mamm.* 26 (3), 241–252.

Wells, R. S., Allen, J. B., Hofmann, S., Bassos-Hull, K., Fauquier, D. A., Barros, N. B., et al. (2008). Consequences of Injuries on Survival and Reproduction of Common Bottlenose Dolphins (Tursiops Truncatus) Along the West Coast of Florida. *Mar. Mamm. Sci.* 24 (4), 774–794. doi: 10.1111/j.1748-7692.2008.00212.x

Werner, T. B., Northridge, S., Press, K. M., and Young, N. (2015). Mitigating Bycatch and Depredation of Marine Mammals in Longline Fisheries. *ICES J. Mar. Sci.* 72 (5), 1576–1586. doi: 10.1093/icesjms/fsv092

Whitehead, H., Rendell, L., Osborne, R. W., and Würsig, B. (2004). Culture and Conservation of Non-Humans With Reference to Whales and Dolphins: Review and New Directions. *Biol. Conserv.* 120 (3), 427–437. doi: 10.1016/j.biocon.2004.03.017

Williams, A. J., Petersen, S. L., Goren, M., and Watkins, B. P. (2009). Sightings of Killer Whales Orcinus Orca From Longline Vessels in South African Waters, and Consideration of the Regional Conservation Status. *Afr. J. Mar. Sci.* 31 (1), 81–86. doi: 10.2989/AJMS.2009.31.1.7.778

Würtz, M., and Marrale, D. (1993). Food of Striped Dolphin, Stenella Coeruleoalba, in the Ligurian Sea. *J. Mar. Biol. Assoc. Unit. Kingd.* 73 (3), 571–578. doi: 10.1017/S0025315400033117

Yuan, K., Cheng, X., Gui, Z., Li, F., and Wu, H. (2019). A Quad-Tree-Based Fast and Adaptive Kernel Density Estimation Algorithm for Heat-Map Generation. *Int. J. Geograph. Inf. Sci.* 33 (12), 2455–2476. doi: 10.1080/13658816.2018.1555831

Zappes, C. A., Simões-Lopes, P. C., Andriolo, A., and Di Beneditto, A. P. M. (2016). Traditional Knowledge Identifies Causes of Bycatch on Bottlenose Dolphins (Tursiops Truncatus Montagu 1821): An Ethnobiological Approach. *Ocean. Coast. Manage.* 120, 160–169. doi: 10.1016/j.ocecoaman.2015.12.006

Zeller, D., and Pauly, D. (2005). Good News, Bad News: Global Fisheries Discards are Declining, But So are Total Catches. *Fish. Fish.* 6 (2), 156–159. doi: 10.1111/j.1467-2979.2005.00177.x

Zollett, E. A., and Read, A. J. (2006). Depredation of Catch by Bottlenose Dolphins (Tursiops Truncatus) in the Florida King Mackerel (Scomberomorus Cavalla) Troll Fishery. *Fish. Bull.* 104 (3), 343–349.

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Spatial Distribution and Encounter Rates of Delphinids and Deep Diving Cetaceans in the Eastern Mediterranean Sea of Turkey and the Extent of Overlap With Areas of Dense Marine Traffic

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Marine traffic has been identified as a serious threat to Mediterranean cetaceans with few mitigation strategies in place. With only limited research effort within the Eastern Basin, neither baseline species knowledge nor the magnitude of threats have been comprehensively assessed. Delineating the extent of overlap between marine traffic and cetaceans provides decision makers with important information to facilitate management. The current study employed the first seasonal boat surveys within the Eastern Mediterranean Sea of Turkey, incorporating visual and acoustic survey techniques between 2018 and 2020 to understand the spatial distribution of cetacean species. Additionally, marine traffic density data were retrieved to assess the overlap with marine traffic. Encounter rates of cetaceans and marine traffic density were recorded for each 100 km^2 cell within a grid. Subsequently, encounter and marine traffic density data were used to create a potential risk index to establish where the potential for marine traffic and cetacean overlap was high. Overall, eight surveys were undertaken with a survey coverage of 21,899 km^2 between the Rhodes and Antalya Basins. Deep diving cetaceans (sperm and beaked whales) were detected on 28 occasions, with 166 encounters of delphinids of which bottlenose, striped and common dolphins were visually confirmed. Spatially, delphinids were distributed throughout the survey area but encounter rates for both deep diving cetaceans and delphinids were highest between the Rhodes and Finike Basins. While sperm whales were generally detected around the 1000m contour, delphinids were encountered at varying depths. Overall, two years of monthly marine traffic density were retrieved with an average density of 0.37 hours of monthly vessel activity per square kilometer during the study period. The mean density of vessels was

0.32 and 1.03 hours of monthly vessel activity per square kilometer in non-coastal and coastal waters respectively. The Eastern Mediterranean Sea has several important shipping lanes within the study area. Two priority areas for deep diving cetacean and a large priority area for cetaceans were identified in the waters between Marmaris and Finike where high cetacean encounters and dense marine traffic overlapped. The current study revealed important habitats for cetaceans within the data deficient Eastern Mediterranean Sea and delineated potential risk area where marine traffic should be limited.

Keywords: cetacean, spatial distribution, sperm whale, beaked whale, delphinids, marine traffic, AIS

INTRODUCTION

The Mediterranean Sea is a semi-enclosed body of water that contains extensive areas of abyssal waters, deep basins and trenches bounded by steep slopes. The most easterly part of the Mediterranean Sea is also known as the Levantine Sea, of which Turkey's coastlines covers 1577km. Previous studies have reported seven species of cetaceans commonly observed within the Eastern Mediterranean Sea of Turkey; sperm whales *(Physeter macrocephalus)*, Cuvier's beaked whales *(Ziphius cavirostris)*, fin whales *(Balaenoptera physalus)*, bottlenose dolphins *(Tursiops truncatus)*, striped dolphins *(Stenella coeruleoalba)*, common dolphins *(Delphinus delphis)*, and Risso's dolphins *(Grampus griseus)* (Güçlüsoy et al., 2014; Akkaya Baş et al., 2016; Öztürk et al., 2016; Akkaya et al., 2020).

Sperm whales and Cuvier's beaked whales are deep-diving species that generally live in pelagic areas characterized by deep basins and trenches or steep slopes (Heyning, 1989; Praca and Gannier, 2008; Praca et al., 2009; Notarbartolo di Sciara and Birkun Jr, 2010). As such, their distribution is closely related to bathymetry, with distinctive preference for depths of 1000m and 500-1500m for sperm whales and Cuvier's beaked whales, respectively (Frantzis et al., 2003; Boisseau et al., 2010; Frantzis et al., 2014). Sperm whales in the Mediterranean have been described as genetically different from the Atlantic population, with estimates of less than 300 individuals for the Eastern Mediterranean (Drouot et al., 2004; Engelhaupt et al., 2009; Frantzis et al., 2014; Lewis et al., 2018; Frantzis et al., 2019). Ozturk et al. (2013) reported 43 sperm whale sightings in Turkish waters between 1999 and 2014, with the majority of the sightings occurring between Fethiye and the Rhodes Basin in the Anadolu Submarine Canyon, one of the deepest parts of the Mediterranean Sea. The whales were, however, present from Gökçeada in the northern Aegean to an eastern limit of Alanya in Turkey (Öztürk et al., 2013). Evidence suggests that the Mediterranean subpopulation is declining and is currently listed as 'Endangered' under the IUCN Red List (Reeves and Notarbartolo di Sciara, 2006; Notarbartolo di Sciara et al., 2012; Pirotta et al., 2021). Cuvier's beaked whales are also regularly observed in the Eastern Mediterranean Sea, specifically in the Finike (Anaximander) Seamounts, the Antalya Canyon and the Adana Trough (Akkaya Baş et al., 2016; Cañadas and Notarbartolo di Sciara, 2018) and strandings have occurred from Gökçeada to Karataş in the east of Turkey (Öztürk et al., 2011). The species have been recently categorized as 'Vulnerable' on the IUCN Red List (Cañadas and Notarbartolo di Sciara, 2018). Fin whales are also considered to be 'Vulnerable' with a declining population (Panigada and Notarbartolo di Sciara, 2012) and scattered sightings and strandings have been reported along the Turkish Mediterranean coast (Stephens et al., 2021). In addition to deep diving species, four species of delphinid have been recorded in the Turkish waters. Populations of bottlenose dolphins have become increasingly fragmented and are listed together with striped dolphins as 'Vulnerable' by the IUCN (Bearzi et al., 2012; Aguilar and Gaspari, 2012). Similarly, common dolphins have a patchy distribution in the Mediterranean and are considered 'Endangered' on the IUCN Red List (Bearzi, 2003; Bearzi et al., 2021). Additionally, Risso's dolphins were recently recategorized from 'Data Deficient' to 'Endangered' by the IUCN (Lanfredi et al., 2021) due in part to an estimated 50% reduction in individuals over a ten year-period in some areas of the Mediterranean (Airoldi et al., 2015; Azzellino et al., 2016). Their abundance appears even scarcer in the Eastern Mediterranean Sea, with just a handful of documented sightings and strandings (Öztürk et al., 2011; Dede et al., 2012; Kerem et al., 2012; Ryan et al., 2014).

Anthropogenic threats and their impacts on the conservation status of cetacean species in the Mediterranean are of significant concern (Boisseau et al., 2010). An increasing population has developed the necessity for increased development along the coast (both for housing and for tourism), increased exploitation of natural resources (both for food and for fuel), and increased shipping for trade. This has resulted in threats to marine mammals in the Turkish waters of the Eastern Mediterranean Sea from habitat destruction, unsustainable fishing practices (Reeves and Notarbartolo di Sciara, 2006; Notarbartolo di Sciara and Birkun Jr., 2010), chemical and noise pollution (Frantzis, 1998; Frantzis, 2004; Notarbartolo di Sciara and Birkun Jr., 2010; Notarbartolo di Sciara et al., 2012; Fylaktos and Papanicolas, 2019) and ship strikes (Laist et al., 2001; Notarbartolo di Sciara and Birkun Jr., 2010; Frantzis et al., 2014). Marine traffic may contribute to the degradation and loss of cetacean habitat through temporal or permanent changes in habitat preference (Bejder et al., 2006a; Rako et al., 2013; Campana et al., 2015), short-term changes in behavior (Jahoda et al., 2003; Aguilar Soto et al., 2006; Bejder et al., 2006b; Tyack et al., 2011), or direct physical injuries due to collisions (Panigada et al., 2006). While local traffic tends to present a threat to coastal populations, maritime transport tends to follow direct routes around land masses with a safe distance from shore, often around the 1000m depth contour (Frantzis et al., 2019). As a consequence, deep-diving cetacean species are susceptible to the high intensity of shipping in the Mediterranean (Notarbartolo di Sciara and Birkun Jr., 2010; Frantzis et al., 2019). Over 6.2% of stranded sperm whales in Italy and Greece were caused by collisions with ships, as well as 6.6% of photo-identified individuals having visible injuries attributed to ship strikes (Pesante et al., 2002; Abdulla and Linden, 2008). Additionally, in the Pelagos Sanctuary, 3% of strandings were attributed to ship strikes between 1972 and 2018 with 59% of observed injured whales showing evidence of collisions with ships (Panigada et al., 2020).

Thus far, there has been an inequality in the collection of data between the eastern and western Mediterranean (Frantzis et al., 2003; Akkaya et al., 2020) which means that it is likely that patches of cetacean habitat remain unidentified and may overlap with a range of the aforementioned anthropogenic disturbances (Akkaya et al., 2020). The current study maps the distribution of the coastal and offshore cetacean species and identifies potential risk habitats that are likely to be under pressure from maritime traffic. The Eastern Mediterranean Sea suffers from a dearth of knowledge surrounding marine megafauna. Providing more knowledge on cetacean presence, critical habitats and anthropogenic threats will offer guidance to stakeholders forming policy and aid the development of species conservation action plans for cetaceans in the Turkish waters of the Eastern Mediterranean Sea.

MATERIALS AND METHODS

Study Area

The study area includes coastal zones, the continental shelf and the high seas surrounding the Turkish coast within the Eastern Mediterranean Sea (**Figure 1**), extending 55 km offshore and approximately 470 km along the Turkish Mediterranean coast from Marmaris to Anamur. The main survey area covered an area of 16,396 km² in the Rhodes and Finike Basins with an additional section to the east of 5,503 km² in the Antalya Basin. In the south, there was partial overlap with the most northerly section of the Special Environmental Protection Area, “Finike Seamount Special Protected Area”, and in the west, a small area of overlap with the eastern edge of the Marmaris National Park. It also overlaps with two Important Marine Mammal Areas

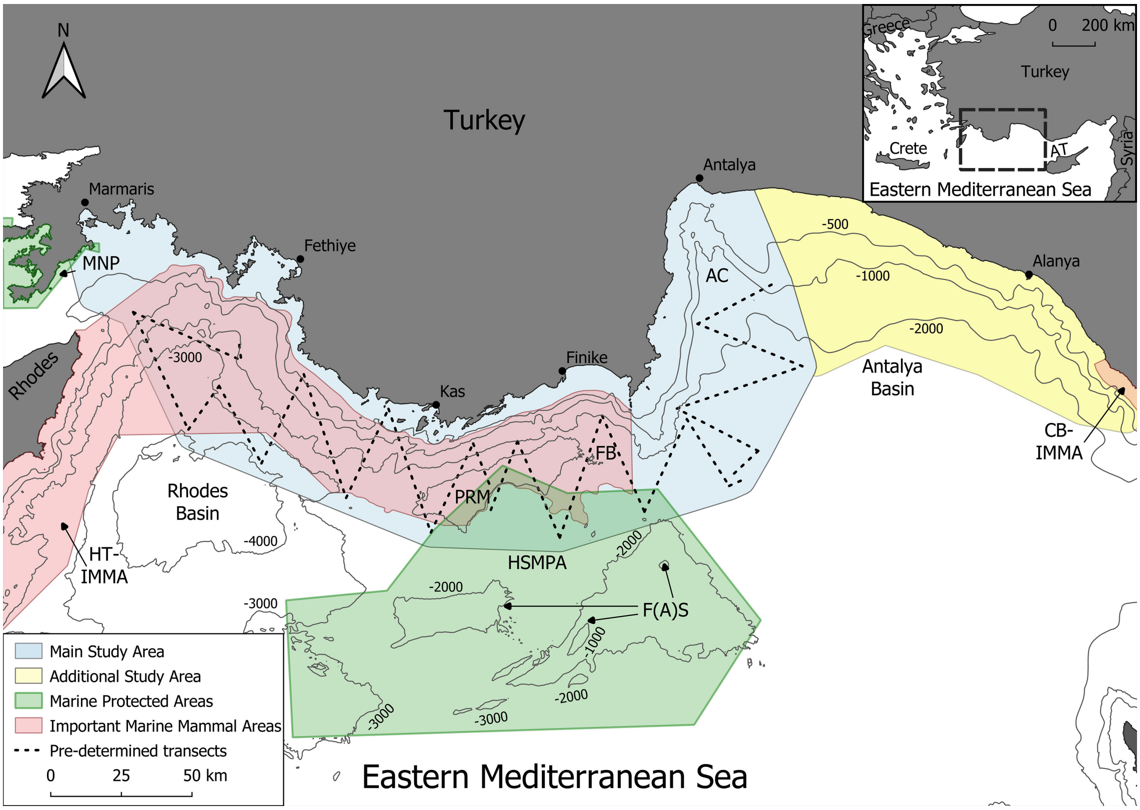

FIGURE 1 | The main study area in the Eastern Mediterranean Sea which was surveyed using predetermined transects (shown in light blue) as well as the additional survey area (yellow), current marine protected areas (green) and Important Marine Mammal Areas (pink). The pre-determined transects are overlaid as dashed lines. Abbreviations: FB: Finike Basin, AC: Antalya Canyon, AT (inset): Antalya Trough, F(A)S: Finike (Anaximander) Seamounts, PRM: Piri Reis Sea Mountains, HSMPA: High Seas Marine Protected Area, MNP: Marmaris National Park, HT-IMMA: Hellenic Trench, Deep Divers’ Sanctuary Important Marine Mammal Area, CB-IMMA: Cilician Basin Important Marine Mammal Area.

(IMMAs). The Hellenic Trench IMMA covers the entirety of the Hellenic trench in Greece as well as the Turkish submarine canyons (IUCN-MMPATF, 2017a; Notarbatolo di Sciara and Hoyt, 2020). A small portion of the Cilician Basin IMMA (IUCN-MMPATF, 2017b), designated for the presence of the Mediterranean monk seals, overlaps the survey area in the east (**Figure 1**).

Data Collection

Survey data were collected from a 13.95m sailing vessel with a 100hp diesel engine which typically travelled at 4 knots during search efforts. This speed was selected to avoid introducing significant hydrophone flow, propeller, or engine noise. The vessel followed equally spaced zigzag transects designed using DISTANCE software, Version 7.3 as well as the 1000m bathymetric contour line. The predetermined tracklines comprised 22 transects, with a total route of 644 km. The series of visual and acoustic surveys along these transect lines were carried out seasonally between 16th April 2018 and 14th January 2020. In addition, the eastern section of the survey area (**Figure 1**) was surveyed in April 2019 only.

Visual surveys were conducted during daylight hours, starting half an hour before sunrise and finishing half an hour after sunset, in Beaufort Sea states ≤4. Two observers with binoculars were stationed at the bow of the vessel, one scanning to port (270 to 10 degrees) and the other to starboard (350 to 90 degrees). The data logging software, Logger, Version 2010 was run continuously for the full duration of each survey. Effort status and environmental information were entered on an hourly basis or when conditions changed considerably. During the visual data collection, species identification, group size, group composition, behavior, group cohesion, group bearing and distance, photo-identification data and anthropogenic presence in the area were recorded.

The acoustic surveys were conducted 24 hours per day for the full duration of the survey, using a four omni-directional broadband hydrophone array towed 200m behind the vessel. The hydrophone elements can be used for high and low frequency monitoring and have a range between 10Hz – 200kHz. Acoustic signals were digitized using a Behringer U-Phoria UMC404HD sound card sampling up to 192kHz. The acoustic software PAMGuard, Version 1.15.15 Core (www.pamguard.org), was used throughout all surveys, scanning incoming signals for clicks and whistles. The PAM operators on the vessel were responsible for logging acoustic detections of cetaceans. The operator filled out an acoustic form at 15-minute time intervals for species presence, acoustic type and strength, and background noise. A scale from 0 (nothing heard) to 5 (nothing else can be heard) was used to quantify acoustic signals as in Ryan et al. (2014). Both visual and acoustic data were used in the subsequent analysis of the relative abundance of each species.

Data Analysis

During analysis, both acoustic and visual detections were classed as encounters and detected species were mapped at point of first detection for each cetacean group in QGIS (version 3.14). If a cetacean group was logged both visually and acoustically (determined by a difference in detection time of less than 2 minutes), then only the visual detection was used. This was to ensure that there was no overestimation of abundance. In order to perform spatial comparisons of different areas, a grid of hexagonal cells was created spanning the survey area. Hexagonal tessellation has shown advantages over the traditionally used square cells when visualizing data (Birch et al., 2007), as it offers a better representation of the visual and acoustic detection area around the vessel (Paradell et al., 2019). Further, hexagonal cells show a better connectivity, as the central point is the same distance from the central point of all neighboring cells (Birch et al., 2007; Paradell et al., 2019). The grid generated 286 hexagonal cells of height 10.746 km and width 10.746 km, corresponding to an area of 100 km^2 per cell.

Cetacean Encounter Rates

When calculating the encounter rate, the number of groups in each hexagonal grid cell were summed using the 'Count points in polygon' tool in QGIS. The total distance travelled by the survey vessel in each cell was calculated using the 'Sum line lengths' tool in QGIS. In order calculate the encounter rates (ER), the following formula was used:

$$ER = \quad n/L$$

where n is the number of group encounters per cell and L is the survey effort (distance travelled by the survey vessel in kilometers). This value was then multiplied by 100 to get the encounter rate per 100km to make it comparable with other studies in the region (e.g., Boisseau et al., 2010; Ryan et al., 2014). The majority of encounters were detected acoustically, making group size more ambiguous as not all individuals in a group will necessarily be vocalizing at the same time. Thus, encounter rates were calculated as 'number of groups of cetaceans per hundred kilometers' rather than 'number of individuals per hundred kilometers'. It is generally accepted that in order to avoid artificial inflation of encounter rates due to small sample biases, cells where a distance less than the diagonal of the cell has been covered by the research vessel (in this case 12.408km) should be removed from further analysis (e.g., Bearzi et al., 2006; Dinis et al., 2016). The total encounter rate was further pooled into delphinids and deep-diving cetaceans.

Depth data for each encounter was derived and integrated from the General Bathymetric Chart of the Oceans (GEBCO) using the 'Sample from raster' tool and distance to the nearest coast was calculated using the 'Distance to nearest hub' tool in QGIS.

Marine Traffic

Vessel density data were retrieved from the European Marine Observation and Data Network (EMODnet)'s Human Activities Data Portal (www.emodnet-humanactivities.eu). Full details on how vessel density by EMODnet is calculated are given in EMODnet (2019). In brief, EMODnet converts received Automatic Identification System messages from vessels (at a three-minute resolution) into reconstructions of ship track lines between these points. Each line is then intersected with a grid of cells at a 1km x 1km resolution and the length and duration attributes attached to each line are used to calculate the length of time spent by a vessel in each cell. As a result, vessel density for EMODnet data is expressed as hours per square kilometer per month (EMODnet, 2019).

Monthly raster data was retrieved from April 2018 until March 2020 (n=24 files) and data was mean averaged using the 'Raster' package in R (version 4.1.2) resulting in a single raster averaged across the two-year period. The zonal statistics tool in QGIS was then used to down-sample these data to match the resolution of the grid of 100km^2 hexagonal cells to give the mean hours per square kilometer per month of each cell. In order to identify any seasonal variation present, monthly raster data was grouped into seasons [defined as spring: March, April, May; summer: June, July, August; autumn: September, October, November and winter: December, January, February (n = 6 files per season)]. For each season, the zonal statistics analysis used for the overall data was repeated to create four seasonal marine traffic density vector layers.

When comparing between coastal and offshore cells, coastal cells were considered those directly touching land or with a centroid that was 200m or shallower, whilst offshore cells did not intersect with land and had a centroid with a depth greater than 200m. In order to investigate which species were detected in cells with the most marine traffic, each detection was intersected with the corresponding seasonal marine traffic density vector layer using the 'Join attributes by location' tool in QGIS.

Potential Risk Areas

As this study represents the first two years of systematic cetacean surveys in this region, the dataset collected was not large enough to create species habitat suitability models as has previously been done when analyzing collision risk for larger datasets (e.g., Blondin et al., 2020). Thus, a simpler method was created to identify potential risk areas where both cetacean encounter rates and levels of marine traffic were high.

As deep diving cetaceans and delphinids travel considerable distances, a kernel density analysis was undertaken to create a raster surface first of deep diving cetaceans and then delphinids across the survey area. In order to do this, the centroid of each encounter rate cell created in 2.3.1 was created in QGIS. Using the encounter rate of each point as a weighting, a density raster was created using a ten-kilometer search radius and a 1km2 cell size (so as to correspond with the cell size of the existing marine traffic density raster).

Due to the skew of both marine traffic density and encounter rate data, a log-transformation was performed to coerce the data towards a normal distribution. During the log-transformation, raster cells with a value of zero for any raster, were given a 'null' value by QGIS as a result of trying to log-transform zero. As a zero value in any raster cell indicates there was no spatial overlap between cetaceans and marine traffic in this cell, it indicates there is no risk and so would have been given a null value in a later stage of the analysis, and therefore this was not considered an issue.

So that encounter rate and marine traffic had an approximately equal influence on the potential risk index, both encounter rate rasters were normalized between 0 and 1 using:

$$z_i = (x_i - min(x))/ \quad (max(x) - min(x))$$

where z_i is the ith normalized cell value in the raster, x_i is the ith cell value in the original raster, min(x) is the minimum cell value in the raster and max(x) is the maximum value in the dataset. Potential risk index for each cell was then calculated as

$$PRI_i = ER_i * MTD_i$$

where PRI_i refers to the potential risk index for the ith cell, ER_i refers to the transformed encounter rate for the ith raster cell is encounter rate >0 and MTD_i refers to the transformed marine traffic density for the ith raster cell with marine traffic density >0. Values for potential risk index were then normalized between 0 and 1 to make them easier to interpret. A potential risk index was created for deep diving cetaceans and delphinids separately. All raster analyses were conducted in the raster calculator in QGIS. Due to the low percentage of cells with sufficient survey effort (>12.408km) at a seasonal scale, the potential risk index was only calculated for the total data and not for each season.

Critical Habitats

In order to identify critical habitats for deep diving cetaceans and delphinids, a kernel density analysis was again run in QGIS using the centroids of each of the hexagonal cells. The kernel density analysis was weighted using the potential risk index and used radii of 24.816km (i.e., twice the diagonal diameter of a hexagonal cell), and a cell size of 1km2. The 'contour' tool in QGIS was used to draw contours around the highest 20% of potential risk cells and then the 'lines to polygons' tool was used to convert these into polygons in order to measure the area of critical habitats for both deep diving cetaceans and for delphinids.

RESULTS

Surveys with a total effort of 52 days were conducted from 16th April 2018 to 14th January 2020 which covered eight separate seasons (two surveys in each spring, summer, autumn and winter). Spring and summer were the seasons with the most survey effort (distance travelled by the research vessel) with 31.4% and 27.2% of the total survey effort, respectively. Autumn and winter were the least surveyed seasons with 16.2% and 24.6% of the survey effort in total (**Table 1**). During the study, 191 visual and acoustic detections were recorded, of which 25 were deep diving cetaceans, 25 were identified delphinids and 141 were unidentified delphinids (**Table 1**).

Survey Effort

Considering the zig-zag nature of transects, survey effort was generally well distributed within the core survey area with a survey effort which was greater than the diagonal of the cell (12.408km) to allow encounter rates to be calculated in 114 out

TABLE 1 | Summary of cetacean encounters by season.

Season	Total Survey Days (km)	Total Encounters	Deep Diving Cetaceans		Delphinids			
			Sperm whales	Cuvier's beaked whales	Bottlenose Dolphins	Common Dolphins	Striped Dolphins	Unidentified Delphinids
Spring	17 (1972km)	41	7	0	6	2	0	26
Summer	14 (1712 km)	63	14	1	7	4	0	37
Autumn	11 (1021 km)	39	1	1	3	0	0	34
Winter	10 (1584 km)	48	0	1	1	0	2	44
Total	**52 (6289km)**	**191**	**22**	**3**	**17**	**6**	**2**	**141**

of 227 cells (50.2%) in the main study area and just 2 cells in the additional survey area (**Figure 2**).

Distribution and Encounter Rates of Deep Diving Cetaceans and Delphinids

Deep diving cetaceans were detected on 25 occasions, mainly to the west of the survey site between Rhodes and Kekova Island and consisted of sperm whales (n=22) and beaked whales (n=3). When accounting for survey effort (with insufficient survey effort removed), the median encounter rate for deep diving cells was 0 groups per 100 km due to a low number of overall detections with a mean of 0.31 groups per hundred kilometers. The highest encounter rate of 8.3 groups of deep diving cetaceans per 100km were in the waters off Fethiye. 60% of sperm whale detections (n = 13) occurred within 3km of the 1000m bathymetric contour. Sperm whale distribution had a median depth of 996.5m and a median distance from shore of 10.9km. Cuvier's beaked whales had a slightly deeper median depth of 1,236m and were found at a similar median distance offshore of 9.6km (**Figures 3**, **4** and **Table 2**).

Delphinids were encountered throughout the entire survey area, from Marmaris to Alanya on 166 occasions (**Figures 3**, **4**; **Table 1**). Bottlenose dolphins (n = 17) were found in shallower waters than other identified cetaceans, with a median depth of 692m and were also detected the closest to shore with a median distance of 7.4km. Common dolphins (n=6) and striped dolphins (n=2) were found at considerably deeper median depths of 1379m and 1899.5m, respectively. Whilst common dolphins were encountered at a similar median distance from shore to bottlenose dolphins (8.3km), striped dolphins had a median distance from shore of 16.2km. Unidentified delphinids had a median depth of 1756m and a median distance from shore of 18.6km (**Figures 3** and band 4; **Table 2**). In comparison to the deep diving cetaceans, there was far less of a pattern in delphinid encounter rates, with encounter rates spread throughout the survey site. The median encounter rate for delphinids of 2.3 groups per 100 km was x times higher than that of the deep diving cetaceans. The median encounter rate for all cetaceans was 2.7 groups per 100 km and the highest encounter rate of 13.4 groups per 100 km was found in offshore waters between Fethiye

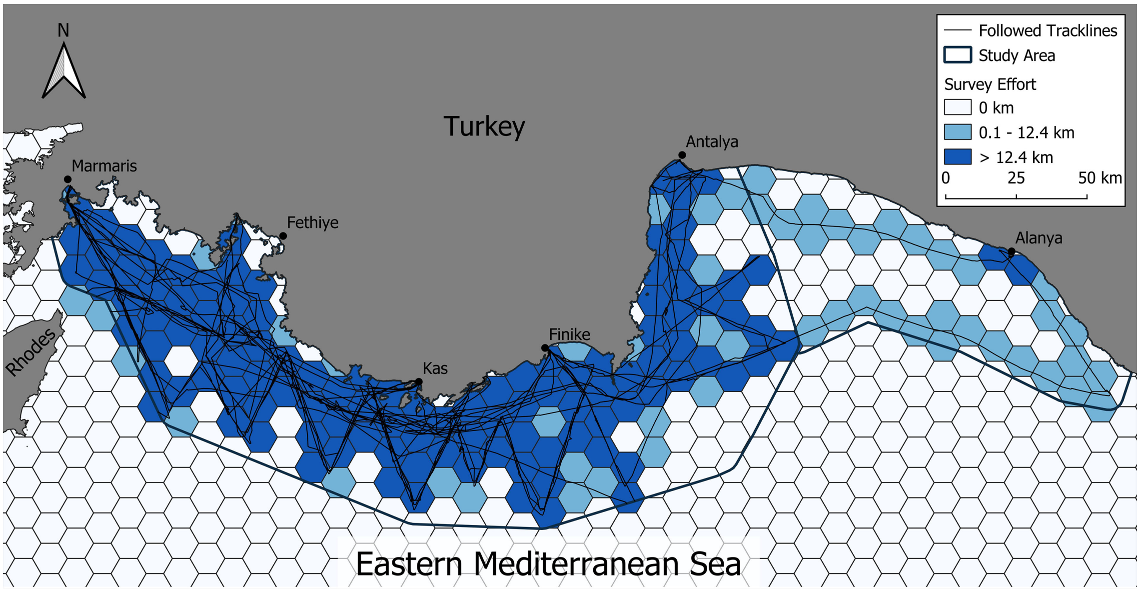

FIGURE 2 | Survey effort in kilometres covered by the research vessel per cell in the Eastern Mediterranean Sea surrounding Turkey with darker blue cells showing areas with more than 12.408km of boat track line (suitable for encounter rate analysis), lighter blue cells showing less than 12.408km and white cells showing no survey effort.

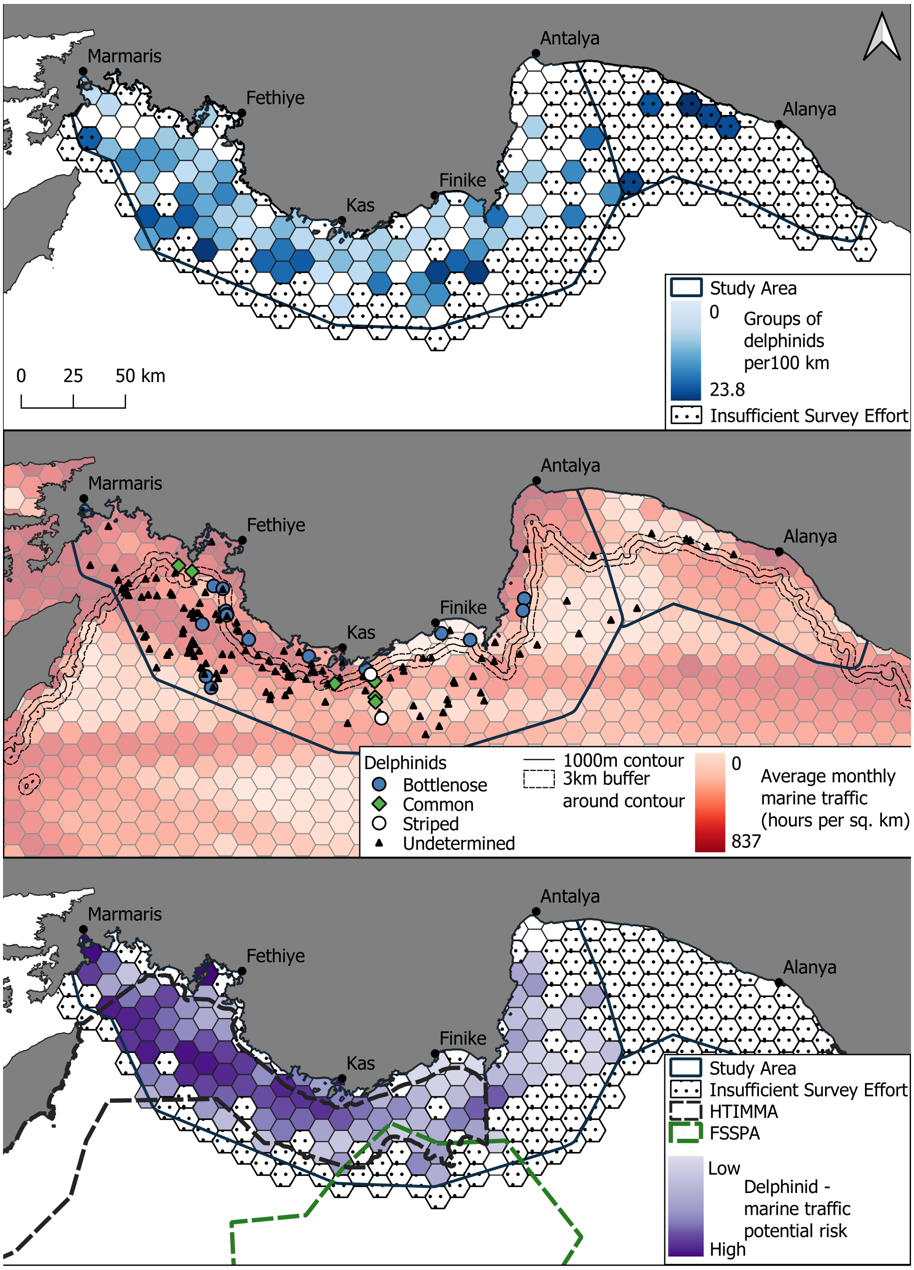

FIGURE 3 | (Upper) Groups of delphinids per 100 km in the Eastern Mediterranean Sea with darkening blue representing an increased number of delphinid groups. (Middle) The distribution of different delphinid species with bottlenose, striped, common and undetermined groups of delphinids denoted by blue circles, green diamonds, hollow circles and filled triangles respectively. Distribution is superimposed onto the average monthly marine traffic (hours per square kilometre) with darkening red signifying higher traffic. Marine vessel information used in this figure was made available by the EMODnet Human Activities project, www.emodnet-humanactivities.eu, funded by the European Commission Directorate General for Maritime Affairs and Fisheries. The 1000m contour is shown with a 3km buffer area around the contour. (Lower) Potential risk areas where the presence of delphinids and marine traffic were both high during the study period. Darkening purple shows an increased potential for risk.

and Kaş. There was no clear spatial pattern between the highest overall encounter rates, but 16 of the 20 cells with the highest overall cetacean encounter rate were found in the Hellenic Trench Important Marine Mammal Area. Marine Traffic

Retrieved vessel density showed vessel presence across the entire survey area (**Figures 3**, **4**). The average monthly hours of marine traffic per square kilometer was 0.37 (median absolute deviation (MAD): ± 0.29) with density increasing to more than 300 average monthly vessel hours per square kilometer in four cells near major ports in Marmaris, Antalya and Fethiye (n=2). Traffic was also higher around the coastline in general and a shipping lane running east-west as well as either side of Rhodes.

TABLE 2 | Summary of depths and distances from the nearest shore of deep diving cetaceans and delphinid species in the study area.

Species		Median	Mean (± SE)	Range
Sperm whales (n=22)	Depth (m)	996.5	235.8 (± 139.9)	149 - 2391
	Distance (km)	10.9	12.6 (± 1.9)	0.9 - 40.0
Beaked whales (n=3)	Depth (m)	1236.0	1497.3 (± 552.6)	698 - 2558
	Distance (km)	9.6	13.0 (± 4)	8.3 - 21.0
Bottlenose dolphins (n=17)	Depth (m)	692.0	1037.1 (± 276)	23 - 3829
	Distance (km)	7.4	10.4 (± 2.7)	0.2 - 39.1
Common dolphins (n=6)	Depth (m)	1379.0	1713.3 (± 454.8)	574 - 3057
	Distance (km)	8.3	10.3 (± 2.4)	4.5 - 18.5
Striped dolphins (n=2)	Depth (m)	1899.5	1899.5 (± 852.5)	1047 - 2752
	Distance (km)	16.2	16.2 (± 10.7)	5.5 - 26.8
Unidentified delphinids (n=141)	Depth (m)	1756.0	1816.7 (± 87.4)	41 - 3992
	Distance (km)	18.6	19.2 (± 1)	0.4 - 50.3

For the entire survey area, coastal cells had a median of 1.03 average monthly vessel hours per square kilometer (MAD: ± 1.50), whilst offshore cells (those not directly connected to the coast or adjacent to the coast with a depth of less than 200m) had a median of 0.32 (MAD: ± 0.21).

In areas where marine protected areas overlapped the study area, the average monthly vessel hours per square kilometer was 0.30 (MAD: ± 0.13) and 11.53 (MAD: ± 14) for the cells in Finike Special Protected Area and Marmaris National Park, respectively. For cells in the Hellenic Trench IMMA, average monthly vessel hours per square kilometer was 0.43 (MAD: ± 0.31) whilst in the Cilician Basin it was 0.26 (MAD: ± 0.21). There were large seasonal differences in marine traffic with summer having the highest monthly vessel hours per square kilometer with 0.65 (MAD: ± 0.34) and winter the lowest with 0.09 (MAD: ± 0.11). This seasonal difference was most evident in coastal cells where summer marine traffic density was more than 13 times that of winter marine traffic (**Table 3**).

Sperm whales were detected in cells with a median of 0.98 (MAD: ± 0.58) monthly vessel hours per square kilometer, whilst Cuvier's beaked whales were found in areas with a median of 0.59 (MAD: ± 0.08). Of the delphinids, bottlenose dolphins were detected in areas that had the highest levels of marine traffic throughout the study period with a median of 0.80 (MAD: ± 0.87) monthly vessel hours per square kilometer. Common dolphins and unidentified delphinids were found in cells with relatively similar levels of marine traffic with median densities of 0.64 (MAD: ± 0.29) and 0.52 (MAD: ± 0.53) whilst striped dolphins were found in considerably less dense areas of 0.03 (MAD: ± 0.02) monthly vessel hours per square kilometer.

Potential Risk Areas

The highest potential for risk for deep divers were in the channel of water that runs between Turkey and Rhodes and in cells that surround the 1000m contour in Marmaris Bay to the West of Antalya Bay. The majority of the potential high-risk cells overlapped with the shipping lane identified during the marine traffic density analysis and also fell within the Hellenic Trench Important Marine Mammal Area. Cells further to the east had comparatively lower potential risk scores (**Figure 3**). Delphinids followed a similar patter with higher risk cells generally in the west of the survey area, along the same shipping lane. In addition, potential risk for delphinids was high around ports (Marmaris, Fethiye and Kaş). The potential risk index scores in Antalya Bay were generally much lower than in the west of the study area (**Figure 4**).

Critical Habitats

For deep diving cetaceans two critical habitats were delineated, the first in the waters between Marmaris and Fethiye, and the second in the waters off Kaş. The two critical habitats were roughly even in size (511.1 km^2 and 664.3 km^2 respectively). A single large critical habitat was identified spanning the waters from Rhodes Basin to the Piri Reis Sea Mountains (2516.7 km^2) (**Figure 5**). Over 99% (1170.1 km^2) of the deep diving cetacean critical habitat overlapped with the area identified as a critical habitat for delphinids. Nearly 85% (996.2km^2) and 92% (2305.7km^2) of deep diving cetacean and delphinid critical habitat overlapped with the Hellenic Trench IMMA respectively. None of the critical habitats overlapped with any existing protected areas (**Figure 5**).

TABLE 3 | Summary of seasonal differences in vessel density for different regions within the survey area.

Season	All Cells	Coastal Cells	Offshore Cells	Cilician IMMA	Hellenic Trench IMMA
Spring	0.34 (± 0.29)	0.62 (± 0.86)	0.30 (± 0.24)	0.39 (± 0.34)	0.38 (± 0.26)
Summer	0.65 (± 0.54)	2.06 (± 2.96)	0.60 (± 0.42)	0.30 (± 0.22)	0.77 (± 0.59)
Autumn	0.28 (± 0.32)	0.97 (± 1.35)	0.23 (± 0.25)	0.16 (± 0.06)	0.42 (± 0.34)
Winter	0.09 (± 0.11)	0.15 (± 0.23)	0.08 (± 0.08)	0.14 (± 0.14)	0.13 (± 0.15)
Yearly median	0.37 (± 0.29)	1.03 (± 1.50)	0.32 (± 0.31)	0.26 ± (0.21)	0.43 (± 0.31)

Units are median hours of vessel activity per square kilometer per month with median absolute deviations in brackets.

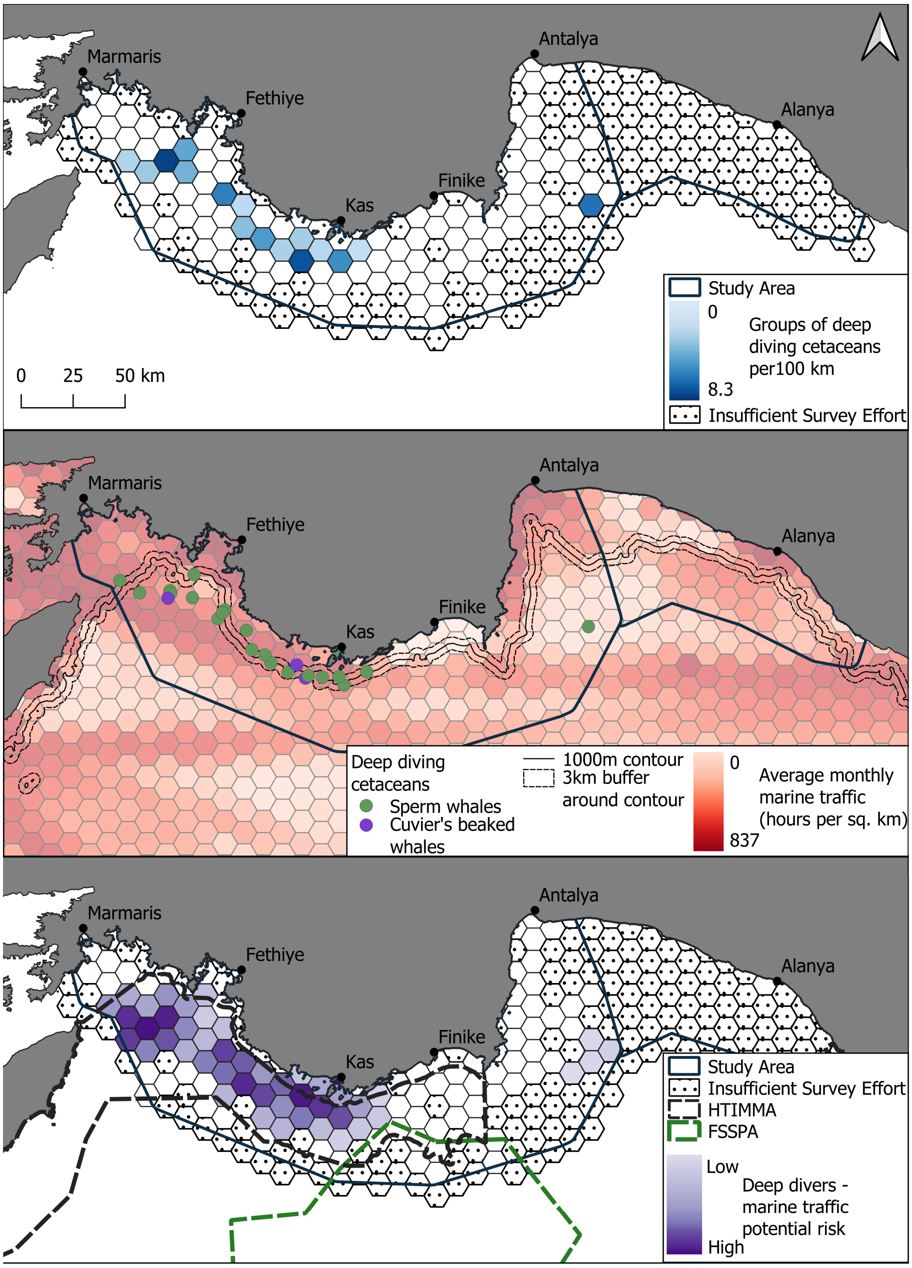

FIGURE 4 | (Upper) Groups of deep diving cetaceans per 100 km in the Eastern Mediterranean Sea with darkening blue representing an increased number of delphinid groups. (Middle) The distribution of different deep diving cetacean species with sperm whales and Cuvier's beaked whales denoted in green and purple circles respectively. Distribution is superimposed onto the average monthly marine traffic (hours per square kilometre) with darkening red signifying higher traffic. Marine vessel information used in this figure was made available by the EMODnet Human Activities project, www.emodnet-humanactivities.eu, funded by the European Commission Directorate General for Maritime Affairs and Fisheries. The 1000m contour is shown with a 3km buffer area around the contour. (Lower) Potential risk areas where the presence of deep diving cetacean and marine traffic were both high during the study period. Darkening purple shows an increased potential for risk.

DISCUSSION

The current study is the longest ongoing cetacean research project, spanning all seasons, in the Turkish Eastern Mediterranean Sea. This study builds off the preliminary results of Akkaya et al. (2020) to include two complete years of data collection. Of the seven cetacean species regularly found in Turkish waters (Öztürk et al., 2016), five were detected during this study, although bottlenose dolphins were the only species to be seen throughout all seasons (**Table 1**).

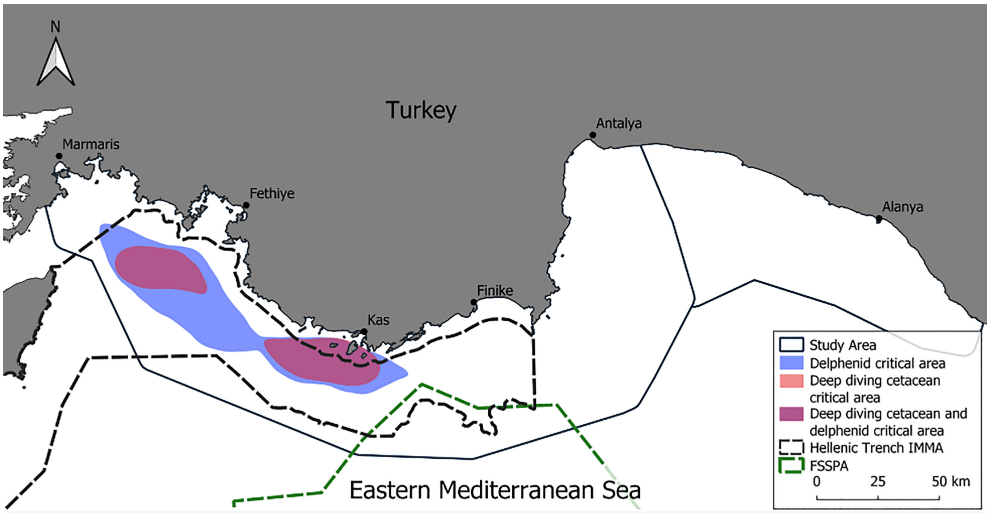

FIGURE 5 | Areas of the Eastern Mediterranean Sea surrounding Turkey deemed critical for delphinids (blue) and deep diving cetaceans (pink). Note that both critical areas for deep diving cetaceans fell within the critical area for delphinids and thus are shown in a purple colour.

Distribution and Encounter Rates

As expected, the different species had varying distribution, albeit with considerable spatial overlap with each other and maritime traffic (**Figures 3**, **4**). Deep diving species were encountered mainly in the west, with 23 out of 25 (92%) of the sightings occurring in the bathymetric irregularities that stretch east from the Rhodes basin to the end of the Finike Basin (**Figure 3**). Bathymetry has been shown to be important in relation to the distribution of the deep diving species (sperm whales and beaked whales) (Frantzis et al., 2003; Frantzis et al., 2019). The Hellenic Trench is considered by Podestà et al. (2016) to be the largest "high-density areas of occurrence" in the Mediterranean for Cuvier's beaked whale as well as a core habitat with year-round presence of the eastern Mediterranean sperm whale population (Frantzis et al., 2011; Frantzis et al., 2019). (Frantzis. et al.'s 2019) study along the Hellenic Trench recorded 74% of the detected sperm whales to be within 3km of the 1000m contour with density decreasing as water depth gets shallower or deeper. The current study found that 60% of the deep diving species were recorded within 3km of the 1000m contour with a median respective depth of 999m, although individuals also occurred in much shallower (149m) and much deeper waters (2391m). Furthermore, in the Greek areas of the Hellenic Trench, Cuvier's beaked whales have also shown a preference for depths of 500 to 1500m depth as well as any steep sloping bathymetry (Frantzis et al., 2003). The median value of 1236m found in this study aligned well with the findings in adjoining waters, although it should be noted that this is for just three recorded individuals, one of which was considerably deeper (2558m). Due to the close proximity and overlap of the Hellenic Trench to the current study area and similarities in depth parameters, it is more than likely that the home range of the sperm whales and Cuvier's beaked whales extends across the waters surrounding both Greece and Turkey (Akkaya et al., 2020).

Delphinids were distributed throughout the entire survey area from Rhodes Basin to Antalya Basin, ranging from coastal waters to 50.3km offshore. In the Mediterranean, bottlenose dolphins are generally found in shallow waters along the continental shelf and in productive waters up to 600m depth (Bearzi, 2003; de Stephanis et al., 2008). However, the current study reported a median depth preference of 692m with detections of the species occurring up to a depth of 3,829m. Therefore, it is likely that as well as the commonly occurring coastal population, the Eastern Mediterranean Sea may also hold an offshore distribution of the bottlenose dolphins. Further research is needed to understand the reasons behind these offshore sightings as they may indicate that the home range of the coastal population extends to the deeper waters, that the species has separate offshore populations or that the species may show long-distance movement patterns, none of which have previously been reported in the Mediterranean Sea. Additionally, the majority of the delphinids which could not be identified to species level, were detected with a median depth of 1756m. Some delphinids depend on certain cephalopod species and abundance of these species needs to be investigated. Öztürk et al. (2007) examined the cephalopod remains from the stomachs of three striped dolphins and two Risso's dolphins (*Grampus griseus*) taken as bycatch in the swordfish industry in the eastern Mediterranean Sea off the Turkish coast. In total, 478 lower beaks were identified as belonging to 14 cephalopod species, some of which are only found in deep seas. This stresses the importance of not just coastal waters, but also deep-sea ecosystems for delphinids within the Eastern Mediterranean Sea of Turkey.

In terms of seasonal distribution, sperm whales were mostly detected in spring and summer months with only one individual identified in autumn and none in winter (**Table 1**). The additional two seasons (autumn and winter) of data here, were further confirmation of (Akkaya *et al.*'s 2020) findings of seasonal presence of sperm whales in Turkish waters and corresponds with previous works in Turkey (Ozturk et al., 2013) and in Greece (Diogou et al., 2019) which both found higher numbers of sperm whales in spring and summer. When coupled with the fact that social units have been observed with calves in this area (Akkaya et al., 2020), it suggests that this area of the Eastern Mediterranean Sea may be an important breeding or nursing ground in warmer seasons. Although limited data meant that it was not possible to perform a potential risk index analysis for each season, the trends for increased marine traffic found in summer are concerning.

In contrast to Baş et al. (2016), who detected bottlenose dolphins, in just spring and summer in the coastal water of the Gulf of Antalya, this study detected bottlenose dolphins across all seasons. Common dolphins were only seen in summer months, however, this is possibly a result of limited data collection as they have been recorded year-round in the adjoining Greek (Milani et al., 2019) and Turkish (Akkaya et al., in review) areas of the Aegean Sea. There were not enough beaked whale or striped dolphin detections recorded to analyze their seasonal distribution but filling this important data gap should be considered a priority in the future.

The median encounter rate of cetaceans was 2.7 groups per 100km (2.3 groups and 0 groups per 100km for delphinids and deep diving cetaceans respectively) in the study area. This is much higher than previous recordings in the region with cetacean encounter rates of 0.68 (Boisseau et al., 2010) and 0.008 per 100km (Ryan et al., 2014). This is likely due to these studies covering different survey areas to this study and the fact that they consisted of single summer survey efforts, highlighting the importance of multi-seasonal, multi-year, local efforts (Akkaya et al., 2020).

Marine Traffic and Potential Risk

The Mediterranean is one of the world's busiest waterways (Notarbartolo di Sciara and Birkun Jr., 2010), and thus it is important to understand the magnitude of overlap between cetaceans and marine traffic. This study highlighted the overlap in spatial usage of both the delphinids and the deep diving species with areas of heavy maritime traffic (**Figure 3**). The highest levels of marine traffic were along the coast, however, there was a notable presence of a shipping lane in the west of the study area along the 1000m contour of the Finike Canyon, presumably due to it being a safe depth for large boats (Frantzis et al., 2019). Whilst the median monthly hours of vessel traffic/km^2 was much lower in the Finike Seamount Marine Protected Area than the rest of the core study area (0.3 as opposed to 0.37), this is likely due to the Finike Seamounts being further offshore rather than any specific protective measures. What is more concerning, is that the monthly median hours of vessel traffic/km^2 within the Hellenic Trench IMMA was 0.43. When compared to the median for the entire survey area, this does not seem too high (~16% higher) but compared to other non-coastal cells (those cells not directly connected to land and with a centroid depth greater than 200m), it is a worrying 34% higher. The Hellenic Trench IMMA was ranked in the top ten most at risk of ship strike IMMAs of 114 IMMAs assessed globally (WWF-IUCN-IWC-OceanMind, 2019). The marine traffic density results in this study confirm the potential for increased ship strike. Thus, unsurprisingly, a high proportion of the cells considered to have a high potential risk index score were found within the Hellenic Trench IMMA. The western part of this IMMA has previously been proposed for a MPA (Agardy et al., 2007). The current study has now demonstrated the importance of the extension of Hellenic Trench and Anadolu Seamounts, and future protective measures must span across the international border between Greece and Turkey.

When considering the overlap between cetaceans and maritime traffic, sperm whales followed by bottlenose dolphins were detected in areas with the highest density of marine traffic. Sperm whales (Frantzis et al., 2019) and Cuvier's beaked whales (Carrillo and Ritter, 2010) are known to be particularly vulnerable to shipping collisions (Panigada et al., 2006). The work of Frantzis et al. (2019) indicated that as a result of the major shipping route along the Hellenic Trench there is increased mortality rates of the sperm whale population due to ship strikes in this area. As there is only a low recruitment rate of sperm whales (~2.5/year) in the Eastern Mediterranean, even a small number of ship strikes would likely have population level effects (Frantzis et al., 2019). It has been found, however, that small changes in shipping routes could dramatically reduce risk (Frantzis et al., 2019). By rerouting or reducing vessel speed within these areas, the collision risk and noise pollution for sperm whales and beaked whales could be considerably reduced with minimal inconvenience for the shipping industry (Vanderlaan and Taggart, 2009; Frantzis et al., 2019). While ship strikes may be less of a direct threat to the bottlenose dolphins' population, increased noise and pollution levels may come with direct and indirect consequences to the dolphin populations such as habitat shifts and behavioral alterations (Papale et al., 2012; Akkaya Bas et al., 2017).

Marine traffic considered within this study were retrieved from vessels using AIS which is only mandatory for ships with 300 or greater gross tonnage (International Maritime Organisation, 2021). According to the FAO, 82.9% of the fishing within the Mediterranean and Black Sea is done from small scale vessels (FAO, 2020), and this along with other small recreational boats suggest that the actual marine traffic is a lot higher than seen here, with smaller boats more likely to have a predominantly coastal distribution. The impact of small boats compared to large freight ships may vary and so it would be beneficial to have further studies looking into the combined impacts.

As well as the threat of increasing marine traffic, cetaceans are also subject to other anthropogenic disturbances. These include unsustainable fishery practices, sonar use, naval exercises and hydrocarbon exploration (Notarbartolo di Sciara and Birkun Jr, 2010; Fylaktos and Papanicolas, 2019). The latter is of particular concern with a recent rise in the number of oil and gas explorations taking place within the Eastern Mediterranean Sea. The year-round distribution and habitat use of cetaceans within Turkish waters of the Mediterranean encourages a reassessment of current levels of protections of the region to counteract excessive anthropogenic impacts.

Recommendations

The current study has delineated two habitats for deep diving cetaceans and one for delphinids that the authors deem critical where the area usage of cetaceans and marine traffic overlaps (**Figure 5**). The first deep diving cetacean is between Marmaris and Fethiye, the second between the Fethiye and Kaş and the delphinid critical habitat effectively encompasses the first two. These two deep diving habitats are different in terms of nutrient availability, which is important for primary production, thus consequently affecting the top predators. The cyclonic Rhodes Gyre causes nutrient enrichment, concentration of larval food distribution and local retention of eggs and larvae (Agostini and Bakun, 2002). In contrast to this, the Levantine Basin is oligotrophic, which results in extremely low values of phytoplankton abundance (Herut et al., 2017). Therefore, a research priority is to investigate the relation between oceanographic conditions and cetacean presence, especially within the Finike Basin.

Well-managed MPAs have been found to be a key tool for conservation through the regulation of anthropogenic activities in biologically valuable areas and can simultaneously be used as study sites for scientists (Edgar et al., 2007). According to Woodside et al. (2006) and Öztürk et al. (2013), the Finike (Anaximander) Seamount MPA is an important area for deep diving mammal species like sperm whales and Cuvier's beaked whales. Whilst the current study did not detect these deep-diving species and no potential risk areas were identified within the MPA, small cetaceans were both acoustically and visually detected in this area. The lack of detections of deep diving cetaceans may be a result of very limited vessel coverage in this area and efforts should be made in the future to study these unique deep-sea habitats.

Another future research priority is the investigation of seasonal variation in the overlap between cetaceans and marine traffic as well as studying the magnitude of the impact of marine traffic on different cetacean species, particularly with the variation in seasonal marine traffic identified. Efforts have recently been made to study the Turkish waters to the east of the current study area to assess the species range and densities from west to east (Akkaya, 2021) and incorporate species like fin whales which are sighted only in the eastern part of Turkish waters (Stephens et al., 2021). Despite this, there are still few data available on potential risk areas where cetaceans are exposed to high shipping densities in the Eastern basin of the Mediterranean Sea. Dedicated long-term survey efforts prove essential to provide information on population, density patterns, and movement of cetaceans throughout the entire year and therefore reveal important cetacean habitats along the Turkish Mediterranean coast (Akkaya et al., 2020). In the meantime, this study has clearly shown the importance of the Eastern Mediterranean Sea for the cetaceans. Despite Turkey becoming a member of the Agreement on the Conservation of the Cetaceans of the Black Sea, Mediterranean Sea and contiguous Atlantic Area (ACCOBAMS), which commits the country to protect cetaceans from unregulated and uncontrolled anthropogenic activities, a lack of scientific knowledge and threat assessment persists resulting in unsustainable practices continuing within these waters (Akkaya et al., 2020). Several important international agreements including the Convention on Biological Diversity, which Turkey is a party to, require a precautionary approach to be taken and clearly there is enough evidence of the distribution of a range of cetaceans throughout this area, as well as evidence of potential risks to their prolonged survival.

Despite this study filling a considerable data gap, there is still a great dearth of information within Turkish waters. The findings of this work indicate a need for further study on cetacean response to different seasons and oceanographic variables to allow more advanced risk analyses to be performed as well as investigation into the magnitude of impact of the marine traffic on different species. It is imperative that Species Conservation Action Plans are created as soon as possible for each of these species based on current information and updated as data gaps are filled. This will allow relevant management measures such as rerouting marine traffic or creating marine protected areas to minimize exposure of cetaceans to anthropogenic threats. For highly migratory species such as cetaceans, transboundary cooperation is also essential for the sake of better conservation in this understudied area.

DATA AVAILABILITY STATEMENT

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

ETHICS STATEMENT

The animal study was reviewed and approved by Turkish Ministry of Agriculture and Forestry.

AUTHOR CONTRIBUTIONS

TA, AAk, and PL participated in the field survey. Analysis was conducted by TA, AAk, GH, MN, and DK and the manuscript was written by all the authors. All authors contributed to the article and approved the submitted version.

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REFERENCES

Abdulla, A., and Linden, O. (Eds.) (2008). *Maritime Traffic Effects on Biodiversity in the Mediterranean Sea: Review of Impacts, Priority Areas and Mitigation Measures* (Malaga, Spain: IUCN Centre for Mediterranean Cooperation), 184 pp.

Agardy, T., Aguilar, N., Cañadas, A., Engel, M., Frantzis, A., Hatch, L., et al. (2007). "A Global Scientific Workshop on Spatio-Temporal Management of Noise," in *Report of the Scientific Workshop*, 20–21. Puerto Calero, Lanzarote, Leviathan Sciences

Agostini, V. N., and Bakun, A. (2002). 'Ocean Triads' İn the Mediterranean Sea: Physical Mechanisms Potentially Structuring Reproductive Habitat Suitability (With Example Application to European Anchovy (*Engraulis Encrasicolus*). *Fish. Oceanog.* 11 (3), 129–142. doi: 10.1046/j.1365-2419.2002.00201.x

Aguilar, A., and Gaspari, S. (2012). Stenella Coeruleoalba *(Mediterranean Subpopulation). IUCN. Red. List. Threat. Specie.* 2012, e.T16674437A16674052. doi: 10.2305/IUCN.UK.2012-1.RLTS.T16674437A16674052.en

Aguilar Soto, N., Johnson, M., Madsen, P. T., Tyack, P. L., Bocconcelli, A., and Borsani, J. F. (2006). Does Intense Ship Noise Disrupt Foraging in Deep-Diving Cuvier's Beaked Whales (*Ziphius Cavirostris)*? *Mar. Mam. Sci.* 22 (3), 690–699.

Airoldi, S., Azzellino, A., David, L., Dhermain, F., Di-Meglio, N., Jimenez, V., et al (2015). Final Report "Absolute Abundance of Risso's Dolphin *(Grampus Griseus)* in the North-Western Part of the Pelagos Sanctuary. *Pelagos. Sanctuary. Convent. No. 03/2014* 59 Istituto Tethys /EcoOcean Institut/ GECEM/Fondazione CIMA/GIS3M. Available online at: https://www.gis3m.org/sites/gis3m.org/files/doc/final_report_grampus_pelagos.pdf

Akkaya, A. (2021). "Investigating the Impact of Anthropogenic Activities on Cetaceans Within the Eastern Mediterranean Sea of Turkey," in *5th Conference on the Conservation of Cetaceans in South Mediterranean Countries*, Lebanon, 15th April.

Akkaya, A., Awbery, T., Lyne, P., Schartmann, H., Nease, K., Hass, C., et al. (In review) Short-Beaked Common Dolphins of Dilek Peninsula National Park, South Aegean Sea: Preliminary Results on Group Structure, Spatial Distribution, BehaviouralPatterns, Whistle Characteristics and its Behavioural Context.

Akkaya Baş, A., Lagoa, J., and Atchoi, E. (2016). New Records of Cuvier's Beaked Whales (*Ziphius Cavirostris*) From the Turkish Levantine Sea. *Turkish. J. Zool.* 40 (3), 454–460. doi: 10.3906/zoo-1509-19

Akkaya Bas, A., Christiansen, F., Amaha Öztürk, A., Öztürk,, and B. and McIntosh, C. (2017). The Effects of Marine Traffic on the Behaviour of Black Sea Harbour Porpoises (Phocoena Phocoena Relicta) Within the Istanbul Strait, Turkey. *PloS One* 12 (3), e0172970. doi: 10.1371/journal.pone.0172970

Akkaya, A., Lyne, P., Schulz, X., Awbery, T., Capitain, S., Rosell, B. F., et al. (2020). Preliminary Results of Cetacean Sightings in the Eastern Mediterranean Sea of Turkey. *J. Black. Sea/Mediterranean. Environ.* 26 (1). https://blackmeditjournal.org/volumes-archive/vol-26-2020/vol-26-2020-no-1/5329-2/

Azzellino, A., Airoldi, S., Gaspari, S., Lanfredi, C., Moulins, A., Podestà, M., et al. (2016). "Risso's Dolphin, Grampus Griseus, in the Western Ligurian Sea: Trends in Population Size and Habitat Use," in *Advances in Marine Biology* 75, 205–232. Eds. G. Notarbartolo Di Sciara, M. Podestà and B. E. Curry (Academic Press), 205–223.

Baş, A. A., Erdoğan, M. A., Morris, N. R. C., Yeoman, K., Humphrey, O., Gaggioli, E., et al. (2016). Seasonal Encounter Rates and Residency Patterns of an Unstudied Population of Bottlenose Dolphin *(Tursiops Truncatus)* in the Northwestern Levantine Sea, Turkey. *Hyla.: Herpetol. Bull.* 2016 (1), 1–13. https://hrcak.srce.hr/file/260618

Bearzi, G., Reeves, R.R., Notarbartolo Di Sciara, G., Politi, E., Cañadas, A., Frantzis, A., et al (2003). *Delphinus Delphis* (Mediterranean Subpopulation). *IUCN. Red. List. Threat. Specie.* 2003, e.T41762A10557372. doi: 10.2305/IUCN.UK.2003.RLTS.T41762A10557372.en

Bearzi, G., Politi, E., Agazzi, S., and Azzellino, A. (2006). Prey depletion caused by overfishing and the decline of marine megafauna in eastern Ionian Sea coastal waters (central Mediterranean). *Biological Conservation*, 127 (4), 373–382. doi: 10.1016/j.biocon.2005.08.017

Bearzi, G., Fortuna, C., and Reeves, R. (2012). Tursiops truncatus. *The IUCN Red List of Threatened Species 2012: e.T22563A2782611*. Accessed on 27 May 2022. Available online at: https://www.iucnredlist.org/species/22563/2782611

Bearzi, G., Genov, T., Natoli, A., Gonzalvo, J., and Pierce, G. J. (2021). *Delphinus Delphis* (Inner Mediterranean Subpopulation). *IUCN. Red. List. Threat. Specie.* 2021, e.T189865869A189865884. doi: 10.2305/IUCN.UK.2021-3.RLTS.T189865869A189865884.en

Bearzi, G., Reeves, R. R., Notarbartolo Di Sciara, G., Politi, E., Cañadas, A., et al. (2003). Ecology, Status and Conservation of Short-Beaked Common Dolphins, *Delphinus Delphis* in the Mediterranean Sea. *Mam. Rev.* 33 (3-4), 224–252. doi: 10.1046/j.1365-2907.2003.00032.x

Bejder, L., Samuels, A., Whitehead, H., and Gales, N. (2006b). Interpreting Short-Term Behavioural Responses to Disturbance Within a Longitudinal Perspective. *Anim. Behav.* 72 (5), 1149–1158. doi: 10.1016/j.anbehav.2006.04.003

Bejder, L., Samuels, A., Whitehead, H., Gales, N., Mann, J., Connor, R., et al. (2006a). Decline in Relative Abundance of Bottlenose Dolphins Exposed to Long-Term Disturbance. *Conserv. Biol.* 20 (6), 1791–1798. doi: 10.1111/j.1523-1739.2006.00540.x

Birch, C. P., Oom, S. P., and Beecham, J. A. (2007). Rectangular and Hexagonal Grids Used for Observation, Experiment and Simulation in Ecology. *Ecol. Model.* 206 (3-4), 347–359. doi: 10.1016/j.ecolmodel.2007.03.041

Blondin, H., Abrahms, B., Crowder, L. B., and Hazen, E. L. (2020). Combining High Temporal Resolution Whale Distribution and Vessel Tracking Data Improves Estimates of Ship Strike Risk. *Biol. Conserv.* 250, 108757. doi: 10.1016/j.biocon.2020.108757

Boisseau, O., Lacey, C., Lewis, T., Moscrop, A., Danbolt, M., McLanaghan, R., et al. (2010). Encounter Rates of Cetaceans in the Mediterranean Sea and Contiguous Atlantic Area. *J. Mar. Biol. Assoc. Unite. Kingdom.* 90 (8), 1589–1599. doi: 10.1017/S0025315410000342

Campana, I., Crosti, R., Angeletti, D., Carosso, L., David, L., Di-Méglio, N., et al. (2015). Cetacean Response to Summer Maritime Traffic in the Western Mediterranean Sea. *Mar. Environ. Res.* 109, 1–8. doi: 10.1016/j.marenvres.2015.05.009

Cañadas, A., and Notarbartolo di Sciara, G. (2018). "Ziphius Cavirostris (Mediterranean Subpopulation)," in *The IUCN Red List of Threatened Species 2018.*

Carrillo, M., and Ritter, F. (2010). Increasing Numbers of Ship Strikes in the Canary Islands: Proposals for Immediate Action to Reduce Risk of Vessel-Whale Collisions. *J. Cetacean. Res. Manage.* 11 (2), 131–138.

Dede, A., Saad, A., Fakhri, M., and Öztürk, B. (2012). Cetacean Sightings in the Eastern Mediterranean Sea During the Cruise in Summer 2008. *J. Black. Sea/ Mediterranean. Environ.* 18 (1), 49–57.

De Stephanis, R., Cornulier, T., Verborgh, P., Salazar Sierra, J., Pérez Gimeno, N., and Guinet, C. (2008). Summer Spatial Distribution of Cetaceans in the Strait of Gibraltar in Relation to the Oceanographic Context. *Mar. Ecol. Prog. Ser.* 353, 275–288. doi: 10.3354/meps07164

Dinis, A., Carvalho, A., Alves, F., Nicolau, C., Ribeiro, C., Kaufmann, M., et al. (2016). "Spatial and Temporal Distribution of Bottlenose Dolphins, Tursiops Truncatus, in the Madeira Archipelago, NE Atlantic," in *Arquipélago-Life and Marine Sciences*, vol. 33, pp. 45–pp. 54.

Diogou, N., Klinck, H., Frantzis, A., Nystuen, J. A., Papathanassiou, E., and Katsanevakis, S. (2019). Year-Round Acoustic Presence of Sperm Whales (Physeter Macrocephalus) and Baseline Ambient Ocean Sound Levels in the Greek Seas. *Mediterranean. Mar. Sci.* 20 (1), 208–221. doi: 10.12681/mms.18769

Drouot, V., Bérubé, M., Gannier, A., Goold, J. C., Reid, R. D., Palsbøll, P., et al. (2004). A Note on Genetic Isolation of Mediterranean Sperm Whales (Physeter Macrocephalus) Suggested by Mitochondrial DNA. *J. Cetacean. Res. Manage.* 6 (1), 29–32. https://www.cetaces.org/wp-content/uploads/2009/07/Drouot04_gen-jcrm.pdf

Edgar, G. J., Russ, G. R., and Babcock, R. C. (2007). "Marine Protected Areas". in *Mar. Ecol.* eds S. D. Connell and B. M. Gillanders (Oxford: Oxford University Press) 27, 533–555.

Emodnet-humanactivities.eu (2019) *EU Vessel Density Map. Detailed Method*. Available at: https://www.emodnet-humanactivities.eu/documents/Vessel%20density%20maps_method_v1.5.pdf (Accessed 1 March 2022).

Engelhaupt, D., Hoelzel, R. A., Nicholson, C., Frantzis, A., Mesnick, S., Gero, S, et al. (2009). Female Philopatry in Coastal Basins and Male Dispersion Across the North Atlantic in a Highly Mobile Marine Species, the Sperm Whale (Physeter Macrocephalus). *Mol. Ecol.* 18 (20), 4193–4205. doi: 10.1111/j.1365-294X.2009.04355.x

FAO (2020). *The State of Mediterranean and Black Sea Fisheries 2020* (Rome: General Fisheries Commission for the Mediterranean). doi: 10.4060/cb2429en Last updated 10/03/2021

Frantzis, A. (1998). Does Acoustic Testing Strand Whales? *Nature* 392 (6671), 29. doi: 10.1038/32068

Frantzis, A. (2004). The First Mass Stranding That was Associated With the Use of Active Sonar (Kyparissiakos Gulf, Greece 1996). *ECS. Newslett.* 42 (Special Issue), 14–20. https://www.europeancetaceansociety.eu/system/files/ECS%20Special%20Publication%20Series/ECS2004_News42_Active_Sonar_Workshop.pdf#page=18

Frantzis, A., Airoldi, S., Notarbartolo-di-Sciara, G., Johnson, C., and Mazzariol, S. (2011). Inter-Basin Movements of Mediterranean Sperm Whales Provide Insight Into Their Population Structure and Conservation. *Deep. Sea. Res. Part I.: Oceanog. Res. Pap.* 58 (4), 454–459. doi: 10.1016/j.dsr.2011.02.005

Frantzis, A., Alexiadou, P., and Gkikopoulou, K. (2014). Sperm Whale Occurrence, Site Fidelity and Population Structure Along the Hellenic Trench (Greece, Mediterranean Sea). *Aquat. Conservat.: Mar. Freshwat. Ecosyst.* 24 (1), 83–102. doi: 10.1002/aqc.2435

Frantzis, A., Alexiadou, P., Paximadis, G., Politi, E., Gannier, A., et al. (2003). Current Knowledge of the Cetacean Fauna of the Greek Seas. *J. Cetacean. Res. Manage.* 5 (3), 219–232. http://www.pelagosinstitute.gr/gr/pelagos/pdfs/Greek%20Cetacean%20Fauna.pdf

Frantzis, A., Leaper, R., Alexiadou, P., Prospathopoulos, A., and Lekkas, D. (2019). Shipping Routes Through Core Habitat of Endangered Sperm Whales Along the Hellenic Trench, Greece: Can We Reduce Collision Risks? *PloS One* 14 (2), e0212016. doi: 10.1371/journal.pone.0212016

Fylaktos, N., and Papanicolas, C. N. (2019)New Technologies for Eastern Mediterranean Offshore Gas Exploration. In: *European Parliamentary Research Services*. Available at: http://www.europarl.europa.eu/RegData/etudes/STUD/2019/634419/EPRS_STU(2019)634419_EN.pdf (Accessed 01 November 2020).

Güçlüsoy, H., Karauz, E. S., Kirac, C. O., and Bilecenoğlu, M. (2014). Checklist of Marine Tetrapods (Reptiles, Seabirds, and Mammals) of Turkey. *Turkish. J. Zool.* 38 (6), 930–938. doi: 10.3906/zoo-1405-81

Herut, B., Segal, Y., and Gertner, Y. (2018). *The National Monitoring Program of Israel's Mediterranean Waters—Scientific Report on Marine Pollution for 2017, Israel Oceanographic and Limnological Research; IOLR Report H50/2018; IOLR* (Israel Oceanographic and Limnological Research): Haifa, Israel pp.10–102.

Heyning, J. E. (1989). "Cuvier's Beaked Whale Ziphius Cavirostris G. Cuvier," in *Handbook of Marine Mammals Vol. 4. River Dolphins and the Larger Toothed Whales*. Eds. S. H. Ridgway and R. J. Harrison (London and San Diego: Academic Press), 289–308.

International Maritime Organization (2021) *AIS Transponders*. Available at: https://www.imo.org/en/OurWork/Safety/Pages/AIS.aspx (Accessed 25/04/2021).

IUCN-MMPATF (2017a)Hellenic Trench IMMA Factsheet. In: *IUCN Joint SSC/WCPA Marine Mammal Protected Areas Task Force*. Available at: https://www.marinemammalhabitat.org/portfolio-item/hellenic-trench/ (Accessed 27/04/2021).

IUCN-MMPATF (2017b)Cilician Basin IMMA Factsheet. In: *IUCN Joint SSC/WCPA Marine Mammal Protected Areas Task Force*. Available at: https://www.marinemammalhabitat.org/portfolio-item/hellenic-trench/ (Accessed 03/03/2021).

Jahoda, M., Lafortuna, C. L., Biassoni, N., Almirante, C., Azzellino, A., Panigada, S., et al. (2003). Mediterranean Fin Whales (Balaenoptera Physalus) Response to Small Vessels and Biopsy Sampling Assessed Through Passive Tracking and Timing of Respiration. *Mar. Mam. Sci.* 19 (1), 96–110. doi: 10.1111/j.1748-7692.2003.tb01095.x

Kerem, D., Hadar, N., Goffman, O., Scheinin, A., Kent, R., Boisseau, O., et al. (2012). Update on the Cetacean Fauna of the Mediterranean Levantine Basin. *Open Mar. Biol. J.* 6 (1), 6–27. doi: 10.2174/1874450801206010006

Laist, D. W., Knowlton, A. R., Mead, J. G., Collet, A. S., and Podesta, M. (2001). Collisions Between Ships and Whales. *Mar. Mam. Sci.* 17 (1), 35–75. doi: 10.1111/j.1748-7692.2001.tb00980.x

Lanfredi, C., Arcangeli, A., David, L., Holcer, D., Rosso, M., and Natoli, A. (2021). Grampus Griseus (Mediterranean Subpopulation). *IUCN. Red. List. Threat. Specie.*, e.T16378423A190737150. doi: 10.2305/IUCN.UK.2021-3.RLTS.T16378423A190737150.en

Lewis, T., Boisseau, O., Danbolt, M., Gillespie, D., Lacey, C., Leaper, R., et al. (2018). Abundance Estimates for Sperm Whales in the Mediterranean Sea From Acoustic Line-Transect Surveys. *J. Cetacean. Res. Manage.* 18, 103–117. https://view.officeapps.live.com/op/view.aspx?src=https%3A%2F%2Fore.exeter.ac.uk%2Frepository%2Fbitstream%2Fhandle%2F10871%2F27538%2F139-Abundance%2520estimates%2520for%2520sperm%2520whales%2520in%2520the%2520Med-Edited%2520Manuscript.doc%3Fsequence%3D1%26isAllowed%3Dy&wdOrigin=BROWSELINK

Milani, C., Vella, A., Vidoris, P., Christidis, A., and Koutrakis, E. (2019). Abundance, Distribution and Diet of the Common Dolphin, Delphinus Delphis, in the Northern Aegean Sea (Greece). *Aquat. Conservat.: Mar. Freshwat. Ecosyst.* 31, 76–86. doi: 10.1002/aqc.3081

Notarbartolo di Sciara, G., and Birkun, A. Jr. (2010). *Conserving Whales, Dolphins and Porpoises in the Mediterranean and Black Seas: An ACCOBAMS Status Report* (Monaco: ACCOBAMS), 212.

Notarbartolo di Sciara, G., Frantzis, A., Bearzi, G., and Reeves, R. R. (2006). "Sperm Whale Physeter Macrocephalus (Mediterranean Subpopulation)," in *The Status and Distribution of Cetaceans in the Black Sea and Mediterranean Sea*. Eds. R. R. Reeves and G. Notarbartolo di Sciara (Malaga, Spain:IUCN Centre for Mediterranean Cooperation, Workshop report), 45–56.

Notarbartolo di Sciara, G., Frantzis, A., Bearzi, G., and Reeves, R. (2012). Physeter Macrocephalus Mediterranean Subpopulation. In: *The IUCN Red List of Threatened Species 2012*. Available at: https://ww.iucnredlist.org/species/16370739/16370477 (Accessed 26 April 2021).

Notarbatolo di Sciara, G., and Hoyt, E. (2020). Healing the Wounds of Marine Mammals by Protecting Their Habitat. *Ethics. Sci. Environ. Politics.* 20, 15–23. doi: 10.3354/esep00190

Öztürk, A., Ayhan, D., and Tonay, A. (2016). "Cetaceans in the Turkish Waters of the Mediterranean Sea," in *The Turkish Part of the Mediterranean Sea Marine Biodiversity, Fisheries, Conservation and Governance*. Eds. C. Turan, B. Salihoglu, E. O. Özbek and B. Öztürk (Turkish Marine Research Foundation), 566–571.

Öztürk, B., Salman, A., Ozturk, A. A., and Tonay, A. (2007). Cephalopod Remains in the Diet of Striped Dolphins (Stenella Coeruleoalba) and Risso's Dolphins (Grampus Griseus) in the Eastern Mediterranean Sea. *Vie. Milieu.* 57, 57–63.

Öztürk, A., Tonay, A., and Ayhan, D. (2013). Sperm Whale (Physeter Macrocephalus) Sightings in the Aegean and Mediterranean Part of Turkish Waters. *J. Black. Sea/Mediterranean. Environ.* 19 (2), 169?177.

Öztürk, A. A., Tonay, A. M., and Dede, A. (2011). Strandings of the Beaked Whales, Risso's Dolphins, and a Minke Whale on the Turkish Coast of the Eastern Mediterranean Sea. *J. Black. Sea/Mediterr. Environ.* 17, 269–274.

Panigada, S., Azzellino, A., Cubaynes, H., Folegot, T., Fretwell, P., Jacob, T., et al. (2020). *Proposal to Develop and Evaluate Mitigation Strategies to Reduce the Risk of Ship Strikes to Fin and Sperm Whales in the Pelagos Sanctuary - Final Report* (Pelagos Secretariat - Convention), 67 pp. Available at: https://www.sanctuaire-pelagos.org/it/tous-les-telechargements/etudes-scientifiques-studi-scientifici-studies/studi-italiani/1657-2018-04/file

Panigada, S., and Notarbartolo di Sciara, G. (2012). Balaenoptera Physalus (Mediterranean Subpopulation). *IUCN. Red. List. Threat. Specie. 2012.*, e.T16208224A17549588. doi: 10.2305/IUCN.UK.2012.RLTS.T16208224A17549588.en

Panigada, S., Pesante, G., Zanardelli, M., Capoulade, F., Gannier, A., Weinrich, M.T., et al. (2006). Mediterranean Fin Whales at Risk From Fatal Ship Strikes. *Mar. Pollut. Bull.* 52 (10), 1287–1298. doi: 10.1016/j.marpolbul.2006.03.014

Papale, E., Azzolin, M., and Giacoma, C. (2012a). Vessel Traffic Affects Bottlenose Dolphin (Tursiops Truncatus) Behaviour in Waters Surrounding Lampedusa Island, South Italy. Marine Biological Association of the United Kingdom. *J. Mar. Biol. Assoc. Unite. Kingdom.* 92 (8), 1877. doi: 10.1017/S002531541100083X

Papale, E., Azzolin, M., and Giacoma, C. (2012b). Vessel Traffic Affects Bottlenose Dolphin (Tursiops Truncatus) Behaviour in Waters Surrounding Lampedusa Island, South Italy. *J. Mar. Biol. Assoc. Unite. Kingdom.* 92 (8), 1877–1885. doi: 10.1017/S002531541100083X

Paradell, O. G., López, B. D., and Methion, S. (2019). Modelling Common Dolphin (Delphinus Delphis) Coastal Distribution and Habitat Use: Insights for Conservation. *Ocean. Coast. Manage.* 179, 104836. doi: 10.1016/j.ocecoaman.2019.104836

Pesante, G., Collet, A., Dhermain, F., Frantzis, A., Panigada, S., Podestà, M., et al (2002). "Review of Collisions in the Mediterranean Sea," in *Proceedings of the Workshop "Collisions Between Cetaceans and Vessels: Can We find Solutions?"* Eds. G. Pesante, S. Panigada and M. Zanardelli (15th Annual Meeting of the European cetacean Society, Roma: ECS Newsletter).

Pirotta, E., Carpinelli, E., Frantzis, A., Gauffier, P., Lanfredi, C., Pace, D. S., et al. (2021). Physeter Macrocephalus (Mediterranean Subpopulation). *IUCN. Red. List. Threat. Specie.* e.T16370739A50285671. doi: 10.2305/IUCN.UK.2021-3.RLTS.T16370739A50285671.en

Podestà, M., Azzellino, A., Cañadas, A., Frantzis, A., Moulins, A., Rosso, M., et al. (2016). Cuvier's Beaked Whale, Ziphius Cavirostris, Distribution and Occurrence in the Mediterranean Sea: High-Use Areas and Conservation Threats. *Adv. Mar. Biol.* 75, 103–140. doi: 10.1016/bs.amb.2016.07.007

Praca, E., and Gannier, A. (2008). Ecological Niches of Three Teuthophageous Odontocetes in the Northwestern Mediterranean Sea. *Ocean. Sci.* 4, 49–59. doi: 10.5194/os-4-49-2008

Praca, E., Gannier, A., Das, K., and Laran, S. (2009). Modelling the Habitat Suitability of Cetaceans: Example of the Sperm Whale in the Northwestern Mediterranean Sea. *Deep-Sea. Res. Part I.* 56, 648–657. doi: 10.1016/j.dsr.2008.11.001

Rako, N., Fortuna, C. M., Holcer, D., Mackelworth, P., Nimak-Wood, M., Pleslić, G., et al. (2013). Leisure Boating Noise as a Trigger for the Displacement of the Bottlenose Dolphins of the Cres-Lošinj Archipelago (Northern Adriatic Sea, Croatia). *Mar. Pollut. Bull.* 68 (1), 77–84. doi: 10.1016/j.marpolbul.2012.12.019

R. Reeves and G. Noterbartolo di Sciara (Eds.) (2006). *The Status and Distribution of Cetaceans in the Black Sea and Mediterranean Sea* (Malaga, Spain: IUCN Centre for Mediterranean Cooperation), 137 pp.

Ryan, C., Cucknell, A. C., Romagosa, M., Boisseau, O., Moscrop, A., Frantzis, A., et al. (2014). "A Visual and Acoustic Survey for Marine Mammals in the Eastern Mediterranean Sea During Summer 2013," in *Unpublished Report to the International Fund for Animal Welfare* (Kelvedon, UK: Marine Conservation Research International).

Stephens, G., Akkaya, A., Hardy, J., Awbery, T., Rudd, L., Abbiss, L., et al. (2021). Sightings and Stranding Reports of Fin Whales (Balaenoptera Physalus) in the Levantine Sea. *J. Cetacean. Res. Manage.* 22 (1), 55–60. doi: 10.47536/jcrm.v22i1.212

Taylor, B. L., Baird, R., Barlow, J., Dawson, S. M., Ford, J. K. B., et al. (2012) Grampus Griseus. In: *IUCN 2013. IUCN Red List of Threatened Species*. Available at: www.iucnredlist.org (Accessed 26 April 2021).

Tyack, P. L., Zimmer, W. M. X., Moretti, D., Southall, B. L., Claridge, D. E., Durban, J.W., et al. (2011). Beaked Whales Respond to Simulated and Actual Navy Sonar. *PloS One* 6 (3), e17009. doi: 10.1371/journal.pone.0017009

Vanderlaan, A. S. M., and Taggart, C. T. (2009). Efficacy of a Voluntary Area to be Avoided to Reduce Risk of Lethal Vessel Strikes to Endangered Whales. *Conserv. Biol.* 23 (6), 1467–1474. doi: 10.1111/j.1523-1739.2009.01329.x

Woodside, J. M., David, L., Frantzis, A., and Hooker, S. K. (2006). Gouge Marks on Deep-Sea Mud Volcanoes in the Eastern Mediterranean: Caused by Cuvier's Beaked Whales? *Deep-Sea. Res.* 53 (11), 1762–1771. doi: 10.1016/j.dsr.2006.08.011

WWF-IUCN-IWC-OceanMind (2019) *WWF-IUCN-IWC-OceanMind: A Geospatial Analysis of Vessel Traffic in Important Marine Mammal Areas*. Available at: https://www.marinemammalhabitat.org/mmpatf/activities/geospatial-analysis-of-threats/ (Accessed 27/04/2021).

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Plastic additive di(2-ethylhexyl) phthalate (DEHP) causes cell death and micronucleus induction on a bottlenose dolphin's (*Tursiops truncatus*) *in vitro*-exposed skin cell line

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Marine plastic pollution is one of the most concerning worldwide environmental issues, and research is day by day demonstrating its adverse effects on marine ecosystems. Nevertheless, little is still known about the toxic potential on marine fauna of chemical additives released by plastic debris. Here we investigated the cyto- and genotoxicity of the most used plasticizer in plastic production, di(2-ethylhexyl)phthalate (DEHP), on a skin cell line (TT) derived from the bottlenose dolphin (*Tursiops truncatus*), a species particularly exposed to the accumulation of this lipophilic pollutant, being a coastal top predator rich in fatty subcutaneous tissues. Dolphin cell cultures were exposed to increasing DEHP doses (0.01–5 mM) to evaluate effects on cell viability, cell death, and induction of DNA damage. On the hypothesis that bottlenose dolphin cells show greater resistance to DEHP toxicity than terrestrial mammals, as already shown for other pollutants, the same parameters were analyzed on exposed Chinese hamster ovary (CHO) cell lines. Both MTT and Trypan Blue assays showed no significant decrease in dolphin's cell viability after 24-h DEHP exposure. No induction of primary DNA damage was detected by the comet assay, whereas the cytokinesis-block micronucleus assay revealed significant micronuclei induction and inhibition of cell proliferation starting from the lowest DEHP doses. DEHP had similar but sharper and significant effects on cell viability in CHO cells, also causing a much greater induction of necrosis than that recorded on dolphin cells. For both cell lines, the lack of induction of primary DNA damage (i.e., strand breaks) together with the increase of micronuclei yield after DEHP treatment suggests an aneugenic effect of the phthalate, that is, the loss of entire chromosomes during cell division. Overall, the potential chromosome loss detected could constitute a threat for species of marine mammals constantly exposed to plastic marine litter.

KEYWORDS

plastic additives, DEHP, DNA damage, cytotoxicity, genotoxicity, bottlenose dolphin cell line

1 Introduction

Marine debris contaminates the world's oceans from polar regions to the equator (Zarfl and Matthies, 2010; Jambeck and Johnsen, 2015; Rangel-Buitrago et al., 2020). This debris can be found floating on the sea surface (Campana et al., 2018), on the seafloor (Hardesty et al., 2021), and on shorelines (Galgani et al., 2015). Plastic has been produced and used in large quantities by humans in the last decades, and, to date, it represents the main source of anthropogenic debris in the oceans (Law et al., 2010), contributing for 70%–90% to the marine litter (Andrady, 2011).

A wide range of marine taxa, including birds, sea turtles, and marine mammals, are affected by entanglement and ingestion of macroplastic debris, with consequences including impaired movement, decreased feeding ability, reduced reproductive fitness, gastrointestinal lesions, ulcerations, and, in the most severe cases, also death (Moore, 2008; Gregory, 2009; Parker et al., 2021). Due to physical and chemical degradation, work of atmospheric agents, and seawater, plastic is fragmented in micro (<5 mm) and nano debris (<20 µm) (Koelmans et al., 2015). Impacts caused by microplastic debris in the oceans derive from both fragmentation (i.e., secondary microplastic) and microplastics produced as such (i.e., primary microplastic). Fragments of small size are facilitated to enter the trophic chains, becoming a serious threat to aquatic organisms inhabiting both continental and marine ecosystems (Eriksen et al., 2013; Castañeda et al., 2014; do Sul and Costa, 2014; Gambardella et al., 2018; Costa et al., 2020). Indeed, laboratory studies have shown that invertebrates such as crustaceans, barnacles, polychaetes worms, mussels, and amphipods can ingest microplastic fragments (Browne et al., 2008; Graham and Thompson, 2009; Gambardella et al., 2017), and there is increasing evidence documenting the ingestion of plastic fragments by invertebrates also in the natural environment (Boerger et al., 2010; Murray and Cowie, 2011; Macali et al., 2018).

Although plastic polymers are considered to be chemically inert, in the last years, the scientific community has focused attention on the smallest plastic debris, since they can be vectors of lipophilic pollutants collected in the sea and then absorbed by feeding organisms (Koelmans et al., 2021). Moreover, plastic can contain plasticizer additives whose release is facilitated by plastic degradation in seawater (Paluselli et al., 2019). Phthalate esters (PAEs) are widely used as plasticizers in polyvinyl chloride (PVC) and other plastic polymers formulation, such as in the manufacture of construction products, medical devices, pharmaceuticals, and personal care products (Heudorf et al., 2007). PAEs are easily leached into the aquatic environment, as they are not chemically bound to polymers (Fromme et al., 2002). Moreover, wastewaters often contain relevant concentrations of PAEs (Tran et al., 2022), which are partly transported to the sea by river runoff. Among PAEs, di(2-ethylhexyl)phthalate (DEHP) is the most widely produced and used, as well as the most persistent phthalate found in seawaters (Chaler et al., 2004; Bergé et al., 2013). Indeed, DEHP was reported to reach a high concentration in the marine environment, up to 11,500 µg/kg dw in marine sediments, 4.35 µg/L in marine water, and 1,573 µg/kg ww in marine fishes (Stewart et al., 2014; Hermabessiere et al., 2017; Zhang et al., 2018; Malem et al., 2019; Hu et al., 2020). DEHP-related adverse effects on biota have been demonstrated for many organisms. DEHP has been reported to impair development and reproductive function through activation of the estrogen receptor (ER) and peroxisome proliferator-activated receptor (PPAR) in mammals (Lyche et al., 2009; Magdouli et al., 2013). The European Scientific Committee on Toxicity, Ecotoxicity and Environment (CSTEA) indicated DEHP as an endocrine disruptor, dangerous for reproduction in both mammals and aquatic species. Other studies focused on aquatic organisms, such as shellfish, crustaceans, annelids, and fishes (Oehlmann et al., 2009; Zhang et al., 2021). DEHP showed antiestrogenic activity in female medaka (*Oryzias latipes*), causing retardation of oocyte development (Kim et al., 2002), and it deeply impaired fecundity, oogenesis, and embryo production in female zebrafish (*Danio rerio*) by affecting signals involved in oocyte maturation (Carnevali et al., 2010). Furthermore, DEHP disrupted spermatogenesis by interfering with signaling pathways in the testis and the liver of adult male zebrafish (Uren-Webster et al., 2010). Additionally, DEHP caused endocrine-disrupting effects, altering sex hormone levels in some freshwater fish species, such as the Chinese rare minnow (*Gobiocypris rarus*) (Wang et al., 2013), carp (*Cyprinus carpio*) (Thibaut and Porte, 2004), and fathead minnows (*Pimephales promelas*) (Crago and Klaper, 2012). Finally, recent *in vitro* studies demonstrated DEHP hazard, emphasizing its cytotoxic, genotoxic, and aneugenic effects on marine organisms (Hermabessiere et al., 2017; Molino et al., 2019; Zhang et al., 2021). Although marine environments are heavily prone to phthalate pollution, studies on DEHP-related adverse effects on marine organisms are still relatively scarce. In particular, marine mammals can be subjected to such an accumulation of phthalates, which some authors consider concentrations of both DEHP and its metabolite MEHP in their tissues as a measure for assessing the levels of plastic pollution in the sea (Fossi et al., 2014). Indeed, marine mammals, such as cetaceans, are top predators of large dimensions, rich in subcutaneous adipose tissues in which pollutants tend to concentrate, due to the great quantities of contaminated food they ingest and to the processes of bioaccumulation and biomagnification of lipophilic xenobiotics (Zantis et al., 2021). However, marine mammals may exhibit higher resistance to the toxic effects of xenobiotics as compared to terrestrial mammals. For example, as reported by Chen and coworkers (2012), the exposure to chromium induced lower cytotoxicity and clastogenic effect in sperm whales' skin fibroblast with respect to human fibroblast. Similarly, Taddei and coworkers (2001) detected a greater resistance of bottlenose dolphin leukocytes to the genotoxic effect of methyl mercury chloride when compared to human cells, demonstrating a higher DNA repair efficiency of dolphin cells. These results suggest the possible development of defense strategies to contrast both dietary and environmental exposure to pollutants in marine mammals (Taddei et al., 2001; Chen et al., 2012).

Among cetaceans, the bottlenose dolphin (*Tursiops truncatus*) is particularly exposed to pollution by plastic and its additives, inhabiting highly contaminated coastal areas, often in the proximity of the river mouths, where primary and secondary productivity is high (Cafaro et al., 2015). Despite this, to the best of our knowledge, there are no studies evaluating the toxic potential of phthalates on cetaceans.

This study aims to test the possible cytotoxic and genotoxic effects of DEHP in a bottlenose dolphin's skin cell line. Cells were exposed to increasing doses of DEHP for 24 h, and effects were evaluated in terms of cell viability, cell death, primary DNA damage induction, and micronucleus formation. As a term of comparison, the same parameters were analyzed on Chinese hamster ovary (CHO) cells, an established cell line widely used in genotoxicity testing. This had the dual purpose of verifying whether DEHP produced comparable effects on different cell lines and whether bottlenose dolphin cells were more resistant also to the toxic effects of phthalates than those of a terrestrial mammal.

2 Materials and methods

2.1 Chemicals

Dulbecco's modified Eagle's medium (DMEM), Ham's F10 Nutrient mix (F-10), phosphate-buffered saline (PBS) without Ca^{2+} and Mg^{+}, and L-glutamine were purchased from Lonza (Rome, Italy). Penicillin/streptomycin and trypsin-EDTA were purchased from EuroClone (Pero, Italy). Bis(2-ethylhexyl) phthalate (DEHP, ≥98.0% purity, CAS: 117-81-7), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), cytochalasin B, Trypan Blue solution (0.4%), dimethyl sulfoxide (DMSO), and sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (Milan, Italy). Fetal bovine serum (FBS) was purchased from Invitrogen (Milan, Italy).

2.2 Cell lines and culture conditions

Primary cell culture of *T. truncatus* (TT) derived from fresh skin tissue samples was collected during the post-mortem examination of a freshly dead stranded adult bottlenose dolphin. Primary cell culture was performed following an established laboratory protocol (Peruffo et al., 2004). To immortalize the cells, the primary cell culture was transfected with pSV3neo plasmid (LGC Promochem, Teddington, UK) by using the cationic lipid Lipofectamine 2000 (Invitrogen, Carlsbad, CA, USA), following the protocol by Suman et al. (2012). Resistant cells were selected with the antibiotic G418 (400 µg/ml; Gibco, Life Technologies BRL, Gaithersburg, MD, USA).

TT cells were grown in DMEM supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin.

The CHO cell line was grown in Ham's F10 supplemented with 10% FBS, 1% L-glutamine, and 1% penicillin/streptomycin. This epithelial-like cell line is routinely used in mutagenicity testing as recommended by the guidelines of European Economic Community (EEC) Council 79/831 and Organisation for Economic Co-operation and Development (OECD) for the test of chemical No. 471.

Both cell lines were maintained in an incubator at 37°C, 5% CO_2, and 95% humidity.

2.3 Cell culture treatments

DEHP, purchased at a concentration of 100 mM, was diluted at 5 mM with DMSO. Further dilutions were freshly made before

DEHP treatments in order to have a DMSO concentration not exceeding 1% in the culture medium. DEHP treatments were performed for 24 h in TT and CHO cell lines, and DEHP was used in a range between 0.01 and 5 mM in both cell lines.

The solvent sample was treated with 1% DMSO for 24 h, while the positive control was treated with 100 μM of H_2O_2 for 1 h. Each experiment was repeated at least two times, and the results are displayed as the mean of two independent experiments, showing good reproducibility and comparable outcomes.

2.4 Cytotoxicity

2.4.1 Cell viability assay

To study cell viability after DEHP treatment, the MTT assay was performed in accordance with Botta and collaborators (2019), with minor modifications. Briefly, for each experimental point, cells were seeded onto 96-well microplates at a density of 5,000 cells/100 μl for TT and 2,000 cells/100 μl for CHO and incubated for 24 h to allow cell adherence. The growth medium was then replaced by a fresh medium containing DEHP and incubated for a further 24 h. At the end of treatments, MTT was added to each well (0.5 mg/ml), and cells were incubated for an additional 3 h at 37°C. After incubation, the supernatant was replaced with 100 μl of lysis solution (10% SDS and 0.6% acetic acid in DMSO) to dissolve the formazan crystals and produce a purple solution. Optical density measurements were obtained using a scanning spectrophotometer DTX 880 Multimode Detector (Beckman Coulter, Brea, CA, USA). Readings were made using 630-nm (background) and 570-nm filters.

Cell viability, presented as the relative optical density (OD) at 570, was calculated using the following formula:

$$\mathrm{OD} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of control cells}}$$

2.4.2 Trypan Blue exclusion assay

For each experimental point, cells were seeded onto 35-mm Petri dishes with 2 ml of the medium at a density of 1.5×10^5 cells/dish for TT and 1×10^5 cells/dish for CHO and incubated for 24 h. Cell lines were then treated with DEHP and incubated for a further 24 h. At the end of treatments, cells were harvested, and 10 μl of cell suspension was mixed with 10 μl of Trypan Blue Solution (1:1; w:w) for 5 min to allow cell staining; cells were then seeded on a slide and counted under an optical microscope (Molino et al., 2019). For each experimental point, 250 cells were counted by two different operators. The percentage of cell viability was calculated using the following formula:

$$\text{Cell viability}(\%) = \frac{viable\ \ cells}{total\ \ cells} \times 100$$

2.4.3 Detection of DEHP-induced cell death by fluorescence staining

To evaluate DEHP-induced cell death, cells were seeded onto 35-mm Petri dishes at the same density of Trypan Blue exclusion (TBE) assay, incubated for 24 h, and then treated with DEHP for a further 24 h. At the end of treatments, cells were harvested, and to distinguish apoptotic and necrotic cells from viable cells, a combination of fluorescein di-acetate (FDA, 0.75 mg/ml), propidium iodide (PI, 0.25 mg/ml), and Hoechst (HO, 0.1 mg/ml) dyes were used (Proietti De Santis et al., 2001; Filippi et al., 2022). FDA and HO are vital dyes that stain, respectively, the cytoplasm and the nucleus of viable cells; PI staining identifies the necrotic and late stage of apoptotic cells; cells in the early phase (viable HO stained) and late phase (dead PI stained) of apoptosis displayed the characteristic pattern of chromatin fragmentation. For each experimental point, 500 randomly selected cells were counted by two different operators.

2.5 Genotoxicity

2.5.1 Single-cell gel electrophoresis analysis

To quantify primary DNA damage in terms of single-strand breaks, the alkaline version of the single-cell gel electrophoresis (Comet assay) was performed. Cells were seeded onto 35-mm Petri dishes with 2 ml of the medium at the same density of TBE assay, incubated for 24 h to allow cell adherence, and then treated for 24 h with DEHP. The test procedure was performed according to previous works (Egidi et al., 2018; Molino et al., 2019). After slide preparation and cell lysis, electrophoresis was conducted for 20 min at 25 V and 300 mA at 4°C preceded by a 15-min incubation in electrophoresis buffer to allow DNA unwinding. Slides were neutralized and stained with ethidium bromide (20 μg/ml, 50 μl), and nucleoids were analyzed at ×400 magnification with an automatic image analyzer (Comet Assay III, Perceptive Instruments, St Edmunds, UK) connected to a fluorescence microscope (Axioskop 2, Zeiss, Oberkochen, Germany). To evaluate the amount of DNA damage, computer-generated % DNA in the tail (tail intensity (TI)) values were used. For each experimental point, a total of 200 randomly selected cells were scored by two different operators.

2.5.2 Cytokinesis-block micronucleus assay

The cytokinesis-block micronucleus (CBMN) assay was carried out with the standard technique proposed by Fenech (1993), with minor adjustments (Meschini et al., 2018). For each experimental point, cells were seeded onto 60-mm Petri dishes with 3 ml of the medium at a density respectively of 2.5×10^5 cells/dish for TT and 3×10^5 cells/dish for CHO, incubated for 24 h, and then treated with DEHP for 24 h. At the end of DEHP treatment, cells were washed with PBS, and a fresh medium containing 6 μg/ml of cytochalasin B was added for a further

24 h in order to arrest cytokinesis. For each experimental point, 1,000 binucleated cells with intact cytoplasm were scored by three different operators for the presence of micronuclei (MN), a biomarker of chromosome breakage or loss. For the analysis of cell cycle progression, 1,000 cells for each sample were scored for the presence of one, two, or more than two nuclei.

Cytokinesis-block proliferation index (CBPI) was calculated using the following formula: $\frac{[1N+(2\ x\ 2N)+(3\ x\ >2N)]}{Total\ cells\ examinated}$ ($1N$ means cells with 1 nucleus; $2N$, cells with 2 nuclei; and $>2N$, cells with more than 2 nuclei).

The percentage of cytostasis was calculated with the following formula: $100 - 100\frac{CBPIt-1}{CBPIc-1}$,where t and c are treated and control samples, respectively (Lorge et al., 2008).

2.6 Statistical analysis

For viability tests (MTT, TBE, and cell death assays), statistical significance of raw data between treated samples and their relative solvent in each cell line was evaluated using one-way ANOVA followed by Sidak's or Tukey's multiple comparisons post-test. Data belonging to different experiments were represented and averaged in the same graph. The GraphPad Prism software package (GraphPad Software, San Diego, CA, USA) was used. Results were expressed as means ± SD. For the cytostatic effect (CBMN assay), the chi-squared test (χ^2) was used to compare treated samples and their relative solvent for each cell line. For the Comet assay and the yield of micronuclei per cell, Student's t-test was applied. For the comparison of cytotoxicity between the two cell lines, the data were analyzed with the Kruskal–Wallis test (KW). For pairwise comparisons (apoptosis, necrosis, and CBPI), the data were analyzed with the Wilcoxon test (WT). For comparisons, data were normalized with respect to the relative solvent, and the results of medium, positive, and solvent control were excluded. All analyses for the comparison of the two cell lines were performed with R. The levels for statistical significance were set at $p \leq 0.05$ and $p \leq 0.01$.

3 Results

3.1 Cytotoxicity

3.1.1 MTT assay

Figure 1A shows the results of cell viability measured by MTT assay in TT and CHO cell lines after 24 h of treatment with DEHP. The analysis revealed no significant effects of the solvent, whereas H_2O_2 (100 μM) caused a significant decrease in cell viability of about 70% in both cell lines ($p \leq 0.01$). After DEHP treatments, a reduction in cell viability was observed in both cell lines. Only for the CHO cells the reduction was statistically significant ($p \leq 0.05$; $p \leq 0.01$), with respect to the solvent, starting at 0.02 mM. Conversely, in the TT cell line, no significant effect on the decrease of cell viability was detected. In both cell lines, the reduction reached a plateau at the three highest doses.

3.1.2 Trypan Blue exclusion assay

Figure 1B shows the results of cell viability measured by TBE assay in TT and CHO cell lines, after 24 h of treatment with increasing DEHP concentrations. The solvent did not affect cell viability in both cell lines, whereas the treatment with H_2O_2 (100 μM) caused a decrease in cell viability, which was 24.5% and 20.9% in TT and CHO, respectively. DEHP treatments caused a dose-dependent reduction of cell viability in both cell lines but of a greater and statistically significant ($p \leq 0.05$; $p \leq 0.01$) magnitude only for the CHO cell line.

3.1.3 Detection of DEHP-induced cell death by fluorescence staining

Results obtained by fluorescence staining are shown in Figures 2A, B for TT and CHO cell lines, respectively. TT cells showed about 10% of dead cells in both control and solvent samples, mostly due to necrosis (8.7% and 7.9%, respectively), whereas total cell mortality of CHO cells resulted in approximately 1% and 2% for the two aforementioned samples. In both cell lines, the positive control (H_2O_2, 100 μM) showed a significant increase in necrotic cells, with respect to the control ($p \leq 0.05$ for both TT and CHO cells). After 24 h of DEHP treatments, the TT cell line displayed no significant increases in either apoptosis or necrosis in treated cells with respect to the solvent. Conversely, the CHO cell line showed a significant increase of necrotic cells at all DEHP concentrations ($p \leq 0.05$; $p \leq 0.01$), which appears to be dose-dependent at the four higher concentrations. No increases in apoptosis occurred at any DEHP concentration.

3.2 Genotoxicity

3.2.1 Comet assay

Comet assay results are shown in Table 1. In both TT and CHO cell lines, control cells showed a mean TI of 6.70 and 5.76, respectively. In both cell lines, treatment with solvent did not increase TI with respect to the control, while treatment with H_2O_2 (100 μM) caused a significant increase in both cell lines ($p \leq 0.01$), reaching 12.27 and 11.95 in TT and CHO cell lines, respectively. Conversely, DEHP treatments did not exert any increase of primary DNA damage with respect to the solvent, neither in the TT nor in the CHO cell line.

3.2.2 Cytokinesis-block micronucleus assay

Table 2 shows the results obtained performing the CBMN assay in TT and CHO cell lines after 24 h of DEHP treatments.

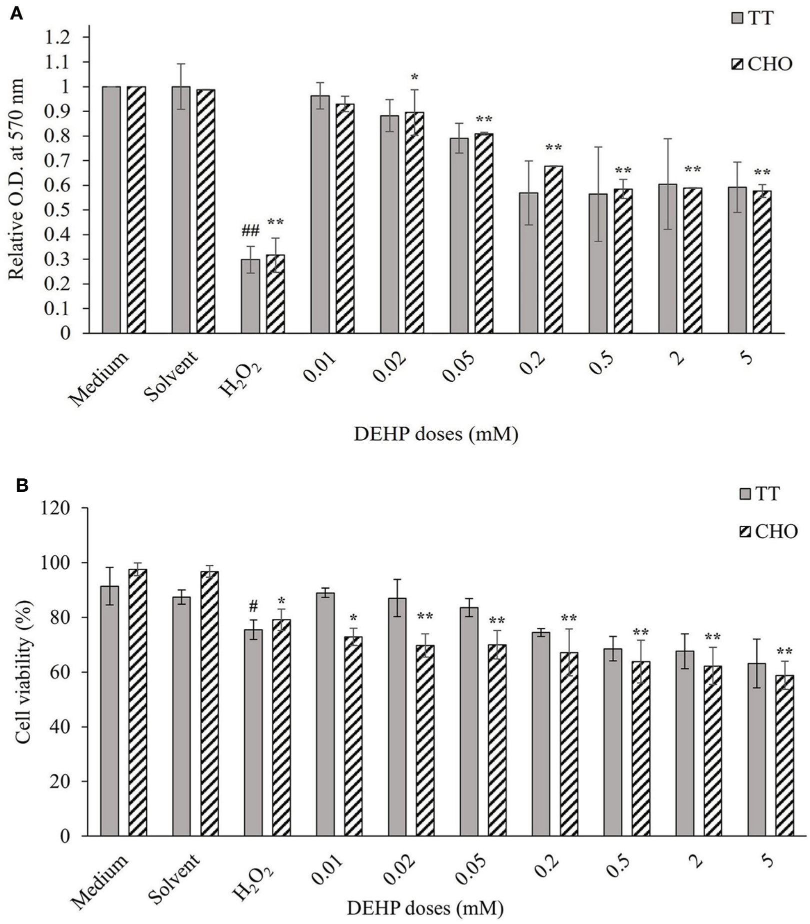

FIGURE 1

Cellular viability in DEHP-exposed TT and CHO cell lines. **(A)** MTT assay results are displayed as a mean of the optical density (OD) at 570 nm, at each treatment level normalized to the relative solvent (DMSO) in each cell line. Data are expressed as mean ± SD of two independent experiments. **(B)** Trypan Blue exclusion assay results are expressed as the percentage of viable cells out of the total cells at each treatment level. Data are expressed as mean ± SD of two independent experiments. One-way ANOVA significance: $^{\#}p \leq 0.05$ H_2O_2 vs medium in TT cell line; ##$p \leq 0.01$ H_2O_2 vs medium in TT cell line; *$p \leq 0.05$ H_2O_2 vs medium and treated vs solvent in CHO cell line; **$p \leq 0.01$ H_2O_2 vs medium and treated vs solvent in CHO cell line. DEHP, di(2-ethylhexyl)phthalate; TT, *Tursiops truncatus*; CHO, Chinese hamster ovary; DMSO, dimethyl sulfoxide.

In both cell lines, no differences in either MN frequencies or CBPI values emerged in the solvent samples, when compared to controls. Treatment with H_2O_2 (100 μM) caused significant increases in MN frequencies ($p \leq 0.01$) and significant decreases in CBPI values ($p \leq 0.01$) in both cell lines, coupled with an increase in the percentage of cytostasis.

After DEHP treatments, in the TT cell line, the CBMN assay revealed a statistically significant increase in the frequencies of MN at all DEHP concentrations ($p \leq 0.01$), when compared to the solvent. Moreover, a plateau trending increase in the yield of MN was observed, as well as a dose-dependent decrease of CBPI values and a statistically significant ($p \leq 0.01$) increase in the percentage of cytostasis with respect to the solvent.

In the CHO cell line, DEHP treatments induced a statistically significant ($p \leq 0.01$) and dose-dependent increase in the frequency of MN when compared with the solvent. A dose-dependent decrease of CBPI values and a statistically significant increase in the percentage of cytostasis at all DEHP concentrations ($p \leq 0.01$) with respect to the solvent were observed.

3.2.3 Statistical comparison of cytotoxic effects in the two cell lines

The comparison of the cumulative cytotoxic effect (MTT + TBE) detected in the two cell lines highlighted the greater effect exerted by DEHP on the CHO cell line in terms of cell viability reduction and cell death induction, which resulted in statistical

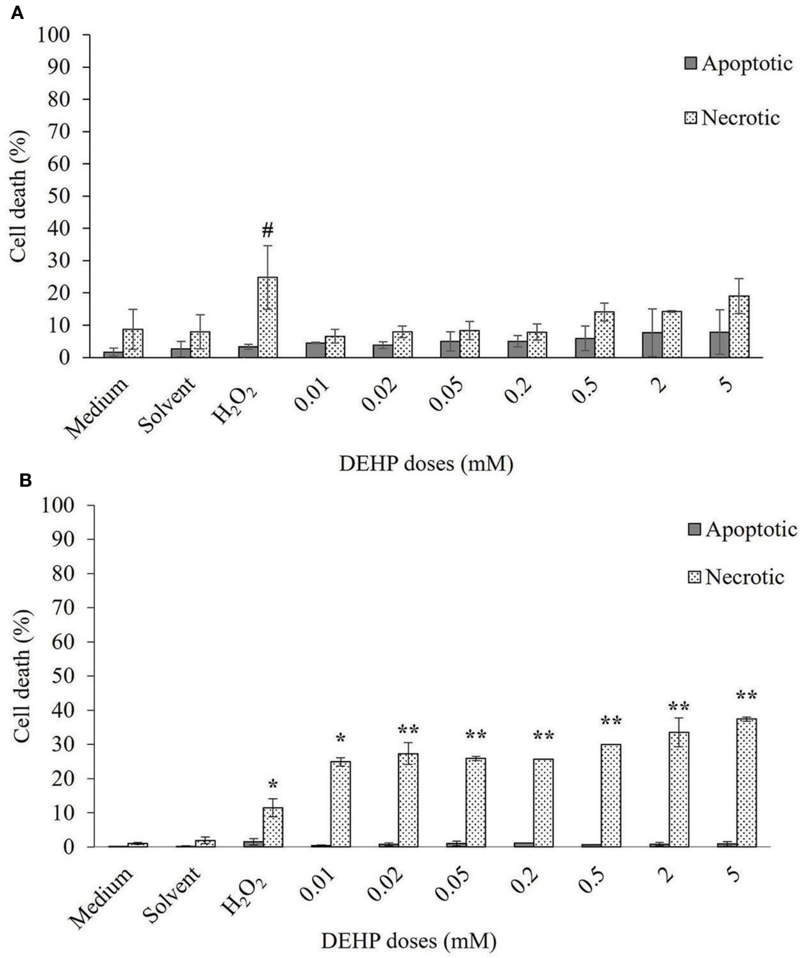

FIGURE 2
Induction of apoptosis and necrosis in TT **(A)** and CHO **(B)** cell lines after 24 h of exposure at increasing doses of DEHP. Data are expressed as means ± SD of two independent experiments. One-way ANOVA significance: # $p \leq 0.05$ H_2O_2 vs medium in TT cell line; * $p \leq 0.05$ H_2O_2 vs medium and treated vs solvent in CHO cell line; ** $p \leq 0.01$ treated vs solvent in CHO cell line. TT, *Tursiops truncatus*; CHO, Chinese hamster ovary; DEHP, di(2-ethylhexyl)phthalate.

significance (KW $p \leq 0.01$) when compared to TT cells. Nevertheless, the higher CHO mortality was due to a significantly higher necrosis induction (WT $p \leq 0.01$), whereas the TT cell line still overwhelmed the CHO cell line in terms of induced apoptosis (WT $p \leq 0.01$). Lastly, the comparison of the inhibition of cell proliferation (CBPI) showed a higher effect exerted by DEHP on the TT cell line (WT $p \leq 0.05$).

4 Discussion

In the last few years, plastic pollution has become a global problem. DEHP, a plasticizer used in various plastic products, is one of the most common PAEs detected in the environment and especially in the marine environment, where it derives mainly from rivers' input and chemical and physical degradation of

TABLE 1 Tail intensity (%) values obtained through the Comet assay in TT and CHO cell lines treated for 24 h with DEHP.

Cell line	Medium	Solvent	H_2O_2	DEHP doses (mM)					
				0.02	0.05	0.2	0.5	2	5
TT	6.70 ± 1.74	7.49 ± 0.07	12.27 ± 1.44§§	7.52 ± 0.71	6.76 ± 0.12	5.25 ± 0.21	5.54 ± 0.43	5.85 ± 3.22	5.27 ± 0.66
CHO	5.76 ± 0.10	6.46 ± 1.32	11.95 ± 2.35§§	6.53 ± 1.21	6.40 ± 1.03	6.33 ± 1.74	5.21 ± 0.06	6.31 ± 1.90	5.76 ± 1.02

Data are presented as means ± SD of two independent experiments for each treatment. Significance of Student's *t*-test (ts): §§ $p \leq 0.01$ H_2O_2 vs medium.
TT, *Tursiops truncatus*; CHO, Chinese hamster ovary; DEHP, di(2-ethylhexyl)phthalate.

TABLE 2 Induction of micronuclei (MN), cytokinesis-block proliferation index (CBPI), and % of cytostasis in TT and CHO cell lines treated for 24 h with DEHP.

Treatment	Harvesting time after cyto-B	MN/1000 BN ± SE Student's *t*-test (ts)		CBPI ± SE Chi-squared test (χ_2)		% Cytostasis ± SE	
		TT	CHO	TT	CHO	TT	CHO
Medium	24 h	21.5 ± 0.03	17 ± 0.08	1.65 ± 0.0006	1.69 ± 0.002	0 ± 0.00	0 ± 0.00
Solvent	24 h	19.5 ± 0.08^{NS}	18.3 ± 0.05^{NS}	1.64 ± 0.0007^{NS}	1.67 ± 0.003^{NS}	2.4 ± 0.04	3.4 ± 0.1
H_2O_2	24 h	75.2 ± 0.72$^{\$\$}$	84.6 ± 0.04$^{\$\$}$	1.31 ± 0.002$^{\$\$}$	1.28 ± 0.0006$^{\$\$}$	53.0 ± 0.40	59.6 ± 0.06
0.01 mM	24 h	35.8 ± 0.18**	39.9 ± 0.12**	1.54 ± 0.001**	1.67 ± 0.002**	16.3 ± 0.13	3.6 ± 0.03
0.02 mM	24 h	49.0 ± 0.28**	44.7 ± 0.15**	1.52 ± 0.002**	1.62 ± 0.003**	16.6 ± 0.19	8.1 ± 0.02
0.05 mM	24 h	45.8 ± 0.09**	51.9 ± 0.06**	1.49 ± 0.001**	1.57 ± 0.003**	22.4 ± 0.10	16.2 ± 0.04

Data are presented as means ± SE of two independent experiments for each treatment. Significance of Student's *t*-test (ts) and chi-squared test (χ_2): NS, not significant; ** $p \leq 0.01$ treated vs solvent; $^{\$\$}$ $p \leq 0.01$ H_2O_2 vs medium.
TT, *Tursiops truncatus*; CHO, Chinese hamster ovary; DEHP, di(2-ethylhexyl)phthalate.

plastic litter (Selvaraj et al., 2015; Paluselli et al., 2018; Zhang et al., 2021). DEHP causes immunotoxicity, metabolic toxicity, neurotoxicity, and endocrine toxicity in both terrestrial mammals (Chang et al., 2017; Radke et al., 2020; Weaver et al., 2020) and aquatic organisms (Molino et al., 2019; Yu et al., 2019; Xu et al., 2021; Zhang et al., 2021). In the current study, results of *in vitro* exposure to DEHP on TT and CHO cell lines highlighted cytotoxic and genotoxic effects, confirming that this phthalate constitutes an important threat to both marine and terrestrial mammals.

Both cytotoxicity assays revealed an effect of DEHP treatments in both cell lines, which resulted in statistical significance only in CHO cells. Therefore, both tests highlighted a higher sensitivity in terms of DEHP cytotoxicity on the CHO cell line; results were also supported by the analysis of cell death. It is worth noting that the differences that occurred between the two cell lines were likely due to a different sensitivity to DEHP, given that treatments with H_2O_2 caused nearly the same detrimental effect in terms of both cell viability and cell death in TT and CHO lines. Indeed, hydrogen peroxide can have immediate cellular effects when diffusing through cells and tissues (Sies, 2017). Similarly, previous studies have shown that DEHP has direct cytotoxic and genotoxic effects on several eukaryotic cell lines (Caldwell, 2012; Peropadre et al., 2013; Erkekoglu and Kocer-Gumusel, 2014; Pournejati et al., 2021). DEHP is rather stable in the aqueous phase, degrading under the specific condition of UV radiation intensity, pH, and temperature (Chen, 2010; Huang et al., 2017). Therefore, considering the purity of the DEHP tested (≥98%) and that the experiments were carried out in stable environmental conditions, our results could be explained by a direct effect of DEHP on both TT and CHO cell lines with a greater sensitivity of the latter. Cytotoxicity results are in agreement with previous studies, which demonstrated the cytotoxic effect of DEHP on both fishes (Zheng et al., 2013; Molino et al., 2019; Wang et al., 2020) and mammals, including humans (Eljezi et al., 2017; Eljezi et al., 2019; Weaver et al., 2020; Radke et al., 2020; Zhang et al., 2021). Furthermore, it has been observed a greater resistance of marine mammal cells to the cytotoxic effect of persistent pollutants, which might be due to adaptations and the development of more efficient detoxification mechanisms (Chen et al., 2009; Chen et al., 2012; Gui et al., 2014). In this context, the results obtained *in vitro* in this study also suggest a higher resistance to the effect of DEHP treatment on the fibroblasts of the bottlenose dolphin cell line compared to the CHO epithelial-like cells.

As for genotoxicity, in both TT and CHO cells, a clear genotoxic effect of DEHP was observed, as revealed by micronuclei induction. The Comet assay, carried out to verify the potential clastogenic effect of DEHP, detected no effect in either cell line, whereas the positive control with H_2O_2 always induced a statistically significant increase in DNA strand breaks. Conversely, in both cell lines, the CBMN assay showed an effect of DEHP with an increase in the frequency of micronuclei coupled with an increment of cytostasis and a dose-dependent decrease in cell proliferation. Moreover, when compared with CHO cells, the TT cell line displayed a higher cytostatic effect of DEHP. This affects the expression of chromosomal damage in terms of micronuclei that are dose-dependent in the CHO cell line while reaching a plateau in TT cells. Thus, the higher cytostasis detected in TT probably did not allow to fully detect cytogenetic damage induced by DEHP on the TT cell line. Performing both the Comet assay and the CBMN assay allows to detect with high sensitivity clastogenic and aneugenic substances (Araldi et al., 2015); therefore, their comparison suggests that micronuclei formation after DEHP exposure was not due to chromosome breakage but potentially by the loss of the entire chromosomes, due to an aneugenic effect of DEHP. This result indicates that DEHP may represent a greater risk for the bottlenose dolphin.

Previous genotoxic investigation of DEHP effects in mammals (Caldwell, 2012; Erkekoglu and Kocer-Gumusel, 2014) and fishes (Khalil et al., 2016; Ma et al., 2018; Molino et al., 2019) demonstrated both an increase in DNA strand breaks and an induction of micronuclei. The comparison with other data on aquatic organisms, like the European seabass (Molino et al., 2019) and the zebrafish (Chen et al., 2014), emphasizes a higher sensitivity of teleosts when compared to the bottlenose dolphin. Higher resistance of marine mammals, compared to other aquatic organisms, has already been suggested for other xenobiotics (e.g., PCB and heavy metals), which has been ascribed as an adaptation in response to high levels of contamination to which marine mammals are subjected (Chen et al., 2009; Desforges et al., 2016). With regard to PAEs, it has been shown that their concentration in the body of high-trophic-level organisms is often lower than that of organisms at the lower trophic levels (Sun et al., 2016; Zhang et al., 2021). This suggests a higher metabolic capacity of high-trophic-level organisms to produce PAE metabolites, which usually results in less toxicity than their parent compounds (Ye et al., 2014; Zhang et al., 2021).

The concentration of anthropogenic xenobiotics in marine mammals' blubber can be used as an indicator of the sea's contamination levels since marine mammals play an important role at the top of the food webs (Bossart, 2011; Desforges et al., 2016). However, due to obvious ethical and conservation reasons, there is severe legislation aimed at protecting marine mammals (Habitat Directive, 1992; ACCOBAMS, 2001; Marine Strategy Framework Directive, 2008), which prevents *in vivo* studies on these organisms. Thus, *in vitro* experiments afford the opportunity to evaluate marine mammals' cellular response to xenobiotics and to make hypotheses regarding ecotoxicology hazards to wild organisms. The present work pays attention to one of the main and, to date, still poorly investigated threats of marine plastic litter: the ecotoxicological risk of plastic additives and its potential threat to marine mammals. Our approach permitted the detection of the cytotoxic and genotoxic effects caused by DEHP exposure, showing its effects on both cellular viability and DNA integrity in *T. truncatus*. Therefore, DEHP might be considered an additional stressor to the multiple threats that act synergistically and undermine marine mammals' conservation. It is also relevant to pay attention to the potential chromosome loss detected since it is considered a mutation, which could be particularly insidious for the species and the conservation of genetic patterns (Fenech, 2008; Fan et al., 2019). Indeed, genetic damage can potentially extend from the individual to the population (Fan et al., 2019). Moreover, a link between marine mammal death and chemical pollution in the sea cannot be excluded, as in the past epizootic events (e.g., *Cetacean morbillivirus*) were connected to immunosuppression caused by high levels of contamination in the organism (Beineke et al., 2005; Mori et al., 2008; Beineke et al., 2010).

In conclusion, this study showed relevant cytotoxic and genotoxic effects of DEHP on both TT and CHO cell lines, occurring mainly as cell death, inhibition of cell proliferation, and induction of micronuclei. Moreover, data indicated also a different effect of DEHP treatment on the two cell lines such as a higher cytostasis on TT cells and stronger cytotoxicity on the CHO cell line as well as a greater resistance of the former to the toxic effects of phthalates. Although relatively high, the DEHP concentrations applied in the present study are similar to those recently found in *T. truncatus* blubber samples of 26,068 ng/g (about 0.07 mM), which is in the lower range of our treatments (Baini et al., 2017). Therefore, the current study underlines the importance of learning more about DEHP's potential threat to the bottlenose dolphin and possibly other marine mammals, which are constantly exposed to plastic marine litter. In this respect, *ex vivo* studies could represent an additional approach to further assess the effects of DEHP.

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: http://hdl.handle.net/2067/43634 - University of Tuscia Archive of research doctorate thesis.

Author contributions

GG contributed by conducting both cytotoxicity and genotoxicity assays, drawing figures and tables, performing data analysis, statistical analysis with the R software, and writing the first draft of the manuscript. SF contributed to designing the experimental work, collaborating in both cytotoxicity and genotoxicity assays, performing data analysis, and revising the final draft of the manuscript. CM contributed by conducting both cytotoxicity and genotoxicity assays, drawing figures and tables, performing data analysis, and revising the first and the final draft of the manuscript. AP and CC contributed by kindly providing the *T. truncatus* cell line and revising the final draft of the manuscript. RM contributed to the conception and design of the work, interpretation of the data, and revising the final draft of the manuscript. DA contributed to the conception and design of the work, interpretation of the data, and writing the first draft of the manuscript. All the authors approved the final version of the manuscript to be published.

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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.

Publisher's note

References

Peropadre, A., Freire, P. F., Herrero, O., Martín, J. P., and Hazen, M. J. (2013). Cytotoxic effects of di (2-ethylhexyl) phthalate on cultured mammalian cells. *Toxicology* 9, 35–42.

Peruffo, A, Massimino, M.L., Ballarin, C., Carmignoto, G., Rota, A., and Cozzi, B. (2004). Primary cultures from fetal bovine brain. *Neuroreport* 15 (11), 1719–1722. doi: 10.1097/01.wnr.0000136036.15977.3a

ACCOBAMS (2001). *The agreement on the conservation of cetaceans of the black Sea, Mediterranean Sea and contiguous Atlantic area*. Available at: http://www.accobams.org.

Andrady, A. L. (2011). Microplastics in the marine environment. *Mar. pollut. Bull.* 62 (8), 1596–1605. doi: 10.1016/j.marpolbul.2011.05.030

Araldi, R. P., de Melo, T. C., Mendes, T. B., de Sá Júnior, P. L., Nozima, B. H. N., Ito, E. T., et al. (2015). Using the comet and micronucleus assays for genotoxicity studies: A review. *BioMed. Pharmacother.* 72, 74–82. doi: 10.1016/j.biopha.2015.04.004

Baini, M., Martellini, T., Cincinelli, A., Campani, T., Minutoli, R., Panti, C., et al. (2017). First detection of seven phthalate esters (PAEs) as plastic tracers in superficial neustonic/planktonic samples and cetacean blubber. *Anal. Methods* 9 (9), 1512–1520. doi: 10.1039/C6AY02674E

Beineke, A., Siebert, U., McLachlan, M., Bruhn, R., Thron, K., Failing, K., et al. (2005). Investigations of the potential influence of environmental contaminants on the thymus and spleen of harbor porpoises (*Phocoena phocoena*). *Environ. Sci. Technol.* 39 (11), 3933–3938. doi: 10.1021/es048709j

Beineke, A., Siebert, U., Wohlsein, P., and Baumgärtner, W. (2010). Immunology of whales and dolphins. *Vet. Immunol. Immunop.* 133 (2-4), 81–94. doi: 10.1016/j.vetimm.2009.06.019

Bergé, A., Cladière, M., Gasperi, J., Coursimault, A., Tassin, B., and Moilleron, R. (2013). Meta-analysis of environmental contamination by phthalates. *Environ. Sci. pollut. R.* 20 (11), 8057–8076. doi: 10.1007/s11356-013-1982-5

Boerger, C. M., Lattin, G. L., Moore, S. L., and Moore, C. J. (2010). Plastic ingestion by planktivorous fishes in the north pacific central gyre. *Mar. pollut. Bull.* 60 (12), 2275–2278. doi: 10.1016/j.marpolbul.2010.08.007

Bossart, G. D. (2011). Marine mammals as sentinel species for oceans and human health. *Vet. Pathol.* 48 (3), 676–690. doi: 10.1177/0300985810388525

Botta, L., Filippi, S., Bizzarri, B. M., Meschini, R., Caputo, M., Proietti-De-Santis, L., et al. (2019). Oxidative nucleophilic substitution selectively produces cambinol derivatives with antiproliferative activity on bladder cancer cell lines. *Bioorg. Med. Chem. Lett.* 29 (1), 78–82. doi: 10.1016/j.bmcl.2018.11.006

Browne, M. A., Dissanayake, A., Galloway, T. S., Lowe, D. M., and Thompson, R. C. (2008). Ingested microscopic plastic translocates to the circulatory system of the mussel, *Mytilus edulis* (L.). *Environ. Sci. Technol.* 42 (13), 5026–5031. doi: 10.1021/es800249a

Cafaro, V., Angeletti, D., Bellisario, B., Macali, A., Carere, C., and Alessi, J. (2015). Habitat overlap between bottlenose dolphins and seabirds: a pilot study to identify high-presence coastal areas in the tyrrhenian Sea. *J. Mar. Biol. Assoc. UK.* 96 (4), 891–901. doi: 10.1017/S0025315415001447

Caldwell, J. C. (2012). DEHP: Genotoxicity and potential carcinogenic mechanisms - a review. *Mut. Res-Rev. Mutat.* 751 (2), 82–157. doi: 10.1016/j.mrrev.2012.03.001

Campana, I., Angeletti, D., Crosti, R., Di Miccoli, V., and Arcangeli, A. (2018). Seasonal patterns of floating macro-litter across the Western Mediterranean Sea: a potential threat for cetacean species. *Rend. Lincei-Sci. Fis.* 29 (2), 453–467. doi: 10.1007/s12210-018-0680-0

Carnevali, O., Tosti, L., Speciale, C., Peng, C., Zhu, Y., and Maradonna, F. (2010). DEHP impairs zebrafish reproduction by affecting critical factors in oogenesis. *PloS One* 5 (4), e10201. doi: 10.1371/journal.pone.0010201

Castañeda, R. A., Avlijas, S., Simard, M. A., and Ricciardi, A. (2014). Microplastic pollution in st. Lawrence river sediments. *Can. J. Fish. Aquat. Sci.* 71 (12), 1767–1771. doi: 10.1139/cjfas-2014-0281

Chaler, R., Cantón, L., Vaquero, M., and Grimalt, J. O. (2004). Identification and quantification of n-octyl esters of alkanoic and hexanedioic acids and phthalates as urban wastewater markers in biota and sediments from estuarine areas. *J. Chromatogr. A.* 1046 (1-2), 203–210. doi: 10.1016/j.chroma.2004.06.098

Chang, Y. J., Tseng, C. Y., Lin, P. Y., Chuang, Y. C., and Chao, M. W. (2017). Acute exposure to DEHP metabolite, MEHP cause genotoxicity, mutagenesis and carcinogenicity in mammalian Chinese hamster ovary cells. *Carcinogenesis* 38 (3), 336–345. doi: 10.1093/carcin/bgx009

Chen, C. Y. (2010). The oxidation of di-(2-Ethylhexyl) phthalate (DEHP) in aqueous solution by UV/H_2O_2 photolysis. *Water Air. Soil pollut.* 209, 411–417. doi: 10.1007/s11270-009-0209-3

Chen, T. L., LaCerte, C., Wise, S. S., Holmes, A., Martino, J., Wise, J. P.Jr., et al. (2012). Comparative cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human and sperm whale (*Physeter macrocephalus*) skin cells. *Comp. Biochem. Phys. C.* 155 (1), 143–150. doi: 10.1016/j.cbpc.2011.03.011

Chen, T. L., Wise, S. S., Kraus, S., Shaffiey, F., Levine, K. M., Thompson, W. D., et al. (2009). Particulate hexavalent chromium is cytotoxic and genotoxic to the north Atlantic right whale (*Eubalaena glacialis*) lung and skin fibroblasts. *Environ. Mol. Mutagen.* 50 (5), 387–393. doi: 10.1002/em.20471

Chen, X., Xu, S., Tan, T., Lee, S. T., Cheng, S. H., Lee, F. W. F., et al. (2014). Toxicity and estrogenic endocrine disrupting activity of phthalates and their mixtures. *Int. J. Env. Res. Pub. He.* 11 (3), 3156–3168. doi: 10.3390/ijerph110303156

Costa, E., Piazza, V., Lavorano, S., Faimali, M., Garaventa, F., and Gambardella, C. (2020). Trophic transfer of microplastics from copepods to jellyfish in the marine environment. *Front. Environ. Sci.* 158. doi: 10.3389/fenvs.2020.571732

Council Directive 92/43/EEC (1992). *Conservation of natural habitats and of wild fauna and flora*. Available at: http://data.europa.eu/eli/dir/1992/43/oj.

Crago, J., and Klaper, R. (2012). A mixture of an environmentally realistic concentration of a phthalate and herbicide reduces testosterone in male fathead minnow (*Pimephales promelas*) through a novel mechanism of action. *Aquat. Toxicol.* 110, 74–83. doi: 10.1016/j.aquatox.2011.12.021

De Santis, L. P., Garcia, C. L., Balajee, A. S., Calvo, G. T. B., Bassi, L., and Palitti, F. (2001). Transcription coupled repair deficiency results in increased chromosomal aberrations and apoptotic death in the UV61 cell line, the Chinese hamster homologue of cockayne's syndrome b. *Mutat. Res-DNA. Repair* 485 (2), 121–132. doi: 10.1016/S0921-8777(00)00065-3

Desforges, J. P. W., Sonne, C., Levin, M., Siebert, U., De Guise, S., and Dietz, R. (2016). Immunotoxic effects of environmental pollutants in marine mammals. *Environ. Int.* 86, 126–139. doi: 10.1016/j.envint.2015.10.007

Directive 2008/56/EC (2008). *The European parliament and the council establishing a framework for community action in the field of marine environmental policy (Marine Strategy Framework Directive)*. Available at: http://data.europa.eu/eli/dir/2008/56/oj.

do Sul, J. A. I., and Costa, M. F. (2014). The present and future of microplastic pollution in the marine environment. *Environ. pollut.* 185, 352–364. doi: 10.1016/j.envpol.2013.10.036

Egidi, A., Filippi, S., Manganello, F., Lopez-Martinez, W., and Meschini, R. (2018). Modulation of chromatin conformation by the histone deacetylase inhibitor trichostatin. a promotes the removal of radiation-induced lesions in ataxia telangiectasia cell lines. *Mutat. Res-Gen. Tox. En. Mutagen.* 836, 109–116. doi: 10.1016/j.mrgentox.2018.06.016

Eljezi, T., Pinta, P., Nativel, F., Richard, D., Pinguet, J., Roy, O., et al. (2019). *In vitro* cytotoxic effects of secondary metabolites of DEHP and its alternative plasticizers DINCH and DINP on a L929 cell line. *Int. J. Hyg. Envir. Heal.* 222 (3), 583–589. doi: 10.1016/j.ijheh.2019.03.005

Eljezi, T., Pinta, P., Richard, D., Pinguet, J., Chezal, J. M., Chagnon, M. C., et al. (2017). *In vitro* cytotoxic effects of DEHP-alternative plasticizers and their primary metabolites on a L929 cell line. *Chemosphere* 173, 452–459. doi: 10.1016/j.chemosphere.2017.01.026

Eriksen, M., Mason, S., Wilson, S., Box, C., Zellers, A., Edwards, W., et al. (2013). Microplastic pollution in the surface waters of the laurentian great lakes. *Mar. pollut. Bull.* 77 (1-2), 177–182. doi: 10.1016/j.marpolbul.2013.10.007

Erkekoglu, P., and Kocer-Gumusel, B. (2014). Genotoxicity of phthalates. *Toxicol. Mech. Method.* 24 (9), 616–626. doi: 10.3109/15376516.2014.960987

Fan, G., Zhang, Y., Liu, X., Wang, J., Sun, Z., Sun, S., et al. (2019). The first chromosome-level genome for a marine mammal as a resource to study ecology and evolution. *Mol. Ecol. Res.* 19 (4), 944–956. doi: 10.1111/1755-0998.13003

Fenech, M. (1993). The cytokinesis-block micronucleus technique: a detailed description of the method and its application to genotoxicity studies in human populations. *Mutat. Res-Fund. Mol. M.* 285, 35–44. doi: 10.1016/0027-5107(93)90049-L

Fenech, M. (2008). The micronucleus assay determination of chromosomal level DNA damage. *Methods Mol. Biol.* 410, 185–216. doi: 10.1007/978-1-59745-548-0_12

Filippi, S., Paccosi, E., Balzerano, A., Ferretti, M., Poli, G., Taborri, J., et al. (2022). CSA Antisense targeting enhances anticancer drug sensitivity in breast cancer cells, including the triple-negative subtype. *Cancers* 14 (7), 1687. doi: 10.3390/cancers14071687

Fossi, M. C., Coppola, D., Baini, M., Giannetti, M., Guerranti, C., Marsili, L., et al. (2014). Large Filter feeding marine organisms as indicators of microplastic in the pelagic environment: The case studies of the Mediterranean basking shark (*Cetorhinus maximus*) and fin whale (*Balaenoptera physalus*). *Mar. Environ. Res.* 100, 17–24. doi: 10.1016/j.marenvres.2014.02.002

Fromme, H., Küchler, T., Otto, T., Pilz, K., Müller, J., and Wenzel, A. (2002). Occurrence of phthalates and bisphenol a and f in the environment. *Water Res.* 36 (6), 1429–1438. doi: 10.1016/S0043-1354(01)00367-0

Galgani, F., Hanke, G., and Maes, T. (2015). "Global distribution, composition and abundance of marine litter," in *Marine anthropogenic litter*. Eds. M. Bergmann, L. Gutow and M. Klages (Cham: Springer), 29–56. doi: 10.1007/978-3-319-16510-3_2

Gambardella, C., Morgana, S., Bramini, M., Rotini, A., Manfra, L., Migliore, L., et al. (2018). Ecotoxicological effects of polystyrene microbeads in a battery of marine organisms belonging to different trophic levels. *Mar. Environ. Res.* 141, 313–321. doi: 10.1016/j.marenvres.2018.09.023

Gambardella, C., Morgana, S., Ferrando, S., Bramini, M., Piazza, V., Costa, E., et al. (2017). Effects of polystyrene microbeads in marine planktonic crustaceans. *Eco. Environ. Safe.* 145, 250–257. doi: 10.1016/j.ecoenv.2017.07.036

Graham, E. R., and Thompson, J. T. (2009). Deposit-and suspension-feeding sea cucumbers (Echinodermata) ingest plastic fragments. *J. Exp. Mar. Biol. Ecol.* 368 (1), 22–29. doi: 10.1016/j.jembe.2008.09.007

Gregory, M. R. (2009). Environmental implications of plastic debris in marine settings–entanglement, ingestion, smothering, hangers-on, hitch-hiking and alien invasions. *Philos. T. Roy. Soc. B.* 364 (1526), 2013–2025. doi: 10.1098/rstb.2008.0265

Gui, D., Yu, R., He, X., Tu, Q., Chen, L., and Wu, Y. (2014). Bioaccumulation and biomagnification of persistent organic pollutants in indo-pacific humpback dolphins (*Sousa chinensis*) from the pearl river estuary, China. *Chemosphere* 114, 106–113. doi: 10.1016/j.chemosphere.2014.04.028

Hardesty, B. D., Roman, L., Leonard, G. H., Mallos, N., Pragnell-Raasch, H., Campbell, I., et al. (2021). Socioeconomics effects on global hotspots of common debris items on land and the seafloor. *Global Environ. Chang.* 71, 102360. doi: 10.1016/j.gloenvcha.2021.102360

Hermabessiere, L., Dehaut, A., Paul-Pont, I., Lacroix, C., Jezequel, R., Soudant, P., et al. (2017). Occurrence and effects of plastic additives on marine environments and organisms: a review. *Chemosphere* 182, 781–793. doi: 10.1016/j.chemosphere.2017.05.096

Heudorf, U., Mersch-Sundermann, V., and Angerer, J. (2007). Phthalates: toxicology and exposure. *Int. J. Hyg. Envir. Heal.* 210 (5), 623–634. doi: 10.1016/j.ijheh.2007.07.011

Huang, J., Li, X., Ma, M., and Li, D. (2017). Removal of di-(2-ethylhexyl) phthalate from aqueous solution by UV/peroxymonosulfate: Influencing factors and reaction pathways. *Chem. Eng. J.* 314, 182–191. doi: 10.1016/j.cej.2016.12.095

Hu, H., Mao, L., Fang, S., Xie, J., Zhao, M., and Jin, H. (2020). Occurrence of phthalic acid esters in marine organisms from hangzhou bay, China: implications for human exposure. *Sci. Tot. Environ.* 721, 137605. doi: 10.1016/j.scitotenv.2020.137605

Jambeck, J. R., and Johnsen, K. (2015). Citizen-based litter and marine debris data collection and mapping. *Comput. Sci. Eng.* 17 (4), 20–26. doi: 10.1109/MCSE.2015.67

Khalil, S. R., Elhakim, Y. A., and EL-Murr, A. E. (2016). Sublethal concentrations of di-n-butyl phthalate promote biochemical changes and DNA damage in juvenile Nile tilapia (*Oreochromis niloticus*). *Jpn. J. Vet. Res.* 64 (1), 67–80. doi: 10.14943/jjvr.64.1.67

Kim, E. J., Kim, J. W., and Lee, S. K. (2002). Inhibition of oocyte development in Japanese medaka (*Oryzias latipes*) exposed to di-2-ethylhexyl phthalate. *Environ. Int.* 28 (5), 359–365. doi: 10.1016/S0160-4120(02)00058-2

Koelmans, A. A., Besseling, E., and Shim, W. J. (2015). ""Nanoplastics in the aquatic environment. critical review"," in *Marine anthropogenic litter*. Eds. M. Bergmann, L. Gutow and M. Klages (Cham: Springer), 325–340. doi: 10.1007/978-3-319-16510-3_12

Koelmans, A. A., Diepens, N. J., and Mohamed Nor, N. H. (2021). ""Weight of evidence for the microplastic vector effect in the context of chemical risk assessment"," in *Microplastic in the environment: pattern and process. environmental contamination remediation and management*. Ed. M. S. Bank (Cham: Springer), 155–197. doi: 10.1007/978-3-030-78627-4_6

Law, K. L., Morét-Ferguson, S., Maximenko, N. A., Proskurowski, G., Peacock, E. E., Hafner, J., et al. (2010). Plastic accumulation in the north Atlantic subtropical gyre. *Science* 329 (5996), 1185–1188. doi: 10.1126/science.1192321

Lorge, E., Hayashi, M., Albertini, S., and Kirkland, D. (2008). Comparison of different methods for an accurate assessment of cytotoxicity in the in vitro micronucleus test. I. Theoretical aspects. *Mutat. Res. - Genet. Toxicol. Environ Mutagen* 655 , 1–3. doi: 10.1016/j.mrgentox.2008.06.003

Lyche, J. L., Gutleb, A. C., Bergman, Å., Eriksen, G. S., Murk, A. J., Ropstad, E., et al. (2009). Reproductive and developmental toxicity of phthalates. *J. Toxicol. Env. Heal. B.* 12 (4), 225–249. doi: 10.1080/10937400903094091

Macali, A., Semenov, A., Venuti, V., Crupi, V., D'Amico, F., Rossi, B., et al. (2018). Episodic records of jellyfish ingestion of plastic items reveal a novel pathway for trophic transference of marine litter. *Sci. Rep.* 8 (1), 1–5. doi: 10.1038/s41598-018-24427-7

Magdouli, S., Daghrir, R., Brar, S. K., Drogui, P., and Tyagi, R. D. (2013). Di 2-ethylhexylphtalate in the aquatic and terrestrial environment: A critical review. *J. Environ. Manag.* 127, 36–49. doi: 10.1016/j.jenvman.2013.04.013

Ma, Y. B., Jia, P. P., Junaid, M., Yang, L., Lu, C. J., and Pei, D. S. (2018). Reproductive effects linked to DNA methylation in male zebrafish chronically exposed to environmentally relevant concentrations of di-(2-ethylhexyl) phthalate. *Environ. pollut.* 237, 1050–1061. doi: 10.1016/j.envpol.2017.11.025

Malem, F., Soonthondecha, P., Khawmodjod, P., Chunhakorn, V., Whitlow, H. J., and Chienthavorn, O. (2019). Occurrence of phthalate esters in the eastern coast of Thailand. *Environ. Monit. Assess.* 191 (10), 1–13. doi: 10.1007/s10661-019-7785-5

Meschini, R., D'Eliseo, D., Filippi, S., Bertini, L., Bizzarri, B. M., Botta, L., et al. (2018). Tyrosinase-treated hydroxytyrosol-enriched olive vegetation waste with increased antioxidant activity promotes autophagy and inhibits the inflammatory response in human THP-1 monocytes. *J. Agr. Food Chem.* 66 (46), 12274–12284. doi: 10.1021/acs.jafc.8b03630

Molino, C., Filippi, S., Stoppiello, G. A., Meschini, R., and Angeletti, D. (2019). *In vitro* evaluation of cytotoxic and genotoxic effects of di (2-ethylhexyl)-phthalate (DEHP) on European sea bass (*Dicentrarchus labrax*) embryonic cell line. *Toxicol. in Vitro* 56, 118–125. doi: 10.1016/j.tiv.2019.01.017

Moore, C. J. (2008). Synthetic polymers in the marine environment: a rapidly increasing, long-term threat. *Environ. Res.* 108 (2), 131–139. doi: 10.1016/j.envres.2008.07.025

Mori, C., Morsey, B., Levin, M., Gorton, T. S., and De Guise, S. (2008). Effects of organochlorines, individually and in mixtures, on b-cell proliferation in marine mammals and mice. *J. Toxicol. Env. Healt. A.* 71 (4), 266–275. doi: 10.1080/15287390701612860

Murray, F., and Cowie, P. R. (2011). Plastic contamination in the decapod crustacean *Nephrops norvegicus* (Linnaeus 1758). *Mar. pollut. Bull.* 62 (6), 1207–1217. doi: 10.1016/j.marpolbul.2011.03.032

Oehlmann, J., Schulte-Oehlmann, U., Kloas, W., Jagnytsch, O., Lutz, I., Kusk, K. O., et al. (2009). A critical analysis of the biological impacts of plasticizers on wildlife. *Philos. T. Roy. Soc. B.* 364 (1526), 2047–2062. doi: 10.1098/rstb.2008.0242

Paluselli, A., Aminot, Y., Galgani, F., Net, S., and Sempere, R. (2018). Occurrence of phthalate acid esters (PAEs) in the northwestern Mediterranean Sea and the Rhone river. *Prog. Oceanogr.* 163, 221–231. doi: 10.1016/j.pocean.2017.06.002

Paluselli, A., Fauvelle, V., Galgani, F., and Sempéré, R. (2019). Phthalates release and biodegradation from plastic fragments in seawater. *Env. Sci. Technol.* 53 (1), 166–175. doi: 10.1021/acs.est.8b05083

Parker, B., Andreou, D., Green, I. D., and Britton, J. R. (2021). Microplastics in freshwater fishes: Occurrence, impacts and future perspectives. *Fish.* 22 (3), 467–488. doi: 10.1111/faf.12528

Pournejati, R., Gust, R., Sagasser, J., Kircher, B., Jöhrer, K., Ghanbari, M. M., et al. (2021). *In vitro* evaluation of cytotoxic effects of di (2-ethylhexyl) phthalate (DEHP) produced by bacillus velezensis strain RP137 isolated from Persian gulf. *Toxicol. In. Vitro* 73, 105148. doi: 10.1016/j.tiv.2021.105148

Proietti De Santis, L., Lorenti Garcia, C., Balajee, A. S., Brea Calvo, G. T., Bassi, L., and Palitti, F. (2001). Transcription coupled repair deficiency results in increased chromosomal aberrations and apoptotic death in the UV61 cell line, the Chinese hamster homologue of cockayne's syndrome. *B. Mutat. Res.* 485, 121–132. doi: 10.1016/S0921-8777(00)00065-3

Radke, E. G., Braun, J. M., Nachman, R. M., and Cooper, G. S. (2020). Phthalate exposure and neurodevelopment: A systematic review and meta-analysis of human epidemiological evidence. *Environ. Int.* 137, 105408. doi: 10.1016/j.envint.2019.105408

Rangel-Buitrago, N., Williams, A., Costa, M. F., and de Jonge, V. (2020). Curbing the inexorable rising in marine litter: An overview. *Ocean. Coast. Manag.* 188, 105133. doi: 10.1016/j.ocecoaman.2020.105133

Selvaraj, K. K., Sundaramoorthy, G., Ravichandran, P. K., Girijan, G. K., Sampath, S., and Ramaswamy, B. R. (2015). Phthalate esters in water and sediments of the kaveri river, India: environmental levels and ecotoxicological evaluations. *Environ. Geochem. Hlth.* 37 (1), 83–96. doi: 10.1007/s10653-014-9632-5

Sies, H. (2017). Hydrogen peroxide as a central redox signaling molecule in physiological oxidative stress: Oxidative eustress. *Redox Biol.* 11, 613–619. doi: 10.1016/j.redox.2016.12.035

Stewart, M., Olsen, G., Hickey, C. W., Ferreira, B., Jelić, A., Petrović, M., et al. (2014). A survey of emerging contaminants in the estuarine receiving environment around Auckland, new Zealand. *Sci. Tot. Environ.* 468, 202–210. doi: 10.1016/j.scitotenv.2013.08.039

Suman, M., Giacomello, M., Corain, L., Ballarin, C., Montelli, S., Cozzi, B., et al. (2012). Estradiol effects on intracellular Ca^{2+} homeostasis in bovine brain-derived endothelial cells. *Cell Tissue Res.* 350, 109–118. doi: 10.1007/s00441-012-1460-2

Sun, J., Pan, L., Zhan, Y., Lu, H., Tsang, D. C., Liu, W., et al. (2016). Contamination of phthalate esters, organochlorine pesticides and polybrominated diphenyl ethers in agricultural soils from the Yangtze river delta of China. *Sci. Tot. Environ.* 544, 670–676. doi: 10.1016/j.scitotenv.2015.12.012

Taddei, F., Scarcelli, V., Frenzilli, G., and Nigro, M. (2001). Genotoxic hazard of pollutants in cetaceans: DNA damage and repair evaluated in the bottlenose dolphin (*Tursiops truncatus*) by the comet assay. *Mar. pollut. Bull.* 42 (4), 324–328. doi: 10.1016/S0025-326X(00)00159-4

Thibaut, R., and Porte, C. (2004). Effects of endocrine disrupters on sex steroid synthesis and metabolism pathways in fish. *J. Steroid Biochem. Mol. Biol.* 92 (5), 485–494. doi: 10.1016/j.jsbmb.2004.10.008

Tran, H. T., Lin, C., Bui, X. T., Nguyen, M. K., Cao, N. D. T., Mukhtar, H., et al. (2022). Phthalates in the environment: characteristics, fate and transport, and advanced wastewater treatment technologies. *Biores. Technol.* 344, 126249. doi: 10.1016/j.biortech.2021.126249

Uren-Webster, T. M., Lewis, C., Filby, A. L., Paull, G. C., and Santos, E. M. (2010). Mechanisms of toxicity of di (2-ethylhexyl) phthalate on the reproductive health of male zebrafish. *Aquat. Toxicol.* 99 (3), 360–369. doi: 10.1016/j.aquatox.2010.05.015

Wang, S., Cao, Y., Wang, S., Cai, J., and Zhang, Z. (2020). DEHP induces immunosuppression through disturbing inflammatory factors and CYPs system homeostasis in common carp neutrophils. *Fish. Shellfish Immun.* 96, 26–31. doi: 10.1016/j.fsi.2019.11.073

Wang, X., Yang, Y., Zhang, L., Ma, Y., Han, J., Yang, L., et al. (2013). Endocrine disruption by di-(2-ethylhexyl)-phthalate in Chinese rare minnow (Gobiocypris rarus). *Environ. Toxicol. Chem.* 32 (8), 1846–1854 doi: 10.1002/etc.2261

Wang, Y. Q., Li, Y. W., Chen, Q. L., and Liu, Z. H. (2013). Long-term exposure of xenoestrogens with environmental relevant concentrations disrupted spermatogenesis of zebrafish through altering sex hormone balance, stimulating germ cell proliferation, meiosis and enhancing apoptosis. *Environ. pollut.* 244, 486–494. doi: 10.1016/j.envpol.2018.10.079

Weaver, J. A., Beverly, B. E., Keshava, N., Mudipalli, A., Arzuaga, X., Cai, C., et al. (2020). Hazards of diethyl phthalate (DEP) exposure: A systematic review of animal toxicology studies. *Environ. Internat.* 145, 105848. doi: 10.1016/j.envint.2020.105848

Xu, H. E., Cao, W., Sun, H., Zhang, S., Li, P., Jiang, S., et al. (2021). Dose-dependent effects of di-(2-Ethylhexyl) phthalate (DEHP) in mussel *Mytilus galloprovincialis*. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.658361

Ye, T., Kang, M., Huang, Q., Fang, C., Chen, Y., Shen, H., et al. (2014). Exposure to DEHP and MEHP from hatching to adulthood causes reproductive dysfunction and endocrine disruption in marine medaka (*Oryzias melastigma*). *Aquat. Toxicol.* 146, 115–126. doi: 10.1016/j.aquatox.2013.10.025

Yu, H., Caldwell, D. J., and Suri, R. P. (2019). *In vitro* estrogenic activity of representative endocrine disrupting chemicals mixtures at environmentally relevant concentrations. *Chemosphere* 215, 396–403. doi: 10.1016/j.chemosphere.2018.10.067

Zantis, L. J., Carroll, E. L., Nelms, S. E., and Bosker, T. (2021). Marine mammals and microplastics: A systematic review and call for standardisation. *Environ. pollut.* 269, 116142. doi: 10.1016/j.envpol.2020.116142

Zarfl, C., and Matthies, M. (2010). Are marine plastic particles transport vectors for organic pollutants to the Arctic? *Mar. pollut. Bull.* 60 (10), 1810–1814. doi: 10.1016/j.marpolbul.2010.05.026

Zhang, Y., Jiao, Y., Li, Z., Tao, Y., and Yang, Y. (2021). Hazards of phthalates (PAEs) exposure: A review of aquatic animal toxicology studies. *Sci. Tot. Environ.* 771, 145418. doi: 10.1016/j.scitotenv.2021.145418

Zhang, Z. M., Zhang, H. H., Zou, Y. W., and Yang, G. P. (2018). Distribution and ecotoxicological state of phthalate esters in the sea-surface microlayer, seawater and sediment of the bohai Sea and the yellow Sea. *Environ. pollut.* 240, 235–247. doi: 10.1016/j.scitotenv.2021.145418

Zheng, Q., Feng, M., and Dai, Y. (2013). Comparative antioxidant responses in liver of carassius auratus exposed to phthalates: An integrated biomarker approach. *Environ. Toxicol. Phar.* 36 (3), 741–749. doi: 10.1016/j.etap.2013.07.008

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Resources and population traits modulate the association patterns in the common bottlenose dolphin living nearby the Tiber River estuary (Mediterranean Sea)

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Sociality and ecological drivers that can influence individual association patterns are infrequently considered in wildlife management, although they are essential aspects affecting animals' responses to both human-related pressures and conservation strategies. In common bottlenose dolphins (*Tursiops truncatus*), sex-specific social dynamics and interactions with anthropogenic activities may affect grouping and induce changes in relationships between individuals. Out of a total of 347 individuals, we assessed the level of association among 68 bottlenose dolphins that have been sighted more than five times near the Roman coast (central Mediterranean Sea, Italy). The half-weight index (HWI) of dyadic associations, their network relations, and stability over time were investigated by using the SOCPROG software. Outcomes showed that females were more strongly associated than other individuals, with both preferred constant short-term associations and random long-term associations, possibly resulting in greater success in rearing young. Individuals interacting with the bottom trawl fishery showed weaker and short-term associations. Temporary disruption of individual associations during interaction with fishery and the relatively low number of females with calves participating in depredation seem to denote both the opportunistic nature of interactions with fishing vessels and the offspring-related protection strategy. The results show that the dolphins in this region maintain a complex but flexible social structure that varies with local biological requirements and is resilient to anthropogenic pressures.

KEYWORDS

social structure, *Tursiops truncatus*, trawling fishery, conservation, ecology, Tyrrhenian Sea

Introduction

Differences in gregariousness and social relationships are features of many group-living mammalian species, influencing numerous traits of an individual's life, from feeding success, mate selection, and grouping in different life stages, to habitat preference (Pace et al., 2014a; Majolo and Huang, 2018; Pace et al., 2018) and defense against predators (Heithaus and Dill, 2006; Wirsing et al., 2008; Miller et al., 2022). Group formation is a highly dynamic process subjected to many variations in size and member composition over different temporal scales. Groups can separate (fission) or merge (fusion) (Couzin, 2006; Couzin and Laidre, 2009), and this fluid joining and splitting possibly evolved as an adaptive strategy to minimize competition in relation to the fickleness of resources, the mating access, and the habitat type (Majolo and Huang, 2018). The flexible scheme, within fission–fusion societies, is assumed to enable mammals to react to highly mobile resources and/or rapid-changing pressures. It allows to adjust the number of associates and to shift the identity of the individuals with which they relate (Archie and Chiyo, 2012). However, 'strategic' non-random association patterns and long-term social preferences may emerge in specific conditions or contexts. They can guide, for example, the development of sympatric communities, as in some common bottlenose dolphin (*Tursiops truncatus*, hereinafter 'bottlenose dolphins') populations with specializations in hunting/foraging techniques (Chilvers and Corkeron, 2001; Wiszniewski et al., 2009; Daura–Jorge et al., 2012; Pace et al., 2012). In the fluid fission–fusion societies of bottlenose dolphins, the composition of group members changes over hours, days, or seasons (Gowans et al., 2007). Differences in interaction patterns and social affinity between and within genders are also recognized (Moreno and Acevedo-Gutiérrez, 2016). For example, sex-specific social dynamics, such as sex-age segregation (in Florida: Wells et al., 1987), hierarchical male alliances (in Australia: Randić et al., 2012; Connor and Krützen, 2015), strong or preferred associations between/within sexes (in New Zealand and Australia: Lusseau et al., 2003; Gero et al., 2005, respectively), and female–male affiliations with the absence of male alliances (in Ireland: Baker et al., 2020), may have effects on social structure.

Bottlenose dolphin social structure shows a high degree of flexibility and adaptations, with changes in the association patterns relative to different pressures acting on local populations (Papale et al., 2017; Louis et al., 2018). The environmental conditions, habitat features (Lusseau et al., 2003; Wiszniewski et al., 2009), behavioral states (Moreno and Acevedo-Gutiérrez, 2016), and prey availability and predictability (Gowans et al., 2007; Wiszniewski et al., 2009), are considered drivers for variations in bottlenose dolphin social structure (Díaz López and Shirai, 2008; Blumstein, 2010; Pace et al., 2012; Blasi and Boitani, 2014; Genov et al., 2019; Bonizzoni et al., 2021; Frau et al., 2021). Furthermore, anthropogenic factors and other mediating forces that alter resource accessibility and distribution can influence the association pattern among individuals. For example, individuals opportunistically exploiting both aquaculture cages and trawling fishery may form long-term preferred companionship (Pace et al., 2012). However, during opportunistic feeding behavior at marine fish farms, the number of dolphin associations is described to decrease, as it is easier to capture prey, and cooperation is not as necessary (Díaz López and Shirai, 2008). A trawler efficiently herds species that are part of the bottlenose dolphin diet, letting dolphins decrease the effort spent feeding, in both energy and time, thus enhancing foraging effectiveness (Fertl and Leatherwood, 1997; Pace et al., 2012). The energetic benefit of depredation (Tixier et al., 2015) comes with an increased risk of injury for individuals, incidental capture (bycatch), and/or mortality during the interaction with the fishing gear, leading to a risk–reward trade-off that can modify individual behavior and social dynamics (Santana–Garcon et al., 2018; Buscaino et al., 2021). Several common bottlenose dolphin populations are known to interact with different fishing gears (e.g., Blasi and Boitani, 2014; Pennino et al., 2015; Buscaino et al., 2021), and a number of reports are related to trawling boats (e.g., Pace et al., 2012; Genov et al., 2019; Bonizzoni et al., 2021; Bonizzoni et al., 2022), with individuals intentionally entering the nets and actively take advantage of fisheries through depredation (i.e., injuries or removal of captured fish from a fishing gear; Chilvers and Corkeron, 2001; Hamer et al., 2012).

The interaction with trawling vessels highlights conservation issues and management implications as well (e.g., Chilvers and Corkeron, 2001; Pace et al., 2012; Bonizzoni et al., 2021; Vella et al., 2021; Bonizzoni et al., 2022). It clearly influences social dynamics and changes the pattern of interactions at the group level, possibly affecting demographic parameters such as survival and reproduction (Maldonado-Chaparro and Chaverri, 2021). The disruption of groups and the loss of individuals that may play central roles within a social network, possibly holding key information (e.g., the location of food resources or the fulfillment of specific feeding strategies), could induce the loss of behavioral diversity (Kühl et al., 2019) and may result in a reduction of adaptability to changing conditions (He et al., 2019). Sociality and ecological drivers that affect population dynamics and select for individual association patterns are infrequently considered in wildlife management (Bolaños-Jiménez et al., 2021), although they affect animals' responses to both human-related pressures and conservation strategies (Díaz López, 2019). Considering that different geographic units may have highly variable sizes, distribution patterns, degrees of exposure to potential anthropogenic threats, and flexible social structures, understanding drivers affecting association patterns could be crucial for dolphin conservation (Avila et al., 2018; Díaz López, 2019). Information on the social structure of bottlenose dolphin units in the Mediterranean Sea is scattered and not fully reported yet (Blasi and Boitani, 2014). Some of the available data seem to suggest that sex composition (Blasi and Boitani, 2014) and operational trawlers (Pace et al., 2012; Genov et al., 2019; Bonizzoni et al., 2021; Bonizzoni et al., 2022) may play a pivotal role in shaping social structure and patterns of individual arrangements. Here, the social structure of the bottlenose dolphins living nearby the Tiber River estuary (central Mediterranean Sea, Italy) is reported, with the aim of providing an initial assessment of the relationships between individuals in this geographical unit under local conditions. Furthermore, the following goals are achieved: a) evaluating the level of association between individuals within groups, b) examining if any evidence occurred for sex-specific patterns, c) assessing eventual changes in groups opportunistically interacting with trawlers, and d) discussing ecological implications and associated conservation issues.

Materials and methods

Study site

The study area (Figure 1) is located in the central Tyrrhenian Sea (Mediterranean Sea, Italy). The area is characterized by a variety of habitats (seagrass meadows; hard and soft bottom communities within coastal banks and cliffs; Ventura et al., 2015; Ardizzone et al., 2018; Ventura et al., 2018; Bonifazi et al., 2019; Casoli et al., 2019) and includes the Tiber River estuary. The southern portion of the seabed of the Tiber River's mouths presents several habitats of biological importance (i.e., coralligenous outcrops and *Posidonia oceanica* meadows, extending on both sandy and rocky substrata), which are included in the EU Natura 2000 network Sites of Community Importance and the Marine Protected Area of Secche di Tor Paterno (MPA IT6000010, 1,387 ha).

At about 3 nautical miles off the two Tiber River mouths is situated a terminal including two single-point moorings (SPMs) handling crude and petroleum products. Navigation, anchoring, diving, and fishing are banned within a radius of 750 m from

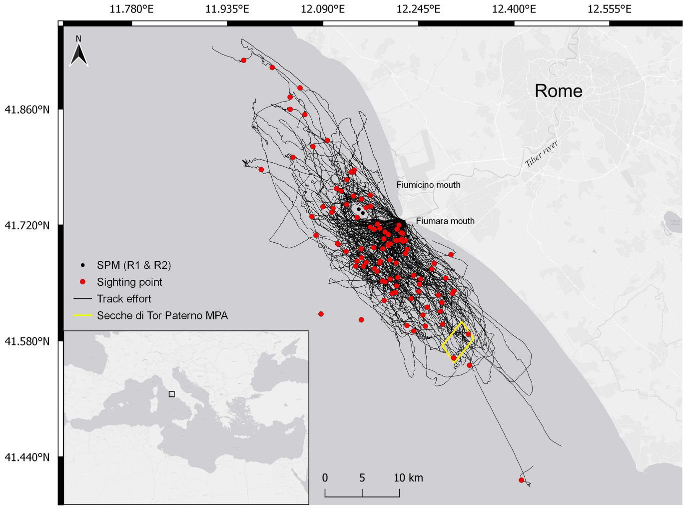


FIGURE 1
Study area in Central Tyrrhenian Sea (Mediterranean Sea, Italy). Black lines represent the 2017–2020 survey tracks, red dots correspond to the location of the bottlenose dolphin encounters, black dots identify the two single-point moorings (SPMs), and the yellow square delimits the Secche di Tor Paterno marine protected area (MPA).

each SPM. These structures are reported to attract some dolphin species (Triossi et al., 2013; Todd et al., 2020), as confirmed by the regular presence of groups of bottlenose dolphins around them (Pace et al., 2019; Pace et al., 2021). Bottlenose dolphins are also commonly reported at the mouths of the Tiber River and the Secche di Tor Paterno MPA (Pace et al., 2019; Pace et al., 2021; Martino et al., 2021). Three hundred forty-seven unique individuals have been photo identified, with females showing a high degree of site fidelity, low levels of dispersion, and localized movements (Pace et al., 2021). Mother–calf dyads have been observed throughout all survey seasons (May–November). Bottlenose dolphin occurrence is possibly favored by the ecological conditions and heterogeneity of morphological features of the region (Ardizzone et al., 2018). Water mass circulation generates upwellings that support high productivity, making the area a suitable site for both feeding and nursing bottlenose dolphins (Pace et al., 2019; Pedrazzi et al., 2022). The study area is considered a valuable ground for the commercial fishery as well (Ardizzone et al., 2018) and is characterized by a high proportion of small-scale artisanal fishery and larger trawling vessels (Ardizzone et al., 2018). Bottom trawlers belonging to the Fiumicino fleet, consisting of 25–30 vessels typically 18–25 m long, operate on both the continental shelf and the slope, running 1-day fishing trips from Monday to Friday year-round. Interactions with fishery were commonly observed in the bottlenose dolphin population frequenting the area (Pace et al., 2019; Pace et al., 2021; Pace et al., 2022). Dolphins have been observed foraging almost evenly across all the study sites, where target prey species (Blanco et al., 2001; Bearzi et al., 2009) are distributed. These include demersal species (European hake, *Merluccius merluccius*; red mullets, *Mullus barbatus* and *Mullus surmuletus*), small pelagic fishes (sardine, *Sardina pilchardus*; anchovy, *Engraulis encrasicolus*), cephalopods (common octopus, *Octopus vulgaris*; horned octopus *Eledone cirrhosa*), and crustaceans (shrimps, *Parapenaeus longirostris*; Norway lobster, *Nephrops norvegicus*), which are also main fishery targets (Ardizzone et al., 2018). Prey abundance appears to peak in August, with anchovies representing more than 20% of the fishery catches (EMODnet, 2022; EUMOFA, 2022).

Data collection

Focal follows and photo-identification protocols over four survey seasons, from August 2017 to November 2020, were conducted. Data were collected onboard a sailing vessel Beneteau, model Oceanis, length 41.1 ft, powered by a 55-hp Volvo diesel engine. Daily surveys were carried out principally during summer, in favorable weather conditions (i.e., sea state ≤ 3 Douglas, wind force ≤ 3 Beaufort, no rain, and no fog). Surveys were conducted by three to six observers alternating between 7 × 50 and 7 × 80 binoculars and the naked eye, at a steady speed of 4–6 knots. Survey track lines did not follow a standardized scheme but an adaptive procedure (Dawson et al., 2008; La Manna et al., 2016; La Manna et al., 2020; Martino et al., 2021). Acoustic data were also collected (Papale et al., 2021; Pace et al., 2022).

A group of dolphins were defined as a number of individuals with relatively close spatial cohesion (i.e., each member within 10 m of any other member) engaged in similar predominant behavioral activities (Parra et al., 2006). When a dolphin group was sighted, location, time, direction, behavior, size, age classes (see below), and the presence of concomitant anthropogenic activities (e.g., fishing vessels, fishing gears, and pleasure boats) were recorded. More specifically, the occurrence of trawlers and the presence of dolphins interacting with the fishing vessels were evaluated. Dolphins were considered to interact with trawlers when following the operating vessel at a variable distance (from less than 100 to 400 m; similarly to Pace et al., 2012; Genov et al., 2019), alternating sequences of short surfacing with dives of different duration (from 2 to 6 min), and often showing surface behaviors (e.g., rushes and leaps).

Two observers collected photographs of the dorsal fins using Canon digital 5D and 6D cameras and Canon 70–300 and 100–400 mm f/4.5–5.6L lenses. Once observers were confident that the best possible photographs had been acquired, or the animals were lost, dolphin sighting ended. Total group size and age class composition were estimated in the field and then corrected (if needed) *via* photo-identification analysis. Age class was defined following Pace et al. (2021): adult = an individual generally of a length of about 2.8–3.0 m; juvenile = a poorly scarred and rarely nicked individual of about 2/3 the length of an adult; calf = an individual of about 1/2 the length of an adult, with often visible fetal folds, always in echelon swimming position close to an adult mid-lateral flank; and newborn = an individual of about 1/3 the length of an adult, with visible fetal folds, swimming uncoordinatedly always in echelon position, very close to an adult. Sex was determined whenever possible using the following procedures (Pace et al., 2021): the collection of photographs of the genital area of individuals or the observation of constant adult–offspring associations during one or more encounters (the adult was assumed to be a female). Each sighting and related photo-identification analysis were considered an independent sample.

Photo-identification analysis

Photographs were classified considering their quality (see Würsig and Jefferson, 1990), and a quality grade (G) of between 1 and 5 was assigned to each image. Only high-quality photos with G ≥ 4 were used. The occurrence and position of permanent natural markings on the dorsal fins (such as nicks and notches) and on the body were used to univocally recognize dolphins (Pulcini et al., 2014; Urian et al., 2015; Mariani et al., 2016; Mussi et al., 2021). The individual distinctiveness was scored as well-marked (individuals with highly distinctive dorsal fins and scars on the body), fairly marked (individuals with moderately distinctive dorsal fins), and unmarked (individuals with no distinctive features on dorsal fins) (see Pace et al., 2021 for further details).

Association patterns, social network analysis, and temporal patterns

The 'gambit of the group' (GoG) assumption, i.e., each animal in a group or a cluster is associating and interacting with every other animal in that group, was adopted to examine dolphins' association patterns (Whitehead and Dufault, 1999; Whitehead, 2008a; Franks et al., 2010). To reduce biases (Chilvers and Corkeron, 2001; Bouveroux et al., 2019), and to include bottlenose dolphins regularly frequenting the study area, only distinctive individuals encountered on ≥5 occasions in two to four different years were used for the association analysis (all individuals, AI dataset; n = 68). The number of 68 individuals resulted in a powerful sample size considering an estimated abundance of 80 resident bottlenose dolphins in the study area (see Pace et al., 2021, for details on population abundance). The sample size was calculated using G*Power 3 software (Faul and Erdfelder, 1992; Faul et al., 2007). Then, association patterns were specifically examined in a) the subgroup of individuals classified as 'females' (females only, FO dataset; n = 23), b) the subgroup of individuals interacting with trawling vessels at least 70% of their total encounter occasions (individuals in the presence of trawls, PT dataset; n = 23), and c) the subgroup of individuals not interacting with trawling vessels at least 70% of their total encounter occasions (individuals in the absence of trawls, AT dataset; n = 27). We used the 70% threshold since about 30% of the sightings occurred when trawling vessels were not operating (every Saturday and Sunday, and during the fishing break period for biological recovery each year). Daily sampling periods were used to remove demographic effects occurring during the study period, such as birth, death, immigration, and emigration (Whitehead, 2008b; Bouveroux et al., 2019).

Three basic approaches were considered using the software SOCPROG version 2.9 (Whitehead, 2009a): 1) the dyadic association levels, 2) the network metrics, and 3) the type and temporal stability of the associations. Dyadic associations were evaluated with the half-weight index (HWI) (Cairns and Schwager, 1987). HWI measures the proportion of times a pair of individuals was associated, ranging from 0 to 1 (with 0 indicating a pair never observed together and 1 a pair always observed together). Following Quintana–Rizzo and Wells (2001), HWIs were classified into categories based on strength of associations. Mean and maximum levels of association (HWI_{ave} and HWI_{max}, respectively) were examined for each individual. The social differentiation (S), i.e., the coefficient of variation (CV) of the true association indices, represents how varied the social system is. This was estimated by maximizing the likelihood of observed dyadic associations using the algorithm available in SOCPROG. S values close to 0 reveal a very homogeneous society, S close to 0.5 indicates quite well-differentiated societies, and S > 2 indicates extremely differentiated societies (Whitehead, 2008b).

To characterize social bonds between individuals, a mean linkage hierarchical cluster analysis was performed. Results were represented as dendrogram only if the cophenetic correlation coeficient (CCC, i.e., the correlation between real HWIs and the levels of clustering between individuals)—which ranges from 0 to 1—was greater than about 0.8 (which indicates a reliable separation among clusters; Whitehead, 2008b). The modularity clustering technique (Newman, 2006) was then applied to understand whether the population is divided into clusters of individuals based on social affiliations. The modularity coefficient (Q), i.e., the difference between the observed and the expected proportion of the total of the HWIs within clusters (Newman, 2006; Dungan et al., 2016), was calculated in SOCPROG. Q values ≥ 0.3 reveal strong divisions in the population (Newman, 2006).

The presence of preferred (non-random) associations among dolphins was tested through a modified permutation test against the null hypothesis that the dolphins were randomly associated. The Manly and Bëjder permutation technique (Manly, 1995; Bëjder et al., 1998) in SOCPROG—with extensions advanced by Whitehead (1999); Whitehead et al. (2005) and corrections introduced by Krause et al. (2009)—was used. The association matrices were randomly permutated 10,000 times with 1,000 flips per permutation, with HWIs being calculated after each permutation, at which point the *p*-values stabilized (Whitehead, 2009a and b). Since the *p*-value cannot be considered as a statistical threshold to identify significant associations (Whitehead, 2008a), an arbitrary threshold was fixed to identify the significant associations at twice the mean association index of the population, including zero values (Gero et al., 2005; Frau et al., 2021). The hypothesis of non-random associations (i.e., preferred companionships in the population) in the observed matrix was accepted if the value of the standard deviation (SD) and the coefficient of variation (CV) were significantly higher than those computed from the randomly permuted data (Whitehead and Dufault, 1999; Whitehead, 2008b).

Then, the social structure was examined through specific network metrics (Wey et al., 2008; Croft et al., 2011). To measure how individuals were connected and/or central in the groups (Whitehead, 2008b), the following parameters were estimated in SOCPROG. a) The *strength*: it indicates the gregariousness of each individual, so larger values suggest a broad preference for larger groups. b) The *affinity*: it measures if individuals are strongly connected to other individuals that have also strong connections, so an individual with high affinity has relatively high associations with individuals that have high strength. c) The *eigenvector centrality*: it determines an individual's relevance (connectedness) in the network, so higher values indicate that individuals generally have high gregariousness and/or are connected to individuals with high gregariousness. d) The *reach*: it evaluates the indirect connectedness of an individual, so a high value indicates that individuals are indirectly linked to many others in the population. e) The *clustering coefficient*: it measures how well the associates of an individual are themselves associated, so a value of 0 indicates none of an individual's associates are associated with each other, and a value of 1 indicates that are all associates of each other with equal weight (Whitehead, 2008b; Titcomb et al., 2015; Dungan et al., 2016). In a well-connected network, all these measurements are likely significantly higher than expected at random. To graphically display network relationships and illustrate the structure of each network, sociograms were obtained with NetDraw 2.123 (Borgatti et al., 2002) using double HWI_{ave} values and their multiples (Diaz-Aguirre et al., 2020).

Finally, to determine the stability over time of associations, the standardized lagged association rate (SLAR; Whitehead, 1995) was calculated for the AI, FO, PT, and AT datasets. The SLAR estimates the probability of resighting two individuals in association at t(x), after having found them associated at t(0). The following four exponential models in SOCPROG were fitted to SLAR to describe the temporal patterning of bottlenose dolphin associations at the Tiber River estuary. 1) *Preferred companions*: some pairs of individuals have a preference for associating, which is constant over time, suggesting permanent associations. 2) *Casual acquaintances*: some pairs of individuals associate for some time, disassociate, and may reassociate. 3) *Constant companions and casual acquaintances*: association followed by disassociation at some time lag to a lower level of associations where associations stabilize. 4) *Two levels of casual acquaintances:* association and disassociation occurring on two different time scales. The best-fitting model was chosen according to the lowest quasi-Akaike information criterion (QAIC) (Burnham and Anderson, 2002).

Results

A total of 137 surveys were conducted between 2017 and 2020, covering a total of 4,967 km on-effort within the study area (Figure 1). As reported in Pace et al. (2021), 105 bottlenose dolphin groups were encountered during surveys; their distribution within the study area is shown in Figure 1. Group size ranged from one to 65 animals, with an average value of 15. The typical group composition consisted of several adults, mostly accompanied by calves (70% of sightings). Three hundred forty-seven (347) unique individuals were identified from 104,781 high-quality images (40% of the total photographs collected). The maximum number of re-sighting was 30 for a single animal, while 65% of individuals (n = 226) were recorded only once or twice (Figure 2A). The discovery curve for the overall number of identified individuals regularly increased throughout the study, while the curve of the 68 identified at least five times (AI dataset) showed a stabilization over time (Figure 2B).

Association level

The estimate of social differentiation (S) indicates a good representation of the social pattern, with a quite well-differentiated population (S = 0.54, SE = 0.06, n = 100 bootstrap replicates). The overall HWI_{ave} obtained for the individuals in the AI dataset (n = 68) was 0.18 ± 0.06, suggesting very low levels of associations. However, the overall HWI_{max} was considerably higher (0.58 ± 0.13), with 14 individuals (21% of the total) showing $HWI_{max} \geq 0.70$. Five of

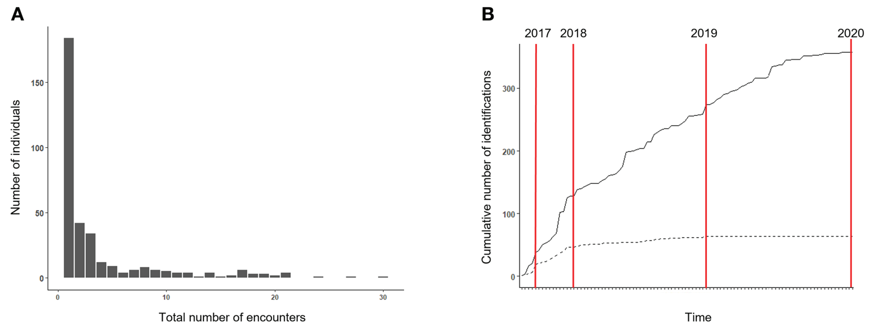


FIGURE 2
(A) Frequency distribution of the number of encounters of individual bottlenose dolphins. **(B)** Cumulative discovery curves for bottlenose dolphin individuals over the study period. The solid line shows the discovery curve for the overall 347 individuals identified; the broken line represents the discovery curve for the 68 individuals seen at least five times.

these individuals with higher HWI_{max} were females. Indeed, females (FO dataset, n = 23) showed a higher HWI_{ave} (0.25 ± 0.08) than the overall individuals, with 13 individuals (57%) strongly associated ($HWI_{ave} > 0.5$), including a pair (seen between 18 and 27 times) having $HWI_{ave} > 0.7$ (Figure 3, top panel, CCC ≥ 0.80). The modularity coeficient (Q = 0.11) also indicates divisions in the female network (the best division into clusters was at an association index of 0.3), resulting in two main clusters of six and 10 individuals.

Monte Carlo permutation tests for equal variances (n = 10,000) showed a significant lower HWI_{ave} (F = 6.565; p = 0.0001) and HWI_{max} (F = 2.418; p = 0.0087) for individuals mostly seen in association with trawling vessels (PT dataset; HWI_{ave} = 0.21 ± 0.04; HWI_{max} = 0.57 ± 0.12) compared to dolphins preferentially observed without interactions (AT dataset; HWI_{ave} = 0.25 ± 0.11; HWI_{max} = 0.65 ± 0.18). The modularity coeficient without trawling vessels (AT dataset; Q = 0.15) indicates divisions in the network (the best division into clusters was at an association index of 0.3), resulting in four main clusters of two, three, four, and 15 individuals; Figure 3, bottom panel; CCC ≥ 0.80).

Preferred/avoided associations

The results of the permutation tests on possible preferred or avoided associations in the population are reported in Table 1. In the overall population (AI dataset), in female individuals (FO dataset), and dolphins preferentially observed without interactions with trawling vessels (AT dataset), permutation tests hinted at preferred companionships, as revealed by a significantly higher SD and CV of the observed data compared with random data. The significantly smaller proportion of non-zero HWIs in the observed data (AI, FO, and AT datasets) seem to indicate that avoidance between individuals may also occur within the investigated population. Short-term preferred associations emerged in individuals mostly seen in association with trawling vessels (PT dataset), whereby random HWI_{ave} was lower than the observed data.

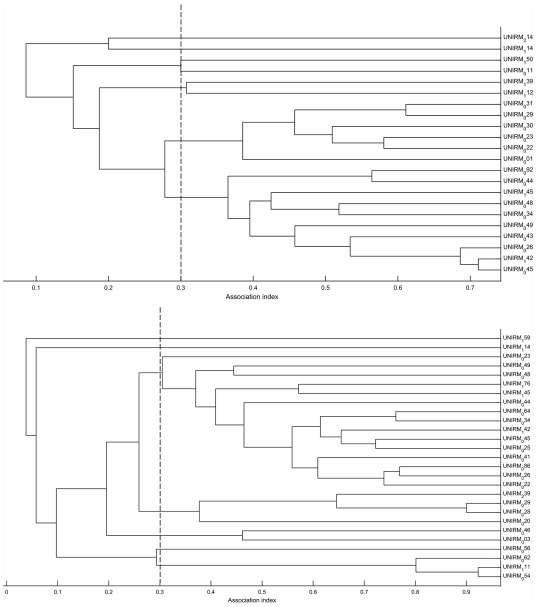


FIGURE 3
Hierarchical cluster analysis of female bottlenose dolphins (FO dataset, n = 23; top panel) and individuals mostly seen without interactions with trawling vessels (AT dataset, n = 27; bottom panel) using the half-weight index.

Social network

The values of the social metric parameters in the investigated datasets are shown in Table 2. The characterization of the social network of overall individuals (AI dataset) emphasized a well-connected network. All metrics (strength, affinity, eigen-centrality, reach, and clustering coeficient) were significantly different from random, indicating a general preference for larger groups, with individuals strongly connected to each other and indirectly linked to many others in the population. The same pattern was observed for female individuals (FO dataset) and dolphins preferentially observed without interactions with trawling vessels (AT dataset) as well, while all network metrics were not significantly different from random for individuals mostly observed in association with trawling vessels (PT dataset).

The sociograms generated with levels of double HWI_{ave} showed the well-connected bottlenose dolphin network at the Tiber River estuary and confirmed several strong associations among females (Figure 4, upper panels). The networks in the absence or presence of fishing vessels (Figure 4, lower panels, respectively) were different, with the former characterized by multiple connections between female individuals and the latter by a lower number of links between individuals of unknown sex. Both networks showed a triad separated from the principal ones.

Type and temporal stability of the associations

The best-fitting model (Table 3) obtained for overall (AI dataset) and female individuals (FO dataset) was '*two levels of casual acquaintance*' (a short, casual level of association and a longer-term one). However, in the female dataset, a model containing '*constant companions and casual acquaintances*' strongly supported the SLAR as well, suggesting that sex-specific patterns of association may persist over time between females at two levels of association, one of '*constant companions*'(preferred and constant short-term associations)

TABLE 1 Permutation tests for preferred and avoided associations of bottlenose dolphins.

	Observed data	Random data	*p*-Value
Overall individuals (AI dataset)			
HWI_{ave}	0.18	0.19	**0.0003**
SD of HWI_{ave}	0.15	0.12	**0.0002**
CV of HWI_{ave}	0.80	0.61	**0.0002**
Proportion of non-0 element	0.81	0.88	**0.0002**
Females (FO dataset)			
HWI_{ave}	0.25	0.26	**0.0004**
SD of HWI_{ave}	0.16	0.12	**0.0003**
CV of HWI_{ave}	0.66	0.49	**0.0000**
Proportion of non-0 element	0.91	0.95	**0.0142**
Individuals interacting with trawls (PT dataset)			
HWI_{ave}	0.21	0.20	0.0873
SD of HWI_{ave}	0.17	0.16	0.2222
CV of HWI_{ave}	0.78	0.74	0.1348
Proportion of non-0 element	0.76	0.76	0.4554
Individuals not interacting with trawls (AT dataset)			
HWI_{ave}	0.25	0.26	**0.0004**
SD of HWI_{ave}	0.22	0.14	**0.0002**
CV of HWI_{ave}	0.90	0.54	**0.0000**
Proportion of non-0 element	0.72	0.91	**0.0002**

CV, coefficient of variation; HWI, half-weight index.
Significant result ($p < 0.025$ or $p > 0.975$) are shown in bold.

and one of '*casual acquaintances*' (random long-term associations). Thus, although the social structure appeared to be driven by short-term relationships, female individuals also had longer-term and constant companions over the 4-year study period. A similar result was highlighted for individuals mainly observed in the presence of trawling vessels (PT dataset). The model containing '*casual acquaintances*'(random long-term associations) was the best-fitting one in the case of individuals principally sighted without trawls (AT dataset), although a model containing '*constant companions and casual acquaintances*' strongly supported the SLAR as well.

Discussion

This study investigated for the first time the social structure of *T. truncatus* in the Tiber River estuary (Italy) over 4 years. The discovery curve for these individuals (AI dataset) showed a stabilization after 15 encounters, highlighting that the portion of individuals within the population showing a considerable degree of site fidelity was captured, similarly to bottlenose dolphins at the Shannon Estuary (Baker et al., 2018) and in a lagoon in the Gulf of Mexico (Morteo et al., 2017). Results also showed that common bottlenose dolphins in the Tiber River estuary were organized into a quite well-differentiated fission–fusion society encompassing both extremely fluid and stable associations between individuals, which seem to be a common pattern among bottlenose dolphin populations (e.g., Moreno and Acevedo-Gutiérrez, 2016; Morales-Rincon et al., 2019). HWI_{ave} was significantly higher in both females and individuals not interacting with trawling vessels if compared to the overall individuals. Association levels in female bottlenose dolphins are generally related to reproductive state (Connor et al., 2000), calf protection and food access (Mann et al., 2000; Möller et al., 2006), defense against predators and male coercion (Connor et al., 2000; Galezo et al., 2018), or lifetime fitness (Möller et al., 2006). Here, the stronger associations between females seem analogous to other populations (e.g., Papale et al., 2017), where females tend to associate with other females sharing similar energy requirements to obtain greater success in rearing the young and maximize the chances of offspring survival (Wells, 2003; Möller and Harcourt, 2008; Rendell et al., 2019; Diaz-Aguirre et al., 2020).

From a behavioral ecology perspective, the higher levels of associations here observed between individuals in the absence of trawling vessels could be related to possible benefits from 1) increased cooperation and reduced intragroup competition to advance information sharing when the patchy and uneven distribution of prey occur, since cooperative foraging strategies may increase the foraging efficiency (Methion and Díaz López, 2020); 2) safer contexts to improve calf care, social behaviors, or resting in females; and 3) foraging specializations preferences (not related to bottom trawling fishery) possibly transferred from mother to offspring through the social learning process

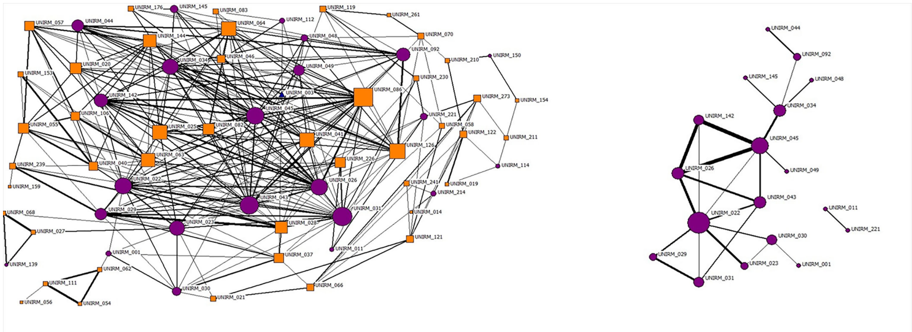
TABLE 2 Network metrics for the different groups of individuals considered in this study.

	Mean ± SD	*p*-Value
Overall individuals (AI dataset)		
Strength	12.39 ± 3.80	**<0.001**
Affinity	13.33 ± 0.88	**0.01**
Eigenvector centrality	0.11 ± 0.04	**<0.001**
Reach	167.65 ± 58.56	**0.02**
Clustering coefficient	0.27 ± 0.03	**<0.001**
Females (FO dataset)		
Strength	5.43 ± 1.77	**<0.001**
Affinity	5.91 ± 0.31	0.24
Eigenvector centrality	0.20 ± 0.07	**<0.001**
Reach	32.52 ± 11.35	0.03
Clustering coefficient	0.42 ± 0.04	**0.02**
Individuals interacting with trawls (PT dataset)		
Strength	4.77 ± 0.96	0.08
Affinity	4.93 ± 0.21	0.72
Eigenvector centrality	0.20 ± 0.05	0.29
Reach	23.59 ± 5.22	0.23
Clustering coefficient	0.31 ± 0.03	0.40
Individuals not interacting with trawls (AT dataset)		
Strength	6.54 ± 2.83	**<0.001**
Affinity	7.52 ± 0.83	**1.00**
Eigenvector centrality	0.17 ± 0.08	**<0.001**
Reach	50.47 ± 24.09	**<0.001**
Clustering coefficient	0.40 ± 0.04	0.08

Significant result ($p < 0.025$ or $p > 0.975$) are shown in bold.

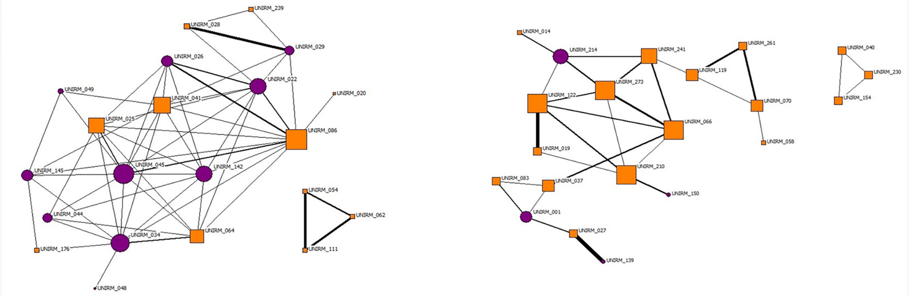
(vertical transmission; Rendell and Whitehead, 2001; Weiss, 2006). Although our study did not find separate bottlenose dolphin 'trawling' and 'non-trawling' communities (Chilvers and Corkeron, 2001; Genov et al., 2019), despite overlapping spatial ranges, significantly lower association levels were obtained when individuals interact with trawls. The opportunistic interaction behind the trawling vessel makes it possible to feed on organisms captured by the trawl, picking out fish entangled in the nets or possibly feeding on fish passing through the net meshes (Fertl and Leatherwood, 1997), even behaviorally impaired (Ryer et al., 2004). Concentrated food sources and increased prey availability are key attracting factors for bottlenose dolphins (Fertl and Leatherwood, 1997), which may act individually with lower association levels. However, despite these positive aspects, animals are exposed to the risk of bycatch (dolphins that spend more time in the vicinity of fishing nets are more likely to get caught than dolphins that avoid the interaction; Fortuna et al., 2010), although there is no reported evidence of entanglement or bycatch in the study area (Carpentieri et al., 2021).

Similarly, to the estuarine population of bottlenose dolphins in the Indian River (Titcomb et al., 2015), social network structures governed by preferred and avoided companionships were not homogeneous. The networks varied from a few vertices with multiple links to only one or two links. The overall dataset (AI) network appeared cohesive and well-connected, showing multiple links between individuals. Females seemed to have a central role in this network, being strongly associated with each other. This pattern of association between females appeared clearer in the female-only network (FO) and in the network of individuals not preferentially interacting with trawls (AT), where central positions were occupied almost by the same female individuals. However, individuals not identified as females composed the network of animals mostly seen in association with trawling vessels. Non-random associations are common in many terrestrial and marine mammals that exhibit fission–fusion grouping patterns [e.g., African elephants, *Loxodonta africana* (Wittemyer et al., 2005); Indian ocean humpback dolphins, *Sousa plumbea* (Bouveroux et al., 2019); killer whale, *Orcinus orca* (Ford et al., 2000), particularly in female clusters [e.g., bottlenose dolphins (Connor et al., 2000); grey kangaroos, *Macropus giganteus* (Best et al., 2014); zebras, *Equus grevyi* (Sundaresan et al., 2007); and giraffes, *Giraffa camelopardalis* (Carter et al., 2013)]. Here, non-random associations within different groups may indicate that not all individuals have the same role in this society or play a similar part in the network's



All individuals (AI dataset)

Females only (FO dataset)



Individuals not preferentially interacting with trawling fishery (AT dataset)

Individuals preferentially interacting with trawling fishery (PT dataset)

FIGURE 4
Sociograms of individuals in the different datasets. Sociograms were set with HWI values at least twice the HWI_{ave} for all datasets. Top left: sociogram of overall individuals (AI dataset, HWI $\geq$ 0.36). Top right: sociogram of female individuals (FO dataset, HWI $\geq$ 0.50). Bottom left: sociogram of individuals not preferentially interacting with trawlers (AT dataset, HWI $\geq$ 0.50). Bottom right: individuals preferentially interacting with trawlers (PT dataset, HWI $\geq$ 0.42). Purple circles indicate females, and orange squares mark individuals of unknown gender. HWI, half-weight index.

TABLE 3 Exponential decay models fitted to the standardized lagged association rate (SLAR) among bottlenose dolphin individuals at the Tiber River estuary.

Models' explanation	Fitted model	QAIC
Overall (AI dataset)		
Two levels of casual acquaintance	0.015414*exp(−0.11466*td) + 0.021612*exp(−0.00024417*td)	61,660
Female individuals (FO dataset)		
Constant companions and casual acquaintances	0.058268 + 0.13241*exp(−1.1164*td)	23,739
Two levels of casual acquaintance	0.14304*exp(−1.3938*td) + 0.06169*exp(−0.00017703*td)	23,738
Individuals interacting with trawls (PT dataset)		
Constant companions and casual acquaintances	0.044376 + 0.23639*exp(−1.0567*td)	1,334
Two levels of casual acquaintance	0.27686*exp(−1.2707*td) + 0.048625*exp(−0.00030941*td)	1,336
Individuals not interacting with trawls (AT dataset)		
Casual acquaintances	0.060229*exp(−0.0005296*td)	16,907
Two levels of casual acquaintance	0.025394*exp(−1.0002*td) + 0.059235*exp(−0.00049512*td)	16,909

Only the best-fitting model for each group is shown. Note the negligible ΔQAIC between two models in FO, PT, and AT datasets.
QAIC, quasi-Akaike information criterion.

cohesion (Lusseau et al., 2003). Individual female centrality and the strength of associations with other females, for example, are likely to change over time because of variable interbirth intervals (Mann et al., 2000; Barrett and Henzi, 2002), thus altering the networks' configuration. It is known that kinship may also play an important role in shaping female associations (Diaz-Aguirre et al., 2020), but it is not known the degree of genetic relatedness between females in the study area. This aspect needs further investigations in the future.

The type and temporal stability of the associations of the bottlenose dolphin groups in the Tiber River estuary were best described by models containing a) associations or dissociations at two different time scales ('*two levels of casual acquaintances*'), where the associations eventually decay completely, and b) short-term preferred mates and occasional long-term acquaintances with individuals that associate over a period of time, disassociate, and re-associate later ('*constant companions and casual acquaintances*'). In females, the tendency to form strong temporary associations with other females appears to be a defense technique to reduce harassment by groups of males (Moreno and Acevedo-Gutiérrez, 2016; Galezo et al., 2018), but prey type may play an important role in the decision-making regarding leaving and/or bonding specific individuals in a group as well (Lusseau et al., 2004). Indeed, the wide-open habitat at the Tiber River mouths allows bottlenose dolphins both to pursue and circle schooling fish, with a few individuals at a time preying (Connor et al., 2000) and to individually target isolated prey items throughout the water column. These strategies may favor associations or disassociations at different temporal scales depending on changing foraging opportunities (Gregorietti et al., 2021).

Conservation implications

In the Tiber River estuary, bottlenose dolphins appear to be organized in a fission–fusion society characterized by both free and fluctuating, but also strong and preferred associations. Strong social bonds can be attributed to differences in habitat use and residency patterns of dolphin groups inhabiting the study region (Pace et al., 2021) and to the regular presence of females with their recent offspring (Pedrazzi et al., 2022). The River mouths are likely to represent a key nursery ground and a valuable habitat with an important availability of suitable food sources for bottlenose dolphins since nutrient transport influences primary production and the whole trophic web. These favorable local conditions also support the exploitation by trawling fishery, which in turn affects the social dynamics of the population. Furthermore, the presence of trawling vessels can influence relationships and bonds between individuals because the behavioral complexity required to advantageously complete this opportunistic feeding activity possibly implies a specific type of cooperation (Pace et al., 2012). Nevertheless, in this study, common bottlenose dolphins established weaker associations in the presence of trawling, letting us presume that they might prefer limiting the risk of bycatch by avoiding associating during this activity. This hypothesis seems to be also supported by the lower presence of identified females (prevalently recognized by the occurrence of associated calves) following fishing trawlers, suggesting that females with calves are possibly using other foraging strategies (not related to operating bottom trawlers) to secure food more easily when resources are generally abundant (as in the study area), in order to meet their daily energetic requirement and prevent unnecessary risk (Kovacs et al., 2017). From a conservation perspective, both aspects (weaker associations between individuals and mother–calf pairs avoidance) may be pivotal since they represent a temporary disruption of adult social bonds due to fishing activity but may also denote that dolphin's social structure may be a complex adaptive system resilient to anthropogenic disturbance (Ansmann et al., 2012; Díaz López, 2019; Genov et al., 2019; Frau et al., 2021), as bonds are restored when fishing trawlers are absent. Different anthropogenic activities have been demonstrated to possibly alter population structure in terms of age and sex composition, by influencing the survival rate (Senigaglia et al., 2019; Tenan et al., 2020) or affecting the relationships among individuals (Marley et al., 2017), with the potential of eventually influencing population dynamics (Tenan et al., 2020). Thus, assessing how social structure changes and adapts in response to human activities is essential to investigate the possible consequences of anthropogenic disturbance on a population level. This study reports information on a bottlenose dolphin population over a 4-year period, which is a short time frame that does not allow for analyzing interannual or even generational changes. Further, long-term data collection is therefore needed to investigate population dynamics over a wider time frame (Pace et al., 2014b).

The present work provides new evidence on the common bottlenose dolphin that could be useful for future management plans and practical conservation efforts for the species. To date, current management approaches focus on the conservation of numbers of animals, yet this study emphasizes the importance of individual variations and the necessity to preserve behaviors that allow adaptation to the local environment. The bottlenose dolphin is included in Annex II of the EU Habitats Directive (92/43/CEE) as priority species and listed as Least Concern in the last International Union for Conservation of Nature (IUCN) Red List of Threatened species regional assessment (Natoli et al., 2021). This new assessment strongly indicated to monitor the effects of human-related stressors, to guarantee the preservation of intra-species diversity and the survival across its range (Natoli et al., 2021). In addition, the Tiber River estuary area was identified as an 'Area of Interest' for bottlenose dolphin during the first Important Marine Mammal Areas (IMMA) Mediterranean workshop organized by the IUCN Marine Mammal Protected Areas Task Force (IUCN, 2017). This was the first significant step toward the recognition of this discrete area as important for feeding and reproduction of the common bottlenose dolphin, thus having the potential to be managed for conservation.

Data availability statement

The data supporting the conclusions of this article will be made available by the authors upon request.

Ethics statement

Ethical review and approval was not required for the animal study because this is a non-invasive, observational study.

Author contributions

DSP, GG, and GA designed the study and managed the funding acquisition. DSP, GG, SF, CD, MS, GP, EC, and DV performed the field work. SF, CD, and DSP completed the photo-identification analysis. SF and DSP analyzed the data. DSP and EP wrote the first version of the manuscript. All authors discussed the results and implications, commented on the manuscript at all stages, and contributed extensively to the work here presented.

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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.

Publisher's note

References

Ansmann, I. C., Parra, G. J., Chilvers, B. L., and Lanyon, J. M. (2012). Dolphins restructure social system after reduction of commercial fisheries. *Anim. Behav.* 84, 575–581. doi: 10.1016/j.anbehav.2012.06.009

Archie, E. A., and Chiyo, P. I. (2012). Elephant behaviour and conservation: social relationships, the effects of poaching, and genetic tools for management. *Mol. Ecol.* 21, 765–778. doi: 10.1111/j.1365–294X.2011.05237

Ardizzone, G. D., Belluscio, A., and Criscoli, A. (2018). *Atlante degli habitat dei fondali marini del Lazio* (Rome, Italy: Sapienza Università Editrice).

Avila, I. C., Kaschner, C., and Dormann, C. F. (2018). Current global risks to marine mammals: Taking stock of the threats. *Biol. Conservat.* 221, 44–58. doi: 10.1016/j.biocon.2018.02.021

Baker, I., O'Brien, J., McHugh, K., and Berrow, S. (2020). Fine-scale sociality reveals female–male affiliations and absence of male alliances in bottlenose dolphins (*Tursiops truncatus*) in the Shannon estuary, Ireland. *Mar. Mam. Sci.* 36, 66–88. doi: 10.1111/mms.12631

Baker, I., O'Brien, J., McHugh, K., Ingram, S. N., and Berrow, S. (2018). Bottlenose dolphin (*Tursiops truncatus*) social structure in the Shannon estuary, Ireland, is distinguished by age– and area–related associations. *Mar. Mam. Sci.* 34, 458–487. doi: 10.1111/mms.12462

Barrett, L., and Henzi, S. P. (2002). Constraints of relationship formation among female primates. *Behaviour* 139, 263–289. doi: 10.1163/156853902760102672

Bearzi, G., Fortuna, C. M., and Reeves, R. R. (2009). Ecology and conservation of common bottlenose dolphins *Tursiops truncatus* in the Mediterranean Sea. *Mammal. Rev.* 39, 92–123. doi: 10.1111/j.1365–2907.2008.00133.x

Bĕjder, L., Fletcher, D., and Bräger, S. (1998). A method for testing association patterns of social animals. *Anim. Behav.* 56, 719–725. doi: 10.1006/anbe.1998.0802

Best, E. C., Dwyer, R. G., Seddon, J. M., and Goldize, A. W. (2014). Associations are more strongly correlated with space use than kinship in female eastern grey kangaroos. *Anim. Behav.* 89, 1–10. doi: 10.1016/j.anbehav.2013.12.011

Blanco, C., Salomón, O., and Raga, J. A. (2001). Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. *J. Mar. Biol. Assoc. UK* 81, 1053–1058. doi: 10.1017/S0025315401005057

Blasi, M., and Boitani, L. (2014). Complex social structure of an endangered population of bottlenose dolphins (*Tursiops truncatus*) in the aeolian archipelago (Italy). *PloS One* 9 (12), e114849. doi: 10.1371/journal.pone.0114849

Blumstein, D. T. (2010). "Social behaviour in conservation" in *Social behaviour: genes, ecology and evolution*. Eds. T. Szèkely, A. J. Moore and J. Komdeur (Cambridge: Cambridge University Press), 654–672.

Bolaños-Jiménez, J., Morteo, E., Delfín-Alfonso, C. A., Fruet, P. F., Secchi, E. R., and Bello-Pineda, J. (2021). Population dynamics reveal a core community of the common bottlenose dolphin (Tursiops truncatus) in open waters of the South-Western Gulf of Mexico. *Frontiers in Marine Science*, 1610. doi: 10.3389/fmars.2021.753484

Bonifazi, A., Lezzi, M., Ventura, D., Lisco, S., Cardone, F., and Gravina, M. F. (2019). Macrofaunal biodiversity associated with different developmental phases of a threatened Mediterranean *Sabellaria alveolata* (Linnaeus 1767) reef. *Mar. Environ. Res.* 145, 97–111. doi: 10.1016/j.marenvres.2019.02.009

Bonizzoni, S., Furey, N. B., and Bearzi, G. (2021). Bottlenose dolphins (*Tursiops truncatus*) in the north-western Adriatic Sea: Spatial distribution and effects of trawling. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 635–650. doi: 10.1002/aqc.3433

Bonizzoni, S., Hamilton, S., Reeves, R. R., Genov, T., and Bearzi, G. (2022). Odontocete cetaceans foraging behind trawlers, worldwide. *Rev. Fish Biol. Fish.* 32, 827–877. doi: 10.1007/s11160–022–09712–z

Borgatti, S. P., Everett, M. G., and Freeman, L. C. (2002). *Ucinet 6 for Windows: Software for Social Network Analysis*. Harvard, MA: Analytic Technologies.

Bouveroux, T., Kirkman, S. P., Conry, D., Vargas–Fonseca, O. A., and Pistorius, P. A. (2019). The first assessment of social organisation of the Indian ocean humpback dolphin (*Sousa plumbea*) along the south coast of south Africa. *Can. J. Zool.* 97, 855–865. doi: 10.1139/cjz–2018–0244

Burnham, K. P., and Anderson, D. R. (2002). *Model selection and multimodel inference: a practical information–theoretic approach* (New York: Springer).

Buscaino, G., Ceraulo, M., Alonge, G., Pace, D. S., Grammauta, R., Maccarrone, V., et al. (2021). Artisanal fishing, dolphins, and interactive pinger: A study from a passive acoustic perspective. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 2241–2256. doi: 10.1002/aqc.3588

Cairns, S. J., and Schwager, S. J. (1987). A comparison of association indices. *Anim. Behav.* 35, 1454–1469. doi: 10.1016/S0003–3472(87)80018–0

P. Carpentieri, A. Nastasi, M. Sessa and A. Srour (Eds.) (2021). "Incidental catch of vulnerable species in Mediterranean and black Sea fisheries – a review," in *Studies and reviews no. 101* (Rome: General Fisheries Commission for the Mediterranean, FAO). doi: 10.4060/cb5405en

Carter, K. D., Seddon, J. M., Frere, C. H., Carter, J. K., and Goldizen, A. W. (2013). Fission–fusion dynamics in wild giraffes may be driven by kinship, spatial overlap and individual social preferences. *Anim. Behav.* 85, 385–394. doi: 10.1016/j.anbehav.2012.11.011

Casoli, E., Bonifazi, A., Ardizzone, G., Gravina, M. F., Russo, G. F., Sandulli, R., et al. (2019). Comparative analysis of mollusc assemblages from different hard bottom habitats in the central tyrrhenian Sea. *Diversity* 11, 74. doi: 10.3390/d11050074

Chilvers, B. L., and Corkeron, P. J. (2001). Trawling and bottlenose dolphins' social structure. *Proc. R. Soc B* 268, 1901–1905. doi: 10.1098/rspb.2001.1732

Connor, R. C., and Krützen, M. (2015). Male Dolphin alliances in shark bay: changing perspectives in a 30–year study. *Anim. Behav.* 103, 223–235. doi: 10.1016/j.anbehav.2015.02.019

Connor, R. C., Wells, R. S., Mann, J., and Read, A. J. (2000). "The bottlenose dolphin: social relationships in a fission–fusion society", in *Cetacean Societies: Field Studies of Dolphins and Whales*. Eds. J. Mann, R. C. Connor, P. L. Tyack and H. Whitehead (Chicago: The University of Chicago Press), 91–126.

Couzin, I. D. (2006). Behavioral ecology: social organization in fission–fusion societies. *Curr. Biol.* 16, R169–R171. doi: 10.1016/j.cub.2006.02.042

Couzin, I. D., and Laidre, M. E. (2009). Fission–fusion populations. *Curr. Biol.* 19, R633–R635. doi: 10.1016/j.cub.2009.05.034

Croft, D. P., Madden, J. R., Franks, D. W., and James, R. (2011). Hypothesis testing in animal social networks. *Trends Ecol. Evol.* 26, 502–507. doi: 10.1016/j.tree.2011.05.012

Daura–Jorge, F. G., Cantor, M., Ingram, S. N., Lusseau, D., and Simões–Lopes, P. C. (2012). The structure of a bottlenose dolphin society is coupled to a unique foraging cooperation with artisanal fishermen. *Biol. Lett.* 8, 702–705. doi: 10.1098/rsbl.2012.0174

Dawson, S., Wade, P., Slooten, E., and Barlow, J. (2008). Design and field methods for sightings surveys of cetaceans in coastal and riverine habitats. *Mammal Rev.* 38, 19–49. doi: 10.1111/j.1365-2907.2008.00119.x

Diaz-Aguirre, F., Parra, G. J., and Passadore, C. (2020). Kinship and reproductive condition correlate with affiliation patterns in female southern Australian bottlenose dolphins. *Sci. Rep.* 10, 189. doi: 10.1038/s41598–020–58800–2

Díaz López, B. (2019). "Hot deals at sea": responses of a top predator (Bottlenose dolphin, *Tursiops truncatus*) to human–induced changes in the coastal ecosystem. *Behav. Ecol.* 30 (2), 291–300. doi: 10.1007/s00265–007–0512–1

Díaz López, B., and Shirai, J. A. B. (2008). Marine aquaculture and bottlenose dolphins' (*Tursiops truncatus*) social structure. *Behav. Ecol. Sociobiol.* 62, 887–894. doi: 10.1093/beheco/ary162

Dungan, S. Z., Wang, J. Y., Araujo, C. C., Yang, S. C., and White, B. N. (2016). Social structure in a critically endangered indo–pacific humpback dolphin (*Sousa chinensis*) population. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 26, 517–529. doi: 10.1002/aqc.2562

European Marine Observation and Data Network (2022) *Human activities*. Available at: https://www.emodnet-humanactivities.eu (Accessed June 6, 2022).

European Market Observatory for Fisheries and Aquaculture products (2022) *Data*. Available at: https://eumofa.eu/web/eumofa/data (Accessed June 6, 2022]).

Faul, F., and Erdfelder, E. (1992). *GPOWER a priori-, post hoc-, and compromise power analyses for MS-DOS* (Bonn, Germany: Bonn University). Available at: https://www.psychologie.hhu.de/fileadmin/redaktion/Fakultaeten/Mathematisch-Naturwissenschaftliche_Fakultaet/Psychologie/AAP/gpower/GPowerManual.pdf.

Faul, F., Erdfelder, E., Lang, A. G., and Buchner, A. (2007). G*Power 3: A flexible statistical power analysis program for the social, behavioral, and biomedical sciences. *Behav. Res. Methods* 39, 175–191. doi: 10.3758/BF03193146

Fertl, D., and Leatherwood, S. (1997). Cetacean interactions with trawls: A preliminary review. *J. Northw. Atl. Fish. Sci.* 22, 219–248. doi: 10.2960/J.v22.a17

Ford, J. K. B., Ellis, G. M., and Balcomb, K. C. (2000). *Killer whales: The natural history and genealogy of orcinus orca in British Columbia and Washington* (Vancouver, Canada: UBC Press).

Fortuna, C. M., Vallini, C., Filidei, E., Ruffino, M., Consalvo, I., Di Muccio, S., et al. (2010). By–catch of cetaceans and other species of conservation concern during pair trawl fishing operations in the Adriatic Sea (Italy). *Chem. Ecol.* 26, 65–76. doi: 10.1080/02757541003627662

Franks, D. W., Ruxton, G. D., and James, R. (2010). Sampling animal association networks with the gambit of the group. *Behav. Ecol. Sociobiol.* 64, 493–503. doi: 10.1007/s00265–009–0865–8

Frau, S., Ronchetti, F., Perretti, F., Addis, A., Ceccherelli, G., and La Manna, G. (2021). The influence of fish farm activity on the social structure of the common bottlenose dolphin in Sardinia (Italy). *Peer J* 9, e10960. doi: 10.7717/peerj.10960

Galezo, A. A., Krzyszczyk, E., and Mann, J. (2018). Sexual segregation in indo–pacific bottlenose dolphins is driven by female avoidance of males. *Behav. Ecol.* 29, 377–386. doi: 10.1093/beheco/arx177

Genov, T., Centrih, T., Kotnjek, P., and Hace, A. (2019). Behavioural and temporal partitioning of dolphin social groups in the northern Adriatic Sea. *Mar. Biol.* 166, 11. doi: 10.1007/s00227–018–3450–8

Gero, S., Bejder, L., Whitehead, H., Mann, J., and Connor, R. C. (2005). Behaviourally specific preferred associations in bottlenose dolphins, tursiops spp. *Can. J. Zool.* 83, 1566–1573. doi: 10.1139/z05–155

Gowans, S., Würsig, B., and Karczmarski, L. (2007). The social structure and strategies of delphinids: predictions based on an ecological framework. *Adv. Mar. Biol.* 53, 195–294. doi: 10.1016/S0065–2881(07)53003–8

Gregorietti, M., Papale, E., Ceraulo, M., de Vita, C., Pace, D. S., Tranchida, G., et al. (2021). Acoustic presence of dolphins through whistles detection in Mediterranean shallow waters. *J. Mar. Sci. Eng.* 9, 78. doi: 10.3390/jmse9010078

Hamer, D. J., Childerhouse, S. J., and Gales, N. J. (2012). Odontocete bycatch and depredation in longline fisheries: A review of available literature and of potential solutions. *Mar. Mamm. Sci.* 28, E345–E374. doi: 10.1111/j.1748–7692.2011.00544.x

Heithaus, M. R., and Dill, L. M. (2006). Does tiger shark predation risk influence foraging habitat use by bottlenose dolphins at multiple spatial scales? *Oikos* 114 (2), 257–264. doi: 10.1111/j.2006.0030-1299.14443.x

He, P., Maldonado–Chaparro, A. A., and Farine, D. R. (2019). The role of habitat configuration in shaping social structure: a gap in studies of animal social complexity. *Behav. Ecol. Sociobiol.* 73, 9. doi: 10.1007/s00265–018–2602–7

IUCN Marine Mammal Protected Areas Task Force (2017) *Final report of the workshop: First IMMA regional workshop for the Mediterranean, chania, Greece, 24–28 October 2016*. Available at: https://www.marinemammalhabitat.org/download/final–report–regional–workshop–mediterranean–sea–important–marine–mammal–areas/?ind=1501742106612&filename=First%20IMMA%20Regional%20Workshop%20Mediterranean%20Region%20Final%20Report_01.08.17_rev.2.pdf&wpdmdl=1648&refresh=62661bf8240ba1650859000.

Kovacs, C. J., Perrtree, R. M., and Cox, T. M. (2017). Social differentiation in common bottlenose dolphins (*Tursiops truncatus*) that engage in human-related foraging behaviors. *PloS One* 12 (2), e0170151. doi: 10.1371/journal.pone.0170151

Krause, S., Mattner, L., James, R., Guttridge, T., Corcoran, M. J., Gruber, S. H., et al. (2009). Social network analysis and valid Markov chain Monte Carlo tests of null models. *Behav. Ecol. Sociobiol.* 63, 1089–1096. doi: 10.1007/s00265-009-0746-1

Kühl, H. S., Boesch, C., Kulik, L., Haas, F., Arandjelovic, M., Dieguez, P., et al. (2019). Human impact erodes chimpanzee behavioral diversity. *Science* 363, 1453–1455. doi: 10.1126/science.aau4532

La Manna, G., Ronchetti, F., and Sarà, G. (2016). Predicting common bottlenose dolphin habitat preference to dynamically adapt management measures from a marine spatial planning perspective. *Ocean Coast. Manage.* 130, 317–327. doi: 10.1016/j.ocecoaman.2016.07.004

La Manna, G., Ronchetti, F., Sarà, G., Ruiu, A., and Ceccherelli, G. (2020). Common bottlenose dolphin protection and sustainable boating: Species distribution modeling for effective coastal planning. *Front. Mar. Sci.* 7. doi: 10.3389/fmars.2020.542648

Louis, M., Simon–Bouhet, B., Viricel, A., Lucas, T., Gally, F., Cherel, Y., et al. (2018). Evaluating the influence of ecology, sex and kinship on the social structure of resident coastal bottlenose dolphins. *Mar. Biol.* 165 (5), 80. doi: 10.1007/s00227–018–3341–z

Lusseau, D., Schneider, K., Boisseau, O. J., Haase, P., Slooten, E., and Dawson, S. M. (2003). The bottlenose dolphin community of doubtful sound features a large proportion of long–lasting associations. can geographic isolation explain this unique trait? *Behav. Ecol. Sociobiol.* 54, 396–405. doi: 10.1007/s00265–003–0651–y

Lusseau, D., Williams, R. J., Wilson, B., Grellier, K., Barton, T. R., Hammond, P. S., et al. (2004). Parallel influence of climate on the behaviour of pacific killer whales and Atlantic bottlenose dolphins. *Ecol. Lett.* 7, 1068–1076. doi: 10.1111/j.1461–0248.2004.00669.x

Majolo, B., and Huang, P. (2018). "Group living" in *Encyclopedia of animal cognition and behaviour*. Eds. J. Vonks and T. Shackelford (New York: Springer International Publishing).

Maldonado-Chaparro, A. A., and Chaverri, G. (2021). Why do animal groups matter for conservation and management? *Conserv. Sci. Pract.* 3 (12), e550. doi: 10.1111/csp2.550

Manly, B. F. J. (1995). A note on the analysis of species co–occurrences. *Ecology* 76, 1109–1115. doi: 10.2307/1940919

Mann, J., Connor, R. C., Barre, L. M., and Heithaus, M. R. (2000). Female reproductive success in bottlenose dolphins (*Tursiops* sp.): life history, habitat, provisioning, and group–size effects. *Behav. Ecol.* 11, 210–219. doi: 10.1093/beheco/11.2.210

Mariani, M., Miragliuolo, A., Mussi, B., Russo, G. F., Ardizzone, G., and Pace, D. S. (2016). Analysis of the natural markings of risso's dolphins (*Grampus griseus*) in the central Mediterranean Sea. *J. Mammal.* 97, 1512–1524. doi: 10.1093/jmammal/gyw109

Marley, S. A., Salgado Kent, C. P., Erbe, C., and Parnum, I. M. (2017). Effects of vessel traffic and underwater noise on the movement, behaviour and vocalisations of bottlenose dolphins in an urbanised estuary. *Sci. Rep.* 7, 13437. doi: 10.1038/s41598-017-13252-z

Martino, S., Pace, D. S., Moro, S., Casoli, E., Ventura, D., Frachea, A., et al. (2021). Integration of presence–only data from several sources: a case study on dolphins' spatial distribution. *Ecography* 44, 1533–1543. doi: 10.1111/ecog.05843

Methion, S., and Díaz López, B. (2020). Individual foraging variation drives social organization in bottlenose dolphins. *Behav. Ecol.* 31, 97–106. doi: 10.1093/beheco/arz160

Miller, P. J., Isojunno, S., Siegal, E., Lam, F. P. A., Kvadsheim, P. H., and Curé, C. (2022). Behavioral responses to predatory sounds predict sensitivity of cetaceans to anthropogenic noise within a soundscape of fear. *PNAS* 119 (13), e2114932119. doi: 10.1073/pnas.2114932119

Möller, L. M., Beheregaray, L. B., Allen, S. J., and Harcourt, R. G. (2006). Association patterns and kinship in female indo–pacific bottlenose dolphins (*Tursiops aduncus*) of south–eastern Australia. *Behav. Ecol. Sociobiol.* 61, 109–177. doi: 10.1007/s00265–006–0241–x

Möller, L. M., and Harcourt, R. G. (2008). Shared reproductive state enhances female associations in dolphin. *Res. Lett. Ecol.* 5, 498390. doi: 10.1155/2008/498390

Morales-Rincon, N., Morteo, E., and Delfín–Alfonso, C. A. (2019). Influence of artisanal fisheries on the behaviour and social structure of *Tursiops truncatus* in the south–western gulf of Mexico. *J. Mar. Biolog. Assoc.* UK 9, 1841–1849. doi: 10.1017/S002531541900078X

Moreno, K., and Acevedo-Gutiérrez, A. (2016). The social structure of golfo dulce bottlenose dolphins (*Tursiops truncatus*) and the influence of behavioural state. *R. Soc Open Sci.* 3, 160010. doi: 10.1098/rsos.160010

Morteo, E., Rocha–Olivares, A., and Abarca–Arenares, L. G. (2017). Abundance, residency, and potential hazards for coastal bottlenose dolphins (*Tursiops truncatus*) off a productive lagoon in the gulf of Mexico. *Aquat. Mamm.* 43, 308–319. doi: 10.1578/AM.43.3.2017.308

Mussi, B., Vivaldi, C., Zucchini, A., Miragliuolo, A., and Pace, D. S. (2021). The decline of short-beaked common dolphin (*Delphinus delphis*) in the waters off the island of Ischia (Gulf of Naples, Italy). *Aquat. Conserv. Mar. Freshw Ecosyst.* 31, 87–100. doi: 10.1002/aqc.3061

Natoli, A., Genov, T., Kerem, D., Gonzalvo, J., Holcer, D., Labach, H., et al. (2021). Tursiops truncatus (Mediterranean subpopulation). *The IUCN Red List of Threatened Species* 2021, e.T16369383A50285287. https://dx.doi.org/10.2305/IUCN.UK.2021–3.RLTS.T16369383A50285287.en. Accessed on 1 May 2022.

Newman, M. E. J. (2006). Modularity and community structure in networks. *Proc. Natl. Acad. Sci. U.S.A.* 103, 8577–8582. doi: 10.1073/pnas.0601602103

Pace, D. S., Arcangeli, A., Mussi, B., Vivaldi, C., Ledon, C., Lagorio, S., et al. (2018). Habitat suitability modelling in sperm whale (*Physeter macrocephalus*) social groups. *Jour. Wild. Manage.* 82, 1062–1073. doi: 10.1002/jwmg.21453

Pace, D. S., Di Marco, C., Giacomini, G., Ferri, S., Silvestri, M., Papale, E., et al. (2021). Capitoline dolphins: residency patterns and abundance estimate of *Tursiops truncatus* at the Tiber river estuary (Mediterranean Sea). *Biology* 10, 275. doi: 10.3390/biology10040275

Pace, D. S., Giacomini, G., Campana, I., Paraboschi, M., Pellegrino, G., Silvestri, M., et al. (2019). An integrated approach for cetacean knowledge and conservation in the central Mediterranean Sea using research and social media data sources. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 29, 1302–1323. doi: 10.1002/aqc.3117

Pace, D. S., Miragliuolo, A., Mariani, M., Vivaldi, C., and Mussi, B. (2014a). Sociality of sperm whale off ischia island (Tyrrhenian Sea, Italy). *Aquat. Conserv. Mar. Freshw. Ecosyst.* 24, 71–82. doi: 10.1002/aqc.2459

Pace, D. S., Mussi, B., Wurtz, M., and Gordon, J. D. C. (2014b). Foreword. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 24, 1–3. doi: 10.1002/aqc.2457

Pace, D. S., Pulcini, M., and Triossi, F. (2012). Anthropogenic food patches and association patterns of bottlenose dolphin (*Tursiops truncatus*) at lampedusa island, Italy. *Behav. Ecol.* 23, 254–264. doi: 10.1093/beheco/arr180

Pace, D. S., Tumino, C., Giacomini, G., Silvestri, M., Pedrazzi, G., Ceraulo, M., et al. (2022). Bray–call sequences in the Mediterranean common bottlenose dolphin (*Tursiops truncatus*) acoustic repertoire. *Biology* 11 (3), 367. doi: 10.3390/biology11030367

Papale, E., Ceraulo, M., Giardino, G., Buffa, G., Filiciotto, F., Grammauta, R., et al. (2017). Association patterns and population dynamics of bottlenose dolphins in the strait of Sicily (Central Mediterranean sea): implication for management. *Popul. Ecol.* 59, 55–64. doi: 10.1007/s10144–016–0566–x

Papale, E., Alonge, G., Caruso, F., Grammauta, R., Mazzola, S., Mussi, B., et al (2021). The higher, the closer, the better? influence of sampling frequency and distance on the acoustic properties of short-beaked common dolphins burst pulses in the Mediterranean Sea. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 51–60. doi: 10.1002/aqc.3158

Parra, G. J., Corkeron, P. J., and Marsh, H. (2006). Population sizes, site fidelity and residence patterns of Australian snubfin and indo–pacific humpback dolphins: implications for conservation. *Biol. Conserv.* 129, 167–180. doi: 10.1016/j.biocon.2005.10.031

Pedrazzi, G., Giacomini, G., and Pace, D. S. (2022). First report of epimeletic and acoustic behaviour in Mediterranean common bottlenose dolphins (*Tursiops truncatus*) carrying dead calves. *Biology* 11 (2), 337. doi: 10.3390/biology11020337

Pennino, M. G., Rotta, A., Pierce, G. J., and Bellido, J. M. (2015) Interaction between Bottlenose Dolphin (Tursiops truncatus) and Trammel Nets in the Archipelago de La Maddalena, Italy. *Hydrobiologia* 747, 69–82 doi: 10.1007/s10750-014-2127-7

Pulcini, M., Pace, D. S., Triossi, F., La Manna, G., Galante, I., and Fortuna, M. C. (2014). Distribution and abundance estimates of bottlenose dolphins (*Tursiops truncatus*) around lampedusa island (Sicily channel, Italy) – implications for their management. *J. Mar. Biol. Assoc. UK* 94, 1175–1184. doi: 10.1017/S0025315413000842

Quintana–Rizzo, E., and Wells, R. S. (2001). Resighting and association patterns of bottlenose dolphins (Tursiops truncatus) in the cedar keys, Florida: insights into social organization. *Can. J. Zool.* 79, 447–456. doi: 10.1139/cjz–79–3–447

Randić, S., Conno, R. C., Sherwin, W. B., and Krützen, M. (2012). A novel mammalian social structure in indo–pacific bottlenose dolphins (*Tursiops* sp.): complex male alliances in an open social network. *Proc. Math. Phys. Eng. Sci.* 279, 3083–3090. doi: 10.1098/rspb.2012.0264

Rendell, L., Cantor, M., Gero, S., Whitehead, H., and Mann, J. (2019). Causes and consequences of female centrality in cetacean societies. *Phil. Trans. R. Soc B.* 374, 20180066. doi: 10.1098/rstb.2018.0066

Rendell, L., and Whitehead, H. (2001). Culture in whales and dolphins. *Behav. Brain Sci.* 24 (2), 309–324. doi: 10.1017/S0140525X0100396X

Ryer, C. H., Ottmar, M. L., and Sturm, E. A. (2004). Behavioral impairment after escape from trawl cod ends may not be limited to fragile fish species. *Fish. Res.* 66, 261–269. doi: 10.1016/S0165-7836(03)00197-8

Santana–Garcon, J., Wakefield, C. B., Dorman, S. R., Denham, A., Blight, S., Molony, B. W., et al. (2018). Risk versus reward: interactions, depredation rates, and bycatch mitigation of dolphins in demersal fish trawls. *Can. J. Fish. Aquat. Sci.* 75, 2233–2240. doi: 10.1139/cjfas–2017–0203

Senigaglia, V., Christiansen, F., Sprogis, K. R., Symons, J., and Bejder, L. (2019). Food-provisioning negatively affects calf survival and female reproductive success in bottlenose dolphins. *Sci. Rep.* 9 (1), 8981. doi: 10.1038/s41598-019-45395-6

Sundaresan, S. R., Fischhoff, I. R., Dushoff, J., and Rubenstein, D. I. (2007). Network metrics reveal differences in social organization between two fission–

fusion species, grevy's zebra and onager. *Oecologia* 151, 140–149. doi: 10.1007/s00442–006–0553–6

Tenan, S., Hernández, N., Fearnbach, H., de Stephanis, R., Verborgh, P., and Oro, D. (2020). Impact of maritime traffic and whale-watching on apparent survival of bottlenose dolphins in the strait of Gibraltar. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 30 (5), 949–958. doi: 10.1002/aqc.3292

Titcomb, E. M., O'Corry–Crowe, G., Hartel, E. F., and Mazzoil, M. S. (2015). Social communities and spatiotemporal dynamics of association patterns in estuarine bottlenose dolphins. *Mar. Mamm. Sci.* 31, 1314–1337. doi: 10.1111/mms.12222

Tixier, P., Authier, M., Gasco, N., and Guinet, C. (2015). Influence of artificial food provisioning from fisheries on killer whale reproductive output. *Anim. Conserv.* 18, 207–218. doi: 10.1111/acv.12161

Todd, V. L. G., Lazar, L., Williamson, L. D., Peters, I. T., Hoover, A. L., Cox, S. E., et al. (2020). Underwater visual records of marine megafauna around offshore anthropogenic structures. *Front. Mar. Sci.* 7. doi: 10.3389/fmars.2020.00230

Triossi, F., Willis, T., and Pace, D. S. (2013). Occurrence of bottlenose dolphins *Tursiops truncatus* in natural gas fields of the northwestern Adriatic Sea. *Mar. Ecol.* 34, 373–379. doi: 10.1111/maec.12020

Urian, K., Gorgone, A., Read, A., Balmer, B., Wells, R. S., and Berggren, P. (2015). Recommendations for photo–identification methods used in capture–recapture models with cetaceans. *Mar. Mamm. Sci.* 31, 298–321. doi: 10.1111/mms.12141

Vella, A., Murphy, S., Giménez, J., De Stephanis, R., Mussi, B., Vella, J. G., et al. (2021). The conservation of the endangered Mediterranean common dolphin (*Delphinus delphis*). *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 110–136. doi: 10.1002/aqc.3538

Ventura, D., Bonifazi, A., Gravina, M. F., Belluscio, A., and Ardizzone, G. (2018). Mapping and classification of ecologically sensitive marine habitats using unmanned aerial vehicle (UAV) imagery and object–based image analysis (OBIA). *Remote Sens.* 10, 1331. doi: 10.3390/rs10091331

Ventura, D., Jona Lasinio, G., and Ardizzone, G. (2015). Temporal partitioning of microhabitat use among four juvenile fish species of the genus *Diplodus* (Pisces: Perciformes, sparidae). *Mar. Ecol.* 36, 1013–1032. doi: 10.1111/maec.12198

Weiss, J. (2006). Foraging habitats and associated preferential foraging specializations of bottlenose dolphin (*Tursiops truncatus*) mother-calf pairs. *Aquat. Mamm.* 32 (1), 10–19. doi: 10.1578/AM.32.1.2006.10

Wells, R. S. (2003). "Dolphin social complexity: Lessons from long–term study and life history" in *Animal social complexity: Intelligence, culture, and individualized societies*. Eds. F. B. M. de Waal and P. L. Tyack (Harvard: Harvard University Press), 32–56. doi: 10.4159/harvard.9780674419131.c4

Wells, R. S., Scott, M. D., and Irvine, A. B. (1987). The social structure of free–ranging bottlenose dolphins. *Curr. Mammal.* 1, 247–305. doi: 10.1007/978–1–4757–9909–5_7

Wey, T., Blumstein, D. T., Shen, W., and Jordán, F. (2008). Social network analysis of animal behaviour:a promising tool for the study of sociality. *Anim. Behav.* 75, 333–344. doi: 10.1016/j.anbehav.2007.06.020

Whitehead, H. (1995). Investigating structure and temporal scale in social organizations using identified individuals. *Behaviour* 6, 199–208. doi: 10.1093/beheco/6.2.199

Whitehead, H. (2008a). *Analyzing animal societies: quantitative methods for vertebrate social analysis* (Chicago, IL: University of Chicago Press).

Whitehead, H. (2008b). Precision and power in the analysis of social structure using associations. *Anim. Behav.* 75, 1093–1099. doi: 10.1016/j.anbehav.2007.08.022

Whitehead, H. (2009a). *SOCPROG: programs for analyzing social structure* (Halifax: Nova Scotia, Dalhousie University). Available at: http://whitelab.biology.dal.ca/SOCPROG/Manual.pdf.

Whitehead, H. (2009b). SOCPROG programs: analysing animal social structures. *Behav* 63, 765–778. doi: 10.1007/s00265–008–0697–y

Whitehead, H., Bejder, L., and Ottensmeyer, A. C. (2005). Testing association patterns: issues arising and extensions. *Anim. Behav.* 69, e1–e6. doi: 10.1016/j.anbehav.2004.11.004

Whitehead, H. A. L. (1999). Testing association patterns of social animals. *Anim. Behav* 57(6), F26–F29.

Whitehead, H., and Dufault, S. (1999). Techniques for analyzing vertebrate social structure using identified individuals. *Adv. Study Behav.* 28, 33–74. doi: 10.1016/S0065–3454(08)60215–6

Wirsing, A. J., Heithaus, M. R., Frid, A., and Dill, L. M. (2008). Seascapes of fear: evaluating sublethal predator effects experienced and generated by marine mammals. *Mar. Mamm. Sci.* 24 (1), 1–15. doi: 10.1111/j.1748-7692.2007.00167.x

Wiszniewski, J., Allen, S. J., and Möller, L. M. (2009). Social cohesion in a hierarchically structured embayment population of indo–pacific bottlenose dolphins. *Anim. Behav.* 77, 1449–1457. doi: 10.1016/j.anbehav.2009.02.025

Wittemyer, G., Douglas–Hamilton, I., and Getz, M. (2005). The socioecology of elephants: analysis of the processes creating multitiered social structures. *Anim. Behav.* 69, 1357–1371. doi: 10.1016/j.anbehav.2004.08.018

Würsig, B., and Jefferson, T. A. (1990). Methods of photo–identification for small cetaceans. *Rep. Int. Whal. Commun.* 12, 43–52. doi: 10.1111/j.1365-2907.2008.00119.x

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Assessment of the interactions between cetaceans and fisheries at the south of the Cetacean Migration Corridor and neighboring waters (Western Mediterranean)

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The Cetacean Migration Corridor is an important marine protected area for cetacean species in the Western Mediterranean, and part of its waters constitute the main fishing grounds for the Valencia region (East Spain) fishing fleet. Here we aim to assess the interactions between all active fisheries operating in the waters of the Valencia region and the cetacean species inhabiting this area. A total of 282 face-to-face interviews (51.37% of the total fleet) to fishers were held on 20 ports at the study area to gather information about cetacean by-catch and all types of interactions between fisheries and cetaceans. The interviewed bottom trawlers (n = 148 boats) reported a monthly by-catch rate (C) of 0.01 dolphins per vessel. Bottom trawling vessels operating at neritic zones only reported by-catch of common bottlenose dolphin (C = 0.009), while those fishing at oceanic zones by-caught striped dolphin (C = 0.006) and common bottlenose dolphin (C = 0.003). The interviewed artisanal fishers (n = 114 boats), the second most important fishery in the study area, only reported one dolphin by-catch event, but 90 of these boats communicated continued negative interactions with the common bottlenose dolphin, causing gear damage and catch take. The interviewed vessels gave an estimation of their annual economic loss produced by this interaction (mean ± SD: 2,998.10 ± 2095.02 € per boat). Other fisheries operating in the Valencia region were purse-seiners (n = 15 boats) and pelagic longlines, a fishery that has almost disappeared in the study area (n = 5 boats). The first one had the highest estimated dolphin by-catch rate (C = 0.04) and mainly interacted with common bottlenose dolphin, but also with striped dolphin. The information gathered from interviews was complimented with long-term stranding data (1990–2020). Despite the fact that only 7.80% of all recorded stranded cetaceans showed fisheries interaction signs, 26.72% of the common bottlenose dolphins recorded showed evidence of this interaction, thus confirming that it is the cetacean species most affected by fisheries in the area. Records of stranded striped dolphin (6.45%) also showed evidence of fisheries interaction. Based on both dolphin by-catch and gear damage, management plans are needed in the area to ensure cetacean conservation and also fisheries sustainability in waters inside and around marine protected areas at the Spain's Mediterranean.

KEYWORDS

Cetacean, fisheries interactions, common bottlenose dolphin, striped dolphin, interviews, strandings, Valencia region

1. Introduction

The Western Mediterranean basin hosts up to eight resident cetacean species. The striped dolphin (*Stenella coeruleoalba*) is the most abundant species in the area, but common bottlenose dolphin (*Tursiops truncatus*), short-beaked common dolphin (*Delphinus delphis*), Risso's dolphin (*Grampus griseus*), long-finned pilot whale (*Globicephala melas*), fin whale (*Balaenoptera physalus*), sperm whale (*Physeter macrocephalus*), and Cuviers' beaked whale (*Ziphius cavirostris*) also have stable populations in the area (Gómez de Segura et al., 2006; Boisseau et al., 2010). Moreover, other vagrant species have been reported in this area, such as the minke whale (*Balaenoptera acutorostrata*; Fraija-Fernández et al., 2015, and references therein) or the humpback whale (*Megaptera novaengliae*; Violi et al., 2021) among others. The area between East Iberian Peninsula and Balearic Islands harbors the Cetacean Migration Corridor, a Specially Protected Area of Mediterranean Importance (SPAMI). This marine protected area (MPA) covers 46.385 km^2 and constitutes a key area for cetacean conservation in the Western Mediterranean. Active hydrocarbon prospections and extractive activity are banned at the demarcation of this MPA, except those related to research under permit (OceanCare, 2021). Despite the existence of this MPA, cetaceans still face several threats within the area, such as fisheries interaction. Thus, it is necessary to identify and quantify them to improve the effectiveness of this MPA for helping cetacean species conservation.

Anthropogenic threats affecting cetaceans worldwide include habitat degradation, many types of pollution, acoustic disturbances, marine traffic, and the incidental interaction with fisheries, among others (Nortarbartolo di Sciara, 2016; Nelms et al., 2021, and references therein). Fisheries by-catch is a persistent threat for cetaceans in many seas, with severe impacts on the health and viability of many populations. Cetaceans and fishing fleets usually share niche, and their distribution and fishing activities overlap geographically, entailing negative consequences for cetaceans (Avila et al., 2018; Carpentieri et al., 2021; Moore et al., 2021).

Interactions between cetaceans and different types of fisheries have been described in many places (Lewison et al., 2014). Fishery discards constitute an important food source for cetaceans, attracting them to fishing grounds (Bonizzoni et al., 2022). In areas such as the Atlantic Ocean, bottom trawling entails a high number of cetacean accidental catches (López et al., 2003; Fernández-Contreras et al., 2010). However, absent or low by-catch rates have been reported in the Mediterranean Sea (Gonzalvo et al., 2008; Fortuna et al., 2010). On the other hand, depredation events on artisanal fisheries by dolphins occur frequently, causing damage to nets and to commercial captures (Snape et al., 2018), although low by-catch rates on this fishery have also been reported (Lauriano et al., 2004; Díaz López, 2006; Brotons et al., 2008). Conversely, pelagic longline and purse-seine are fisheries with high levels of cetacean by-catch reported, particularly of small delphinid species, in the Western Mediterranean (Aguilar, 1991; Zahri et al., 2007; Macías-López et al., 2012).

The Valencia region (East Spain, Western Mediterranean) has a big fishing fleet that operates in waters inside and neighboring the Cetacean Migration Corridor SPAMI. This fishing fleet includes bottom trawling, artisanal (using several fishing gears), purse-seine, and pelagic longline vessels (Generalitat Valenciana, 2020). Despite the existing overlapping between fishing grounds and cetacean distribution and habitat use in these waters, there is scarce information about their interactions. To date, only one study described fisheries interactions between one species of cetaceans and artisanal vessels in the area (Revuelta et al., 2018).

Face-to-face interviews to fishers are considered a useful and cost-effective tool for identifying specific problematic interactions between fisheries and threatened marine species, although the results obtained with this methodology are highly dependent on the fishers' reliability (Moore et al., 2010; Goetz et al., 2014). Data from interviews allow to estimate the minimum cetacean by-catch rates in a specific area (López et al., 2003). Prior studies across the Western Spanish Mediterranean denote the importance of such sources of information to analyze marine megafauna by-catch rates and the associated economic loss relative to such interactions (Carreras et al., 2004; Domènech et al., 2015; Revuelta et al., 2018).

Stranding data, although subject to several legal restrictions that constrain access to the animals and also to other caveats, when recorded over long periods and large areas, provide valuable information and have been, for long, a valuable source to determine the species' population status. Considering that working with marine protected species with ocean-wide distribution is often costly and logistically difficult, stranding networks are crucial to evidence the threats to which marine megafauna is exposed, including fisheries interactions (*e.g.*, Leeney et al., 2008; Tomás et al., 2008; Casale et al., 2010). The postmortem examination of stranded animals can unveil the relative mortality consequence of by-catch interactions for both resident and migratory cetacean species (Puig-Lozano et al., 2020; Duras et al., 2021; Peltier et al., 2021). Combining different sources of information, such as face-to-face interviews to fishers and long-term stranding data, can contribute to the obtainment of an accurate assessment of the magnitude at which cetaceans are exposed to fisheries interactions in a specific area.

Here we combine detailed interviews conducted in the fishing ports of the Valencia region with long-term stranding data to gain knowledge about the problem between local fisheries and cetaceans. The specific objectives of the present study are (1) to provide an assessment of the interaction between fisheries operating throughout the Valencia region (East Spain) and the inhabiting cetacean species, and (2) to determine specific areas of cetacean–fisheries interaction in waters inside and neighboring the Cetacean Migration Corridor MPA.

2. Materials and methods

2.1 Study area

The Valencia region coastline extends over 419 km (37°51′ N, 0°45′ W; 40°31′ N, 0°31′ E) across East Spain (Western Mediterranean). The coastal waters are not homogeneous, with a narrower continental shelf at the southernmost province (Alicante) that expands over the central province (Valencia) and is widest at the northernmost province (Castellón, Figure 1). The waters of the Valencia region are included within the

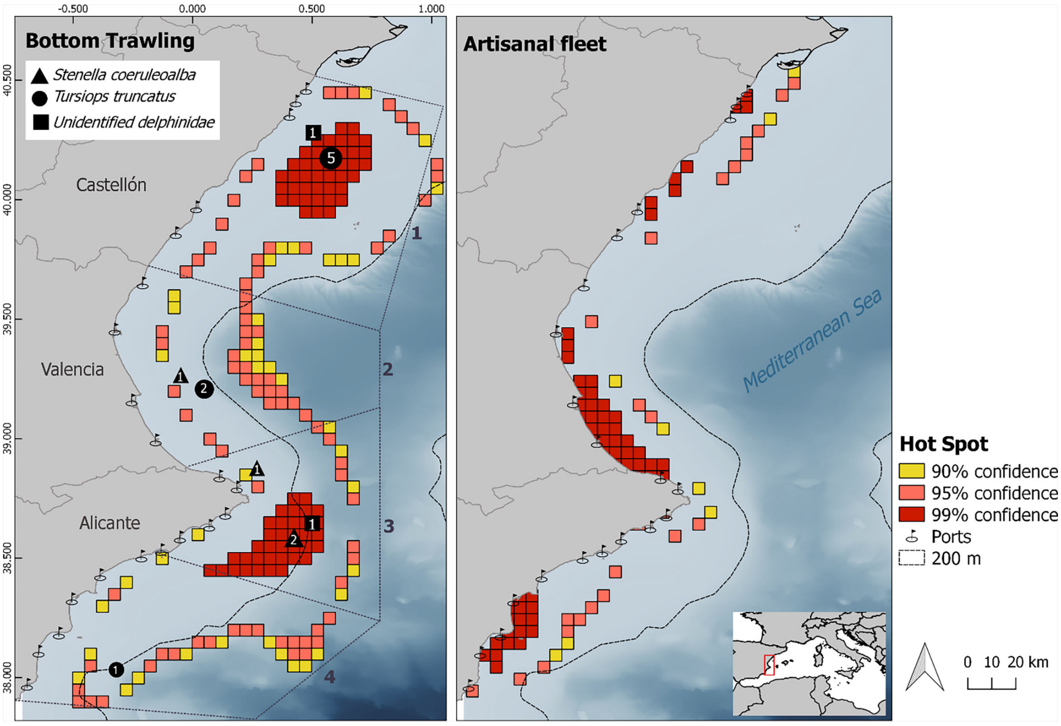

FIGURE 1
Map of the study area (Valencia region, Western Mediterranean) showing the spatial clusters of fishing activity (hot spots) of bottom trawling (left) and artisanal fleets (right) during the interview survey period. Cold spots are not represented. The black symbols show the number of delphinid by-catch events reported by the interviewed bottom trawl vessels. The map includes the distribution of 20 surveyed ports in the three provinces. The bathymetry is depicted with darker shades of blue representing deeper waters and dashed light gray line of the 200-m bathymetric contour. The stratification of the study area in the subzones stablished for bottom trawling fishery is also shown in the map on the left.

Geographical Subarea n°6 from the General Fisheries Commission for the Mediterranean (GFCM GSA06) and comprises part of the Cetacean Migration Corridor MPA. The present study covered 20 ports distributed throughout the three provinces of the Valencia region to provide a heterogeneous sample and analyze potential differences in cetacean–fisheries interactions between provinces.

2.2 Fishing fleet description

The bottom trawling vessels operating in the study area have a mean vessel length of 21.25 ± 3.41 m (range: 16.00–29.00 m) (Ministry of Agriculture, Fisheries and Food, 2020). This fishery has many demersal target species, such as European hake (*Merluccius merluccius*), red mullet (*Mullus barbatus*), monkfish (*Lophius* spp.), and Norway lobster (*Nephrops norvegicus*) (Sala et al., 2019). The Valencian bottom trawling fishing fleet is constituted by 209 vessels (Generalitat Valenciana, 2020), differenciating those fishing in coastal areas all year round from those targeting red shrimp, *Aristeus antennatus*, at deeper waters (400-800 m) at some periods of the year, similarly as described in the adjacent areas of Spain's Mediterranean (Carbonell et al., 1999). This fleet discards a long list of species [see Carbonell et al. (1998)] that can potentially be used as a food resource by marine vertebrates (*e.g.*, Tomás et al., 2001).

Artisanal vessels fish at less than 12 nautical miles from the coast, have a mean vessel length of 10.10 ± 2.00 m (range: 6.00–15.00 m) (Ministry of Agriculture, Fisheries and Food, 2020), and use different gears, mainly trammel nets and also gillnets, pots, and demersal longlines. A total of 294 artisanal vessels of this fleet are active (Generalitat Valenciana, 2020) and are distributed along all fishing ports of the study area. Only vessels using trammel nets and gillnets were included in the present study since they are the most commonly used fishing gears in this fleet and the ones previously described as interacting with cetaceans. The principal target species are common cuttlefish (*Sepia officinalis*), common sole (*Solea solea*), and red mullet (*Mullus* spp.) for trammel nets and Atlantic bonito (*Sarda sarda*), gilt-head bream (*Sparus aurata*), and white seabream (*Diplodus sargus*) for gillnets (Revuelta et al., 2018).

Purse-seine vessels are at least 11 m in length, operate at night in the continental shelf, and target basically European pilchard (*Sardina pilchardus*) and European anchovy (*Engraulis encrasicolus*) (Boubekri et al., 2019). This fishery only has 38 active vessels according to the census of the Generalitat Valenciana (2020). In the Valencia region, pelagic longline is also a minoritarian fishery (*n* = 5 boats) (Generalitat Valenciana, 2020). The pelagic longline boats have variable ranges of vessel length (12–27 m), and its fishing grounds, as purse-seines, cover a big area depending on fish school locations. Pelagic longliners in the Western Mediterranean target swordfish (*Xiphias gladius*), bluefin tuna (*Thunnus thynnus*), and albacore (*Thunnus alalunga*) (Macías-López et al., 2012).

2.3 Interviews to fishers

Interviews were carried out during October–December 2020 at 20 out of 21 ports with fishing vessels at the study area by a trained team of the University of Valencia. Despite the fact that the interviews were conducted after the COVID-19 lockdown situation, the Spanish government considered fishing as an essential activity; hence, fishing activity practically did not stop the year before the interviews. Then, we can assume that the COVID-19 situation did not affect the interview campaign and the results of the present study in the Valencia region. Fishers' associations and port authorities of every port were contacted before conducting the interviews. The interview campaign days were maximized as well as adjusted to the routines of each fishery (Goetz et al., 2014). The interviews were made by large to more than 20% of the active vessels of the two main fisheries (bottom trawling and artisanal) in the study area, following the precedence of other studies in the area [Revuelta et al. (2018) and references therein]. However, in a few ports, the required 20% was narrowly missed: artisanals in Vinaroz and Santa Pola ports and bottom trawlers in Sagunto and Torrevieja ports (Table 1). One questionnaire session was held per boat, with the skipper being preferably the person interviewee, and the interview duration lasted approximately 20 min.

Before the interview was started, the fishers were informed about the confidentiality of their answers and that the personal and professional information asked would be used only for this study. The fishers were asked about their hierarchical level in the crew, number of crew members, fishing gear used (in case of boats using different gears in different periods), and fishing characteristics (vessel length, minimum and maximum depth of fishing operations, five most common target species, spatial information about their fishing activities, and number of months of fishing activity in the previous year to the interview). We also gathered information about gear damage caused by dolphins, estimation of their respective annual economic loss due to this interaction, and cetacean by-catch events in that period. Finally, the interviewees were asked about their perception of cetacean population trends at their fishing grounds. The interviews were conducted with the support of photographs of cetacean species with a higher probability of interacting with fisheries in the area (common bottlenose dolphin, striped dolphin, common short-beaked dolphin, Risso's dolphin, and long-finned pilot whale) to gather accurate information of interacting species (Moore

TABLE 1 Description of the fishing fleet in the Valencia region per port (north to south) at each province, Castellón (C), Valencia (V), and Alicante (A). We represent the number of censed vessels, the number of interviewed vessels and the total engine power (kW) for bottom trawling and artisanal vessels (Generalitat Valenciana, 2020; Ministry of Agriculture, Fisheries and Food, 2020).

Port	Bottom trawling			Artisanals			Total		
	Interviews	Boats	kW	Interviews	Boats	kW	Interviews	Boats	kW
(C) Vinaroz	5	8	1,979.27	4	21	1,219.85	9	29	3,199.12
(C) Benicarló	13	17	4,192.68	6	9	491.39	19	26	4,684.07
(C) Peñíscola	10	21	3,452.43	5	18	844.14	15	39	4,296.57
(C) Castellón	6	14	4,751.93	8	14	949.28	14	28	5,701.21
(C) Burriana	7	8	1,475.95	8	15	914.27	15	23	2,390.22
(V) Sagunto	0	1	–	4	7	322.80	4	8	322.80
(V) Valencia	2	6	539.85	9	14	1,179.69	11	20	1,719.54
(V) Cullera	11	15	2,572.61	10	31	1,507.32	21	46	4,079.93
(V) Gandía	4	5	978.79	15	31	2,160.16	19	36	3,138.95
(A) Denia	13	18	3,159.43	6	10	700.00	19	28	3,859.43
(A) Jávea	4	6	1,586.97	5	7	969.10	9	13	2,556.07
(A) Calpe	9	11	3,259.45	3	7	158.08	12	18	3,417.53
(A) Altea	9	10	3,774.18	2	3	608.10	11	13	4,382.28
(A) Benidorm	–	–	–	1	3	77.94	1	3	77.94
(A) Villajoyosa	26	32	6,807.97	3	14	241.16	29	46	7,049.13
(A) Campello	–	–	–	5	8	476.47	5	8	476.47
(A) Alicante	–	–	–	4	5	184.26	4	5	184.26
(A) Santa Pola	29	36	8,345.41	12	61	2,504.42	41	97	10,849.83
(A) Guardamar	–	–	–	2	10	270.47	2	10	270.47
(A) Torrevieja	0	1	–	2	6	738.93	2	7	738.93

et al., 2010; Revuelta et al., 2018). Only fully completed interviews were considered for further analysis.

2.4 Data analysis

2.4.1 By-catch

By-catch, defined as the unintended capture of marine biota in a fishery targeting different species (Gray and Kennelly, 2018), was related with bottom trawlers' mean fishing depth (m). Mean fishing depth (m) per vessel was calculated using the maximum and minimum depth (m) reported by each interviewee. The waters of the study area where bottom trawlers operate were divided into two marine zones based on bathymetry, neritic (≤200-m depth) and oceanic (>200-m depth) (Hedgpeth, 1957). To analyze differences in the operations of bottom trawlers, we followed Domènech et al. (2015), stratifying information gathered by bottom trawlers in four fishing subareas according to reported fishing zones, the previously stated bathymetrical parameters, trawl fishing depth, and port distribution in these areas (see Figure 1).

Cetacean by-catch rate (C) was determined by dividing the reported annual captures of cetaceans (C_{obs}) by the reported fishing effort of a fishery (F_{obs}), which is the total number of reported months of fishing activity in the previous year to the interview. Annual cetacean by-catch (C_{est}) was estimated by multiplying C by the estimated fishing effort for a fishery (F_{est}).

$$C = \left[\frac{C_{\mathrm{obs}}}{F_{\mathrm{obs}}}\right] \qquad C_{est} = \left[\mathrm{C} \times F_{est}\right]$$

Estimated fishing effort (F_{est}) was extrapolated by multiplying the number of total active censed vessels of a fishery by F_{obs}, which was respectively divided by the number of interviewed vessels.

$$F_{est} = \left[n_t \times \frac{F_{obs}}{n_i}\right]$$

Maximum and minimum 95% confidence intervals (95% CI) were obtained for annual cetacean by-catch estimation (C_{est}) following Greenwood (1996). Cetacean by-catch rates and annual cetacean by-catch estimations were also obtained for bottom trawlers and purse-seiners for those reported species, common bottlenose dolphin and striped dolphin. Additional cetacean by-catch rates were also given for bottom trawlers according to fishing bathymetric zones (Neritic/Oceanic) and fishing subareas (1-4). As artisanal and pelagic longline vessels only reported one capture of cetaceans each, descriptive information was given for both fisheries.

2.4.2 Gear damage: Economic loss in artisanal vessels

In order to evaluate the economic loss produced by cetaceans reported by interviewed artisanal vessels, an ANOVA test (Mangiafico, 2015) was performed to analyze differences between the three provinces. A Spearman correlation test was used to study the correlation between the reported economic loss and the number of months that the artisanal vessels used nets on the previous year to the interviews campaign. A chi-square test was used to compare the artisanal fishers' perception on possible variation in dolphin abundance at their with the one of fishers from other fisheries. In order to establish areas of higher dolphin–fisheries interaction, we considered engine power (kW) of the active artisanal vessels at the 20 surveyed ports, gathered from the Ministry of Agriculture, Fisheries and Food (2020), as a proxy of fishing capacity (Crosti et al., 2017). Then, we related engine power with annual economic loss reported by fishers in each port. Significance for these tests was stablished at $\alpha = 0.05$. Statistical analysis was performed using R 4.0.5.

2.4.3 Spatial analysis of bottom trawling and artisanal fleet

The use of fishers' knowledge through geographical information systems (GISs) allows describing spatially accumulation of fishing activities and fishing grounds (Léopold et al., 2014; Aylesworth et al., 2017). Spatial information about the location of fishing activities was collected during interviews using a base map with a grid of 5 × 5 km cells, including bathymetry, relevant points of reference and localities names. Fishers were asked to draw on the map where they normally fish and each fishing polygon was digitized into GIS vector polygons. Aggregate fishing effort density per cell was expressed as the number of vessels identifying the cell as a fishing ground.

Hot Spot Analysis tool (Getis-Ord Gi*) was used to describe and to map statistically significant spatial clusters for the two main fisheries in the region (bottom trawling and artisanal fleets).

The Getis-Ord Gi$*$ statistic (Getis and Ord, 1992) determines the spatial clustering of grid cell values that are higher (hot spot) than expected by random distribution. It performs significant tests between nearby cells in the surrounding neighborhood area using a *z*-score (Getis and Ord, 1992). The resultant *z*-scores and *p*-values indicate where features with either high or low values cluster spatially. We considered as hot spot the areas where cells with high *z*-score and low *p*-value were spatially clustered.

Distributional maps were created at three levels of confidence (99, 95, and 90%), and all clusters that were within the 99% confidence level were considered for displaying the more intensely hot clusters. All data analysis and a georeferenced distribution map of each fishery fleet were generated using the free open-source Geographic Information System program QGIS, version 3.4.6 (QGIS, 2021).

2.5 Analysis of cetacean strandings

2.5.1 Strandings with evidence of fisheries interaction

We compiled the cases of stranded cetaceans found along the Valencia region coast between 1990 and 2020 displaying fisheries interaction signs. Such stranding events were registered by the cetacean and marine turtle stranding network of the Valencian community that follows the protocol explained in Gozalbes et al. (2010). This network records dead or injured cetaceans and sea turtles stranded on beaches or found floating dead or in a weakened condition. It is coordinated via a 24-h telephone hotline by the Marine Zoology Unit of the University of Valencia (MZU-UV). Since a diagnostic necropsy was not performed in all recorded stranded cetaceans, only stranding cases with available photographs for visual external examination and/or those necropsied or directly examined by the personnel of the MZU-UV were considered to establish fisheries interactions.

The external signs of fisheries interactions in stranded cetaceans considered here were (1) entanglement in fishing gear still attached to the animal in the stranding or by-catch events directly reported by fishers, (2) net marks and superficial skin lesions clearly caused by fishing gear, (3) jaw/skull fracture and broken teeth (these kind of injuries are produced when fishers, particularly trawlers, drop the captures from the net over the ship deck), (4) amputations of different parts of the body with a human cause (*e.g.*, strangulation of flippers by nets), (5) incisions/cuts into the body cavity or over the skin of clear anthropogenic origin, (6) long-term tail entanglement, and (7) remains of fishing gear inside the body cavity and/or the digestive tracts (Duras et al., 2021) (see the examples in Supplementary Figure S1A).

We analyzed trends of common bottlenose dolphin and striped dolphin strandings with fisheries interaction signs (the only two cetacean species with a sufficient sample size) over time through regression analysis. We used a linear regression *t*-test to determine whether the slope of the regression line differs significantly from zero. We explored these trends in two periods—a three-decade study period (1990–2020) and in the last decade (2009–2020)—to gather long-term and recent trends for these two species. We excluded data from the years 1990 and 2007 for striped dolphin since massive stranding of the species occurred in these years due to a *Morbillivirus* epizootic (Duignan et al., 1992; Raga et al., 2008).

2.5.2 Spatial analysis of stranding data

Kernel density maps were produced to describe the spatial distribution of strandings of common bottlenose dolphins and striped dolphins throughout the historical records in the Valencia region coasts between 1990 and 2020. This was only performed for these two species because they are the most abundant cetaceans in the area (Gómez de Segura et al., 2004; Gómez de Segura et al., 2006) and because both account for the vast majority of cetacean interactions reported during the interviews.

The kernel density tool calculates the magnitude per unit of area from point features using the kernel function to produce a more generalized density raster. This allows a visual representation of the density of strandings in an area by creating "hot spots". This required the creation of point shapefiles from geographic coordinates of strandings as an input layer to generate a kernel density estimation (KDE) representing strandings per square kilometer.

Core-stranding areas were identified using fixed kernel density estimations (Worton, 1989) in QGIS version 3.4.6 Geographic Information System Software (QGIS Development Team, 2021). A rule-based *ad hoc* method was applied to estimate the appropriate smoothing parameter (h) for delineating kernel contours (Kie, 2013). We used 90% KDE to estimate the overall stranding range and 50% KDE to represent the core area of dolphin species' strandings.

Since strandings have been previously related to fishing capacity (Byrd et al., 2014; Crosti et al., 2017), we mapped the engine power (kW) of the 21 fishing ports of the Valencia region to visually determine overlapping and assess the spatial relationships of common bottlenose dolphin and striped dolphin stranding (50% KDE) distributions and engine power as a measure of fishing capacity. We represented the proportion of engine power at which each port contributes to the total engine power of the fishing fleet from the study area in Figure 2.

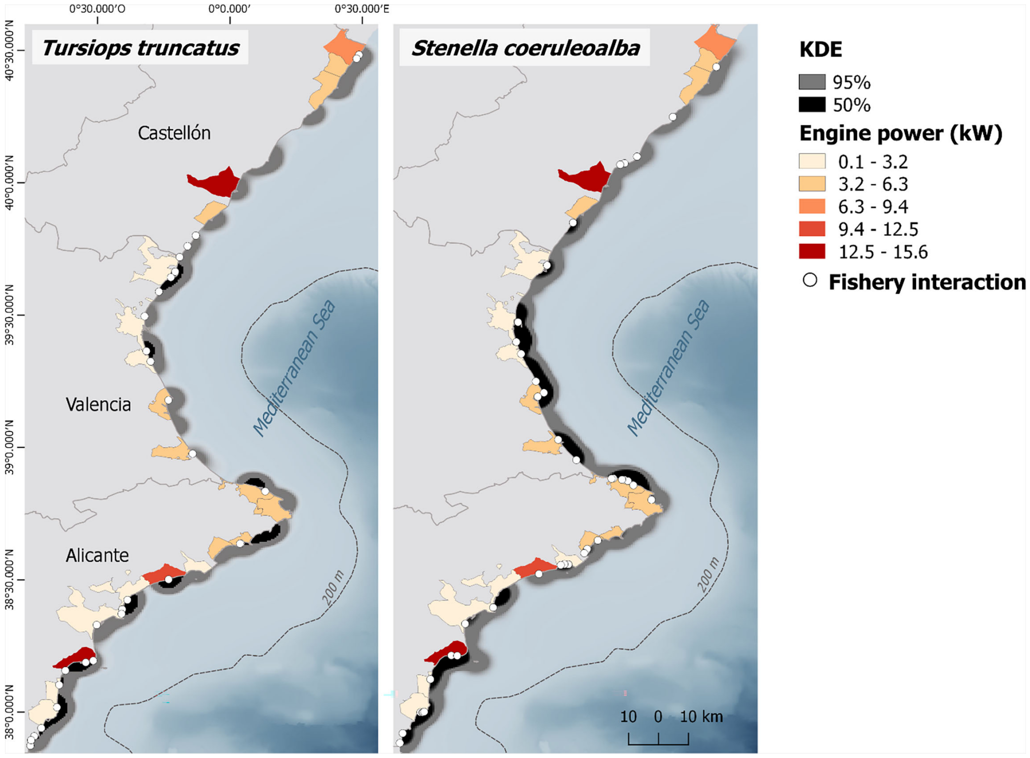

FIGURE 2
Kernel density estimations (KDE) of *Tursiops truncatus* (left) and *Stenella coeruleoalba* (right) stranding events from records of the Valencia region stranding network for the period 1990-2020, according to 5 × 5-km grid cells. The core stranding areas of both species (50% KDE) are represented. The legend inside the figure also shows the ports in five strata representing the proportions of engine power (kW) of each fishing port in relation to the total engine power of the Valencia region fishing fleet. Colored areas represent the municipality of the port. White circles show stranded individuals recorded with fishery interaction signs. Additional details of the study area are shown in Figure 1.

3. Results

3.1 Interview survey

A total of 282 interviews (one per boat) were held during the campaign on 20 out of the 21 ports of the studied area; thus, according to the 2020 census (Generalitat Valenciana, 2020), we surveyed 51.37% of the active censed fleet. The fishers reported 24 accidental catches of small cetaceans, all from the family Delphinidae. There were significant differences regarding by-catch and gear damage events reported among the different gears; therefore, the results per fishery are given below.

3.1.1 Bottom trawling: Fishing ground distribution and cetacean by-catch

The distribution map of the bottom trawling fishing grounds created from the information provided by fishers during interviews (Figure 1) was consistent with the map derived from the VMS data provided by the Spanish Government and North East Atlantic Fisheries Commission (ICES, 2008). The data show that bottom trawlers from the Valencia region ports operated at a mean ± SD depth of 168.30 ± 187. 25 m (range = 20.21–1,203.50 m). For the surveyed bottom trawlers (n = 148), there were significant differences between the mean fishing depth (m) among the four stratified subareas (ANOVA, F = 6.241, $p< 0.001$). Trawlers operating at subarea 3 (mean ± SD: 286.90 ± 223.30 m) and subarea 4 (mean ± SD = 298.70 ± 185.70 m) fished deeper than the ones in subarea 1 (mean ± SD= 141.74 ± 169.25 m). Trawlers operating at subarea 2 fished at a mean ± SD fishing depth of 249.58± 145.03 m, which did not show significant differences with any of the other subareas.

Based on the Getis-Ord Gi* statistic, significant clusters of fishing activity were identified in the waters of the Valencia region. Those higher z-score values defined as hot spots are shown in Figure 1. A hot spot area was found at the northern part of the study area located in the waters over the continental shelf near the 200-m isobaths. A second large hot spot area was detected at the south of the study area, showing that bottom trawling fishing grounds concentrate at both neritic and oceanic zones depending on the area.

Overall, the bottom trawl interviewees reported a by-catch of 16 dolphins for the period October 2019–November 2020, which meant a by-catch rate of 0.01 dolphins captured monthly per vessel. Extrapolating to the entire active fleet of this fishery, this meant an annual cetacean by-catch estimation in the Valencia region of 23 delphinids (95% CI = 7–39). The reported species composition was nine common bottlenose dolphins, four striped dolphins, and three unidentified dolphins by-caught, with differences in the captured species between the neritic and oceanic zones (Table 2). The common bottlenose dolphin by-catch rate was higher in vessels working in the neritic zone (C = 0.009) than in the oceanic zone (C = 0.003). The striped dolphin by-catch events were only reported by fishers operating in the oceanic zone (C = 0.006). There were also differences in by-catch rates between the bottom trawl fishing subareas as the common bottlenose dolphin by-catch rate was higher in subarea 1 (C = 0.012) and subarea 2 (C = 0.018), while for the striped dolphin by-catch rate, it was higher in subareas 2 and 3 (C = 0.006) (Table 2). On this fishery, no substantial gear damage due to dolphin feeding interactions was informed by the interviewees and did not represent a substantial economic loss for this fishery.

3.1.2 Artisanal fleet fishing ground distribution and interactions with cetaceans

A total of 114 interviews were made to artisanal fishers (one per vessel) and were used to map the individual areas of artisanal fishing grounds. The spatial distribution of the interviewed artisanal vessels shows a prevalence of exploitation of the neritic zone. In fact, this fishery showed a mean fishing depth of 34.86 ± 18.80 m (8.52–140.00 m). Figure 1 shows the spatial cluster distribution using the Getis-Ord Gi* and those fishing grounds showing a higher density of artisanal vessels at the

TABLE 2 By-catch of *Tursiops truncatus* and *Stenella coeruleoalba* reported by bottom trawling vessels according to depth zones (neritic/oceanic), fishing subareas (1–4), and totally. The summary of total reported by-catch individuals, by-catch rates, and annual cetacean by-catch estimations with its respective 95% confidence intervals (95% CI) is shown per species. We also present the bottom trawling interviewed vessels, censed vessels, and the estimated fishing effort (Fest) for each stratum and totally.

Species					***Tursiops truncatus***			***Stenella coeruleoalba***		
		Interviews	**Boats**	**Fishing effort**	**Total by-catch**	**By-catch rate**	**Annual by-catch**	**Total by-catch**	**By-catch rate**	**Annual by-catch**
Depth	Neritic	83	116	1,116.64	7	0.009	10	–	–	–
	Oceanic	65	93	962.46	2	0.003	3	4	0.006	6
Fishing subarea	Area 1	44	68	650.66	5	0.012	8	–	–	–
	Area 2	17	27	266.00	3	0.018	5	1	0.006	2
	Area 3	35	45	450.93	1	0.003	1	2	0.006	3
	Area 4	52	69	711.51	–	–	–	1	0.002	1
Total		148	209	2,079.10	9	0.006	13 (0–29)	4	0.003	6 (0–22)

study. The larger areas identified by statistically significant Gi* showed the importance of a variety of fishing zones along the coast. The higher *z*-score values, in red, correspond to the area between Alicante and Santa Pola ports, the area between Denia and Cullera ports, and finally the area around the three northernmost ports (Peñíscola, Vinaróz, and Benicarló). Other hot spots were also evident around the port of Castellón.

Only one by-catch event of a dolphin that the interviewed fisher did not identify at a species level was reported. However, a total of 103 (90.35%) of the interviewed artisanal fishers reported negative interactions with dolphins. In all cases, common bottlenose dolphin was identified as the species causing this interaction. The fishers reported that dolphins cause economic loss by taking or damaging the target species in nets and/or captures and damaging their fishing nets. From all artisanal fishers who reported a negative interaction with dolphins, 90 (87.38%) gave an estimation of their annual economic loss. The mean annual economic loss reported per artisanal vessel was 2,998.10 ± 2,095.02 € (range: 100–9,000 €). Economic loss was significantly different between the three provinces (ANOVA, $F = 3.205$, $p < 0.05$), the lowest mean ± SD being reported at Castellón province (1,716.67 ± 1,617.17 € per vessel) and the highest at Valencia province (3,050.00 ± 2,246.89 € per vessel). This province has ports with high artisanal engine power, such as Gandía, Cullera, and Valencia city (total for Valencia province: 5,169.97 kW) (Table 1). The reported economic loss was also found to be positively correlated ($r^2 = 0.24$) with the number of months that the artisanal fishers used nets (Spearman, $S = 187{,}987$, $p < 0.05$).

3.1.3 Interactions between cetaceans and other fisheries operating in the region

The purse-seine fishers interviewed ($n = 15$ boats) reported four common bottlenose dolphins and two striped dolphins in by-catch events, which supposed a by-catch rate of 0.04 dolphins accidentally caught monthly per vessel. The estimated annual delphinid by-catch for this fishery was 15 dolphin individuals (95% CI: 3–26) for the entire purse-seine fleet. The common bottlenose dolphin by-catch rate was 0.026, and the estimation of the annual by-catch was 10 individuals. The striped dolphin by-catch rate was 0.013, and the estimation of annual by-catch for this species was five individuals. We were able to gather scarce information about mortality due to by-catch for bottom trawling and artisanal and pelagic longline fisheries, although inconsistently. However, all purse-seine fishers interviewed did provide this information. The fishers reported that 33% of cetaceans accidentally caught by this fishery were released alive, while the rest were discharged already dead from nets. Regarding the purse-seiners' gear damage produced by dolphins, four out of 15 interviewees (26.67%) reported sporadic damage on their fishing gear produced by striped dolphin and common bottlenose dolphin, with an associated economic loss (mean ± SD: 9700.00 ± 7596.05 € per vessel). In addition, negative interactions due to depredation by other species, such as tuna species, were reported during the interview campaign.

The pelagic longline vessels interviewed ($n = 5$) operate throughout the study area waters in a mean fishing depth of 201.20 ± 62.70 m (range: 101.86–283.36 m). One pelagic longline vessel reported one accidental capture of a Risso's dolphin, with no gear damage produced by cetaceans at this fishery according to interviewees.

3.1.4 Cetacean population trends according to fishers

In the context of all fisheries, 42.91% of fishers perceived that the cetacean population abundance remains stable at their fishing grounds, while 44.70% assured that the presence of dolphin species has increased. The proportion of artisanal fishers perceiving an increase of dolphin population at their fishing grounds (63.55%) was significantly higher than the one for bottom trawling fishers (33.78%) (chi-square test, $\chi^2 = 32.66$, $p < 0.001$), purse-seines (13.33%) (chi-square test, $\chi^2 = 74.71$, $p < 0.001$), and pelagic longlines (20.00%) (chi-square test, $\chi^2 = 41.40$, $p < 0.001$).

3.2 Cetacean stranding data in the Valencia region

Regarding the information gathered from the stranding network of the Valencia region, a sum of 1,411 cetaceans were stranded at the Valencia region coast between 1990 and 2020. The percentage of stranded cetaceans showing fisheries interactions evidence on the study area and period reaches 7.80% of the total number of cetacean stranding records (Table 3).

3.2.1 Evidences of fisheries interactions in stranded cetaceans

Between 1990 and 2020, a sum of 110 cetaceans recorded by the stranding network of the Valencia region, either stranded or bycaught, showed different evidence of fisheries interactions. Fourteen of them were recorded entangled in fishing gears. One common bottlenose dolphin, one striped dolphin, and two unidentified cetaceans were reported to the network after being having been captured by bottom trawlers. One common bottlenose dolphin, two striped dolphins, and one Risso's dolphin were reported after being entangled in the nets of artisanal vessels. One fin whale and one Cuviers' beaked whale were found entangled in drift nets, and one common short-beaked common dolphin was recorded after being entangled in a longline fishing gear. Three unidentified delphinids were also recorded as by-caught in fishing gears not specified in the records of the network.

3.2.2 Common bottlenose dolphin and striped dolphin strandings' density distribution

Striped dolphin and common bottlenose dolphin species both show a higher number of stranding records at the study area. From 1990 to 2020, the study period, stranding reports were filed for 166 common bottlenose dolphins and 759 striped dolphins throughout the Valencia region coastline. The mean annual common bottlenose dolphin stranding records during the study period was 5.35 ± 3.20, ranging from 0 in 1999 to 14 in 2003. A positive trend in strandings with fisheries interactions signs for the species was detected for the 1990–2020 period (t-test for regression slope, $t = 4.295$, $p < 0.001$) and also for the 2009–2020 period ($t = 3.883$, $p < 0.01$). Common bottlenose dolphin was the species that showed the highest percentage of strandings with fisheries interactions signs (Table 3).

For the striped dolphin, the mean annual stranding records during the study period was 24.50 ± 28.13, ranging from 6 in 1996 to 154 in 1990. For this species, a positive trend in strandings with fisheries interaction signs was found during the whole studied period ($t = 3.355$, $p < 0.01$), but not for the 2009–2020 period ($t = 0.968$, $p > 0.05$). Figure 2 shows the locations of the stranding records of individuals of these two species showing evidences of fisheries interactions from 1990 to 2020.

The spatial distribution of common bottlenose dolphin strandings in the region estimated by KDE indicated that the highest stranding densities occurred along the Alicante coastline (Figure 2). The estimates also showed two isolated clusters in the Valencia province, around Sagunto and at the south of Valencia city. There was no substantial stranding density for common bottlenose dolphins in the Castellón province (Figure 2).

TABLE 3 Number of cetacean individuals stranded on the Valencian Community coast between 1990 and 2020. Individuals showing fisheries' interaction evidence are given per species, with its associated percentage (%) calculated according to the total strandings of each species.

Species	Stranded individuals	Individuals showing fisheries interactions	%
Non-identified cetaceans	374	17	4.54
Tursiops truncatus	116	31	26.72
Stenella coeruleoalba	759	49	6.46
Delphinus delphis	27	3	11.11
Grampus griseus	42	3	7.14
Globicephala melas	23	0	–
Balaenoptera physalus	19	2	10.53
Balaenoptera acutorostrata	4	0	–
Physeter macrocephalus	37	4	10.81
Megaptera novaeangliae	1	0	–
Ziphius cavirostris	9	1	11.11
Total	1,411	110	7.80

The spatial distribution of striped dolphins stranded in the Valencia region coasts also reveals the lowest stranding density along the Castellón province coastline for this species (Figure 2). The highest stranding densities occurred between the ports of Valencia, Cullera, and Gandia. The estimates also showed a small concentration of strandings at the north of the Valencia province and at the southernmost port of the Castellón province. Another area of concentration of strandings was located at the south of the Alicante province, between the ports of Alicante and Torrevieja, and an isolated cluster around El Campello port (Figure 2).

On the other hand, the distribution of engine power contribution of the active censed fleet shows a concentration of ports with higher contribution at the south of the study area (Figure 2), as only Santa Pola and Villajoyosa ports accumulate 28.23% of the engine power of the Valencia region fishing fleet (Ministry of Agriculture, Fisheries and Food, 2020) (Table 1, Figure 2). This concentration of engine power at the south of the region seems to be associated with common bottlenose dolphin and striped dolphin stranding distribution there. On the contrary, some ports at Castellón province highly contributing to the total engine power of the study area seem not to be associated with the strandings of both the common bottlenose dolphin and the striped dolphin due to the absence of KDE core-stranding areas for these species. The lack of association between fishing capacity and stranding distribution can also be seen at ports found at the Valencia province, the province with the lowest fishing capacity contribution value, but with KDE core-stranding areas, especially for striped dolphin.

4. Discussion

4. 1 Problem of cetacean–fisheries interactions according to fishers

Despite the diversity of cetaceans in the Western Mediterranean waters and particularly in the Valencia region (Gozalbes et al., 2010), according to fishers' perception, only two species exhibit an important interaction with fisheries in this area, the common bottlenose dolphins and the striped dolphins. Both species were defined as "Vulnerable" in the Mediterranean Sea, and fisheries interactions were already defined as a threat for them, especially for the common bottlenose dolphin (IUCN, 2021). This interaction seems to be negative in a twofold way. Firstly, fisheries may cause the accidental capture of these species, either because both share the same area or because dolphins may be attracted to fishing grounds by captures in nets or fishing discards, although the by-catch rates seem to be lower in the Valencia region waters compared to other areas (López et al., 2003). Secondly, when predating on captures, dolphins can produce economical loss by reducing the captures of target species and by damaging fishing gears during depredation events, thus increasing the fishers' negative perception of these threatened species. Recent protection figures have been created to protect cetacean species in Spain's Mediterranean waters, such as the Cetacean Migration Corridor (OceanCare, 2021). However, this large marine protected area mainly comprises open waters; thus, little protection may be offered to dolphins inhabiting or migrating throughout the coastal waters. Despite the existence of several marine protected areas in waters over the continental shelf at the Valencia region, even Sites of Community Importance were designated to specifically protect common bottlenose dolphin (Revuelta et al., 2018); those are much smaller than the common bottlenose dolphin distribution in the study area [see Gómez de Segura et al. (2004); Gómez de Segura et al. (2008)]. Marine protected areas also function as nurseries for many target species of regional fisheries, and fishing activity is intense around these areas. All these imply a frequent overlapping between dolphin distribution range and fishing grounds.

Bottom trawling is one of the two main fisheries operating in the area regarding the number of censed vessels, engine power, and fishing effort. Several cetacean species distributions (Gómez de Segura et al., 2004; Gómez de Segura et al., 2008) overlap with the estimated bottom trawling fishing hot spot areas. By-catch events of common bottlenose dolphins and striped dolphins by bottom trawls were reported also in low numbers in other areas of the Mediterranean Sea (Bearzi, 2002; Fortuna et al., 2010). The observed differences in the reported by-catch of cetacean species among subareas could be related to the mean fishing depth of bottom trawlers operating on each subarea. Subarea 1 has a wider continental shelf, which implies that the fishing activities of bottom trawlers concentrate at shallower waters. In fact, bottom trawlers fishing in subarea 1 and in the neritic zone of other subareas reported higher numbers of common bottlenose dolphin accidentally caught, supporting that depth is one factor ruling common bottlenose dolphin interaction with this fishery (Gonzalvo et al., 2008).

On the other hand, bottom trawlers working in the oceanic zone and in waters off subareas with a narrower continental shelf (subareas 2, 3, and 4) work at a higher mean fishing depth (m) and, therefore, reported striped dolphin accidental catches. This result may support the idea about how prey abundance and distribution influence the preferred habitats for cetaceans (Cañadas et al., 2002; Giannoulaki et al., 2016) and the consequent threats associated with the habitat of the species.

Striped dolphin has oceanic distribution in the region (Gómez de Segura et al., 2008, and references therein); however, in the last decade, this species seems to have changed its feeding habits in the Western Mediterranean, increasing the proportion of demersal preys (Aznar et al., 2017), also targeted by bottom trawlers. Moreover, long-term boat-based surveys seemed to indicate a switching trend in the habitat preference of striped dolphin since 2008, expanding its range to the continental shelf (Fraija-Fernández et al., 2015). Despite the fact that more detailed studies on the current distribution and habitat use by striped dolphins in the area are needed, these evidence may explain the interaction of this species with bottom trawling in the present study. Further monitoring of both the striped dolphin habitat use and by-catch by bottom-trawling is necessary to avoid future conservation problems for the species related to its interaction with this fishery.

The artisanal fleet using trammel nets and gillnets also showed hot spot areas of fishing activity throughout the Valencia region coastal waters. In the present study, artisanal fishers reported little dolphin by-catch numbers as in a previous study held in the area (Revuelta et al., 2018) and in other areas of the Mediterranean Sea (Díaz López, 2006; Brotons et al., 2008; Gonzalvo et al., 2015; Pennino et al., 2015). This may be indicative that dolphins are able to detect and avoid the nets in most of the cases. This ability would let the dolphins approach the nets and take captures, therefore causing fishing gear damage and economic loss (Snape et al., 2018). Despite that there are no accurate common bottlenose dolphin population estimations in the studied area since almost two decades (Gómez de Segura et al., 2006), this species seems abundant and has been seen frequently in coastal waters (MZU-UV, studies in progress), often in hot spot areas of artisanal fishing grounds. Moreover, the opportunistic feeding strategy of the species includes demersal target species of this fishing gear (Blanco et al., 2001; Giménez et al., 2017). Overall, the high percentage of artisanal fishers that reported negative interactions with common bottlenose dolphin in our study (see also Revuelta et al., 2018) is not unexpected. The nearshore distribution of this fishery overlaps with common bottlenose dolphin distribution typically found at the continental shelf (Gannier, 2005; Gnone et al., 2011). However, as was described above, changes in striped dolphin distribution in the area could include this species in the conflict with local artisanal fishers, particularly because this fishing hot spot area is found at the south of Valencia province (Figure 1), located west to the most important area for this species in the waters off the Valencia region (Gómez de Segura et al., 2008).

Our results also show that there were differences between the three provinces. Ports at Alicante, as reported by Revuelta et al. (2018), but specially at Valencia province, seemed to have higher economic loss in contrast to those found at Castellón, the northernmost province. There was a generalized fisher's perception that the interaction with dolphins is increasing throughout all the study areas in the last 5 years, which also has been recorded in other regions of Spain and in other areas of the Mediterranean Sea (Mónaco et al., 2020). In fact, the highest proportion of fishers perceiving an increase of dolphin presence in their fishing grounds belonged to artisanal fisheries (63.55%). However, the economic loss reported by artisanal fishers in our study was similar to the values registered by Revuelta et al. (2018) in interviews made in 2015; hence, despite that fishers have become more active in clamming for this issue with dolphins, the problem seems not to have economically worsened in the last years.

In order to have a complete assessment of the interaction between cetaceans and fisheries, we also took into consideration the two other minoritarian fisheries regarding the number of censed vessels: the purse-seines and pelagic longlines. According to interviews, purse-seiners had the highest by-catch rate compared with other fishing gears. The by-catch events of dolphins, more specifically of common bottlenose dolphin and striped dolphin, were reported. These species have also been captured by purse-seines elsewhere (Tudela, 2004; Zahri et al., 2007; Marçalo et al., 2015). The coastal distribution of purse-seiners targeting small pelagic fish schools again explains the interaction with common bottlenose dolphin. Striped dolphin by-catch events in this fishery could be explained because purse-seiners target species are part of the diet of this dolphin (Gómez-Campos et al., 2011; Aznar et al., 2017), and as explained before, by the potential habitat switch of striped dolphin in the area (Aznar et al., 2017).

The pelagic longline fleet at the study area currently has a small representation despite being bigger in the past (Generalitat Valenciana, 2020), and its involvement in by-catch events has decreased due to laws forcing changes in the fishing gear (Tomás et al., 2008). In the present study, one accidental capture of one Risso's dolphin was reported by this pelagic fishery. It is possible that the interaction between this fishery and this species could have been underestimated or undetected in the past since the Risso's dolphin distribution overlaps with the fishing grounds of this fishery in waters over the slope and in open waters, and the species has already been reported being by-caught by pelagic longlines in the Western Mediterranean (Macías-López et al., 2012).

4.2 Assessment of fisheries interactions according to strandings

Cetacean long-term stranding data (1990–2020) show a small percentage of cetaceans stranded with evidence of fisheries interactions. Only in a few of these cases could the specific fishing gear be identified. Common bottlenose dolphin and striped dolphin were the two species showing more strandings with fisheries interaction signs; however, our data gathering revealed that other species may interact with fisheries in the study area, although sporadically. This reduced number of records may be a consequence of the oceanic distribution of most cetacean species inhabiting Spain's Mediterranean waters [see Gómez de Segura et al. (2004)], which makes it difficult for the carcasses of dead animals to reach the coast. Nonetheless, the stranding data support the information provided by fishers during interviews, particularly concerning the interaction with common bottlenose dolphin and also the striped dolphin. The increase in stranding events with fisheries interaction signs of individuals of these two dolphin species over the whole study period may be conditioned by the improvement of the network, particularly during the first 10 years, since no significant trend was found for striped dolphins in the latter years. However, this did happen for the common bottlenose dolphin.

The common bottlenose dolphin has been also reported as the species with a higher percentage of individuals stranded with fisheries interactions signs in a recent study carried out in the Catalonian coast, north to the Valencia region (Cuvertoret-Sanz et al., 2020). In that study, fishery interaction was the most frequent cause of death for the analyzed common bottlenose dolphins, thus confirming the magnitude of this threat in the Western Mediterranean [as informed by Hammond et al. (2012)]. As said before, in the present study, we detected a recent increasing trend in common bottlenose dolphin strandings with signs of fisheries interactions, which may support the perception of increase in abundance of common bottlenose dolphin stocks as reported by fishers during the interviews. This increasing trend in strandings could be linked to an increase in dolphin population size as reported elsewhere (*e.g.*, Leeney et al., 2008) as well as to the increase in associated negative fishery interactions [see also Powell and Wells (2011)]. Moreover, Cuvertoret-Sanz et al. (2020) also reported striped dolphin as a species dying because of fishery interaction. In our study, this species has the highest number of individuals with fisheries interaction signs, even more than the common bottlenose dolphin, but it has a lower percentage probably because it is the most abundant cetacean in the area and, thus, in the stranding network. It has also suffered several episodes of massive strandings caused by a *Morbillivirus* epizootic (Van Bressem et al., 2014). Nonetheless, although for this species a recently increasing trend on stranding individuals with fisheries interaction signs was not detected, the total number of records could confirm the interaction level at which the species is exposed at the study area.

The high stranding density observed for the two species of delphinids at the south of the study area could be related with the location of the ports Santa Pola and Villajoyosa, both contributing to a high percentage of the engine power of the regional fishing fleet, a factor that has been correlated with strandings in other places (Byrd et al., 2014; Crosti et al., 2017). Areas with higher engine power are supposed to contain a larger fleet. Santa Pola and Villajoyosa are the ports with the highest bottom trawling fleet in the Valencia region. However, in other parts of the study area engine power does not match with strandings distribution. The high contribution of fishing capacity found at the northern ports is not associated with the strandings of striped dolphin, probably because this is not the preferred area of distribution for the species in the Valencia region waters (Gómez de Segura et al., 2006) and also because the continental shelf is much wider in this area, and this species is preferably distributed in waters over the slope. The fishing fleet engine power in Castellón is also not related with common bottlenose dolphin stranding distribution, probably because the local stocks can be found at waters further from the coast, over the wide continental shelf near Columbretes Islands (Revuelta et al., 2018). Carcasses of animals dead in open waters are less likely to reach the coast and be recorded (Leeney et al., 2008). The opposite situation seems to happen at Valencia province, where low engine power contribution values do not explain the clusters of striped dolphin strandings, probably associated with the proximity to the preferred area of distribution for striped dolphin in the region. Therefore, fishing capacity alone does not seem to explain the stranding distribution throughout the region The effect of other variables such as species distribution (Leeney et al., 2008), abundance of cetacean species and/or of their prey, or drifting conditions and migration of carcasses due to sea currents and winds (Peltier et al., 2014; Saavedra et al., 2017; Jog et al., 2022) should be also considered to explain cetacean distribution. In fact, at the study area, the stranding clusters of both dolphin species found at the south of the Valencia province could be explained by the existence of a main north–south surface coastal current, already described as a potential factor explaining loggerhead sea turtle stranding distribution in the area (Tomás et al., 2008).

4.3 Combination of methodologies and future management actions

As described in the previous section, stranding-based studies are subject to several gaps, and several factors could explain stranding aggregations at certain locations. As said before, interview-based studies depend on reliability of the interviewed fishers. However, working with marine protected megafauna is often costly and logistically difficult. Hence, combining data obtained from these relatively low-cost methodologies over a long period and large area, despite not fully accurate, may help in gaining knowledge on cetacean species and assessing threats that affect them in a certain area so as to help in their conservation. Through a combination of interviews to fishers, stranding records, and spatial analysis, we could assess cetacean–fisheries interactions happening in waters inside and neighboring the Cetacean Migration Corridor MPA, a key area for cetacean protection. Our results, especially for common bottlenose dolphin and striped dolphin, reflect the need of monitoring and applying management and conservation measures on waters inside and near this protected area.

The current situation of local fisheries is critical due to several factors, including the reduction of target species' stocks, the increase of fuel cost caused by the current global situation, the recent COVID-19 pandemic situation, and the lack of generational handover. The economical loss caused by dolphins is already seen as another problem added to this situation and may result in illegal actions against this protected species. Valencia fishers are still very collaborative with conservation workers and aware of conservation issues; however, management actions are needed for both keeping the fishing activity sustainable and preserving the dolphin populations in the area. Future work must focus on updating extant knowledge in cetacean species abundance, distribution, behavior, and habitat use in order to assess spatial risk (Jog et al., 2022), but it is also necessary to implement management actions for reducing cetacean–fisheries interactions, such as the use of visual deterrent devices, acoustic deterrent devices, and gear modifications, also contributing with compensation funds to fishers in order to reduce these interactions (Jog et al., 2022).

Data availability statement

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

Author contributions

MI-S, JT, JAR, and OR contributed to the conception and design of the study. MI-S, RM-L, and DR-G undertook most of the interviews. MI-S, PG, OR, RM-L, and DR-G created the databases. MI-S, OR, and RM-L performed the data analyses and statistics. MI-S, OR, RM-L, and JT wrote the manuscript. JT and JAR searched for funding for the study. 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.981638/full#supplementary-material

References

Aguilar, A. (1991). Calving and early mortality in the western Mediterranean striped dolphin, *Stenella coeruleoalba*. *Can. J. Zool.* 69 (5), 1408–1412. doi: 10.1139/z91-199

Avila, I. C., Kaschner, K., and Dormann, C. F. (2018). Current global risks to marine mammals: Taking stock of the threats. *Biol. Conserv.* 221, 44–58. doi: 10.1016/j.biocon.2018.02.021

Aylesworth, L., Phoonsawat, R., Suvanachai, P., and Vincent, A. C. J. (2017). Generating spatial data for marine conservation and management. *Biodivers. Conserv.* 26 (2), 383–399. doi: 10.1007/s10531-016-1248-x

Aznar, F. J., Míguez-Lozano, R., Ruiz, B., de Castro, A. B., Raga, J. A., and Blanco, C. (2017). Long-term changes, (1990-2012) in the diet of striped dolphins *Stenella coeruleoalba* from the Western Mediterranean. *Mar. Ecol. Prog. Ser.* 568, 231–247. doi: 10.3354/meps12063

Bearzi, G. (2002). Interactions between cetacean and fisheries in the Mediterranean sea. *Cetaceans of the Mediterranean and black seas: state of knowledge and conservation strategies*, vol. 9. Ed. G.N. di Sciara (Monaco: ACCOBAMS), 78–97.

Blanco, C., Salomón, O., and Raga, J. A. (2001). Diet of the bottlenose dolphin (*Tursiops truncatus*) in the western Mediterranean Sea. *J. Mar. Biol. Assoc. U. K.* 81 (6), 1053–1058. doi: 10.1017/S0025315401005057

Boisseau, O., Lacey, C., Lewis, T., Moscrop, A., Danbolt, M., and McLanaghan, R. (2010). Encounter rates of cetaceans in the Mediterranean Sea and contiguous Atlantic area. *J. Mar. Biol. Assoc. U. K.* 90 (8), 1589–11599. doi: 10.1017/s0025315410000342

Bonizzoni, S., Hamilton, S., Reeves, R. R., Genov, T., and Bearzi, G. (2022). Odontocete cetaceans foraging behind trawlers, worldwide. *Rev. Fish. Biol. Fish.* 32, 827–877. doi: 10.1007/s11160-022-09712-z

Boubekri, I., Amara, R., and Mazurek, H. (2019). Characterization of purse seine fishery of Ziama Bay (Gulf of Béjaia, SW mediterranean): Implications for sustainability management. *Ocean Coast. Manage.* 175, 79–89. doi: 10.1016/j.ocecoaman.2019.03.024

Brotons, J. M., Grau, A. M., and Rendell, L. (2008). Estimating the impact of interactions between bottlenose dolphins and artisanal fisheries around the Balearic islands. *Mar. Mamm. Sci.* 24 (1), 112–127. doi: 10.1111/j.1748-7692.2007.00164.x

Byrd, B. L., Hohn, A. A., Lovewell, G. N., Altman, K. M., Barco, S. G., Friedlaender, A., et al. (2014). Strandings as indicators of marine mammal biodiversity and human interactions off the coast of north Carolina. *Fish. Bull.* 112 (1), 1–23. doi: 10.7755/FB.112.1.1

Cañadas, A., Sagarminaga, R., and Garcıa-Tiscar, S. (2002). Cetacean distribution related with depth and slope in the Mediterranean waters off southern Spain. *Deep-Sea Res.* 49 (11), 2053–2073. doi: 10.1016/s0967-0637(02)00123-1

Carbonell, A., Martin, P., de Ranieri, S.WEDIS team (1998). Discards of the western Mediterranean trawl fleets. *Commun. Int. Mer. Médit.* 35, 392–393.

Carbonell, A., Carbonell, M., Demestre, M., Grau, A., and Monserrat, S. (1999). The red shrimp *Aristeus antennatus* (Risso 1816) fishery and biology in the Balearic islands, Western Mediterranean. *Fish. Res.* 44 (1), 1–13. doi: 10.1016/s0165-7836(99)00079-x

Carpentieri, P., Nastasi, A., Sessa, M., and Srour, A. (2021). *Incidental catch of vulnerable species in Mediterranean and black Sea fisheries – a review*. Rome: FAO. doi: 10.4060/cb5405en

Carreras, C., Cardona, L., and Aguilar, A. (2004). Incidental catch of the loggerhead turtle *Caretta caretta* off the Balearic islands (western Mediterranean). *Biol. Conserv.* 117 (3), 321–329. doi: 10.1016/j.biocon.2003.12.010

Casale, P., Affronte, M., Insacco, G., Freggi, D., Vallini, C., Pino d'Astore, P., et al. (2010). Sea Turtle strandings reveal high anthropogenic mortality in Italian waters. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 20 (6), 611–620. doi: 10.1002/aqc.1133

Crosti, R., Arcangeli, A., Romeo, T., and Andaloro, F. (2017). Assessing the relationship between cetacean strandings (*Tursiops truncatus* and *Stenella coeruleoalba*) and fishery pressure indicators in Sicily (Mediterranean Sea) within the framework of the EU habitats directive. *Eur. J. Wildl Res.* 63 (3), 1–13. doi: 10.1007/s10344-017-1111-8

Cuvertoret-Sanz, M., López-Figueroa, C., O'Byrne, A., Canturri, A., Martí-Garcia, B., Pintado, E., et al. (2020). Causes of cetacean stranding and death on the catalonian coast (western Mediterranean Sea), 2012-2019. *Dis. Aquat. Organ.* 142, 239–253. doi: 10.3354/dao03550

Díaz López, B. (2006). Interactions between Mediterranean bottlenose dolphins (*Tursiops truncatus*) and gillnets off Sardinia, Italy. *ICES J. Mar. Sci.* 63, 946–951. doi: 10.1016/j.icesjms.2005.06.012

Domènech, F., Álvarez de Quevedo, I., Merchán, M., Revuelta, O., Vélez-Rubio, G., Bitón, S., et al. (2015). Incidental catch of marine turtles by Spanish bottom trawlers in the western Mediterranean. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 25 (5), 678–689. doi: 10.1002/aqc.2463

Duignan, P. J., Geraci, J. R., Raga, J. A., and Calzada, N. (1992). Pathology of morbillivirus infection in striped dolphins (*Stenella coeruleoalba*) from Valencia and Murcia, Spain. *Can. J. Vet. Res.* 56 (3), 242–248.

Duras, M., Galov, A., Korpes, K., Kolenc, M., Baburić, M., Gudan Kurilj, A., et al. (2021). Cetacean mortality due to interactions with fisheries and marine litter ingestion in the Croatian part of the Adriatic Sea from 1990 to 2019. *Vet. Arh.* 91 (2), 189–206. doi: 10.24099/vet.arhiv.1254

Fernández-Contreras, M. M., Cardona, L., Lockyer, C. H., and Aguilar, A. (2010). Incidental bycatch of short-beaked common dolphins (*Delphinus delphis*) by pairtrawlers off northwestern Spain. *ICES J. Mar. Sci.* 67 (8), 1732–1738. doi: 10.1093/icesjms/fsq077

Fortuna, C. M., Vallini, C., Filidei, E., Ruffino, M., Consalvo, I., Di Muccio, S., et al. (2010). By-catch of cetaceans and other species of conservation concern during pair trawl fishing operations in the Adriatic Sea (Italy). *Chem. Ecol.* 26 (sup 1), 65–76. doi: 10.1080/02757541003627662

Fraija-Fernández, N., Gozalbes, P., Tomás, J., Balbuena, J. A., Domènech, F., Eymar, J., et al. (2015). Long-term boat-based surveys in the central Spanish

Mediterranean, (2003–2013): cetacean diversity and distribution. *In: Book of abstracts of the 29th*. Malta: European Cetacean Society conference, P 141.

Gannier, A. (2005). Summer distribution and relative abundance of delphinids in the Mediterranean Sea. *Rev. Ecol. (Terre Vie).* 60 (3), 223–238.

Generalitat Valenciana. (2020). Available at: https://agroambient.gva.es/va/web/pesca/flota-pesquera (Accessed July 15, 2021).

Getis, A., and Ord, J. K. (1992). The analysis of spatial association by use of distance statistics. in *Geographical analysis*, vol. 24. (Ohio State University Press), 189–206.

Giannoulaki, M., Markoglou, E., Valavanis, V. D., Alexiadou, P., Cucknell, A., and Frantzis, A. (2016). Linking small pelagic fish and cetacean distribution to model suitable habitat for coastal dolphin species, *Delphinus delphis* and *Tursiops truncatus*, in the Greek seas (Eastern Mediterranean). *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 27 (2), 436–451. doi: 10.1002/aqc.2669

Giménez, J., Marçalo, A., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., et al. (2017). Diet of bottlenose dolphins (*Tursiops truncatus*) from the Gulf of Cádiz: Insights from stomach content and stable isotope analyses. *PloS One* 12 (9), e0184673. doi: 10.1371/journal.pone.0184673

Gnone, G., Bellingeri, M., Dhermain, F., Dupraz, F., Nuti, S., Bedocchi, D., et al. (2011). Distribution, abundance, and movements of the bottlenose dolphin (*Tursiops truncatus*) in the pelagos sanctuary MPA (north-west Mediterranean Sea). *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 21 (4), 372–388. doi: 10.1002/aqc.1191

Goetz, S., Read, F. L., Santos, M. B., Pita, C., and Pierce, G. J. (2014). Cetacean occurrence, habitat preferences and potential for cetacean-fishery interactions in Iberian Atlantic waters: results from cooperative research involving local stakeholders. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 25 (1), 138–154. doi: 10.1002/aqc.2481

Gómez-Campos, E., Borrell, A., Cardona, L., Forcada, J., and Aguilar, A. (2011). Overfishing of small pelagic fishes increases trophic overlap between immature and mature striped dolphins in the Mediterranean Sea. *PloS One* 6 (9), e24554. doi: 10.1371/journal.pone.0024554

Gómez de Segura, A., Tomás, J., and Raga, J. A. (2004) Sector centro (Comunidad Valenciana y región de Murcia). In Proyecto Mediterráneo. Zonas de especial interés para la conservación de los cetáceos en el Mediterráneo español. *Naturaleza y Parques Nacionales Serie Técnica*, vol. 3 . Eds. J., A. Raga and J. Pantoja. (Madrid: Ministerio de Medio Ambiente), 67–131.

Gómez de Segura, A., Crespo, E. A., Pedraza, S. N., Hammond, P. S., and Raga, J. A. (2006). Abundance of small cetaceans in the waters of the central Spanish Mediterranean. *Mar. Biol.* 150 (1), 149–160. doi: 10.1007/s00227-006-0334-0

Gómez de Segura, A., Hammond, P. S., and Raga, J. A. (2008). Influence of environmental factors on small cetacean distribution in the Spanish Mediterranean. *J. Mar. Biol. Assoc. U. K.* 88 (6), 1185–1192. doi: 10.1017/S0025315408000386

Gonzalvo, J., Valls, M., Cardona, L., and Aguilar, A. (2008). Factors determining the interaction between common bottlenose dolphins and bottom trawlers off the Balearic archipelago (western Mediterranean Sea). *J. Exp. Mar. Biol. Ecol.* 367 (1), 47–52. doi: 10.1016/j.jembe.2008.08.013

Gonzalvo, J., Giovos, I., and Moutopoulos, D. K. (2015). Fishermen's perception on the sustainability of small-scale fisheries and dolphin–fisheries interactions in two increasingly fragile coastal ecosystems in western Greece. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 25 (1), 91–106. doi: 10.1002/aqc.2444

Gozalbes, P., Jiménez, J., Raga, J. A., Esteban, J. A., Tomás, J., Gómez, J. A., et al. (2010). Cetáceos y tortugas marinas en la comunitat valenciana. 20 años de seguimiento. In *Collecció treballs tècnics de biodiversitat, 3. ed. conselleria de medio ambiente, agua, urbanismo y vivienda* (Valencia: Generalitat Valenciana), 61 pp.

Gray, C. A., and Kennelly, S. J. (2018). Bycatches of endangered, threatened and protected species in marine fisheries. *Rev. Fish. Biol. Fish.* 28 (3), 521–541. doi: 10.1007/s11160-018-9520-7

Greenwood, J. J. (1996). Basic techniques. In: *Ecological Census Techniques*. Ed. W. J. Sutherland (Cambridge: Cambridge University Press), 11–110.

Hammond, P. S., Bearzi, G., Bjørge, A., Forney, K. A., Karkzmarski, L., Kasuya, T., et al. (2012). *Tursiops truncatus*. In *The IUCN red list of threatened species 2012*. IUCN. doi: 10.2305/IUCN.UK.2012.RLTS.T22563A17347397.en

Hedgpeth, J. W. (1957). Classification of marine environments. In *Treatise on marine ecology and paleoecology* (USA: Geological Society of America Memoirs), vol. 67, 17–28. doi: 10.1130/mem67v1-p17

International Council for the Exploration of the Sea (ICES) (2008). *Report of the ICES advisory committee 2008. ICES advice 2008* (Copenhagen: International Council for the Exploration of the Sea), 842 pp.

IUCN (2021) *The IUCN red list of threatened species. version 2021-3*. Available at: https://www.iucnredlist.org (Accessed July 18, 2022).

Jog, K., Sutaria, P., Diedrich, A., Grech, A., and Marsh, H. (2022). Marine mammal interactions with fisheries: Review of research and management trends across commercial and small-scale fisheries. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.758013

Kie, J. G. (2013). A rule-based *ad hoc* method for selecting a bandwidth in kernel home-range analyses. *Anim. Biotelemetry.* 1 (1), 13. doi: 10.1186/2050-3385-1-13

Lauriano, G., Fortuna, C. M., Moltedo, G., and Notarbartolo di Sciara, G. (2004). Interaction between common bottlenose dolphin (*Tursiops truncatus*) and the artisanal fishery in asinara island national park (Sardinia): Assessment of catch damage and economic loss. *J. Cetacean Res. Manage.* 6, 165–173.

Leeney, R. H., Amies, R., Broderick, A. C., Witt, M. J., Loveridge, J., Doyle, J., et al. (2008). Spatio-temporal analysis of cetacean strandings and bycatch in a UK fisheries hotspot. *Biodiver. Conserv.* 17, 2323–2338. doi: 10.1007/s105331-008-9377-5

Léopold, M., Guillemot, N., Rocklin, D., and Chen, C. (2014). A framework for mapping small-scale coastal fisheries using fishers' knowledge. *ICES J. Mar. Sci.* 71 (7), 1781–1792. doi: 10.1093/icesjms/fst204

Lewison, R. L., Crowder, L. B., Wallace, B. P., Moore, J. E., Cox, T., Zydelis, R., et al. (2014). Global patterns of marine mammal, seabird, and sea turtle bycatch reveal taxa-specific and cumulative megafauna hotspots. *PNAS* 111 (14), 5271–5276. doi: 10.1073/pnas.1318960111

López, A., Pierce, G. J., Santos, M. B., Gracia, J., and Guerra, A. (2003). Fishery by-catches of marine mammals in Galician waters: Results from on-board observations and an interview survey of fishermen. *Biol. Conserv.* 111 (1), 25–40. doi: 10.1016/S0006-3207(02)00244-6

Macías-López, D., Barcelona, S. G., Báez, J. C., de la Serna, J. M., and and Ortiz de Urbina, J. M. (2012). Marine mammal bycatch in Spanish Mediterranean large pelagic longline fisheries, with a focus on Risso's dolphin (*Grampus griseus*). *Aquat. Living Resour.* 25 (4), 321–331. doi: 10.1051/alr/2012038

Mangiafico, S. S. (2015). *An R companion for the handbook of biological statistics, version 1.3.2* (New Brunswick: Rutgers Cooperative Extension).

Marçalo, A., Katara, I., Feijo, D., Araujo, H., Oliveira, I., Santos, J., et al. (2015). Quantification of interactions between the Portuguese sardine purse-seine fishery and cetaceans. *ICES J. Mar. Sci.* 72 (8), 2438–2449. doi: 10.1093/icesjms/fsv076

Ministry of Agriculture, Fisheries and Food (2020) *Fishing fleet census*. Available at: https://servicio.pesca.mapama.es/censo/ConsultaBuqueRegistro/Buques/Search (Accessed August 8, 2021).

Mónaco, C., Aguilera, R., Camiñas, J. A., Laspina, M., Molina, M., Said, A., et al. (2020). Interactions between cetaceans and small-scale fisheries inthe Mediterranean. *Conclusive Report*. Published by Low Impact Fishers of Europe. 24 pp.

Moore, J. E., Cox, T. M., Lewison, R. L., Read, A. J., Bjorkland, R., McDonald, S. L., et al. (2010). An interview-based approach to assess marine mammal and sea turtle captures in artisanal fisheries. *Biol. Conserv.* 143 (3), 795–805. doi: 10.1016/j.biocon.2009.12.023

Moore, J. E., Heinemann, D., Francis, T. B., Hammond, P. S., Long, K. J., Punt, A., et al. (2021). Estimating bycatch mortality for marine mammals: Concepts and best practices. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.752356

Nelms, S. E., Alfaro-Shigueto, J., Arnould, J. P. Y., Avila, I. C., Nash, S. B., Campbell, E., et al. (2021). Marine mammal conservation: over the horizon. *Endanger. Species Res.* 44, 291–325. doi: 10.3354/esr01115

Nortarbartolo di Sciara, G. (2016). Marine mammals in the Mediterranean Sea: An overview. *Medit. Mar. Mam. Ecol. Cons.* 75, 1–36. doi: 10.1016/bs.amb.2016.08.005

OceanCare (2021) *Quiet waters for whales and dolphins: the one-time opportunity to avoid, reduce and mitigate noise-generating activities in the Mediterranean Cetacean Migration Corridor*. Available at: https://www.oceancare.org/wp-content/uploads/2021/11/Quiet-Waters-en-web.pdf (Accessed May 1, 2022).

Peltier, H., Jepson, P. D., Dabin, W., Deaville, R., Daniel, P., Van Canneyt, O., et al. (2014). The contribution of stranding data to monitoring and conservation strategies for cetaceans: developing spatially explicit mortality indicators for common dolphins (*Delphinus delphis*) in the eastern north-Atlantic. *Ecol. Ind.* 39, 203–214. doi: 10.1016/j.ecolind.2013.12.019

Peltier, H., Authier, M., Caurant, F., Dabin, W., Daniel, P., Dars, C., et al. (2021). In the wrong place at the wrong time: Identifying spatiotemporal co-occurrence of bycaught common dolphins and fisheries in the Bay of Biscay (NE Atlantic) from 2010 to 2019. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.704005

Pennino, M. G., Rotta, A., Pierce, G. J., and Bellido, J. M. (2015). Interaction between bottlenose dolphin (*Tursiops truncatus*) and trammel nets in the archipelago de la maddalena, Italy. *Hydrobiologia.* 747 (1), 69–82. doi: 10.1007/s10750-014-2127-7

Powell, J. R., and Wells, R. S. (2011). Recreational fishing depredation and associated behaviors involving common bottlenose dolphins (*Tursiops truncatus*) in Sarasota bay, Florida. *Mar. Mamm. Sci.* 27 (1), 111–129. doi: 10.1111/j.1748-7692.2010.00401.x

Puig-Lozano, R., Fernández, A., Sierra, E., Saavedra, P., Suárez-Santana, C. M., de la Fuente, J., et al. (2020). Retrospective study of fishery interactions in stranded cetaceans, Canary Islands. *Front. Vet. Sci.* 7. doi: 10.3389/fvets.2020.567258

QGIS Development Team (2021) *QGIS geographic information system. open-source geospatial foundation project*. Available at: http://qgis.osgeo.org (Accessed January 10, 2022).

Raga, J. A., Banyard, A., Domingo, M., Corteyn, M., Van Bressem, M. F., Fernández, M., et al. (2008). Dolphin Morbillivirus epizootic resurgence, Mediterranean Sea. *Emerg. Infect. Dis.* 14 (3), 471–473. doi: 10.3201/eid1403.071230

Revuelta, O., Domènech, F., Fraija-Fernández, N., Gozalbes, P., Novillo, O., Penadés-Suay, J., et al. (2018). Interaction between bottlenose dolphins (*Tursiops truncatus*) and artisanal fisheries in the Valencia region (Spanish Mediterranean Sea). *Ocean Coast. Manage.* 165, 117–125. doi: 10.1016/j.ocecoaman.2018.08.001

Saavedra, C., Pierce, G. J., Gago, J., Jusufovski, D., Cabrero, A., Cerviño, S., et al. (2017). Factors driving patterns and trends in strandings of small cetaceans. *Mar. Biol.* 164, 165. doi: 10.1007/s00227-017-3200-3

Sala, A., Notti, E., Bonanomi, S., Pulcinella, J., and Colombelli, A. (2019). Trawling in the Mediterranean: An exploration of empirical relations connecting fishing gears, otterboards and propulsive characteristics of fishing vessels. *Front. Mar. Sci.* 6. doi: 10.3389/fmars.2019.00534

Snape, R. T. E., Broderick, A. C., Çiçek, B. A., Fuller, W. J., Tregenza, N., Witt, M. J., et al. (2018). Conflict between dolphins and a data-scarce fishery of the European union. *Hum. Ecol.* 46 (3), 423–433. doi: 10.1007/s10745-018-9989-7

Tomás, J., Aznar, F. J., and Raga, J. A. (2001). Feeding ecology of the loggerhead turtle *Caretta caretta* in the western Mediterranean. *J. Zool.* 255, 525–532. doi: 10.1017/S0952836901001613

Tomás, J., Gozalbes, P., Raga, J. A., and Godley, B. J. (2008). Bycatch of loggerhead sea turtles: insights from 14 years of stranding data. *Endanger. Species Res.* 5 (2-3), 161–169. doi: 10.3354/esr00116

Tudela, S. (2004). Ecosystem effects of fishing in the Mediterranean: an analysis of the major threats of fishing gear and practices to biodiversity and marine habitats. stud. rev. *General Fisheries Commission for the Mediterranean* Vol. 74 (Rome: FAO), 44p.

Van Bressem, M. F., Duignan, P., Banyard, A., Barbieri, M., Colegrove, K., De Guise, S., et al. (2014). Cetacean Morbillivirus: Current knowledge and future directions. *Viruses.* 6 (12), 5145–5181. doi: 10.3390/v6125145

Violi, B., Verga, A., Jones, L., Calogero, G., Soldano, G., Cheeseman, T., et al. (2021). A wanderer in the Mediterranean Sea: the case of a humpback whale (*Megaptera novaeangliae*) from the West indies. *Aquat. Mamm.* 47 (6), 599–611. doi: 10.1578/AM.47.6.2021.599

Worton, B. J. (1989). Kernel methods for estimating the utilization distribution in home-range studies. *Ecology*. 70 (1), 164–168. doi: 10.2307/1938423

Zahri, Y., Najih, M., El Ouamari, N., Abdellaoui, B., Kada, O., Essekelli, D., et al. (2007). Interactions between the dolphin *Tursiops truncatus* and the purse seine nets in Morocco Mediterranean. *Acoustics Repulsive Tests and Repercussion Evaluation*. (Monaco: CIESM).

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Seasonal distribution of an opportunistic apex predator (*Tursiops truncatus*) in marine coastal habitats of the Western Mediterranean Sea

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Assessing the distribution of marine apex−predators is pivotal to understanding community interactions and defining management goals. However, several challenges arise in both estimates and predictions considering the distinctive and mutable biological/ecological requirements of these species and the influence of human activities. Thus, efforts to study apex−predators' spatial distribution patterns must deal with inherent uncertainty. Relying on different data sources (research programs and social media reports), physiographic and environmental covariates (depth, slope, surface temperature and chlorophyll−*a*), and specific source−related detection functions, this study selected a Spatial Log−Gaussian Cox Process to model the distribution patterns of an opportunistic apex−predator, the common bottlenose dolphin (*Tursiops truncatus*), over 14 years (2008–2021) in the Mediterranean Sea (Italy) using a total of 955 encounters. Both depth and slope showed a significant (95% significance) reduction effect in the encounters when deeper and steeper, respectively. Temperature (parabolic) shows a positive effect (90% significance), while chlorophyll−*a* values did not seem to have a significant effect on encounter intensities within each season. The estimated posterior mean and the coefficient of variation surfaces for the intensity by season showed higher intensity in summer near the Tiber River estuary than other regions. Almost homogeneous predictions were observed in winter, with marginal greater intensities where lower temperatures and higher chlorophyll−*a* concentration were observed. The relatively low variance was predicted in the more coastal parts of the study area within each season, while higher uncertainty was instead revealed in the southernmost offshore area. This study highlighted the persistent presence of the common bottlenose dolphin in the investigated area both winter and summer, with a coherent distribution within each season, and rare transient occurrences in deeper waters (where uncertainty increases). Thanks to its versatile characteristics, the species seems to well adapt to different seasonal conditions and maintain its distributional range.

KEYWORDS

distribution modeling, Spatial Log–Gaussian Cox Process, uncertainty, common bottlenose dolphin, Mediterranean Sea, conservation, ecology

Introduction

Multiple roles are recognized for apex predators in the marine environment, fulfilling key ecological, economic, and cultural functions (Hammerschlag et al., 2019). Apex predators are indeed affected by bottom–up processes and can influence food webs *via* consumptive effects on prey (top–down effects) (Heithaus et al., 2008; Steneck, 2012; Kiszka et al., 2022). Hence, assessing their distribution is pivotal to understanding community interactions and defining management goals to be implemented (Hammerschlag et al., 2019). However, several challenges arise in both distribution estimates and predictions (Pace et al., 2019; Martino et al., 2021), generated by high movement ability and large home–ranges covered by these species, spatio-temporal knowledge gaps and the increasing use of different data sources to model their distribution (Watson et al., 2019). Considering also the distinctive and changing biological and ecological requirements of these species (Forcada, 2018; Pace et al., 2018), and the influence of threats related to human activities (Nelms et al., 2021), efforts to study spatial distribution patterns must deal with inherent uncertainty (Ansong et al., 2017; Stephenson et al., 2021).

The common bottlenose dolphin (*Tursiops truncatus*) is a cosmopolitan marine apex predator inhabiting a variety of habitats, including nearshore waters, harbors, estuaries, and deeper waters over the continental shelf worldwide (Wells and Scott, 2018; Wells et al., 2019). The species range poleward of 45° in northern Europe and southern New Zealand, but it has been reported as far south as 53-55°S in South America and as far north as British Columbia (50°N) (Wells et al., 2019). Different elements seem to drive its distribution in space and time and habitat use, being under the influence of environmental [e.g., sea surface temperature (SST) and bathymetry], ecological [e.g., prey distribution], social [e.g., inter- and intraspecific interactions/ relationships] and anthropogenic variables [e.g., fishing activities and boat traffic] (e.g., see Bennington et al., 2020; Diaz Lopez, 2019; Greller et al., 2021; Haughey et al., 2021; Zanardo et al., 2017 and references herein). Considering their widespread distribution and prominent presence in the coastal marine ecosystems, common bottlenose dolphins could have a significant role in the structure and function of these ecosystems (Diaz Lopez, 2019). However, significant gaps impede our capability to fully determine several critical attributes of the function of these predators, particularly their fine–scale distribution and movements, feeding rates, and prey selection (Kiszka et al., 2022).

In the Mediterranean Sea (30-41°N), the common bottlenose dolphin is widespread across the entire basin, occurring primarily in coastal habitats with depths<100 m (Natoli et al., 2021), often in correspondence with highly productive systems with significant ecological importance in the marine life environment. In the basin, the species shows a remarkable level of ecological and behavioral plasticity, an erratic distribution, and variable habitat use and residency degrees depending on local conditions, resources availability and social factors (Blasi and Boitani, 2012; Giannoulaki et al., 2017; Vassallo et al., 2020; Pace et al., 2021). Several studies reported different distribution patterns, where individuals may present high site fidelity or, conversely, show movements on a scale of hundreds of kilometers (e.g., Gnone et al., 2011; Pulcini et al., 2014; Pleslić et al., 2019; Pace et al., 2021; Labach et al., 2022). Many environmental factors, physiographic characteristics, and seasonal patterns appear to drive the observed fine–scale coastal distribution and seem to best predict suitable habitats for the species (Blasi and Boitani, 2012; Marini et al., 2015; La Manna et al., 2016; Laran et al., 2017; Giannoulaki et al., 2017; Vassallo et al., 2020; Gnone et al., 2022). In addition, the highly opportunistic, versatile, and resilient nature of the common bottlenose dolphin diet and foraging strategies (Giménez et al., 2017; Borrell et al., 2021; Carmen et al., 2021; Natoli et al., 2021) enable the species to also adjust its behavior as a consequence of resources' availability. This opportunistic behavior can be also applied to resources related to human activities (e.g., fisheries and aquaculture) (Bonizzoni et al., 2021; Pace et al., 2012; Pace et al., 2022a; Triossi et al., 2013). All these characteristics may facilitate the development of discrete geographical units in the Mediterranean population (Carnabuci et al., 2016; Vassallo et al., 2020), making distribution assessments more difficult and increasing the uncertainty when predicting scenarios.

The proximity to human activities in the Mediterranean coastal areas makes the common bottlenose dolphin susceptible to various anthropogenic threats (e.g., bycatch, vessel traffic, overfishing, contaminants, and noise pollution) (Natoli et al., 2021). Consequently, the species was included in Annex II of the EU Habitats Directive (92/43/CEE) as priority species and was listed as Least Concern in the last IUCN Red List of Threatened species regional assessment (Natoli et al., 2021). This new assessment imperatively recommended continuously monitoring the effects of human–related stressors on the common bottlenose dolphin to ensure the maintenance of intra–species diversity and the survival across its range (Natoli et al., 2021).

Considering the species characteristics and the variations of the natural and anthropogenic conditions, a large number of occurrence data is required to support studies investigating common bottlenose dolphin distribution, as well as a robust analytical approach capable of coping with heterogeneous data and the variability of predictors (Martino et al., 2021). Relying on different data sources (research programs and social media reports), physiographic and environmental covariates (depth, slope, surface temperature and chlorophyll–*a*), and specific source–related detection functions, this study select a Spatial Log–Gaussian Cox Process to model common bottlenose dolphin distribution patterns using 14-years data (2008–2021) collected in the Mediterranean Sea. Here, to effectively manage and solve the complex issue of presence-only data, the suggestions offered by Warton and Shepherd (2010) and, in the ecological framework, by Renner et al. (2015) were used, adopting a point processes approach where pseudo-absences generation is not required. Since anthropogenic pressures (i.e., coastal population, fishing and tourism; see Figures S5 and S6 in the Supplementary Materials; http://dati.istat.it/, https://www.politicheagricole.it) on the study area were constant within each season in the considered time window of 14 years, a spatial model with a seasonal effect was adopted. Applying the approach and the methodology developed and tested in Martino et al. (2021), this study investigates the common bottlenose dolphin seasonal distribution in a broader area than previously reported and discusses the flexibility of the species to ecological drivers and anthropogenic forces.

Materials and methods

Study site

The study area is located in the western Mediterranean Sea, within the Ligurian and central Tyrrhenian Sea (Figure 1), off the Tuscany and Lazio coasts (Italy). The area covers almost 60,000 km^2 and is a complex marine region including various environmental features (e.g., continental shelf, slope, canyons, seamounts) and various habitats (seagrass meadows, hard-bottom communities with coastal banks, cliffs, sand, and mud). The northern section spans mainly over the continental shelf. It is characterized by shallow waters and coastal shoals surrounding several islands, which form the Tuscan Archipelago (i.e., Capraia, Elba, Giannutri, Giglio, Gorgona, Montecristo, and Pianosa Islands). The Elba, Capraia, and Pianosa Islands area presents mainly shallow waters within the 100 m bathymetry. In contrast, the west–southern area of the Archipelago is characterized by steeper slopes and deep canyons, reaching more than 500 m depths (Ricevuto et al., 2011). Between Capraia and Gorgona Islands, the continental shelf is crossed by the Elba canyon, which descends deeply toward the North–

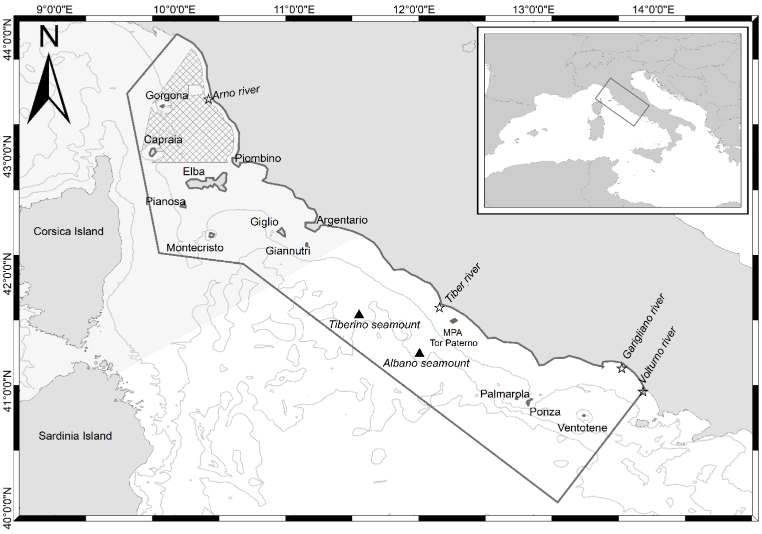

FIGURE 1
Study area in the Mediterranean Sea, Italy.

West. The seabed between Elba and Argentario consists of a single basin, bordered to the west by the Elba ridge, to the east by the Tuscany coast, and to the north by the Piombino canal and Elba. The central–southern section of the study area includes the continental shelf areas off the Lazio coast and, toward the south, the islands of Ponza, Palmarola, and Ventotene (Pontine Archipelago).

Many seamounts are included or border the entire study area, such as the Santa Lucia and Occhiali seamounts in the north, the Cialdi, Etruschi, and Tiberino in the middle, and the Albano seamount in the south (Würtz and Rovere, 2015). Seamounts attract a rich associated fauna and strongly influence the distribution of pelagic top predators, which can find optimal foraging areas around them (Würtz and Rovere, 2015; Bo et al., 2020). The several river mouths present in the area affect the coastal marine ecosystems as they are major sources of organic matter for the adjacent marine waters. They include the Arno river (in the northern part of the study area), the Tiber river (in the middle of the study area), and the Garigliano and Volturno rivers (in the southern part of the study area).

Part of the study area (Figure 1) is included in the international Pelagos Sanctuary for the protection of Marine Mammals and is classified as a Specially Protected Area of Mediterranean Importance (SPAMI) under the Barcelona Convention Protocol for Specially Protected Areas and Biological Diversity in the Mediterranean (SPA/BD Protocol). Additionally, many Marine Protected Areas, including several Sites of Community Importance and Special Protection Areas under the Natura 2000 European network of the Habitat Directive (92/43/EEC), are comprised in the study area. Twelve Natura 2000 sites are found in the northern portion (10 SCZ and 2 SCI), including the new designated Natura 2000 SCI site ‘Tutela del *Tursiops truncatus*’ – IT5160021, located just off the Tuscany coast, for the protection of the common bottlenose dolphin. Two marine protected areas (MPAs) can be found in the southern portion of the study area: the MPA Islands of Ventotene and S. Stefano in the Pontine Archipelago [recently acknowledged by the International Union for Conservation of Nature (IUCN) as Important Marine Mammal Area (IMMA, IUCN-MMPATF, 2017)] and the MPA Tor Paterno bank near Rome. The entire study area is under constant anthropogenic pressures only partially mitigated by the above-mentioned protective measures (see section S5 in the Supplementary Materials for further information on the demographic, fishing and touristic variations in the study area).

Data sources

Following the approach used by Martino et al. (2021), three types of sighting data over 14 years (2008–2021) were used considering two seasons (summer: April–September; winter: October–March). Data included: 1) information derived from conventional visual/acoustic research protocols (adaptive sampling) using motor/sailing boats (Pace et al., 2019; Pace et al., 2021; Papale et al., 2021; Pace et al., 2022b) the resulting dataset was labeled UNIRM; 2) information originated from standardized monitoring protocols (distance sampling) using platforms of opportunity within the project “FLT Mediterranean Monitoring Network” (ISPRA, 2016; Arcangeli et al., 2019; Pace et al., 2019); the resulting dataset was labeled FERRY-FLT; 3) information extracted from social media (Facebook and YouTube) using reports by sea–users (Pace et al., 2019); the resulting dataset was labeled SM. Detailed elements on SM data collection procedures and selection are provided in Pace et al. (2019) and Martino et al. (2021). As the SM dataset also included details on other cetacean species than the common bottlenose dolphin (see Table S1 in the Supplementary Materials), this information was used as a proxy to infer boat densities potentially able to record the animals’ presence (see the following ”Modeling approach” paragraph). The issue of estimating boats’ (especially smaller ones) density was here further developed to explore additional potential sources of information (see section S3 in the Supplementary Materials) to extend and enhance what already reported in Martino et al. (2021).

Physiographic and environmental covariates

The following covariates were initially selected as reasonable proxies for the species’ ecological needs (La Manna et al., 2016): salinity, depth, slope, sea surface temperature (SST), and chlorophyll–*a*. Salinity was not significant for modeling, thus only the last four were used. Depth data were downloaded from GEBCO (General bathymetric Chart of the Ocean – https://www.gebco.net); the slope was computed from depth data through the terrain() function of the R package “terra” (https://www.r–project.org/; Hijmans, 2022); SST and chlorophyll–*a* were retrieved from COPERNICUS platform (https://marine.copernicus.eu/) as monthly average. The retrieved datasets and data handling procedures are reported in Martino et al. (2021) and the Supplementary Materials (see section S1).

Modeling approach

Dolphin sightings were aggregated over time into two seasons (summer and winter) and viewed as two-point patterns over space. Therefore, a point process model was used to describe how those points are generated. To integrate data from all available sources and manage possible detection bias in each dataset (see section S2 in the Supplementary Materials), a Spatial Log–Gaussian Cox Process (LGCP) (Renner et al., 2015) incorporating different detection functions for each data source was built (see Martino et al., 2021 for details). It was assumed that sighting patterns, i.e., locations of dolphin groups in space ($s \in S \subset R^2$) and season t=1, 2 (t is the season), were properly described by a point process whose intensity function (s,t) is additive on the log–scale:

$$\log(\lambda(s,\ t)) = \ \mathbf{X}^{\mathrm{T}}(s,\ t)\boldsymbol{\beta} + \ \omega(s)$$

where $\boldsymbol{X}(s,t)$ is a set of covariates detected at location s and time t with linear effects $\boldsymbol{\beta}$ to be estimated, and $\omega(s)$ is a zero–mean Gaussian process with Matérn covariance function of order 1, describing the residual spatial variation. Notice that both the effect of covariates $\boldsymbol{\beta}$ and the spatial process ω(s) are assumed to be constant and are estimated jointly using data from both seasons.

The vector $\boldsymbol{X}$ included both time constant (depth and slope) and time dependent (SST and chlorophyll–a) covariates. Temperature has been included both as a linear and quadratic term. To distinguish between a possible seasonal effect and the spatial effect of the covariate within one season, we created a standardized version of both temperature and chlorophyll–a as:

$$sst_c(s,t) = \ sst(s,t) \ - \ sst(t)$$

$$chl_c(s,t) = \ chl(s,t) \ - \ chl(t)$$

where $sst(t)$ and $chl(t)$ are the seasonal means of surface temperature and chlorophyll–a over the whole domain of interest, defined as:

$$sst(t) = \int_S sst(t)ds$$

$$chl(t) = \int_S chl(t)ds$$

The model for the log intensity was then formalized as:

$$\begin{aligned} \log(\lambda(s,\ t)) = \ & \beta_0 \ + \ \beta_1 I \quad (summer) \\ & + \ \beta_{depth}\ depth\ \ (s) \\ & + \ \beta_{slope}\ slope(s) + \ \beta_{sst}\ sst_c\ (s,\ t) \\ & + \beta_{sst^2}\ \ sst_c^2(s,\ t) \ + \ \beta_{chl}\ chl_c\ (s,\ t) \\ & + \omega(s),\ s \in\ S,\ t+1,\ 2 \end{aligned} \qquad \text{(Equation 1)}$$

Where β_0 is a global mean, β_1 a seasonal effect and I *(summer)* is an indicator variable for the summer season and the rest of symbols are explained above.

It was assumed that the above process was observed in three different ways, conditionally independent given $\lambda(s,t)$. Thus, three observed intensities were defined as:

$$\lambda_j^*(s,\ t) = \ g_j\ (s,\ t)\lambda(s,\ t), \qquad j = 1,\ 2,\ 3$$

where $g_j(s,t)$ is the detection function (with values between 0 and 1) which determines the thinning of the original process (Martino et al., 2021). The detection functions were defined as follows:

For the adaptive sampling (UNIRM) data:

$$g_1\ (s) = \begin{cases} 1, & d_1\ (s) \le K \\ 0, & d_1(s)\ >\ K \end{cases}$$

where $d_1(s)$ is the distance (Km) between point s and the position of the boat when the groups were sighted. K = 4 Km was defined as the maximum distance measured between the location of the first visual sight of a dolphin group by researchers.

For the distance sampling (FERRY-FLT) data, the half normal detection function was used, defined as:

$$g_2(s) = exp\left(-\frac{d_2^2(s)}{2\xi_2}\right)$$

where, $d_2(s)$ is the perpendicular distance (Km) to the ferry track and ξ_2 is a scale parameter.

Finally, for the SM data, the sighting probability was assumed to be larger with higher number of citizens' small boats, so that the detection function was defined as:

$$g_3(s,t) = \Phi\left(\frac{d_3(s,t)}{\xi_3} - \mu_3\right) \qquad \text{(Equation 2)}$$

Where Φ is the cumulative distribution function of a standard normal distribution, $d_3(s,t)$ is the log–intensity of small boats at point s and time t, and μ_3 and ξ_3 are location and scale parameters.

The intensity of the small boats $d_3(s,t)$ is unknown. Following Martino et al. (2021), this function was estimated using sightings of all cetacean species included in the SM dataset (see Table S1 and Figure S2 in the Supplementary Materials), and accounting for seasonality (more small boats in summer than in winter are expected). The parameters of the detection function ξ_3 and μ_3 were kept constant between seasons (see Supplementary S2 section for details).

An alternative estimation of the density of small boats $d_3(s,t)$ was attempted using images of Copernicus Sentinel–1 satellite radar. Such an approach was proposed in Martino et al. (2021) as a future development but has not been successful in the present study, as the intensity surfaces derived from satellite data reported several artifacts (see section S3 in the Supplementary Materials for details).

The model was fitted in a Bayesian setting using the inlabru R package (Yuan et al., 2017; Bachl et al., 2019). The approach allows for the estimation of all model components, jointly including the parameters in the detection functions, and therefore accounting for all uncertainties in a coherent way (see section S4 in the Supplementary Materials for prior specifications and details about the implementation).

Once the model was fitted, the estimated mean number of sightings was predicted over the whole area of interest as:

$$\Lambda(t) = \int_S \lambda(s, t) ds \qquad \text{(Equation 3)}$$

Such an integral can be estimated using Monte Carlo sampling from the fitted model.

Results

A total of 955 common bottlenose dolphin encounters was collected by research projects and social media reports (Table 1) over a period of 14 years, with a higher number documented in summer (N = 759) than winter (N = 196).

The total research effort in the two research programs (UNIRM and FERRY-FLT datasets) was 23,920 Km during summer (21,479 Km by FERRY-FLT; 2,441 by UNIRM) and 3,477 Km during winter (3,010 Km by FERRY-FLT; 467 by UNIRM). Total effort by season is shown in Figure 2.

Maps showing covariates used within the model are shown in Figure 3 (depth and slope) and Figure 4 (seasonal sea surface temperature and chlorophyll–*a*). Estimated maps of observation process intensity used in the detection functions are presented in Figure 5. The estimated value for the posterior means together with 90 and 95% credible interval (CI) of the model's fixed effects are shown in Table 2. Importance or significance of variables can be deducted by examining the overlap of their 90 or 95% CI with zero. Both depth and slope showed a significant reduction effect in the encounters when deeper and steeper, respectively (90 and 95% CI do not contain zero). The seasonal effect (Seasonal(β_1) has fully positive 90 and 95% CI) indicated an increase in the encounter intensity during the summer season. The two space–time varying covariates, SST and chlorophyll–*a* values, were not significant within each season at the 95% significance level (CI contain zero). However, the parabolic effect of SST was significant at 90% level, suggesting a highly variable, but positive effect of the surface temperature. Spatial field's parameters are reported in the Supplementary Material (see S4 section).

The estimated posterior mean and coefficient of variation (CV) surfaces for the intensity of the common bottlenose dolphin distribution by season are reported in Figure 6. In summer, the Tiber River estuary (nearly in the middle of the study area) showed higher intensity than other coastal regions. In winter, almost homogeneous predictions were observed, with marginal greater intensities at the two extremes of the study area (the Arno estuary in the northern part and Gaeta Gulf in the south), where lower temperatures and slightly higher chlorophyll–*a* concentration were observed. The relatively low variance was predicted in the more coastal parts of the study area within each season, while higher uncertainty was instead revealed in the southernmost offshore area.

Finally, the distribution of the expected number of sightings by each season over the whole area, computed using Equation 3, is reported in Figure 7. While the expected number of sightings is fairly similar during summer and winter, a much larger variance is observed for the winter estimate.

TABLE 1 Total number of common bottlenose dolphins recorded by the two research programs [onboard ferries FLT Net (FERRY-FLT) and a dedicated survey platform UNIRM)] and by social media.

	FERRY-FLT	UNIRM	Social media
Summer	126	137	496
Winter	22	18	156

Discussion

The modeling approach of spatial data integration, able to carefully consider and minimize datasets biases, has been used in this study, offering a more precise picture of the seasonal common bottlenose dolphin distribution in the western Mediterranean Sea. As Dorazio (2014) pointed out, several statistical models have been proposed to integrate presence-only data from different research protocols to obtain reliable predictions of species distribution. However, these models have overlooked the effects of imperfect detectability and survey bias. Recently, Martino et al. (2021) showed that bias in these estimates, induced by multiple detection mechanisms related to data collection, could be reduced by correcting for detectability issues, thus allowing multiple sources of information to be integrated. In this study, new advancements with respect to Martino et al. (2021) were presented, testing a different approach to better define the presence–only (social media data source) detection function (see section S3 in the Supplementary Materials) and describing a possible seasonal effect thanks to a larger amount of data and a more extended study area. However, the relatively low number of sightings currently available, highly scattered in time, makes it difficult to connect to the detailed temporal pattern in the environmental covariates. The number of sightings per year seems insufficient to allow a full space-time modeling to capture possible temporal dynamics in the area.

The model here estimated includes all sources of uncertainty in one framework, allowing for a rigorous evaluation of the overall prediction uncertainty. It is known that when predicting animal distribution, uncertainty – both

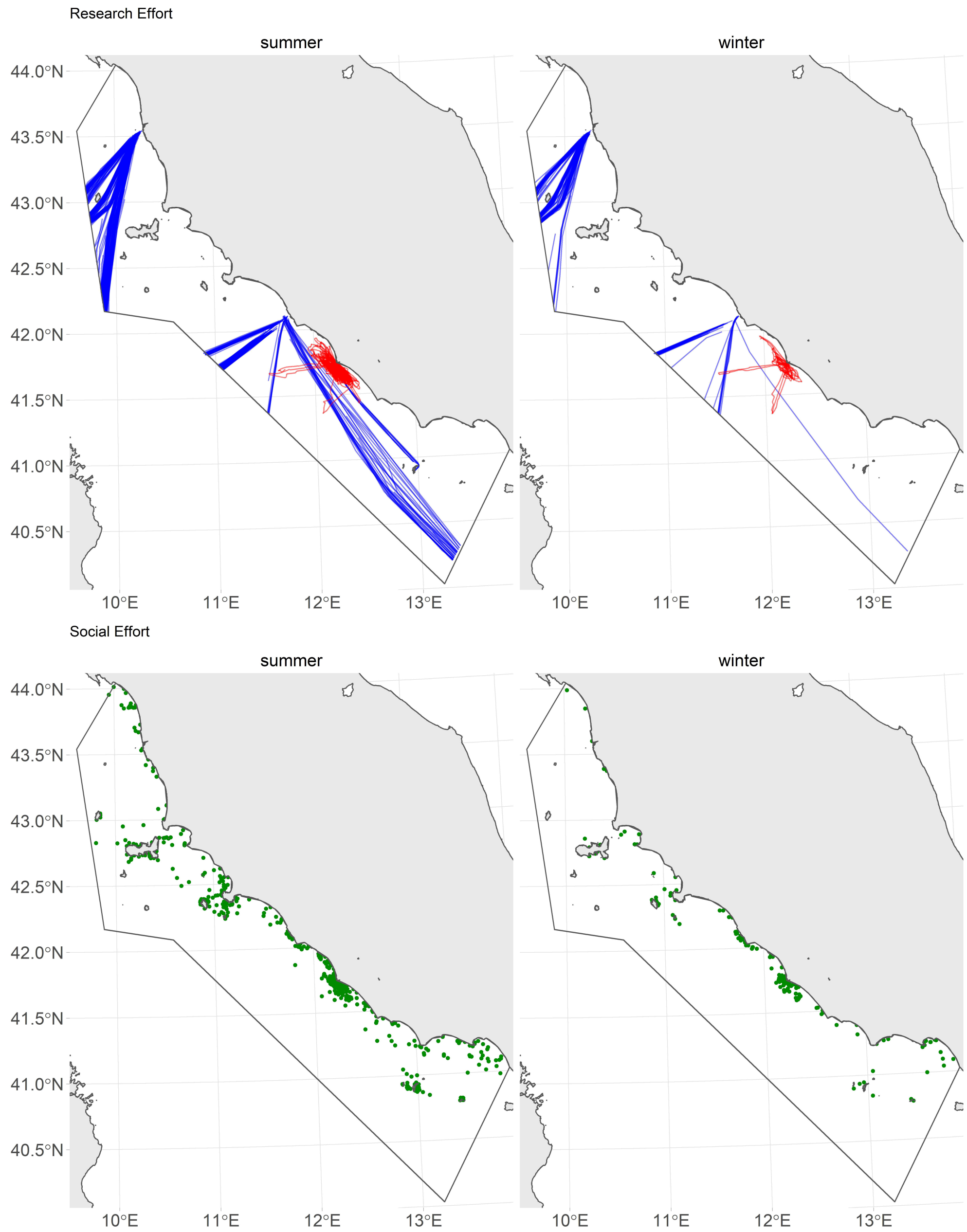

FIGURE 2
Total research effort by UNIRM (red) and FLT Net (blue) research programs and point location of social media sightings (green dots).

epistemic (i.e., the recognized ignorance due to imperfect knowledge; Brown, 2004) and stochastic (i.e., always present when dealing with nature; Walker et al., 2003) – is an unavoidable factor to deal with. This is obviously a critical point in the development of conservation and management measures, as without a proper uncertainty evaluation, weak and inefficient choices could be made (Stephenson et al., 2021). For the distribution of marine apex predators like the common bottlenose dolphin this is a crucial aspect, as these species can travel for great distances or reside in specific coastal locations. These versatile habits entail significant variations in the environmental characteristics and expose the species to a large variety of human pressures overlapping their ecologically important areas.

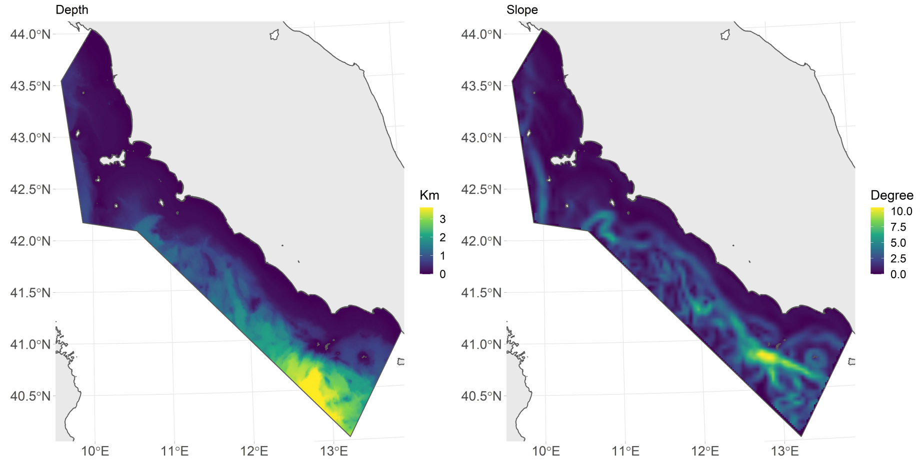

FIGURE 3
Bottom depth (left panel) and slope (right panel) in the study area.

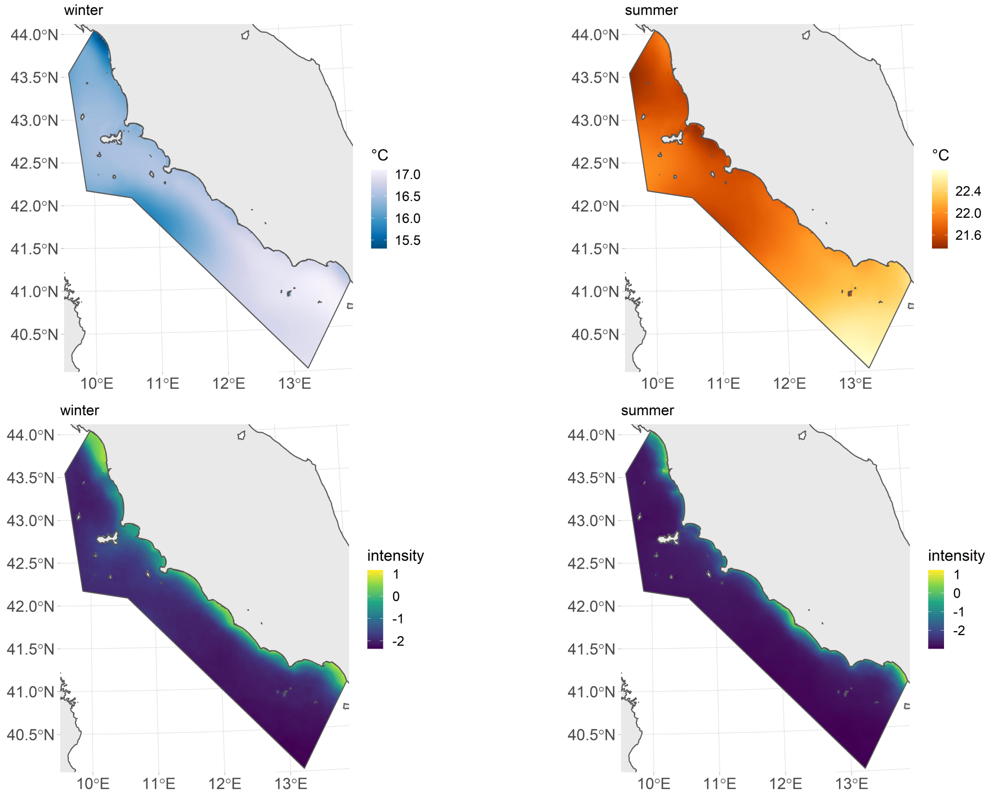

FIGURE 4
Sea surface temperature (SST) distribution in winter (upper left panel) and summer (upper right panel), and chlorophyll–*a* log–scale values in winter (lower left panel) and summer (lower right panel) in the study area.

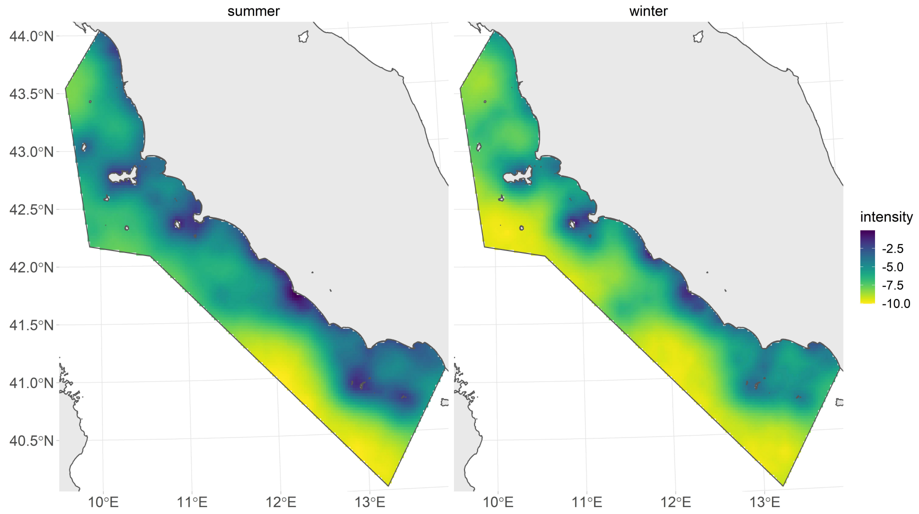

FIGURE 5
Estimated maps of observation process intensity used in the detection functions (summer: left panel; winter: right panel).

This study highlighted that the common bottlenose dolphin seems to well adapt to different seasonal conditions and maintain its distributional range, being constantly present along the coasts of the study area over the investigated 14-years period and showing differences in the intensities of its distribution between the two seasons (winter and summer), while the spatial pattern remain constant. A much larger variance for the winter vs summer estimates emerged, likely related to the reduced observation effort in the winter season due to bad weather conditions. Even not statistically significant, the lowering temperature and the slight rise of chlorophyll–*a* concentration detected in winter seem to increase the probability of dolphin presence. More specifically, the chlorophyll–*a* concentration was higher in two locations, near the Arno estuary in the north and the Garigliano/Volturno estuaries in the south of the study area, respectively. Being responsible for primary production, chlorophyll–*a* could be used as an indicator for other biotic features, such as the zooplankton distribution or plantophagous fish presence (e.g., La Manna et al., 2016). In the southern Mediterranean Sea, chlorophyll–*a* was found to be the second strongest predictor for bottlenose dolphin spatial distribution patterns, representing a good proxy for prey availability and thus a highly useful parameter in identifying relevant aggregation hotspots for dolphins (La Manna et al., 2016). Most likely, it is not by chance that the main estuaries in the study area were sites with the greatest predicted bottlenose dolphin densities, as estuaries have been shown to act as significant habitats for the genus *Tursiops* worldwide (e.g., Sprogis et al., 2016; Hartel et al., 2020). Estuaries are key aspects of the coastal ecosystems because of their unique characteristics and the variability induced by mixing and stratifying fresh and saltwater (McLusky and Elliott, 2004; Lin et al., 2013). Such processes are known to trigger fish aggregations and movements (Krumme, 2004) as they adapt to these changes, and dolphin paths as well, as they follow their prey (i.e., prey availability and distribution in turn influence common bottlenose dolphin distribution; Karczmarski et al., 2000; Soldevilla et al., 2011; Lin et al., 2013). In addition, other

TABLE 2 Estimated posterior means together with 90 and 95% credible interval (CI) for the fixed effects parameters in Equation (1).

	Posterior mean	0.025quantile	0.975quantile	0.05quantile	0.95quantile
Intercept (β_0)	–3.750	–5.165	–2.237	–4.919	–2.58
Season (β_1)	2.812	2.566	3.059	2.605	3.019
β_{sst}	1.330	–0.219	2.884	0.030	2.623
β_{sst^2}	2.068	-0.068	4.225	0.266	3.859
β_{chl}	0.042	–0.121	0.201	-0.093	0.176
β_{depth}	–4.394	–5.450	–3.342	–5.275	–3.509
β_{slope}	–0.460	–0.725	–0.205	–0.678	–0.242

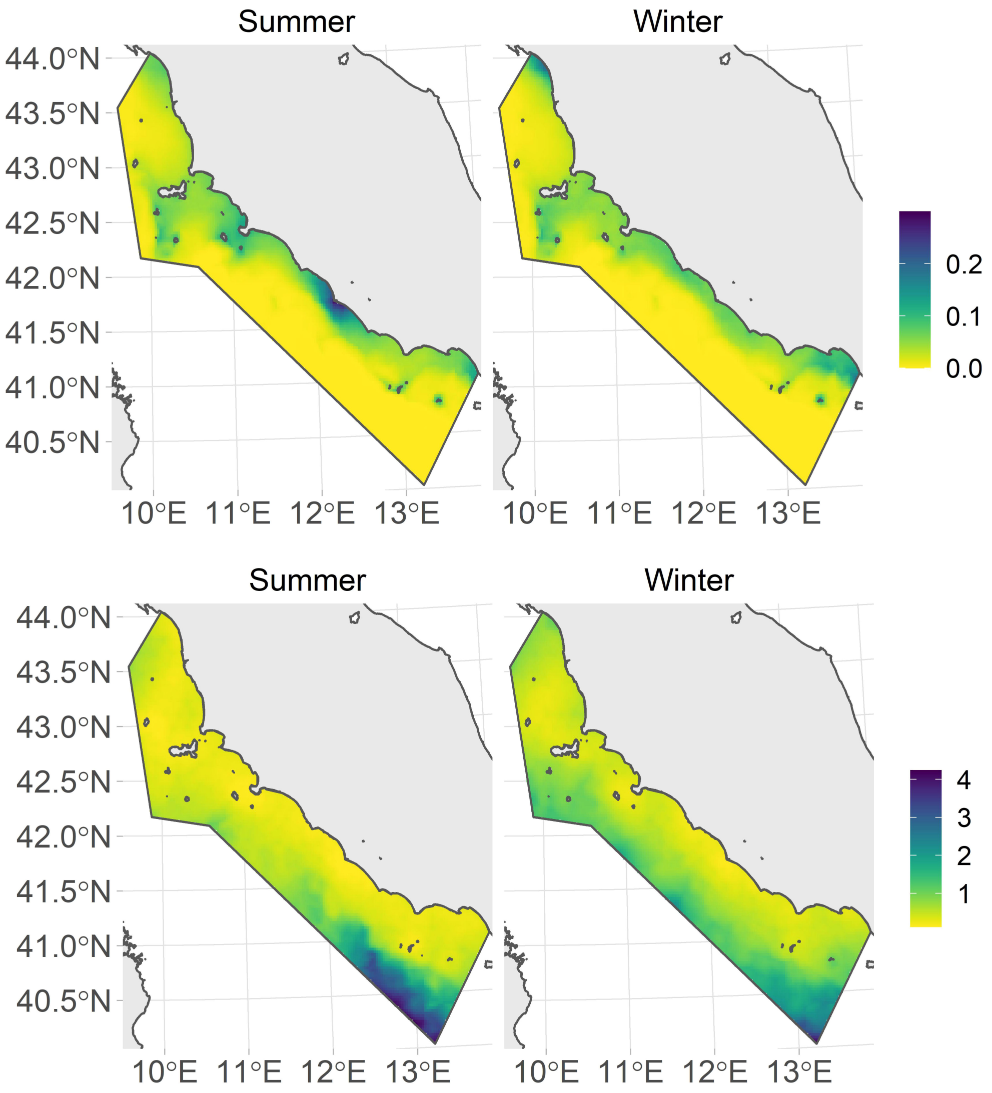

FIGURE 6
Posterior means of the predicted intensity surface for bottlenose dolphin distribution during the summer (upper left panel) and winter (upper right panel) seasons and the coefficient of variation (CV) for each season (summer: lower left panel; winter: lower right panel).

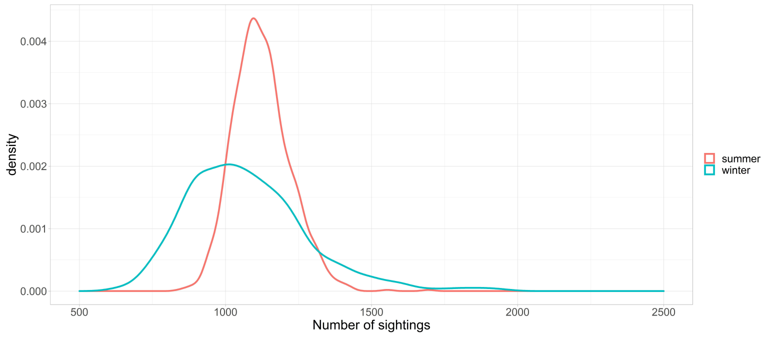

FIGURE 7
Distribution of the expected number of sightings over the whole area during summer (red) and winter (blue).

peculiar, fixed features of the study area such as islands, headlands and seamounts may have played a role in the constant distributional pattern over seasons here observed, as they are known to permanently generate eddies, fronts and water masses circulation, enhancing the amount of available nutrients and aggregating different species able to attract apex predators (Johnston and Read, 2007; Bailey and Thompson, 2010; Dinis et al., 2016).

Bathymetry is considered a proxy for prey availability indirectly linked to the common bottlenose dolphin distribution and habitat selection as well (Marini et al., 2015; Gnone et al., 2022). Here, bathymetry appeared to be the best predictor of the species distribution in the investigated area: the probability of sighting a group of common bottlenose dolphin increased in shallow (coastal) waters and decreased with increasing depth. This result is in accordance with the relatively consistent preferences in terms of bottom topography and water depth observed for the common bottlenose dolphin in the Mediterranean Sea (Natoli et al., 2021), although some transient occurrences in deeper waters, where uncertainty in predictions increases, were detected. Coastal waters may be more suitable habitats for common bottlenose dolphin mother–calf pairs than deeper ones, where females with newborns could form stable resident groups as observed in the study area near the Tiber River estuary by both researchers and sea users (Pace et al., 2019; Pace et al., 2021; Pace et al., 2022a; Pedrazzi et al., 2022).

Marine apex predators are, in general, highly mobile species, which raises issues in identifying their habitat boundaries for conservation actions (Cribb et al., 2015; Pace et al., 2018). The heterogeneous distribution over a wide range of habitats characterizes these species as indicators to estimate the effects of human activities on ecosystem functions (Arcangeli et al., 2015; Carlucci et al., 2016). Coastal dolphins are known to be affected by different anthropogenic threats such as bycatch, entanglement in fishing gears or marine litter, physical disturbance by shipping, unregulated dolphin watching or coastal development, anthropogenic noise, chemical contaminants, or overfishing (Natoli et al., 2021). These multiple pressures are acting in the highly anthropized study area (Gnone et al., 2022), although it encompasses several levels of spatial protection measures such as the SPAMI Pelagos Sanctuary for the protection of Marine Mammals, Marine Protected Areas, and Natura 2000 sites including the 'Tutela del *Tursiops truncatus*' – IT5160021 specifically established for protecting a recognized important site for bottlenose dolphin just off the Tuscany coast. To date, a complete assessment of species conservation status and intervention to assure the favorable status is reached or maintained is mandatory within the recently established Habitat Directive N2000 IT5160021 site (Arcangeli et al., 2021). Our findings underline the importance of the study area for the bottlenose dolphin also well outside the limit of the N2000 site and call for a reinforcement of effective mitigation measures to preserve the species especially along the whole coastal area. The results shown by our integrative modeling effort highlight the importance of using all available data to better understand the distribution of the species (Pace et al., 2014; Pace et al., 2019; Gnone et al., 2022) and, in turn, the characteristics of the marine ecosystem they are part of. Additional efforts to enlarge and reinforce the existing protective regulations in the study area are urgent priorities (to encompass at least the whole coastal area), as well as further investigations and continuous monitoring activities to identify effective mitigation actions for the local common bottlenose dolphin population.

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 we used observational data only.

Author contributions

DSP, AA, GJ, and SMa designed the study and managed the funding acquisition. DSP and AA performed the field work. GP, SMo, GJ, and SMa analyzed data. All authors wrote the first version of the manuscript, discussed the results and implications, commented on the manuscript at all stages and contributed extensively to the work here presented.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.939692/full#supplementary-material

References

Ansong, J., Gissi, E., and Calado, H. (2017). An approach to ecosystem–based management in maritime spatial planning process. *Ocean Coast. Manage.* 141, 65–81. doi: 10.1016/j.ocecoaman.2017.03.005

Arcangeli, A., Aissi, M., Atzori, F., Azzolin, M., Campana, I., Carosso, L., et al. (2019). Fixed line transect mediterranean monitoring network (flt med net), an international collaboration for long term monitoring of macro–mega fauna and main threats fixed line transect mediterranean monitoring network. *Biol. Mar. Mediterr.* 26 (1), 400–401.

Arcangeli, A., Crosti, R., Campana, I., Carosso, L., Gregorietti, M., Mainardi, G., et al. (2021). Long–term monitoring for the surveillance of the conservation status of *Tursiops truncatus* in an EU Natura2000 site in the Mediterranean sea. a pilot study in the Tuscan archipelago. *Mediterr. Mar. Sci.* 22 (2), 340–346. doi: 10.12681/mms.24562

Arcangeli, A., Orasi, A., Carcassi, S., and Crosti, C. (2015). Exploring thermal and trophic preference of balaenoptera physalus in the central tyrrhenian Sea: a new summer feeding ground? *Mar. Biol.* 161, 427–436. doi: 10.1007/s00227-013-2348-8

Bachl, F. E., Lindgren, F., Borchers, D. L., and Illian, J. B. (2019). Inlabru: an r package for bayesian spatial modelling from ecological survey data. *Methods Ecol. Evol.* 10, 760–766. doi: 10.1111/2041-210X.13168

Bailey, H., and Thompson, P. (2010). Effect of oceanographic features on fine–scale foraging movements of bottlenose dolphins. *Mar. Ecol. Prog. Ser.* 418, 223–233. doi: 10.3354/meps08789

Bennington, S., Rayment, W., and Dawson, S. (2020). Putting prey into the picture: improvements to species distribution models for bottlenose dolphins in doubtful sound, new Zealand. *Mar. Ecol. Prog. Ser.* 653, 191–204. doi: 10.3354/meps13492

Blasi, M., and Boitani, L. (2012). Modelling fine–scale distribution of the bottlenose dolphin tursiops truncatus using physiographic features on filicudi (southern thyrrenian Sea, Italy). *Endanger. Species Res.* 17, 269–288. doi: 10.3354/esr00422

Bo, M., Coppari, M., Betti, F., Massa, F., Gay, G., Cattaneo–Vietti, R., et al. (2020). Unveiling the deep biodiversity of the janua seamount (Ligurian sea): first Mediterranean sighting of the rare Atlantic bamboo coral *Chelidonisis aurantiaca* stude. *Deep Sea Res. Part I Oceanogr. Res. Pap.* 156, 103186. doi: 10.1016/j.dsr.2019.103186

Bonizzoni, S., Furey, N. B., and Bearzi, G. (2021). Bottlenose dolphins (*Tursiops truncatus*) in the north-western Adriatic Sea: Spatial distribution and effects of trawling. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 31 (3), 635–650. doi: 10.1002/aqc.3433

Bonizzoni, S., Hamilton, S., Reeves, R. R., Genov, T., and Bearzi, G. (2022). Odontocete cetaceans foraging behind trawlers, worldwide. *Rev. Fish Biol. Fish.* 32, 827–877. doi: 10.1007/s11160-022-09712-z

Borrell, A., Vighi, M., Genov, T., Giovos, I., and Gonzalvo, J. (2021). Feeding ecology of the highly threatened common bottlenose dolphin of the gulf of ambracia, Greece, through stable isotope analysis. *Mar. Mamm. Sci.* 37 (1), 98–110. doi: 10.1111/mms.12725

Brown, J. D. (2004). Knowledge, uncertainty and physical geography: towards the development of methodologies for questioning belief. *Trans. Inst. Br. Geogr.* 29 (3), 367–381. doi: 10.1111/j.0020-2754.2004.00342.x

Carlucci, R., Fanizza, C., Cipriano, G., Paoli, C., Russo, T., and Vassallo, P. (2016). Modeling the spatial distribution of the striped dolphin (*Stenella coeruleoalba*) and common bottlenose dolphin (*Tursiops truncatus*) in the gulf of taranto (Northern Ionian Sea, central–eastern Mediterranean Sea). *Ecol. Indic.* 69, 707–721. doi: 10.1016/j.ecolind.2016.05.035

Carmen, M., Berrow, S. D., and O'Brien, J. M. (2021). Foraging behavior of bottlenose dolphins in the Shannon estuary, Ireland as determined through static acoustic monitoring. *J. Mar. Sci. Eng.* 9, 275. doi: 10.3390/jmse9030275

Carnabuci, M., Schiavon, G., Bellingeri, M., Fossa, F., Paoli, C., Vassallo, P., et al. (2016). Connectivity in the network macrostructure of *Tursiops truncatus* in the pelagos sanctuary (NW Mediterranean sea): does landscape matter? *Popul. Ecol.* 58 (2), 249–264. doi: 10.1007/s10144-016-0540-7

Cribb, N., Miller, C. E., and Seuront, L. (2015). Towards a standardized approach of cetacean habitat: past achievements and future directions. *Open J. Mar. Sci.* 5, 335–357. doi: 10.4236/ojms.2015.53028

Diaz Lopez, B. (2019). "Hot deals at sea": responses of a top predator (Bottlenose dolphin, *Tursiops truncatus*) to human-induced changes in the coastal ecosystem. *Behav. Ecol.* 30 (2), 291–300. doi: 10.1093/beheco/ary162

Dinis, A., Alves, F., Nicolau, C., Ribeiro, C., Kaufmann, M., Cañadas, A., et al. (2016). Bottlenose dolphin *Tursiops truncatus* group dynamics, site fidelity, residency and movement patterns in the Madeira archipelago (North–East Atlantic). *Afr. J. Mar. Sci.* 38 (2), 151–160. doi: 10.2989/1814232X.2016.1167780

Dorazio, R. M. (2014). Accounting for imperfect detection and survey bias in statistical analysis of presence-only data. *Glob. Ecol. Biogeogr.* 23 (12), 1472–1484. doi: 10.1111/geb.12216

Forcada, J. (2018). "Distribution," in *Encyclopedia of marine mammals*. Eds. B. Wursig, J. G. M. Thewissen and K. Kovacs (London: Elsevier), 259–262.

Giannoulaki, M., Markoglou, E., Valavanis, V. D., Alexiadou, P., Cucknell, A., and Frantzis, A. (2017). Linking small pelagic fish and cetacean distribution to model suitable habitat for coastal dolphin species, *Delphinus delphis* and *Tursiops truncatus*, in the Greek seas (Eastern Mediterranean). *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 27 (2), 436–451. doi: 10.1002/aqc.2669

Giménez, J., Marçalo, A., Ramírez, F., Verborgh, P., Gauffier, P., Esteban, R., et al. (2017). Diet of bottlenose dolphins (*Tursiops truncatus*) from the gulf of cadiz: insights from stomach content and stable isotope analyses. *PloS One* 12 (9), e0184673. doi: 10.1371/journal.pone.0184673

Gnone, G., Bellingeri, M., Dhermain, F., Dupraz, F., Nuti, S., Bedocchi, D., et al. (2011). Distribution, abundance, and movements of the bottlenose dolphin (*Tursiops truncatus*) in the pelagos sanctuary MPA (north-west Mediterranean Sea). *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 21 (4), 372–388. doi: 10.1002/aqc.1191

Gnone, G., Bellingeri, M., Molinari, Y., Dhermain, F., Labach, H., Díaz López, B., et al. (2022). The seabed makes the dolphins. physiographic features shape the size and structure of the bottlenose dolphin geographical units. *J. Mar. Sci. Eng.* 10, 1036. doi: 10.3390/jmse10081036

Greller, R., Mazzoli, M., Titcomb, E., Nelson, B., Paperno, R., and Markwith, S. H. (2021). Environmental drivers of habitat use by common bottlenose dolphins (*Tursiops truncatus*) in the Indian river lagoon, Florida, USA. *Mar. Mamm. Sci.* 37 (2), 512–532. doi: 10.1111/mms.12767

Hammerschlag, N., Schmitz, O. J., Flecker, A. S., Lafferty, K. D., Sih, A., Atwood, T. B., et al. (2019). Ecosystem function and services of aquatic predators in the anthropocene. *Trends Ecol. Evol.* 34 (4), 369–383. doi: 10.1016/j.tree.2019.01.005

Hartel, E. F., Durden, W. N., and O'Corry–Crowe, G. (2020). Testing satellite telemetry within narrow ecosystems: nocturnal movements and habitat use of bottlenose dolphins within a convoluted estuarine system. *Anim. Biotelemetry* 8 (1), 1–16. doi: 10.1186/s40317-020-00200-4

Haughey, R., Hunt, T. N., Hanf, D., Passadore, C., Baring, R., and Parra, G. J. (2021). Distribution and habitat preferences of indo-pacific bottlenose dolphins (*Tursiops aduncus*) inhabiting coastal waters with mixed levels of protection. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.617518

Heithaus, M. R., Frid, A., Wirsing, A. J., and Worm, B. (2008). Predicting ecological consequences of marine top predator declines. *TREE* 23 (4), 202–210. doi: 10.1016/j.tree.2008.01.003

Hijmans, R. J. (2022) *Terra: Spatial data analysis*. Available at: https://CRAN.R-project.org/package=terra.

ISPRA (2016). *Fixed line transect monitoring using ferries as platform of observation for marine mega and macro fauna and main threats. monitoring protocol for cetaceans and sea turtles. – ISPRA agreement – technical annex 1* (ISPRA), 19. Available at: https://www.isprambiente.gov.it/files2021/progetti/technical-annex-i_monitoring-protocol_2015.pdf

IUCN-MMPATF (2017) *Campanian and pontino archipelagos IMMA*. Available at: https://drive.google.com/file/d/0BxEdFMHwpjLLa1ZKMkNrYnhldlk/view.

Johnston, D. W., and Read, A. J. (2007). Flow-field observations of a tidally driven island wake used by marine mammals in the bay of fundy, Canada. *Fish. Oceanogr.* 16 (5), 422–435. doi: 10.1111/j.1365-2419.2007.00444.x

Karczmarski, L., Cockcroft, V. G., and Mclachlan, A. (2000). Habitat use and preferences of indo-pacific humpback dolphins *Sousa chinensis* in algoa bay, south Africa. *Mar. Mammal Sci.* 16 (1), 65–79. doi: 10.1111/j.1748-7692.2000.tb00904.x

Kiszka, J. J., Woodstock, M. S., and Heithaus, M. R. (2022). Functional roles and ecological importance of small cetaceans in aquatic ecosystems. *Front. Mar. Sci.* 9, 163. doi: 10.3389/fmars.2022.803173

Krumme, U. (2004). Patterns in tidal migration of fish in a Brazilian mangrove channel as revealed by a split–beam echosounder. *Fish. Res.* 70 (1), 1–15. doi: 10.1016/j.fishres.2004.07.004

Labach, H., Azzinari, C., Barbier, M., Cesarini, C., Daniel, B., David, L., et al. (2022). Distribution and abundance of common bottlenose dolphin (*Tursiops truncatus*) over the French Mediterranean continental shelf. *Mar. Mamm. Sci.* 38 (1), 212–222. doi: 10.1111/mms.12874

La Manna, G., Ronchetti, F., and Sarà, G. (2016). Predicting common bottlenose dolphin habitat preference to dynamically adapt management measures from a marine spatial planning perspective. *Ocean Coast. Manage.* 130, 317–327. doi: 10.1016/j.ocecoaman.2016.07.004

Laran, S., Pettex, E., Authier, M., Blanck, A., David, L., Dorémus, G., et al. (2017). Seasonal distribution and abundance of cetaceans within French waters – part I: The north–Western Mediterranean, including the pelagos sanctuary. *Deep–Sea Res. II: Top. Stud. Oceanogr.* 141, 20–30. doi: 10.1016/j.dsr2.2016.12.011

Lin, T. H., Akamatsu, T., and Chou, L. S. (2013). Tidal influences on the habitat use of indo–pacific humpback dolphins in an estuary. *Mar. Biol.* 160 (6), 1353–1363. doi: 10.1007/s00227-013-2187-7

Marini, C., Fossa, F., Paoli, C., Bellingeri, M., Gnone, G., and Vassallo, P. (2015). Predicting bottlenose dolphin distribution along liguria coast (northwestern Mediterranean Sea) through different modeling techniques and indirect predictors. *J. Environ. Manage.* 150, 9–20. doi: 10.1016/j.jenvman.2014.11.008

Martino, S., Pace, D. S., Moro, S., Casoli, E., Ventura, D., Frachea, A., et al. (2021). Integration of presence-only data from several sources: a case study on dolphins' spatial distribution. *Ecography* 44 (10), 1533–1543. doi: 10.1111/ecog.05843

McLusky, D. S., and Elliott, M. (2004). *The estuarine ecosystem: ecology, threats and management* (Oxford: OUP).

Natoli, A., Genov, T., Kerem, D., Gonzalvo, J., Holcer, D., Labach, H., et al. (2021) *Tursiops truncatus (Mediterranean subpopulation)* (The IUCN Red List of Threatened Species 2021) (Accessed 22 July 2022).

Nelms, S. E., Alfaro-Shigueto, J., Arnould, J. P., Avila, I. C., Nash, S. B., Campbell, E., et al. (2021). Marine mammal conservation: over the horizon. *Endang. Species Res.* 44, 291–325. doi: 10.3354/esr01115

Pace, D. S., Arcangeli, A., Mussi, B., Vivaldi, C., Ledon, C., Lagorio, S., et al. (2018). Habitat suitability modeling in different sperm whale social groups. *J. Wildl. Manage.* 82 (5), 1062–1073. doi: 10.1002/jwmg.21453

Pace, D. S., Di Marco, C., Giacomini, G., Ferri, S., Silvestri, M., Papale, E., et al. (2021). Capitoline dolphins: Residency patterns and abundance estimate of *Tursiops truncatus* at the Tiber river estuary (Mediterranean Sea). *Biology* 10 (4), 275. doi: 10.3390/biology10040275

Pace, D. S., Ferri, S., Giacomini, G., Di Marco, C., Papale, E., Silvestri, M., et al. (2022a). Resources and population traits modulate the association patterns in the common bottlenose dolphin living nearby the Tiber river estuary (Mediterranean Sea). *Front. Mar. Sci* 9, 935235. doi: 10.3389/fmars.2022.935235

Pace, D. S., Giacomini, G., Campana, I., Paraboschi, M., Pellegrino, G., Silvestri, M., et al. (2019). An integrated approach for cetacean knowledge and conservation in the central Mediterranean Sea using research and social media data sources. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 29 (8), 1302–1323. doi: 10.1002/aqc.3117

Pace, D. S., Mussi, B., Wurtz, M., and Gordon, J. D. C. (2014). Foreword. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 24, 1–3. doi: 10.1002/aqc.2457

Pace, D. S., Pulcini, M., and Triossi, F. (2012). Anthropogenic food patches and association patterns of *Tursiops truncatus* at lampedusa island, Italy. *Behav. Ecol.* 23 (2), 254–264. doi: 10.1093/beheco/arr180

Pace, D. S., Tumino, C., Silvestri, M., Giacomini, G., Pedrazzi, G., Pavan, G., et al (2022b). Bray-Call Sequences in the Mediterranean Common Bottlenose Dolphin (Tursiops truncatus) Acoustic Repertoire. *Biology* 11 (3), 367. doi: 10.3390/biology11030367

Papale, E., Alonge, G., Caruso, F., Grammauta, R., Mazzola, S., Mussi, B., et al (2021). The higher, the closer, the better? influence of sampling frequency and distance on the acoustic properties of short-beaked common dolphins burst pulses in the Mediterranean Sea. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 51–60. doi: 10.1002/aqc.3158

Pedrazzi, G., Giacomini, G., and Pace, D. S. (2022). First report of epimeletic and acoustic behaviour in Mediterranean common bottlenose dolphins (*Tursiops truncatus*) carrying dead calves. *Biology* 11 (2), 337. doi: 10.3390/biology11020337

Pleslić, G., Rako-Gospić, N., Miočić-Stošić, J., Blazinić Vučur, T., Radulović, M., Mackelworth, P., et al. (2019). Social structure and spatial distribution of bottlenose dolphins (*Tursiops truncatus*) along the Croatian Adriatic coast. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 29 (12), 2116–2132. doi: 10.1002/aqc.3213

Pulcini, M., Pace, D. S., La Manna, G., Triossi, F., and Fortuna, C. M. (2014). Distribution and abundance estimates of bottlenose dolphins (*Tursiops truncatus*) around lampedusa island (Sicily channel, italy): implications for their management. *J. Mar. Biolog. Assoc. UK* 94 (6), 1175–1184. doi: 10.1017/S0025315413000842

Renner, I. W., Elith, J., Baddeley, A., Fithian, W., Hastie, T., Phillips, S. J., et al. (2015). Point process models for presence-only analysis. *Methods Ecol. Evol.* 6, 366–379. doi: 10.1111/2041-210X.12352

Ricevuto, E., Tognotti, M., and Trombetti, C. (2011). Scientific activities' programme: monitoring species and surveys on anthropogenic impacts on marine–coastal habitats. *Biol. Mar. Medit.* 18, 159–160.

Soldevilla, M. S., Wiggins, S. M., Hildebrand, J. A., Oleson, E. M., and Ferguson, M. C. (2011). Risso's and pacific white–sided dolphin habitat modeling from passive acoustic monitoring. *Mar. Ecol. Prog. Ser.* 423, 247–260. doi: 10.3354/meps08927

Sprogis, K. R., Raudino, H. C., Rankin, R., MacLeod, C. D., and Bejder, L. (2016). Home range size of adult indo-pacific bottlenose dolphins (*Tursiops aduncus*) in a coastal and estuarine system is habitat and sex-specific. *Mar. Mamm. Sci.* 32 (1), 287–308. doi: 10.1111/mms.12260

Steneck, R. S. (2012). Apex predators and trophic cascades in large marine ecosystems: learning from serendipity. *PNAS* 109 (21), 7953–7954. doi: 10.1073/pnas.1205591109

Stephenson, F., Hewitt, J. E., Torres, L. G., Mouton, T. L., Brough, T., Goetz, K. T., et al. (2021). Cetacean conservation planning in a global diversity hotspot: dealing with uncertainty and data deficiencies. *Ecosphere* 12 (7), e03633. doi: 10.1002/ecs2.3633

Triossi, F., Willis, T. J., and Pace, D. S. (2013). Occurrence of bottlenose dolphins *Tursiops truncatus* in natural gas fields of the northwestern Adriatic Sea. *Mar. Ecol.* 34 (3), 373–379. doi: 10.1111/maec.12020

Vassallo, P., Marini, C., Paoli, C., Bellingeri, M., Dhermain, F., Nuti, S., et al. (2020). Species-specific distribution model may be not enough: The case study of bottlenose dolphin (*Tursiops truncatus*) habitat distribution in pelagos sanctuary. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* 30 (8), 1689–1701. doi: 10.1002/aqc.3366

Walker, W. E., Harremoës, P., Rotmans, J., van der Sluijs, J. P., Van Asselt, M. B., Janssen, P., et al. (2003). Defining uncertainty: a conceptual basis for uncertainty management in model–based decision support. *Integr. Assess.* 4 (1), 5–17. doi: 10.1076/iaij.4.1.5.16466

Warton, D. I., and Shepherd, L. C. (2010). Poisson point process models solve the "pseudo-absence problem" for presence-only data in ecology. *Ann. Appl. Stat.* 4, 1383–1402. doi: 10.1214/10-AOAS331

Watson, J., Joy, R., Tollit, D., Thornton, S. J., and Auger–Méthé, M. (2019). Estimating animal utilization distributions from multiple data types: a joint spatio–temporal point process framework. *arXiv preprint arXiv* 1911, 00151. doi: 10.48550/arXiv.1911.00151

Wells, R. S., Natoli, A., and Braulik, G. (2019) *Tursiops truncatus (errata version published in 2019)* (The IUCN Red List of Threatened Species 2019) (Accessed 27 July 2022).

Wells, R. S., and Scott, M. D. (2018). "Bottlenose dolphin, tursiops truncatus, common bottlenose dolphin," in *Encyclopedia of marine mammals*. Eds. B. Wursig, J. G. M. Thewissen and K. Kovacs (London: Elsevier), 118–125.

Würtz, M., and Rovere, M. (2015). *Atlas of the Mediterranean seamounts and seamount–like structures* (Gland, Switzerland: IUCN).

Yuan, Y., Bachl, F. E., Lindgren, F., Borchers, D. L., Illian, J. B., Buckland, S. T., et al. (2017). Point process models for spatio–temporal distance sampling data from a large–scale survey of blue whales. *Ann. Appl. Stat.* 11, 2270–2297. doi: 10.1214/17-AOAS1078

Zanardo, N., Parra, G. J., Passadore, C., and Möller, L. M. (2017). Ensemble modelling of southern Australian bottlenose dolphin tursiops sp. distribution reveals important habitats and their potential ecological function. *Mar. Ecol. Prog. Ser.* 569, 253–266. doi: 10.3354/meps12091

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Application of a multi-species bio-economic modelling approach to explore fishing traits within eligible cetacean conservation areas in the Northern Ionian Sea (Central Mediterranean Sea)

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The assessment of the spatial overlap between eligible cetacean conservation areas (CCAs) and fishing grounds could be a strategic element in the implementation of effective conservation measures in the pelagic offshore areas. A multi-species bio-economic modelling approach has been applied to estimate the fishing traits in eligible CCAs in the Northern Ionian Sea (NIS, Central Mediterranean Sea) between 10-800 m of depth, adopting the Spatial MAnagement of demersal Resources for Trawl fisheries model (SMART). Four possible CCAs were defined according to the distribution of cetacean species, their bio-ecological needs, as well as socio-economic needs of human activities, identifying a Blue, Red, Orange and Green CCAs in the NIS. SMART spatial domain was a grid with 500 square cells (15×15 NM). The analysis was conducted for the period 2016-2019, considering the Otter Trawl Bottom (OTB) fleet activities in the study areas through the Vessel Monitoring System. The spatial extension of fishing activities, hourly fishing effort (h), landings (tons) and economic value (euros) for each CCA and the NIS were estimated as yearly median values. Fishing activities were absent in the Blue CCA, where the presence of the submarine canyon head does not offer accessible fishing grounds. The hourly fishing effort in the Green area accounted for about 22% (3443 h) of the total hourly effort of the NIS, while the Orange and Red areas were about 8% (1226 h) and 2% (295 h), respectively. The Green CCA corresponded to about 14% (36 tons) of the total landings in the NIS, whereas the Orange and Red areas represented about 9% (22 tons) and 6% (16 tons), respectively. The Green CCA accounted for about 13% (156 thousand euros) of the total economic value of the NIS, while the Orange and Red areas represented about 6% (69 thousand euros) and 4% (44thousand euros), respectively. Results showed no or negligible negative effects on trawl activities by potential spatial restrictions due to the establishment of CCAs highlighting the importance to consider spatially integrated information during the establishment process of conservation areas for cetacean biodiversity according to the principles of Ecosystem Based Management.

KEYWORDS

SMART model, fishing effort, fishing production, conservation, MPA, dolphins and whales

Introduction

Spatial analysis of the distribution of key species and their interaction with human activities at sea is a key aspect of any ecosystem-based marine spatial planning (MSP, Foley et al., 2010). Moreover, the MSP approach emphasizes the importance of including both direct and indirect relationships with the legal, socio-economic and ecological complexity of governance when assigning a marine area to a specific use, as there is often a space of overlap between conflicting components that can and must be buffered in advance through measures of appropriate sizing, mitigation and compensation, ensuring greater acceptance and above all a real effectiveness in the conservation of marine biodiversity (Ehler and Douvere, 2009). Being able to harmonize these aspects is crucial if Blue Growth (https://s3platform.jrc.ec.europa.eu/blue-growth) is to be effectively supported. For their bio-ecological traits, the effectiveness of protection measures for cetaceans can be represented by the establishment of conservation areas that encompass large portions of the pelagic domain. Although Marine Protected Areas (MPAs) have been widely adopted in several marine ecosystems (Claudet, 2011), the institution of spatial conservation measures dedicated to the protection of the pelagic domain on a large scale is poorly applied (Wood et al., 2008; Game et al., 2010; Kaplan et al., 2010). On the contrary, the protection of coastal and pelagic offshore areas seems to be a fundamental corner for effective biodiversity conservation, because pelagic MPAs can ensure ecological connectivity between different coastal protected areas, such as those distributed in gulfs and bays (Guidetti et al., 2013). In addition, the ecological benefits derived from coastal MPAs are also often accompanied by positive effects on the rebuilding of fishing stocks, which have economic fallouts on the fishery and other associated activities, such as tourism (Stelzenmüller et al., 2007; Russo et al., 2019). Even more relevant could be the contribution of deep-sea pelagic conservation areas, as the restriction of fishing over large areas could result in minimal economic losses for the sector, but with the advantage of ensuring a more effective remedy against the processes of extinction and loss of diversity and key ecosystem services (Sumaila et al., 2007). In this regard, cetaceans have proven to be of maximum importance in the stability and resiliency of the marine ecosystems (Tromeur and Loeuille, 2017) and in the support of several ecosystem services (Pace et al., 2015), with positive reflection even on climate change (Sergio et al., 2008; Hooker et al., 2011; Roman et al., 2014; Mazzoldi et al., 2019). Therefore, due to threats to and risk of degradation in their status in the Mediterranean Sea (ACCOBAMS, 2020) there is a very urgent need to provide action favouring the maintenance of their critical habitat. However, the planning of cetacean conservation areas represents a real challenge mostly because the spatial overlap between the distribution of cetacean critical habitat and fishing activities is wide. In fact, the feeding preferences of cetaceans and their behavioural strategies could cause conflicts with the fishing activities that are classified as a competition for food resources (Bearzi, 2002; Jusufovski et al., 2019).

Under the umbrella of the European Common fisheries policy (CFP), the management of fishery resources in the Mediterranean Sea is largely based on the regulation of the spatial fishing effort distribution, that is the identification of areas in which to prohibit some or all types of fishing to protect the environment and resources (https://oceans-and-fisheries.ec.europa.eu/policy/common-fisheries-policy-cfp_en). However, the establishment of Fishery-Restricted Area leads to reallocation of fishing effort (displacement from closed areas to adjacent or new ones) that can significantly influence the final effects of this kind of management measures, both in biological and economic aspects (Bastardie et al., 2018; Russo et al., 2019; D'Andrea et al., 2020). Therefore, understanding the allocation of fishing effort displacement is an information to be taken into account when management regulations, which are characterized by temporal and/or spatial banning of fishery, need to be implemented. Several studies have adopted spatial modelling approaches to investigate the fishing exploitation pattern in terms of effort distribution, catches and economic production (Russo et al., 2014; Quijano Quiñones et al., 2021), to simulate possible spatial management scenarios of trawl fishery (Russo et al., 2019) or to investigate the risks of interaction between cetaceans and the fishery (Breen et al., 2017).

In the Northern Ionian Sea (NIS, Central Mediterranean Sea), cetaceans represent key elements in the ecosystem functioning supporting trophic regulations of the entire food web (Ricci et al., 2020a; Carlucci et al., 2021a). Several bio-ecological traits of cetacean species distributed in the NIS (Carlucci et al., 2018a; Carlucci et al., 2018b; Carlucci et al., 2020a; Carlucci et al., 2020b; Cipriano et al., 2022) have been investigated, as well as the potential competition with local fishing activities (Ricci et al., 2020b; Ricci et al., 2021a). This ecological knowledge acquired in the last decade is a focal point in the assessment of cetacean distribution and the interaction with anthropogenic impacts in the Gulf of Taranto, the northernmost part of the NIS (Carlucci et al., 2021b). This information supports the possibility to propose area-based management tools (ABMTs) for cetacean conservation (Notarbartolo di Sciara et al., 2016), such as Cetacean Conservation Areas (CCAs, Carlucci et al., 2021c), aimed at protecting these species and their critical habitats, mitigating anthropogenic impacts and promoting the sustainable development of human maritime activities. In particular, the NIS can be considered an eligible area for the implementation of ABMTs and CCAs, where underwater noise, marine litter, ship collision, and competition for prey by fishery are the main disturbances involved in interacting with cetaceans (Carlucci et al., 2021b). Although direct fishing impacts on the cetaceans (e.g. by-catches) are not recorded in the area (Ricci et al., 2021a), potential competition for food resources could arise with local fishing activities (Carlucci et al., 2021a). However, an investigation into the potential spatial interactions between fishing activities and eligible CCAs has never been explored, although long time series of data are available on species distribution and their life history traits (Maiorano et al., 2010; Capezzuto et al., 2010; Carlucci et al., 2018c; Ricci et al., 2021b) and the characterization of fishing grounds (Russo et al., 2017). Therefore, the main objective of this study is to set up an assessment of the spatial overlap between eligible CCAs and fishing grounds in the NIS using a multi-species bio-economic modelling approach. In particular, the assessment was conducted in the period 2016-2019 using the Spatial MAnagement of demersal Resources for Trawl fisheries model (SMART, Russo et al., 2014; D'Andrea et al., 2020). SMART was selected since it allows reconstruct, using a combination of different data sources, the spatial and temporal origin of catches or landings and their final faith in terms of landing harbour. In addition, given that is a spatial bio-economic model, SMART allows to estimate the economic indicators associated to different patterns of fishing effort, at the scales of both single vessels and fleets. In this way, SMART can be used to assess the economic and the biological value of a given fishing area, supporting quantitative analyses and evaluation in the framework of marine spatial planning. In this paper, SMART has been applied on the harbour-specific fleets of bottom trawlers operating in the study area in order to obtain an assessment of the potential bio-economic impacts of different spatial management actions involving the CCAs. According to Carlucci et al. (2021a), bottom otter trawling (OTB) represents by far the main fishery in the Northern Ionian Sea, both in terms of landings and profits and impacts.

CCAs were described through fishing traits inherent to the otter bottom trawl fleet, by using several indicators, such as fishing effort, landing, economic incomes, and the landing flows from the fishing grounds included within the CCAs towards the main harbours of the study area.

Materials and methods

Study area

The study area extends from Punta Alice to Santa Maria di Leuca covering a surface of about 14000 km^2 and reaching 1500 m in depth in the Northern Ionian Sea (NIS) (Central Mediterranean Sea). The hydrographic features of the area are characterized by up-welling systems (Bakun and Agostini, 2001) and decadal processes of deep-water circulation inversion with effects on the energy exchanges between benthic and pelagic domain (Ricci et al., 2022). The NIS includes several important habitats from a conservation point of view in shallow (including seagrass meadows and coralligenous outcrops), pelagic (upwelling sites) and deep-sea areas (including submarine canyon and cold-water coral banks) (Capezzuto et al., 2010; Bo et al., 2011; D'Onghia et al., 2016; Carlucci et al., 2018c; Castellan et al., 2019; Chimienti et al., 2019) ensuring favourable conditions for the support of a high biological diversity and providing diverse ecological services (Carlucci et al., 2021a). Moreover, the study area has been widely recognized as a critical area for the day-to-day life of striped dolphin *Stenella coeruleoalba* and common bottlenose *Tursiops truncatus* (Carlucci et al., 2016a; Carlucci et al., 2017; Carlucci et al., 2018b; Carlucci et al., 2018d; Ciccarese et al., 2019; Santacesaria et al., 2019; Azzolin et al., 2020). In particular, the spatial distribution and areas where these dolphins realize feeding, resting, socializing, and traveling activities have been identified (Carlucci et al., 2018b; Papale et al., 2020). In addition, other cetacean species occur in the NIS, such as the Risso's dolphin (*Grampus griseus*, Maglietta et al., 2020; Maglietta et al., 2022; Maglietta et al., 2018; Renò et al., 2019; Carlucci et al., 2020a), the sperm whale (*Physeter macrocephalus*, Bellomo et al., 2019), the Cuvier's beaked whale (*Ziphius cavirostris*, Podestà et al., 2016; Carlucci et al., 2020b) and the fin whale (*Balaenoptera physalus*, Dimatteo et al., 2011; Fanizza et al., 2014).

The habitat complexity of this NIS is accompanied by several anthropogenic pressures, which are represented by fishing activity and marine traffic, as well as the occurrence of navy exercises areas, and industrial activities (Carlucci et al., 2021b; Carlucci et al., 2016a). In particular, the geo-morphological and biological heterogeneity described so far, strongly influences the distribution of the fishing effort, distribution and the typologies of fishing gears adopted in the area. Fishing boats are frequently registered as polyvalent fishing vessels, often changing type of fishing according to the season and sea/weather conditions as well as to the variability in the availability of resources and market demand (Carlucci et al., 2016b). In particular, fishing occurs from coastal waters to about 800 m in depth and it is mainly characterized by the bottom otter trawls, that mostly exploit the shelf break and slope, and the small-scale fishery operating on coastal grounds (Russo et al., 2017). The most important fishing resources are the red mullet (*Mullus barbatus*) on the continental shelf, the European hake (*Merluccius merluccius*), the deep-water rose shrimp (*Parapenaeus longirostris*) and the Norway lobster (*Nephrops norvegicus*) on a wide bathymetric range; as regards the bathyal grounds, the shrimps (*Aristeus antennatus* and *Aristaeomorpha foliacea*) are the most important resources (Carlucci et al., 2018c; Maiorano et al., 2010; Carlucci et al., 2016b; Russo et al., 2017).

Information and data on the cetofauna occurring in the NIS have been available since 2009. This knowledge has led to the hypothesis of a delimitation of four possible CCAs based on different assumptions related to the distribution of cetacean species according to their bio-ecological needs as well as socio-economic constraints (Figure 1). The former area, hereafter called the "Red area", has an extension of approximately 715 km^2 and includes the persistent critical habitats of the striped dolphin (Carlucci et al., 2018d). The second, hereafter called the "Orange area", encompasses approximately 1530 km^2 being enlarged to include all the areas where behavioural activities of the striped dolphin population were observed from 2009 to 2017 (Carlucci et al., 2018d). The third, hereafter called the "Green area", covers approximately 3170 km^2 and includes areas where the highest abundances of both striped and common bottlenose dolphins were estimated through habitat modelling techniques (Carlucci et al., 2018a), together with all the sightings recorded up to 2020 for both the Risso's dolphin and the sperm whale. The latter CCA, hereafter called the "Blue area", covers 615 km^2 and has been delimited according to the specific spatial needs indicated by the main stakeholders (e.g. maritime authority, navy, municipality, NGOs, research institutions) operating in the study area.

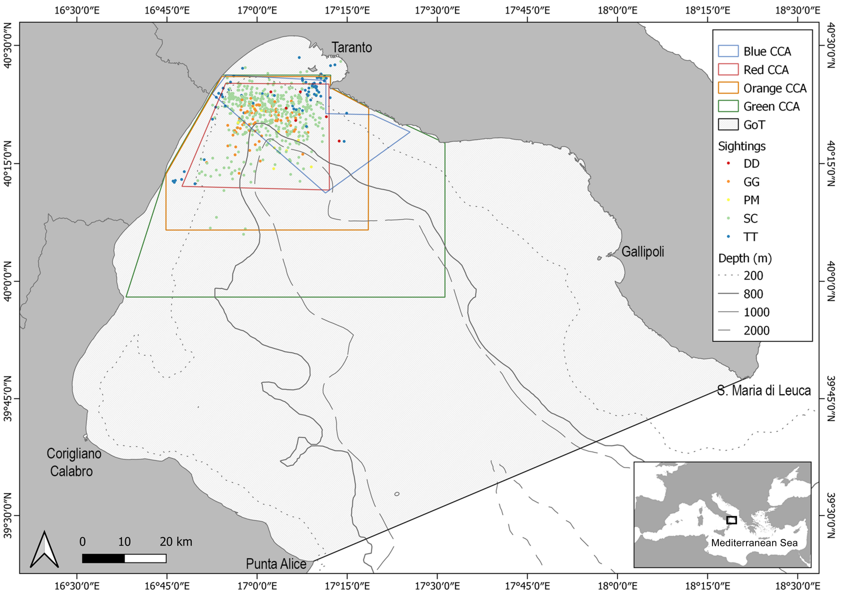

FIGURE 1
Map of the Northern Ionian Sea (Central Mediterranean Sea) with sightings distribution of *S. coeruleoalba* (SC), *D. delphis* (DD), *G. griseus* (GG), *T. truncatus* (TT), *P. macrocephalus* (PM) and the spatial limits of CCAs (the black line indicates the border of the gulf).

SMART Modelling approach and fishing data

The assessment of fishing traits of each CCA and the Northern Ionian Sea was carried out through the SMART modelling approach, a method able to reconstruct the spatial and temporal fluxes of landings coming from well-defined areas (fishing grounds) and times to harbors to which they are delivered for sale (Russo et al., 2018; Russo et al., 2014; D'Andrea et al., 2020). The modelling of spatial fishing effort is based on the use of information obtained by the Vessel Monitoring System (VMS), which is applied to the remote control of fishing vessels with length overall (LOA) ≥ 15 m in European waters (EC, 2011). The VMS data are combined with information on landings acquired from fishing logbooks, where information on landing by species and harbors are reported by the fishers (Gerritsen and Lordan, 2011). All these data are collected within the Data Collection Framework since 2006 and they are provided by the Italian "Ministry of the Agricultural, Alimentary and Forestry Politics" (Russo et al., 2014). Starting from this information on the spatial effort and landings, it is possible to estimate the Landing Per Unit of Effort (LPUE, kg h^{-1} km^{-2}) for each vessel length (D'Andrea et al., 2020). In addition, a reconstruction of the effort and production data for vessels with a LOA < 15 m was carried out using the European Common Fleet Register (EC, 2010) according to the method reported in Russo et al. (2018).

The spatial domain of the SMART model for the investigated area was defined as a grid with 500 square cells (15 × 15 nautical miles). The rationale of the model, as well as the workflow of the smartR R package, can be summarized in the following logical steps:

1. Analysing VMS data to assess the fishing effort by vessel/cell/time;

2. Processing landings data, combined with VMS data, to estimate the spatial/temporal productivity of each cell (or spatial unit), in terms of mean monthly LPUE by species, according to the method described and applied in Russo et al. (2018);

3. Estimating the cost per vessel/time associated with a given effort pattern and the related revenues, as a function of the landings by vessel/species/length class/time;

4. Combining costs and revenues by vessel, on the yearly scale, to obtain the profit, which is the proxy of the vessel performance.

Each of these steps corresponds to a different module of the smartR package (D'Andrea et al., 2020). A detailed description of the SMART workflow is reported in Russo et al. (2019) and D'Andrea et al. (2020), while a diagram of the approach used in this paper is represented in Figure 2.

The analysis was conducted for the period 2016-2019 (48 months), considering the fleets of vessel performing bottom otter trawling (OTB) in the Northern Ionian Sea and belonging to three fleet segments defined by vessel length-over-all (namely LOA <12, LOA between 12-18 and LOA between18-24 m), being the main segments operating in the study areas (Maiorano et al., 2019; Maiorano et al., 2010; Russo et al., 2017). To avoid anomalies in the fishing production induced by fishing effort variations at the national scale which occurred during the SARS-CoV-2 coronavirus pandemic period (Russo et al., 2022) the years 2020-2021 were excluded from the analysis.

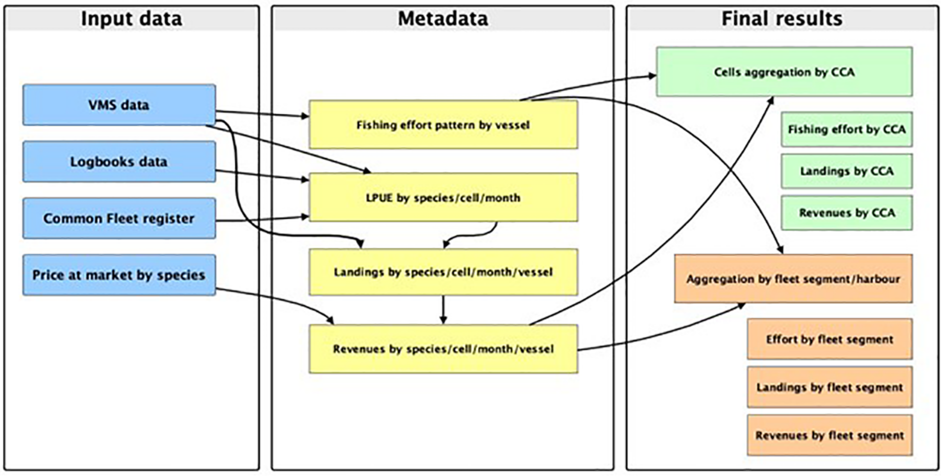

FIGURE 2
Diagram of the workflow, from input data to final output, applied in this study to assess the value of CCAs for the trawl fisheries operating in the area of study.

The combination of the different data sources (i.e. VMS data, Logbooks data, Common Fleet register and Price at market by species) allowed to estimate the Fishing effort and the related landings and revenues for the different CCAs. In addition, being known the harbour of departure/landing of each trawler, it was possible to estimate fishing effort, landings and revenues by harbour-specific fleets. In this way, the results of this modelling approaches represent estimates of the real values but simulations. Moreover, considering that the displacement of effort potentially determined by the CCAs was not predicted, the results of this study represent an assessment of the *status quo* (i.e. the present values of CCAs for trawl fishing).

Assessment of the fishing activities and production within CCAs and in the Northern Ionian Sea

The assessment of the OTB fleet fishing exploitation in the CCAs and the NIS was carried out considering the spatial distribution of fishing activities, the hourly fishing effort, and the production in terms of landing and economic revenue during the period 2016-2019. The spatial extension of fishing activities (expressed as km^2) was calculated as the median of annual values estimated through VMS data for all OTB LOA segments. OTB swept areas by LOA segment were estimated in each year in the range of depth between 10-800 m. In addition, annual trends of the spatial coverage (%) of each VL segment were analysed.

The hourly fishing effort and the yield (landings) were estimated using data provided by the SMART model considering available OTB LOA segments during the investigated period. Therefore, monthly landings were combined with VMS data (using the fishing vessel and temporal range of the fishing activity as references) to estimate the monthly LPUE for each species and cell in the grid (see Russo et al., 2018, for an extensive description of this procedure). The LPUEs obtained were aggregated by the target species of the trawling (Table 1). In addition, data provided by the SMART model were used to calculate several indicators, such as the hourly effort (hours) by OTB LOA segment, the landing, the spatial LPUE and the economic value for each CCA and the NIS. Economic incomes of fishing landing were calculated by multiplying the landing value (kg) of main target species of trawl segment by their price (expressed as mean value in euros kg^{-1} per species) (Table 1).

Further analysis involved the estimation of production flows from the study areas to the main fishing harbours (Crotone, Cariati, Corigliano, Taranto, Gallipoli, Otranto) of the NIS, according to the method reported in Russo et al. (2018). Fishing harbours were aggregated at regional level (Calabrian and Apulian), which are precisely divided by the Taranto valley in the Gulf of Taranto, occupying the southwestern and north-eastern zones, respectively (Rossi and Gabbianelli, 1978). This choice is due to the difference in bottom trawling fleets in the two areas in terms of capacity and effort (Maiorano et al., 2022; Maiorano et al., 2010; Russo et al., 2017), as well as to the structure of the demersal assemblage (Carlucci et al., 2018c) and the food webs (Ricci et al., 2019).

TABLE 1 Mean sales prices for target species indicated by FAO 3alpha code considered in the analysis.

Species	FAO Code	Price (€/kg)
Aristeus antennatus	ARA	18.5
Aristaeomorpha foliacea	ARS	14.0
Boops boops	BOG	0.5
Parapenaeus longirostris	DPS	3.75
Eledone cirrhosa	EOI	4.0
Merluccius merluccius	HKE	6.0
Trachurus trachurus	HOM	1.0
Lophius piscatorius	MON	7.0
Mullus surmuletus	MUR	12.0
Mullus barbatus	MUT	4.0
Nephrops norvegicus	NEP	20.0
Illex coindetii	SQM	4.0

Therefore, median, minimum, and maximum, interquartile range (IR) and the percentage values (% calculated on the median value) of each production indicator for CCAs and the NIS were analysed. A statistical comparison of the fishing and production indicators (hourly effort, landing and economic values) among all CCAs and the NIS was carried out using the multiple non-parametric Mann–Whitney (U) *post hoc* test, based on the Bonferroni correction (McDonald, 2014). The selection of the KW test was due to the non-normal distribution of the data tested by the Shapiro–Wilk test (Shapiro and Wilk, 1965) (Table S1). The statistical analysis was carried out using PAST 4.03 (Hammer et al., 2001).

Results

Spatial and temporal interactions of fishing activities within CCAs

The aggregated fishing footprint distributed in potential fishable areas between 10-800 m of depth is reported, together with the different CCAs of interest, in Figures 3–5 and Supp. Materials (Table S2; Figure S1).

In the Blue CCA, fishing activity was detected for the OTB VL 15-18 only in 2018, with an absolute extent of 4 km^2 (Figure 3). This indicated that the fishing activities are substantially absent in this area. In the Red CCA, the median spatial extent of the entire OTB fleet showed a value of 8 km^2 (IR=8), representing a percentage of spatial extension of 0.9%

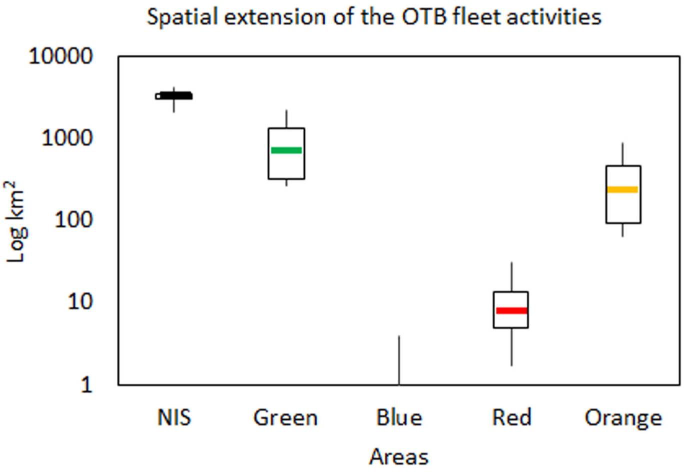

FIGURE 3
The total yearly spatial extension (km^2 in Log scale) of the OTB fleet within CCAs and the NIS during the investigated period (2016-2019). Boxplots report the median (midline), quartiles (box limits); minimum and maximum values (whiskers out of boxes).

with respect to the whole potential fishable area (Figure 3). In addition, the maximum percentage value of spatial extension was estimated for the OTB VL_18-24 in 2016 (32 km^2, 3.7%), while other values in the remaining years were lower than 1.5% (Figure S1). In the Orange CCA, the spatial extension of the OTB fleet showed a median value of 237 km^2 (IR=380; 17.0% of the Orange area total) (Figure 3). The highest spatial extension was estimated for the OTB VL 18-24 with a percentage value of 64.1% in 2016, while the lowest was estimated in 2018 (26.8%) (Figure S1). In the Green area, the median spatial extension was of 704 km^2 (IR=992; 26.5% of the Green CCA total) and the highest value was observed in 2016 (82.9%) for the OTB VL 18-24 (Figure 3). Also in this area, percentage values decreased over time with the lowest value in 2018 (36.5%) (Figure S1). In the NIS, the spatial extension of the OTB fleet showed a median value of 3469 km^2 (IR=522; 36.0% of the total NIS area) (Figure 3). The OTB VL 18-24 segment showed the highest percentage values in 2016, with values of 44.1%, while the lowest was detected in 2019 (28.5%) (Figure S1). In addition, OTB VL 12-18 vessel showed a spatial extension lower than approximately 36% in all years.

In the NIS, the mean total yearly effort of the whole trawl fleet showed a median value of 15925 hours (IR= 5174) (Figure 6A, Table S3). Considering the CC areas, the highest was estimated for the Green area (median value of 3443 hours, IR=3070), followed by the Orange area (median value of 1226 hours, IR=1385) and the Red area (median value of 295 hours; IR=199), which were significantly different between them ($p<0.001$; Table S4). In addition, the fishing effort in the Green area accounted for 21.6% of the total hourly effort of the NIS, and the Orange and Red areas were 7.7% and 1.9%, respectively.

Considering the hourly effort by VL segments, fishing activities in the Red CCA were almost exclusively performed by OTB 18-24 vessels (median value of 295 hours, IR=197; 91.4% of the total hourly effort) (Figure 6B), Differently, other VL segments were absent, as VL <12, or characterized by very negligible activities (less than 30 fishing hours estimated for VL 12-18). Similarly, the hourly effort in the Orange CCA showed the highest median value of 1102 hours (IR=865) for the OTB 18-24 segment, representing 52.7% of the total hourly effort in the CCA (Figure 6C). Lower median values were detected for other segments, where OTB<12 and OTB 12-18 vessels accounted for 18.2% (median value of 380 hours; IR=36) and 29.1% (median value of 609 hours; IR=725), respectively. In the Green CCA, the division of fishing effort by VL segments showed the highest median value for OTB VL 18-24 (2499 hours; IR=3205) accounting for 56.5% of the total fishing hourly effort of the CCA (Figure 6D). In the NIS, the OTB VL<12 vessels showed the highest median value of hourly effort (7535 hours, IR=3269) representing 49.0% of the total effort, followed by OTB 12-18 vessels (median value 4900 hours, IR=3761, 31.9%) and the OTB18-24 fleet (median value 2938, IR=5642, 19.1%).

Fishing production in the Northern Ionian Sea and CCAs

In the NIS, the median estimated production as landings and landing values corresponded to 254.8 tons (IR=124.6) and 1.253 mln euro (IR=580.1), respectively (Table S3). In the CCAs, the highest median value of landings was estimated for the Green

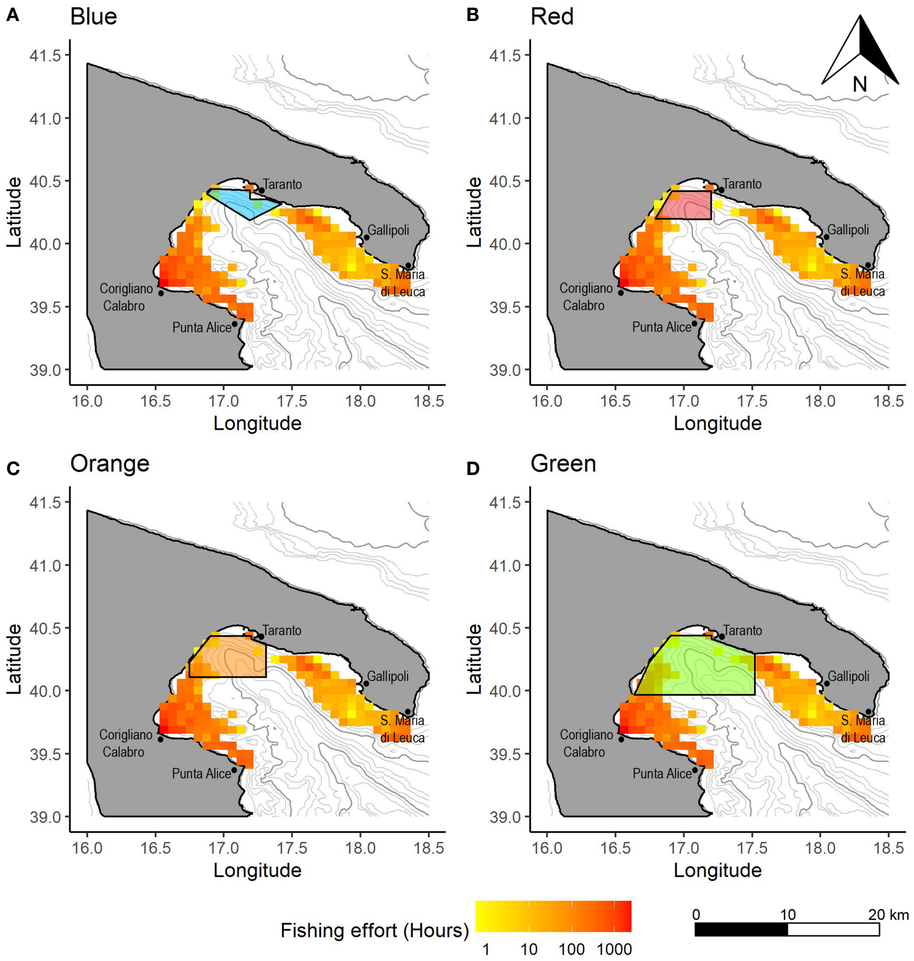

FIGURE 4
Spatial distribution of the OTB effort (in hours) by VL 12-18 showing the overlap with the **(A)** Blue, **(B)** Red, **(C)** Orange and **(D)** Green CCAs. Values in hours are calculated as yearly averages for the period 2016-2019.

area (36.1 tons, IR=26), followed by the Orange area (22.4 tons, IR=13.7) and the Red area (16.1 tons, IR=11.1). Thus, the Green area corresponded to 14.2% of the total landings in the NIS, and the Orange and Red areas represented 8.8% and 6.3%, respectively. Considering the economic revenue, median values estimated for the CCA were 156.3 thousand euros (IR=107.9) for the Green area, 69.2 thousand euros (IR=45.2) for the Orange area and 43.9 thousand euros (IR=25.4) for the Red one. Thus, the Green CCA accounts for 12.5% of the total economic value of the NIS, and the Orange and Red areas represented 5.5% and 3.5%, respectively. All median values calculated for the production indicators were significantly different between the CCAs and NIS (p<0.01) (Table S4).

Considering the composition of landings in the NIS, the most landed species were *B. boops* with a median value of 41.2 tons (IR=19.2; 16.5% of the total landing in the NIS), followed by *T. mediterraneus* (33.6 tons, IR=22.53, 13.4%), *M. merluccius* (32.4 tons, IR=14.37, 13%), *M. barbatus* (29.8 tons, IR=16.87, 11.9%), *I. coindettii* (26.2 tons, IR=20.18, 10.5%) and *P. longirostris* (25.9 tons, IR=12.34, 10.3%) (Figure 7 left plots; Table S5). In term of economic yield, *A. antennatus* was the most important species, with the median revenue value of 254.7 thousand euros (IR=132.47, 20.7%), followed by *M. merluccius* (151.0 tons, IR=67.06, 12.3%) (Figure 7 right plots). In addition, *N. norvegicus*, *M. barbatus* and *I. coindetii* each account for between 8 and 10% of the total economic value of the NIS.

The production pattern showed some differences in the CCAs compared to the NIS. In particular, the Red CCA showed the lowest number of species in the landing. *P. longirostris* was the most important landed and economic species, with median values of 7.2 tons (IR=4; 47.7% of the total landing in the Red CCA) and 26.8 thousand euros (IR=15.04; 65.5% of the total economic yield of Red CCA). Other relevant species in the landing were *T. mediterraneus* (4.0

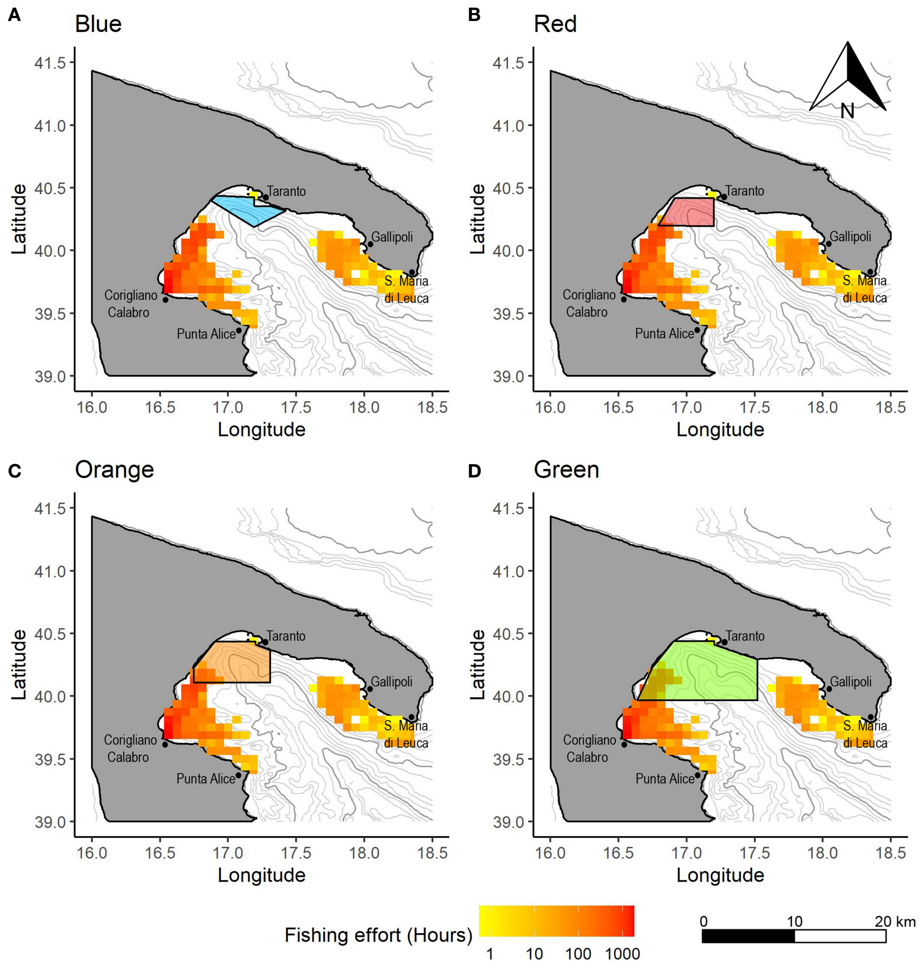

FIGURE 5
Spatial distribution of the OTB effort (in hours) by VL 18-24 showing the overlap with the **(A)** Blue, **(B)** Red, **(C)** Orange and **(D)** Green CCAs. Values in hours are calculated as yearly averages for the period 2016-2019.

tons; 26.5%) and *B. boops* (2.1 tons, 14.1%), but they were characterized by a very low economic value.

In the Orange and Green CCAs, the most important species in the landings were always *P. longirostris*, *T. mediterraneus* and *B. boops*, but an increase in the number of landed species was observed. Deep resources (*A. foliacea*, *A. antennatus* and *N. norvegicus*) were found, as well as commercial cephalopods (*I. coindettii* and *E. cirrhosa*). In the Orange CCA, *P. longirostris* showed the highest median values of landing (7.93 tons, IR=5.28; 36.1% of the total landing in the CCA) and economic yield (29.7 thousand euros, IR=19.8, 37.4% of the total economic value in the Orange CCA). In addition, *A. foliacea* and *A. antennatus* accounted for 12.4% and 11.2% of the total economic value in the Orange CCA (median values of 9.87 thousand euros, IR=5.2 and 8.92 thousand euros, IR=10.4, respectively). In the Green CCA, *P. longirostris* showed the highest median production values account for 25.8% of the landing in the investigated CCA (median value 9.19; IR=5.93) and for 22% of the economic value in the CCA (median value of 34.47 thousand euros, IR=22.25). In addition, *A. antennatus* and *A. foliacea* showed high median revenue values equal to 25.3 thousand euros (IR=24.30, 16.3%) and 23.0 thousand euros (IR=19.26; 14.8%), respectively.

Landing and economic flows from CC areas towards fishing harbours

The analysis of landing flows by species towards the main fishing harbours showed differences in the pattern of production among the CCAs and the NIS (Figures 8, 9; Table S6). Considering landing flows in the NIS, most of the production was landed in the Apulian region, with the highest median total landing of 108.93 tons in Gallipoli (43.4% of the total production

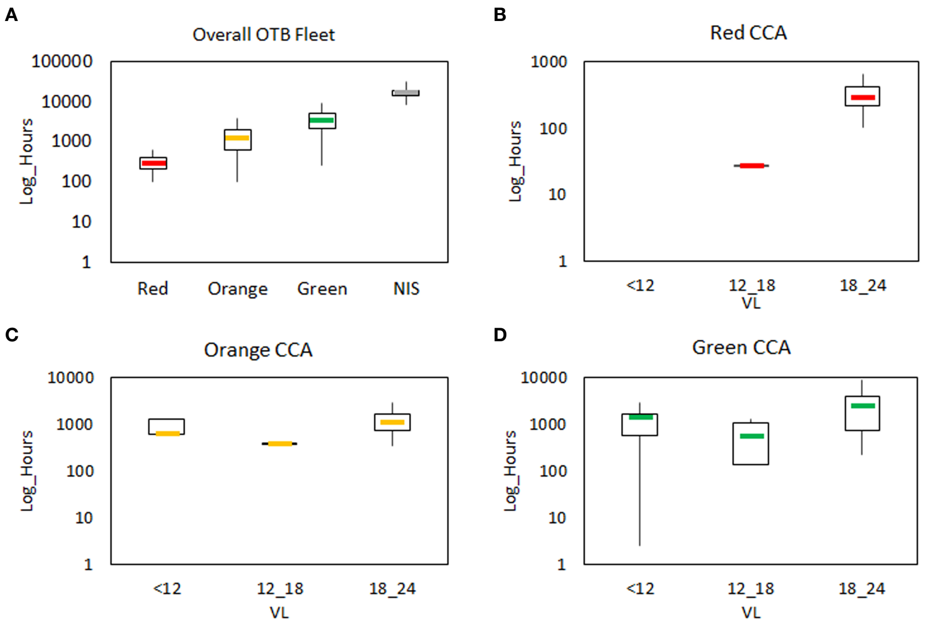

FIGURE 6
The total yearly fishing effort (hours in Log scale) of the OTB fleet estimated by SMART model. In **(A)** the hourly effort is reported for the overall OTB fleet in the CCAs and Northern Ionian Sea. The hourly effort by LOA segments is reported in **(B)** for the Red CCA, in **(C)** for the Orange CCA and in **(D)** for the Green CCA. LOA segments are split into vessels lower than 12 m (<12), vessels between 12-18 m (12_18) and those between 18-24 m (18_24). Boxplots report the median (midline), quartiles (box limits); minimum and maximum values (whiskers out of boxes).

of the NIS), followed by that of Taranto (median total value equal to 43.91 tons; 17.5%), and a very small fraction in Otranto (median total value equal to 6.29 tons; 2.5%) (Figure 9D). In the Calabria region, the landings flows were directed towards Corigliano (median total value equal to 65.82 tons; 26.2%) and Crotone (median total value equal to 26.18 tons; 10.4%). The main landed species in Gallipoli were *B. boops* (median value of 20.87 tons, IR=11.86; 8.3%), *M. merluccius* (median value of 16.22 tons, IR=9.17; 6.5%), *M. barbatus* (median value of 14.33 tons, IR=6.84; 5.7%) and *I. coindetii* and *T. mediterraneus*, each accounting for about 4-5% (Figure 8). A similar pattern was observed for the landing species composition in the remaining harbours with lower percentage values. Economic yields showed similar percentage values to the landing flows, with the highest total median value for the landing in Gallipoli (516.35 thousand euros, 41.6% of the total economic production from the NIS). Considering the species, *A. antennatus* showed the highest economic values in all harbours, excepted for Taranto, where *A. foliacea* showed the highest median value equal to 49.75 thousand euros (18.95). Other relevant species were *M. merluccius*, *N. norvegicus*, *M. barbatus* and *I. coidettii* in all harbours.

In the Red CCA, *P longirostris* showed the highest fraction in the Taranto landings (median value 4.28 tons; IR=3.39) accounted for 27.5% of the total landing flow from this CCA (Figure 8). This species together with *B. boops* represented all species landed in Taranto from the Red CCA. In Corigliano harbour, *T. mediterraneus* showed the highest fraction in the landings (median value 3.99 tons, IR= 6.04; 25.6%), followed by *P longirostris* (median value of 3.03 tons, IR=2.33; 19.5%) and *B. boops* (median value 1.63 tons, IR=1.35; 10.5%). Other species landed in Corigliano were *M. merluccius*, *Lophius* spp. and *M. barbatus*.

Concerning the economic yield, *P. longirostris* was also the most important species in both harbours, showing median values of 16.06 thousand euros (IR=12.73; 38.5% of the total economic value) in Taranto and 11.36 thousand euros (IR=8.72; 27%) in Corigliano, respectively.

Overall, the median total landing from the Red CCA was higher within Corigliano harbour (66.9%) than Taranto harbour (33.1%) (Figure 9A). Similarly, the economic production accounted for 60.5% (25.23 thousand euros) in the Corigliano harbour and 39.5% (16.50 thousand euros) in Taranto, respectively.

In the Orange area, the most important species in the landing flows towards Corigliano harbour were *T. mediterraneus* (median value of 4.65 tons; IR=6.22; 19.8% of the total production of the area), *P. longirostris* (median value of 3.13 tons; IR=2.53; 13.3%) and *B. boops* (median value of 1.84 tons; IR=1.72; 7.8%). In addition, small amounts of *A. foliacea* (median value of 0.71 tons; IR=0.37; 3.0%) and *A. antennatus* (median value of 0.26 tons; IR=0.35; 1.1%) were detected in the

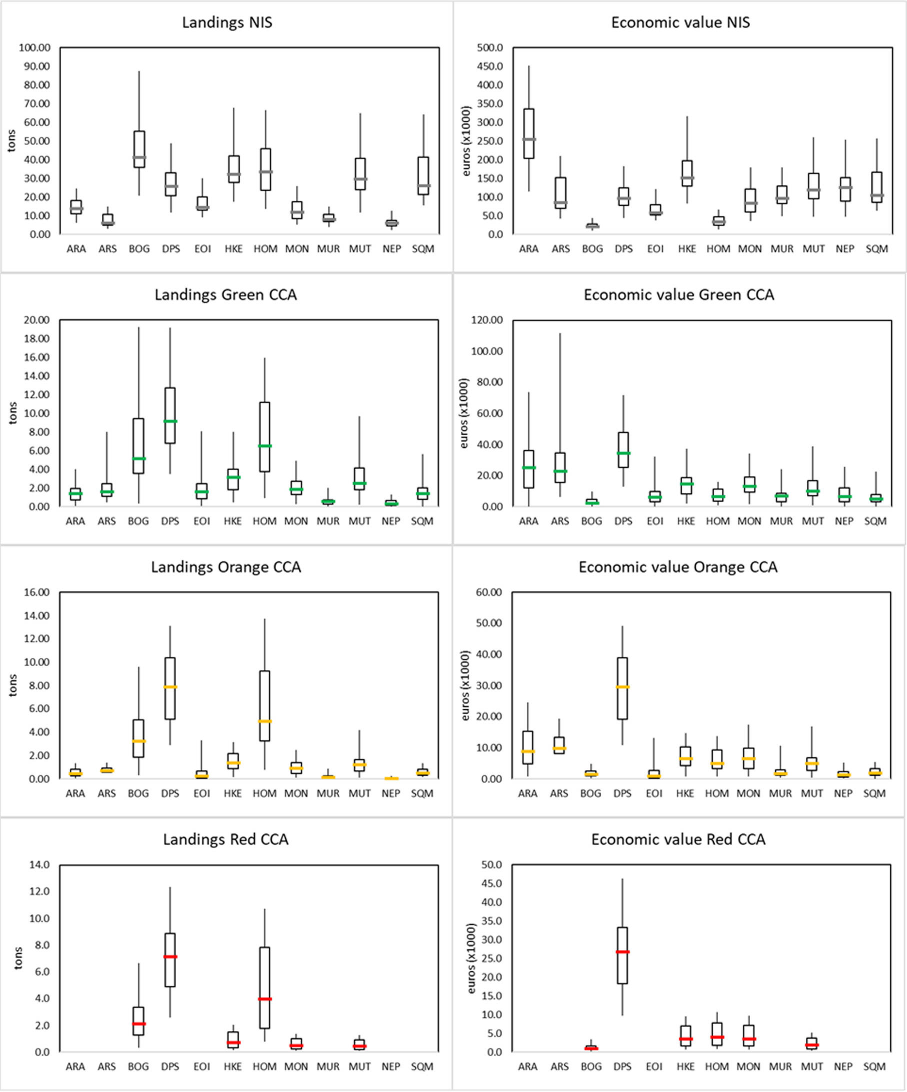

FIGURE 7
Landings (tons) and economic value (mln euro) by commercial species estimated for each CCA and the Northern Ionian Sea (NIS). Code species are reported in the Table 1. Boxplots report the median (midline), quartiles (box limits); minimum and maximum values (whiskers out of boxes).

landings (Figure 8). In Taranto harbour, the main landed species were always *P. longirostris* (median value of 4.70 tons, IR=3.44; 20.1%) and *B. boops* (median value of 2.45 tons, IR=2.52; 10.5%). Other relevant species were *M. barbatus*, *M. merluccius* and *I. coindetii*, and *A. antennatus* was detected in the landing. Concerning the economic revenue, *P. longirostris, A. foliacea* and *A. antennatus* were the main important species in the production flows of both harbours. The former species accounted for 20.5% (17.63 thousand euros) in Taranto and for 13.7% (11.74 thousand euros) in Corigliano harbour, respectively, as well as *A. antennatus* being equal to 8.4% (7.20 thousand euros) for the Taranto production and 5.6% (4.84 thousand euros) in that of the Corigliano. Finally, *A foliacea* represented 11.5% (9.87 thousand euros) in Corigliano. Overall, the median total landing from the Orange CCA was split into 13.66 tons in Corigliano (58.2% of the total landing from the CCA) and 9.80 tons in Taranto (41.8%), respectively (Figure 9B). Median total revenues were slightly higher in the Corigliano (56.7%) than in Taranto (43.3%).

The landings flow from the Green area was mainly directed to the Corigliano harbour (49.5% of the Green CCA total landing) and to Taranto (43.7%), while the lowest fraction was landed in Gallipoli (6.8%) (Figure 9C). In all harbours, *P. longirostris* and *B. boops* were the main landed species (Figure 8). In addition, *T. mediterraneus* showed a high median value exclusively in Corigliano (4.65 tons, IR=5.78;

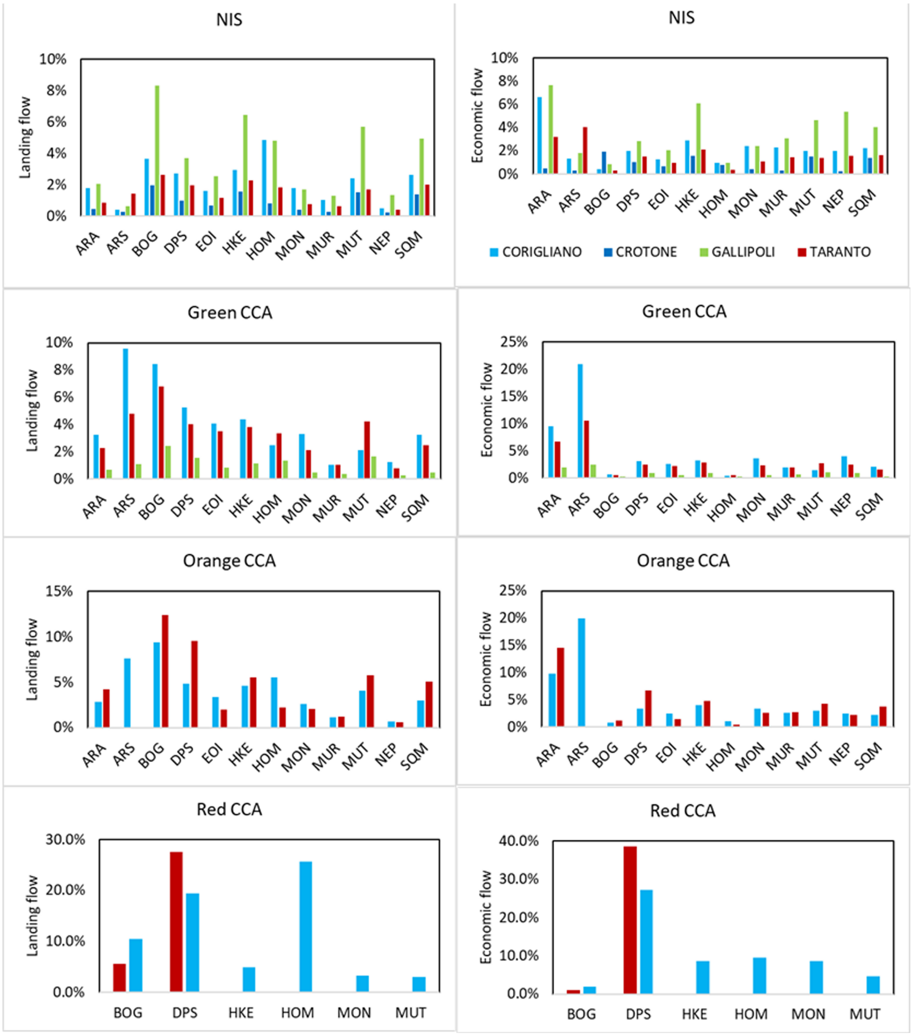

FIGURE 8
Landing flows by species (expressed in %) from the CCAs and NIS towards the main fishing harbours. Species codes are reported in Table 1.

11.3% of the total landing production). Other relevant species were *M. merluccius*, *M. barbatus* and *A. foliacea* with values which ranged between 3-4% in both Corigliano and Taranto harbours. Considering the economic yield, as observed in the Orange CCA, *P. longirostris*, *A. foliacea* and *A. antennatus* were the most important species in all harbours, representing overall about 54% of the total economic revenue (24.2% in Corigliano harbour, 25.8% in Taranto and 3.9% in Gallipoli). Other relevant species were *M. merluccius* and *Lophius* spp. in the production Corigliano harbour, with values of 4.0% and 4.5%, respectively. Overall, the median total economic yield from the Green CCA was slightly higher in Corigliano harbour (92.65 thousand euros, 47.7%), than in Taranto (86.60 thousand euros 44.6%), while Gallipoli accounted for 7.8% (15.10 thousand euros).

Discussion

The analysis developed in this study through a multi-species bio-economic modelling approach is the first attempt to quantify fishing exploitation patterns within eligible CCAs identified with the explicit purpose of protecting cetacean species in the Northern Ionian Sea. The SMART approach was used to obtain a quantitative reconstruction of fishing activities in the study area and to provide a baseline for the planning of spatial conservation measures, as well as for sustainable management of fishery. Indeed, the output obtained could be considered in the application of measures required for a sustainable management of the trawl fishery, as required by the Multi-annual Plan for the Fisheries exploiting demersal stocks (Sánchez Lizaso et al., 2020).

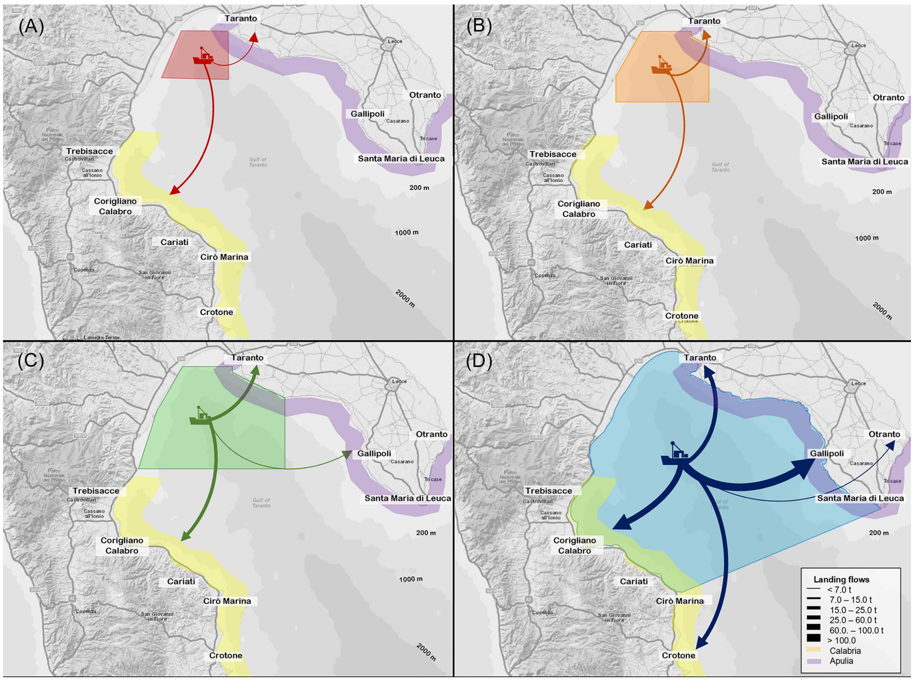

FIGURE 9
Landings flows (tons) from **(A)** the Red CCA, **(B)** Orange CCA, **(C)** Green CCA and **(D)** the Northern Ionian Sea towards the main fishing harbours aggregated in the Calabrian (yellow) and Apulian region (violet). Thickness of arrows is proportional to the magnitude of flows.

In fact, effective management aimed at reducing discards and mortality of both target and non-target species require integrated strategies based on several kinds of regulation (Colloca et al., 2013). Although the main Annual Multiplan Fishery regulations are based on effort reduction in terms of fishing days, other restrictions could be planned to adopt spatial conservation tools able to synthetize multiple targets in the conservation of marine biodiversity and ecosystems (Pérez-Ruzafa et al., 2017; Russo et al., 2019).

The first main relevant output is the absence of fishing activities in the smallest CCA (blue) where the head of the submarine canyon does not offer accessible grounds. Thus, the area can be an interesting space for the planning of spatial conservation actions, without conflicts with the local fishery. In addition, the proximity of the Blue CCA to the coast should be evaluated from the socio-economic perspective, stimulating the involvement of different stakeholders for the planning of regulatory measures (Heck et al., 2011). Indeed, the growing interest in citizen science activities can find opportunities to develop ocean literacy activities in suitable locations such as marine protected areas and to promote Sustainable Development Goals in coastal communities (Ferreira et al., 2021). However, a critical issue could be represented by the small size of this CCA, which partially covers the head of canyons and could not provide and exhaustive protection of some cetacean species, such as *Z. cavirostris*, which is distributed in the offshore area of the NIS (Carlucci et al., 2020b). However, the level of conservation represented by the Blue CCA could be very beneficial for the protection of cetaceans and easily implemented in the area given the involvement of the main stakeholders. From an operational perspective, an increase in knowledge regarding other human pressures impacting in the CCA, such as naval traffic, should be acquired. Indeed, this pressure is an important source of impact for cetaceans, such as the accidental strikes (Pennino et al., 2017), which could be regulated through the adoption of specific spatial measures on the routes and speeds of the naval traffic (Guzman et al., 2020).

Considering the eligible CCAs, the results highlighted a growing fishing exploitation pattern moving from the Red to the Green CCA. This increase in fishing effort and production is expected, because the spatial dimensions of CCAs grow from red to green area, with the consequence of including additional fishing grounds. Furthermore, small changes in the species composition of the landing in each CCA were observed, showing the absence of the exploitation of deep commercial resources in the red CCA. Indeed, its landings composition is exclusively characterized by the shallowest species, such as *P. longirostris*, *T. mediterraneus* and *B. boops*. Though not negligible, the last two species are characterized by a low economic yield, and a high discard rate from trawl catches (Maiorano et al., 2019), and at the same time, they are prey of *T. truncatus* (Bearzi et al., 2010; Ricci et al., 2020a). Moreover, the fishing pressure in this CCA showed the lowest level of spatial coverage and hourly fishing effort, in line with the knowledge on the fishing effort displacement in the Northern Ionian Sea. Here, some fishing grounds are in the south-western zone between Taranto and the Calabrian area and in the south-eastern zone off Gallipoli (Russo et al., 2017). The western border of the CCA is located at the end of the former fishing ground, where the trawl vessels stop their hauls at the head of the canyon slope. This condition forces the trawl vessels towards the shelf platform up to 200 m in depth. Thus, potential fishing interactions in the Red CCA could interest mainly the common bottlenose dolphin, since the species is distributed in shallower areas (Carlucci et al., 2018a; Carlucci et al., 2016a) showing a trophic overlap with commercial species caught by several fishing gears (Ricci et al., 2020a; Carlucci et al., 2021a). A further noteworthy point is that the Red CCA, which defines a conservation level aimed at protecting the persistent critical habitats of the striped dolphin (Carlucci et al., 2018d), is partially overlapping with the blue area, covering the totally canyon head. Thus, the establishment of this CCA does not seem to particularly interfere with local fishing activities and the potential economic losses due to the banning of the area are very scarce. At same time, this CCA represents an efficient conservation level for the life cycle of the striped dolphin, as well as for deep-sea habitats requiring protection actions (Manea et al., 2020). Considering the importance of deep-sea habitats, future studies should investigate the occurrence of other impacts on the area, providing specific regulations of the human activities.

The Green and Orange CCAs differ from the Red one by a higher intensity of fishing activities and in the landing species composition, especially the occurrence of deep commercial species in the catches, which are target species of the Northern Ionian Sea (Maiorano et al., 2022). However, *P. longirostris* is always the main species in terms of landing amount and sale in both CCAs, and only in the Green area do deep-water shrimps seem to achieve an economic yield similar to that of the deep-water rose shrimp. This condition could be affected by the geographic traits and the position of this CCA, which is the only area that partially overlaps with south-eastern grounds, where the main exploited species are *A. foliacea* and *A. antennatus* (Russo et al., 2017; Maiorano et al., 2022). Observations of landings flows to harbours also support this explanation, which in the Green area also show the presence of the Gallipoli fleet, which is known for its exploitation of deep water shrimp on the south-eastern slope the Gulf (D'Onghia et al., 2005; Maiorano et al., 2022). Concerning fishery performances in the Green and Orange CCAs, hourly fishing effort in the former CCA was more than twice that of the latter (3342 against 1226 fishing hours, respectively). However, the landing and economic yield in proportion to the employed effort was lower in the Green CCA than the Orange one. This observation, like that observed at the global scale by Sumaila et al. (2007), should be considered within an overall costs and benefits assessment addressed to planning effective spatial measures to conserve the cetaceans and biodiversity. Indeed, in a scenario of low losses for the fishing industry, other incomes could be acquired by other ecosystem services, such as those performed by small cetaceans (Kiszka et al., 2022), compensating and improving the ecological conditions and the sustainability of the socio-economic systems linked to the marine resources (Hammershalg et al., 2019).

The need to establish conservation areas for cetaceans is a fundamental goal of several international protocols, such as Important Marine Mammal Areas (IMMAs, Notarbartolo di Sciara et al., 2016; Hoyt and Notarbartolo di Sciara, 2021) or the Cetaceans Critical Habitats (CCHs, Notarbartolo di Sciara, 2002). These protocols required several selection criteria of the eligible areas. For instance, IMMA selection criteria are focused on the distribution and abundance of cetacean populations, the key life cycle activities occurring in the considered areas, as well as the assessment of vulnerability of the resident cetacean populations (Tetley et al., 2022). Similarly, CCHs require information on the fishery interactions with the cetaceans to identify suitable habitats for these organisms (ACCOBAMS-ECS-WK Threats, 2017; IUCN Marine Mammal Protected Areas Task Force, 2018). In addition, other international initiatives in the Ionian basin, such as the EU Strategy for the Adriatic-Ionian Region (EUSAIR, 2014), includes among its objectives the implementation of MPAs, with particular attention to the identification of areas to create new MPAs or areas requiring special measures for the conservation of biodiversity, as well as the proposal of complementary measures for sustainable fishing in the conservation areas of the Adriatic-Ionian ecoregion (EUSAIR, 2021). These proposals as a whole should be driven by the application of quantitative methodologies useful to provide both information on the ecological consequences of the establishment of CCAs and the socio-economic effects linked to conservation areas. The quantification of these aspects is important because conservation plans often conflict with fishing activities and other uses of the sea (Grip and Blomqvist, 2020). This is particularly true in such complex exploited systems as the Gulf of Taranto, with multiple human use of the maritime space, relevant sensitive habitats, and high biodiversity at all ecosystem levels (Carlucci et al., 2021b). No less relevant, urgent planning of spatial conservation measures is required in the Adriatic-Ionian region because it is one of the least-protected areas in the Mediterranean Sea (EUSAIR, 2021). Despite all these factors, the analysis shows no or very negligible negative effects on trawling due to potential spatial restrictions on the establishment of CCAs, especially within the Blue and Red area delimitations. At the same time, the ecological benefits for cetofauna diversity provided by a more extensive protection level, such as that of the Orange and Green CCAs, could be accompanied by effects on demersal stock repopulation, reduction of fishing discards, as well as increased ecotourism activities with positive spill-over effects on other economic activities in a more sustainable use of maritime space. Therefore, the planning of spatial conservation measures for cetaceans could find points of agreement with a redefinition of the fishing areas in the Northern Ionian Sea without generating socio-economic conflicts.

The quantification of both fishing pressures and production in terms of economic value from CCAs is an important strength. Indeed, the identification of the level of fishing pressure in space and time, the amounts of landings and their species composition, could provide insight into the intensity of fishing disturbances to the cetaceans, due to competition for food resources (Kaschner and Pauly, 2005). Furthermore, such knowledge may provide data required in the processes of assessing the conservation status of cetaceans and their habitats (ACCOBAMS ECS-WK Threats, 2017; Breen et al., 2017), as well as in the use of indicators that classify the environmental state of the marine ecosystem through cetacean biodiversity (Azzellino et al., 2014). On the other hand, the quantification of economic value represents a way of assessing the ecosystem service represented by the fishing resources production (Holmlund and Hammer, 1999; Pope et al., 2016). Such information could make it possible to manage and mitigate possible conflicts between the need for biodiversity conservation and fishing exploitation, especially in the case of fishing restrictions. In this regard, the results obtained from the SMART model allow for a better understanding of the dynamics of trawling activity in areas important for cetaceans living in the NIS. Not less important, future investigations should be addressed to quantify collateral impacts on cetaceans and the ecosystem derived from trawling activities, such as the underwater noise pollution produced by trawling vessels (Daly and White, 2021) and the spatial redistribution of the trawling fishing effort in response to the establishment of spatial closures (Powers and Abeare, 2009). The former impact represents a critical disturbance for cetaceans, which should be assessed in the framework of the of Marine Strategy Framework Directive (Descriptor 11) (EU, 2017) while the latter could lead to a potential increase in the fishing pressure around the banned areas and on other grounds (Elhani et al., 2018). However, it should be noted that the Blue and Red CCAs investigated in this study should not influence the redistribution of fishing effort, since trawling activity is almost entirely absent. Moreover, potential effects of spatial conservation measures adopted for cetaceans on the population dynamics of demersal resources (e.g. spill-over effects from CCAs) represent an important aspects to investigate through fisheries management scenarios.

The assessment of the value of conservation areas could be considered as part of the broader framework of assessing the ecosystem services provided by specific maritime areas with high biodiversity. To provide spatially integrated information on the fishing effort and the economic value could be a key point in planning based on the principles of EBM (Essington et al., 2018).

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.

Ethics statement

The animal study was reviewed and approved by the Italian Ministry of Ecological Transition.

Author contributions

RC, PR, TR: Conceptualization. RC, PR, TR: methodology. TR, AS, DC, PR: formal analysis. CF, RC, PR, GC: data investigation and sampling design. PR, DC, MI, GC: writing—original draft preparation. RC, PR, TR DC, MI, AS, CF, GC: writing—review and editing. RC, PR: supervision. All authors contributed to the article and approved the submitted version.

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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1005649/full#supplementary-material

References

ACCOBAMS (2020). "Assessment of the conservation status of cetaceans in the ACCOBAMS area," in *Thirteenth meeting of the ACCOBAMS scientific committee, Monaco, ACCOBAMS-SC13/2020/Doc 10Rev1*, 9. (Monaco:ACCOBAMS).

ACCOBAMS-ECS-WK Threats (2017)Inputs to the ACCOBAMS ongoing effort to map human threats on cetaceans in the Mediterranean and black seas ACCOBAMS-ECS-WK Threats/2017/Report. In: . Available at: https://www.accobams.org/wp-content/uploads/2016/06/Report_workshop_Threats_31_ECS.pdf (Accessed June 3, 2020).

Azzellino, A., Fossi, M. C., Gaspari, S., Lanfredi, C., Lauriano, G., Marsili, L., et al. (2014). An index based on the biodiversity of cetacean species to assess the environmental status of marine ecosystems. *Mar. Environ. Res.* 100, 94–111. doi: 10.1016/j.marenvres.2014.06.003

Azzolin, M., Arcangeli, A., Cipriano, G., Crosti, R., Maglietta, R., Pietroluongo, G., et al. (2020). Spatial distribution modelling of striped dolphin (*Stenella coeruleoalba*) at different geographical scales within the EU Adriatic and Ionian Sea region, central-eastern Mediterranean Sea. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 30, 1194–1207. doi: 10.1002/aqc.3314

Bakun, A., and Agostini, V. N. (2001). Seasonal patterns of wind-induced upwelling/downwelling in the Mediterranean Sea. *Sci. Mar.* 65, 243–257. doi: 10.3989/scimar.2001.65n3243

Bastardie, F., Rufener, M., Nielsen, J., Maina, I., Kavadas, S., Vassilopoulou, C., et al. (2018). "Modelling spatial interactions among fish communities, fishers and other marine activities: comparing five European case-studies," in *Conference of the international institute of fisheries economics and trade (IIFET)*(Seattle, US: International Institute of Fisheries Economics & Trade)2018. Session on Marine Spatial Planning and Multiple Use Management.

Bearzi, G. (2002). "Interactions between cetaceans and fisheries in the Mediterranean Sea," in *"Cetaceans of Mediterranean and black seas: State of knowledge and conservation Strategies2 in a report to the ACCOBAMS secretariat*, vol. 20 . Ed. G. Notarbartolo di Sciara (Monaco: ACCOBAMS).

Bearzi, G., Agazzi, S., Gonzalvo, J., Bonizzoni, S., Costa, M., and Petroselli, A. (2010). Biomass removal by dolphins and fisheries in a Mediterranean Sea coastal area: do dolphins have an ecological impact on fisheries? *Aquat. Conserv. Mar. Freshw. Ecosyst.* 20, 549–559. doi: 10.1002/aqc.1123

Bellomo, S., Santacesaria, F. C., Fanizza, C., Cipriano, G., Renò, V., Carlucci, R., et al. (2019). "Photo-identification of physeter macrocephalus in the gulf of taranto (Northern Ionian Sea, central-eastern Mediterranean Sea)," in *Proceedings IEEE metrology for the Sea 2019 IMEKO TC-19 international workshop on metrology for the Sea*(Genoa, Italy:IMEKO), 2019 33–37.

Bo, M., Bavestrello, G., and Canese, S. (2011). Coral assemblage off the calabrian coast (South Italy) with new observations on living colonies of *Antipathes dichotoma*. *Ital J. Zool.* 78, 231–242. doi: 10.1080/11250001003652619

Breen, P., Brown, S., Reid, D., and Rogan, E. (2017). Where is the risk? integrating a spatial distribution model and a risk assessment to identify areas of cetacean interaction with fisheries in the northeast Atlantic. *Ocean Coast. Manage.* 136, 148–155. doi: 10.1016/j.ocecoaman.2016.12.001

Capezzuto, F., Carlucci, R., Maiorano, P., Sion, L., Battista, D., Giove, A., et al. (2010). The bathyal benthopelagic fauna in the north-western Ionian Sea: structure, patterns and interactions". *Chem. Ecol.* 26, 199–217. doi: 10.1080/0275754100-3639188

Carlucci, R., Akkaya Baş, A., Liebig, P., Renò, V., Santacesaria, F. C., Bellomo, S., et al. (2020a). Residency patterns and site fidelity of *Grampus griseus* (Cuvier 1812) in the gulf of taranto (Northern Ionian Sea, central eastern Mediterranean Sea). *Mamm. Res.* 65 (3), 445–455. doi: 10.1007/s13364-02000485-z

Carlucci, R., Akkaya Baş, A., Maglietta, R., Renò, V., Fanizza, C., Rizzo, A., et al. (2018b). "Site fidelity, residency and habitat use of the risso's dolphin grampus griseus in the gulf of taranto (Northern Ionian Sea, central-eastern Mediterranean Sea) by photo-identification," in *Proceedings IEEE metrology for the Sea*, vol. 2018d. (Bari, Italy:IEEE), 173–177.

Carlucci, R., Bandelj, V., Ricci, P., Capezzuto, F., Sion, L., Maiorano, P., et al. (2018c). Exploring spatio-temporal changes of the demersal and benthopelagic assemblages of the north-western Ionian Sea (Central Mediterranean Sea). *Mar. Ecol. Progr. Ser.* 598, 1–19. doi: 10.3354/meps12613

Carlucci, R., Capezzuto, F., Cipriano, G., D'Onghia, G., Fanizza, C., Libralato, S., et al. (2021a). Assessment of cetacean–fishery interactions in the marine food web of the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea). *Rev. Fish Biol. Fish.* 31 (1), 135–156. doi: 10.1007/s11160-020-09623-x

Carlucci, R., Cipriano, G., Fattoruso, G., Oliveri, M. G., Ricci, P., and Squillante, M. (2021c). "Parsimonious AHP applied to the conservation of cetaceans: a case study in the gulf of taranto," in *Proceedings IEEE international workshop on metrology for the Sea: Learning to measure Sea health parameters*, (MetroSea: IEEE) 2021., 1–5.

Carlucci, R., Cipriano, G., Paoli, C., Ricci, P., Fanizza, C., Capezzuto, F., et al. (2018a). Random forest population modelling of striped and common-bottlenose dolphins in the gulf of taranto (Northern Ionian Sea, central-eastern Mediterranean Sea). *Estuar. Coast. Shelf Sci.* 204, 177–192. doi: 10.1016/j.ecss.2018.02.034

Carlucci, R., Cipriano, G., Santacesaria, F. C., Ricci, P., Maglietta, R., Petrella, A., et al. (2020b). Exploring data from an individual stranding of a cuvier's beaked whale in the gulf of taranto (Editoriale Scientifica: Northern Ionian Sea, central-eastern Mediterranean Sea). *J. Exp. Mar. Biol. Ecol.* 553, 151473. doi: 10.1016/j.jembe.2020.151473

Carlucci, R., Fanizza, C., Cipriano, G., Paoli, C., Russo, T., and Vassallo, P. (2016a). Modeling the spatial distribution of the striped dolphin (*Stenella coeruleoalba*) and common bottlenose dolphin (*Tursiops truncatus*) in the gulf of taranto (Editoriale Scientifica: Northern Ionian Sea, central-eastern Mediterranean sea)". *Ecol. Indic.* 69, 707–721. doi: 10.1016/j.ecolind.2016.05.035

Carlucci, R., Maglietta, L., Buscaino, G., Cipriano, G., Milella, A., Pollazzon, V., et al. (2017). "Review on research studies and monitoring system applied to cetaceans in the gulf of taranto (northern Ionian Sea, central-eastern Mediterranean Sea)," in *Proceedings 14th IEEE international conference on advanced video and signal based surveillance (AVSS)*(Lecce:IEEE). doi: 10.1109/AVSS.2017.8078473

Carlucci, R., Maiorano, P., Sion, L., D'Onghia, G., and Tursi, A. (2016b). ""The sustainability of fishing in the southern Adriatic and northern Ionian seas"," in *Governance of the Adriatic and Ionian marine space*. Ed. A. Caligiuri (Editoriale Scientifica), 149–159.

Carlucci, R., Manea, E., Ricci, P., Cipriano, G., Fanizza, C., Maglietta, R., et al. (2021b). Managing multiple pressures for cetaceans' conservation with an ecosystem-based marine spatial planning approach. *J. Environ. Manage.* 287, 112240. doi: 10.1016/j.jenvman.2021.112240

Carlucci, R., Ricci, P., Cipriano, G., and Fanizza, C. (2018d). Abundance, activity and critical habitat of the striped dolphin *Stenella coeruleoalba* in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea). *Aquat. Conserv. Mar. Freshw. Ecosyst.* 28, 324–336. doi: 10.1002/aqc.2867

Castellan, G., Angeletti, L., Taviani, M., and Montagna, P. (2019). The yellow coral *Dendrophyllia cornigera* in a warming ocean. *Front. Mar. Sci.* 6. doi: 10.3389/fmars.2019.00692

Chimienti, G., Bo, M., Taviani, M., and Mastrototaro, F. (2019). "Occurrence and biogeography of Mediterranean cold-water corals," in *Mediterranean Cold-water corals: Past, present and future* (Cham, CH: Springer International Publishing), 213–243.

Ciccarese, S., Carlucci, R., Ciani, E., Corcella, E., Cosentino, A., Fanizza, C., et al. (2019). Cytochrome b marker reveals an independent lineage of *Stenella coeruleoalba* in the gulf of taranto. *PloS One* 14 (3), e0213826. doi: 10.1371/journal.pone.0213826

Cipriano, G., Carlucci, R., Bellomo, S., Santacesaria, F. C., Fanizza, C., Ricci, P., et al. (2022). Behavioral pattern of risso's dolphin (*Grampus griseus*) in the gulf of taranto (Northern Ionian Sea, central-Eastern Mediterranean Sea). *J. Mar. Sci. Eng.* 10 (2), 175. doi: 10.3390/jmse10020175

Claudet, J. (2011). *Marine protected areas - a multidisciplinary approach* (Cambridge: Cambridge University Press).

Colloca, F., Cardinale, M., Maynou, F., Giannoulaki, M., Scarcella, G., Jenko, K., et al. (2013). Rebuilding Mediterranean fisheries: a new paradigm for ecological sustainability. *Fish Fish.* 14, 89–109. doi: 10.1111/j.1467-2979.2011.00453.x

Daly, E., and White, M. (2021). Bottom trawling noise: Are fishing vessels polluting to deeper acoustic habitats? *Mar. pollut. Bull.* 162, 111877. doi: 10.1016/j.marpolbul.2020.111877

D'Andrea, L., Parisi, A., Fiorentino, F., Garofalo, G., Gristina, M., Russo, T., et al. (2020). smartR: a r package for spatial modelling of fisheries and simulation of effort management. *Environ. Monit. Assess.* 192 (12), 1–17. doi: 10.1111/2041-210X.13394

Dimatteo, S., Siniscalchi, M., Esposito, L., Prunella, V., Bondanese, P., Bearzi, G., et al. (2011). Encounters with pelagic and continental slope cetacean species near the northern shore of the gulf of taranto, Italy. *Ital. J. Zool.* 78 (1), 130–132. doi: 10.1080/11250003.2010.532161

D'Onghia, G., Calculli, E., Capezzuto, F., Carlucci, R., Carluccio, A., and Maiorano, P. (2016). New records of cold-water coral sites and fish fauna characterization of a potential network existing in the Mediterranean Sea. *Mar. Ecol.* 37, 1398–1422. doi: 10.1111/maec.12356

D'Onghia, G., Capezzuto, F., Mytilineou, C., Maiorano, P., Kapiris, K., Carlucci, R., et al. (2005). Comparison of the population structure and dynamics of *Aristeus antennatus* (Riss between exploited and unexploited areas in the Mediterranean Sea. *Fish. Res.* 76 (1), 22–38. doi: 10.1016/j.fishres.2005.05.007

EC (2010). *Fleet register on the net*. Available at: http://ec.europa.eu/fisheries/fleet/index.cfm/.

EC. (2011). Commission Implementing Regulation (EU) No. 404/2011 of 8 April 2011 laying down detailed rules for the implementation of Council Regulation (EC) No 1224/2009 establishing a Community control system for ensuring compliance with the rules of the Common Fisheries Policy. *Off. J. Eur. Union* L112, 1–30. Available at: http://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32011R0404&from=EN

Ehler, C., and Douvere, F. (2009). *Marine spatial planning: a step-by-step approach toward ecosystem-based management* (Paris, UNESCO: Intergovernmental Oceanographic Commission and Man and the Biosphere Programme).

Elahi, R., Ferretti, F., Bastari, A., Cerrano, C., Colloca, F., Kowalik, J., et al (2018). Leveraging vessel traffic data and a temporary fishing closure to informmarine management *Front. Ecol. Environ.* 16(8):1–7 doi: 10.1002/fee.19

Essington, T. E., Sanchirico, J. N., and Baskett, M. L. (2018). Economic value of ecological information in ecosystem-based natural resource management depends on exploitation history. *Proc. Natl. Acad. Sci. U.S.A.* 115, 1658–1663. doi: 10.1073/pnas.1716858115

EU (2011). *Impact assessment of discard reducing policies*. Available at: https://ec.europa.eu/fisheries/documentation/studies/discards_en/.

EU (2017). Commission decision (EU) 2017/848 of 17 may 2017 laying down criteria and methodological standards on good environmental status of marine waters and specifications and standardised methods for monitoring and assessment, and repealing decision 2010/477/EU. *Official Journal of the European Union L.* 125 (43), 32

EUSAIR (2014). *Communication from the commission to the European parliament, the council, the European economic and social committee and the committee of the regions* (Brussels:European Commission). 17.6.2014 SWD, 190 final.

EUSAIR (2021). *Analysis of marine (water) protected areas in EUSAIR and proposals for corrective measures*. (Ljubljana, Slovenia:Government Office of the Republic of Slovenia for Development and European Cohesion Policy). 77.

Fanizza, C., Dimatteo, S., Pollazzon, V., Prunella, V., and Carlucci, R. (2014). An update of cetaceans occurrence in the gulf of taranto (Western-central Mediterranean Sea). *Biol. Mar. Mediterr.* 21 (1), 373–374.

Ferreira, J. C., Vasconcelos, L., Monteiro, R., Silva, F. Z., Duarte, C. M., and Ferreira, F. (2021). Ocean literacy to promote sustainable development goals and agenda 2030 in coastal communities. *Educ. Sci.* 11, 62. doi: 10.3390/educsci11020062

Foley, M. M., Halpern, B. S., Micheli, F., Armsby, M. H., Caldwell, M. R., Crain, C. M., et al. (2010). Guiding ecological principles for marine spatial planning. *Mar. Pol.* 34 (5), 955–966. doi: 10.1016/j.marpol.2010.02.001

Game, E. T., Grantham, H. S., Hobday, A. J., Pressey, R. L., Lombard, A. T., Beckley, L. E., et al. (2010). Pelagic MPAs: the devil you know. *Trends Ecol. Evol.* 25, 63–64. doi: 10.1016/j.tree.2009.09.002

Gerritsen, H., and Lordan, C. (2011). Integrating vessel monitoring systems (VMS) data with daily catch data from logbooks to explore the spatial distribution of catch and effort at high resolution. *ICES J. Mar. Sci.* 68, 245–252. doi: 10.1093/icesjms/fsq137

Grip, K., and Blomqvist, S. (2020). Marine nature conservation and conflicts with fisheries. *Ambio* 49, 1328–1340. doi: 10.1007/s13280-019-01279-7

Guidetti, P., Notarbartolo-Di-Sciara, G., and Agardy, T. (2013). Integrating pelagic and coastal MPAs into large-scale ecosystem-wide management. *Aquat. Conserv: Mar. Freshw. Ecosyst.* 23, 179–182. doi: 10.1002/aqc.2314

Guzman, H. M., Hinojosa, N., and Kaiser, S. (2020). Ship's compliance with a traffic separation scheme and speed limit in the gulf of Panama and implications for the risk to humpback whales. *Mar. Policy* 120, 104113. doi: 10.1016/j.marpol.2020.104113

Hammer, Ø., Harper, D. A. T., and Ryan, P. D. (2001). PAST: Paleontological statistics software package for education and data analysis. *Palaeontol. Electron.* 4, 4–9.

Hammerschlag, N., Schmitz, O. J., Flecker, A. S., Lafferty, K. D., Sih, A., Atwood, T. B., et al. (2019). Ecosystem function and services of aquatic predators in the anthropocene. *Trends Ecol. Evol.* 34 (4), 369–383. doi: 10.1016/j.tree.2019.01.005

Heck, N., Dearden, P., and McDonald, A. (2011). Stakeholders' expectations towards a proposed marine protected area: a multi-criteria analysis of MPA performance criteria. *Ocean Coast. Manage.* 54 (9), 687–695. doi: 10.1016/j.ocecoaman.2011.07.003

Holmlund, C. M., and Hammer, M. (1999). Ecosystem services generated by fish populations. *Ecol. Econ.* 29, 253–268. doi: 10.1016/S0921-8009(99)00015-4

Hooker, S. K., Cañadas, A., Hyrenbach, K. D., Corrigan, C., Polovina, J. J., and Reeves, R. R. (2011). Making protected area networks effective for marine top predators. *Endang. Species Res.* 13, 203–218. doi: 10.3354/esr00322

Hoyt, E., and Notarbartolo di Sciara, G. (2021). Important marine mammal areas: a spatial tool for marine mammal conservation. *Oryx* 55, 330. doi: 10.1017/S0030605321000272

IUCN Marine Mammal Protected Areas Task Force (2018). *Guidance on the use of selection criteria for the identification of important marine mammal areas (IMMAs). version.* 82.

Jusufovski, D., Saavedra, C., and Kuparinen, A. (2019). Competition between marine mammals and fisheries in contemporary harvested marine ecosystems. *Mar. Ecol. Prog. Ser.* 627, 207–232. doi: 10.3354/meps13068

Kaplan, D. M., Chassot, E., Gruss, A., and Fonteneau, A. (2010). Pelagic MPAs: the devil is in the details. *Trends Ecol. Evol.* 25, 62–63. doi: 10.1016/j.tree.2009.09.003

Kaschner, K., and Pauly, D. (2005). ""Competition between marine mammals and fisheries: Food for thought"," in *The state of the animals III, eds. D.J. Salem and A.N. Rowan* (Washington, DC: Humane Society Press), 95–117.

Kiszka, J. J., Woodstock, M. S., and Heithaus, M. R. (2022). Functional roles and ecological importance of small cetaceans in aquatic ecosystems. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.803173

Maglietta, R., Carlucci, R., Fanizza, C., and Dimauro, G. (2022). Machine learning and image processing methods for cetacean photo identification: a systematic review. *IEEE Access* 10, 80195–80207. doi: 10.1109/ACCESS.2022.3195218

Maglietta, R., Renò, V., Caccioppoli, R., Seller, E., Bellomo, S., Santacesaria, F. C., et al. (2020). Convolutional neural networks for risso's dolphins identification. *IEEE Access* 8, 80195–80206. doi: 10.1109/ACCESS.2020.2990427

Maglietta, R., Renò, V., Cipriano, G., Fanizza, C., Milella, A., Stella, E., et al. (2018). DolFin: an innovative digital platform for studying risso's dolphins from the northern Ionian Sea (Central-Eastern Mediterranean). *Sci. Rep.* 8, 17185. doi: 10.1038/s41598-018-35492-3

Maiorano, P., Capezzuto, F., Carluccio, A., Calculli, C., Cipriano, G., Carlucci, R., et al. (2022). Food from the depths of the Mediterranean: The role of habitats, changes in the Sea-bottom temperature and fishing pressure. *Foods* 11, 1420. doi: 10.3390/foods11101420

Maiorano, P., Sabatella, R. F., and Marzocchi, B. M. (2019). Annuario sullo stato delle risorse e sulle strutture produttive dei mari italiani. *Biol. Mar. Mediterranea* 22 (1 Suppl.), 358.

Maiorano, P., Sion, L., Carlucci, R., Capezzuto, F., Giove, A., Costantino, G., et al. (2010). The demersal faunal assemblage of the north-Western Ionian Sea (Central mediterranean): present knowledge and perspectives. *Chem. Ecol.* 26 (1), 219–240. doi: 10.1080/02757541003693987

Manea, E., Bianchelli, S., Fanelli, E., Danovaro, R., and Gissi, E. (2020). Towards an ecosystem-based marine spatial planning in the deep Mediterranean Sea. *Sci. Total Environ.* 715, 1–15. doi: 10.1016/j.scitotenv.2020.136884

Mazzoldi, C., Bearzi, G., Brito, C., Carvalho, I., Desiderà, E., Endrizzi, L., et al. (2019). From sea monsters to charismatic megafauna: changes in perception and use of large marine animals. *PloS One* 14 (12), e0226810. doi: 10.1371/journal.pone.0226810

McDonald, J. H. (2014). *Handbook of biological statistics. 3rd edition* (Baltimore, Maryland, U.S.A: Sparky House Publishing).

Notarbartolo di Sciara, G. (2002). *Cetaceans of the Mediterranean and black seas: State of knowledge and conservation strategies. a report to the ACCOBAMS secretariat* (Monaco: ACCOBAMS) Vol. Section 1, 5.

Notarbartolo di Sciara, G., Hoyt, E., Reeves, R., Ardron, J., Marsh, H., Vongraven, D., et al. (2016). Place-based approaches to marine mammal conservation. *Aquat. Conserv.* 26, 85–100. doi: 10.1002/aqc.2642

Pace, D. S., Tizzi, R., and Mussi, B. (2015). Cetaceans value and conservation in the Mediterranean Sea. *J. Biodivers. Endanger. Species* S1, S1004. doi: 10.4172/2332-2543.S1.004

Papale, E., Fanizza, C., Buscaino, G., Maria, C., Cipriano, G., Crugliano, R., et al. (2020). The social role of vocal complexity in striped dolphins. *Front. Mar. Sci.* 7. doi: 10.3389/fmars.2020.584301

Pennino, M. G., Arcangeli, A., Prado Fonseca, V., Campana, I., Pierce, G. J., Rotta, A., et al. (2017). A spatially explicit risk assessment approach: Cetaceans and marine traffic in the pelagos sanctuary (Mediterranean Sea). *PloS One* 12 (6), e0179686. doi: 10.1371/journal.pone.0179686

Pérez-Ruzafa, A., García-Charton, J. A., and Marcos, C. (2017). North East Atlantic vs. Mediterranean marine protected areas as fisheries management tool. *Front. Mar. Sci.* 4. doi: 10.3389/fmars.2017.00245

Podestà, M., Azzellino, A., Cañadas, A., Frantzis, A., Moulins, A., Rosso, M. P., et al. (2016). ""Cuvier's beaked whale, ziphius cavirostris, distribution and occurrence in the Mediterranean Sea: high-use areas and conservation threats"," in *Mediterranean Marine mammal ecology and conservation*, vol. 75 . Eds. G. Notarbartolo di Sciara, M. Podestà and B. E. Curry (Amsterdam: Elsevier), 103–140.

Pope, K. L., Pegg, M. A., Cole, N. W., Siddons, S. F., Fedele, A. D., Harmon, B. S., et al. (2016). Fishing for ecosystem services. *J. Environ. Manage.* 183, 408–417. doi: 10.1016/j.jenvman.2016.04.024

Powers, J. E., and Abeare, S. M. (2009). Fishing effort redistribution in response to area closures. *Fish. Res.* 99, 216–225. doi: 10.1016/j.fishres.2009.06.011

Quijano Quiñones, D. R., López-Rocha, J. A., Hernández-Herrera, I., and Torres-Irineo, E. (2021). Spatial dynamics modeling of small-scale fishing fleets with a random walk approach. *Front. Mar. Sci.* 8. doi: 10.3389/fmars.2021.669112

Renó, V., Dimauro, G., Labate, G., Stella, E., Fanizza, C., Cipriano, G., et al. (2019). A SIFT-based software system for the photo-identification of the risso's dolphin. *Ecol. Inform.* 50, 95–101. doi: 10.1016/j.ecoinf.2019.01.006

Ricci, P., Carlucci, R., Capezzuto, F., Carluccio, A., Cipriano, G., D'Onghia, G., et al. (2022). Contribution of intermediate and high trophic level species to benthic-pelagic coupling: insights from modelling analysis. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.887464

Ricci, P., Ingrosso, M., Carlucci, R., Fanizza, C., Maglietta, R., Santacesaria, F. C., et al. (2020a). "Quantifying the dolphins-fishery competition in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea)," in *Proceedings IMEKO Tc-19 metrology for the Sea*(Naples, Italy:IMEKO).

Ricci, P., Ingrosso, M., Cipriano, G., Fanizza, C., Maglietta, R., Renò, V., et al. (2020b). "Top down cascading effects driven by the odontocetes in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea)," in *Proceedings IMEKO Tc-19 metrology for the Sea*(Naples, Italy:IMEKO).

Ricci, P., Libralato, S., Capezzuto, F., D'Onghia, G., Maiorano, P., Sion, L., et al. (2019). Ecosystem functioning of two marine food webs in the north-Western Ionian Sea (Central Mediterranean Sea). *Ecol. Evol.* 9 (18), 10198–10212. doi: 10.1002/ece3.5527

Ricci, P., Manea, E., Cipriano, G., Cascione, D., D'Onghia, G., Ingrosso, M., et al. (2021a). Addressing cetacean–fishery interactions to inform a deep-sea ecosystem-based management in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea). *J. Mar. Sci. Eng.* 9, 872. doi: 10.3390/jmse9080872

Ricci, P., Sion, L., Capezzuto, F., Cipriano, G., D'Onghia, G., and Libralato, S. (2021b). Modelling the trophic roles of the demersal Chondrichthyes in the northern Ionian Sea (Central Mediterranean Sea). *Ecol. Modell.* 444, 109468. doi: 10.1016/j.ecolmodel.2021.109468

Roman, J., Estes, J. A., Morissette, L., Smith, G., Costa, D., McCarthy, J., et al. (2014). Whales as marine ecosystem engineers. *Front. Ecol. Environ.* 12, 377–385. doi: 10.1890/130220

Rossi, S., and Gabbianelli, G. (1978). Geomorfologia del golfo di taranto. *Boll. Soc Geol. Ital.* 97 (4), 423–437.

Russo, T., Bitetto, E., Carbonara, P., Carlucci, R., D'Andrea, L., Facchini, M. T., et al. (2017). A holistic approach to fishery management: evidence and insights from a central Mediterranean case study (Western Ionian Sea). *Front. Mar. Sci.* 4. doi: 10.3389/fmars.2017.00193

Russo, T., Catucci, E., Franceschini, S., Labanchi, L., Libralato, S., Sabatella, E. C., et al. (2022). Defend as you can, react quickly: the effects of the COVID-19 shock on a large fishery of the Mediterranean Sea. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.824857

Russo, T., D'Andrea, L., Franceschini, S., Accadia, P., Cucco, A., Garofalo, G., et al. (2019). Simulating the effects of alternative management measures of trawl fisheries in the central Mediterranean Sea: Application of a multi-species bio-economic modeling approach. *Front. Mar. Sci.* 6. doi: 10.3389/fmars.2019.00542

Russo, T., Morello, E. B., Parisi, A., Scarcella, G., Angelini, S., Labanchi, L., et al. (2018). A model combining landings and VMS data to estimate landings by fishing ground and harbor. *Fish. Res.* 199, 218–230. doi: 10.1016/j.fishres.2017.11.002

Russo, T., Parisi, A., Garofalo, G., Gristina, M., Cataudella, S., and Fiorentino, F. (2014). SMART: A spatially explicit bio-economic model for assessing and managing demersal fisheries, with an application to Italian trawlers in the strait of Sicily. *PloS One* 9 (1), e86222. doi: 10.1371/journal.pone.0086222

Sánchez Lizaso, J. L., Sola, I., Guijarro-García, E., Bellido, J. M., and Franquesa, R. (2020). A new management framework for western Mediterranean demersal fisheries. *Mar. Policy* 112, 103772. doi: 10.1016/J.MARPOL.2019.103772

Santacesaria, F. C., Bellomo, S., Fanizza, C., Maglietta, R., Renò, V., Cipriano, G., et al. (2019). Long-term residency of Tursiops truncatus in the Gulf of Taranto (Northern Ionian Sea, Central-eastern Mediterranean Sea). *Genoa Italy* 2019, 28–32.

Sergio, F., Caro, T., Brown, D., Clucas, B., Hunter, J., Ketchum, J., et al. (2008). Top predators as conservation tools: ecological rationale, assumptions, and efficacy. *Annu. Rev. Ecol. Evol.* 39, 1–19. doi: 10.1146/annurev.ecolsys.39.110707.173545

Shapiro, S. S., and Wilk, M. B. (1965). An analysis of variance test for normality (complete samples). *Biometrika* 52, 591–611. doi: 10.1093/biomet/52.3-4.591

Stelzenmüller, V., Maynou, F., and Martin, P. (2007). Spatial assessment of benefits of a coastal Mediterranean marine protected area. *Biol. Conserv.* 136, 571–583. doi: 10.1016/j.biocon.2007.01.002

Sumaila, U. R., Zeller, D., Watson, R., Alder, J., and Pauly, D. (2007). Potential costs and benefits of marine reserves in the high seas. *Mar. Ecol. Prog. Ser.* 345, 305–310. doi: 10.3354/meps07065

Tetley, M. J., Braulik, G. T., Lanfredi, C., Minton, G., Panigada, S., Politi, E., et al. (2022). The important marine mammal area network: A tool for systematic spatial planning in response to the marine mammal habitat conservation crisis. *Front. Mar. Sci.* 9. doi: 10.3389/fmars.2022.841789

Tromeur, E., and Loeuille, N. (2017). Balancing yield with resilience and conservation objectives in harvested predator–prey communities. *Oikos* 126 (12), 1780–1789. doi: 10.1111/oik.03985

Wood, L. J., Fish, L., Laughren, J., and Pauly, D. (2008). Assessing progress towards global marine protection targets: shortfalls in information and action. *Oryx* 42, 340–351. doi: 10.1017/S003060530800046X

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Common dolphins (*Delphinus delphis)* in Israel: Unique dynamics of a critically endangered population

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The most eastern population of common dolphins (*Delphinus delphis*) in the Mediterranean Sea inhabits the southern coastal waters of Israel. They are mainly observed in the shallow waters off Ashdod and Ashkelon, between the 15-30 m isobaths, with no reported observations north or west of this area. These dolphins were observed and studied year-round between 2016-2021 using boat-based surveys and photo identification methods. Common dolphins were encountered and photographed 43 times during the study period, resulting in 2,851 identifications of 25 distinctive mature individuals and 12 calves. Most individuals (62%) were sighted over multiple years, with high yearly and monthly sighting rates, indicating long-term site fidelity and residency. Closed population mark-recapture models estimated a total abundance of 25 (95% CI 24 – 37) individuals in 2016 that declined to only 15 (95% CI 15 – 15) individuals in 2021. Social network analysis described these remaining individuals as one closed and well-associated social unit. Survival probabilities for this population appeared lower than those of other delphinid populations. The decrease in their abundance, coupled with their apparent isolation level, qualifies the local population for a re-assessment of their conservation status. This study first describes the Israeli local population of common dolphins, their dynamics and an assessment of their status based on the IUCN Red List framework.

KEYWORDS

common dolphins, IUCN red list, conservation status, extinction risk, abundance, mark-recapture, social structure, delphinus delphis

Introduction

Common dolphins (*Delphinus delphis*) were once one of the most abundant cetaceans in the Mediterranean Sea, inhabiting coastal and deep-water areas (Bearzi et al., 2003; Pace et al., 2016; Vella et al., 2021). However, culling and killing efforts since the early 19th century, followed by a reduction in prey abundance due to overfishing since the mid-20th century (Bearzi et al., 2003; FAO, 2018), has resulted in a dramatic decrease in their numbers. This trend led to their declaration as 'Endangered' in the Mediterranean Sea by the IUCN in 2003 (Bearzi et al., 2003), compared to their global status, classified as 'Least Concern' (Hammond et al., 2008). Furthermore, according to Natoli et al. (2008), genetic evidence of sub-population structure may indicate separate management units in the western and eastern Mediterranean Sea, thus differentiating between the "inner Mediterranean" sub-population of common dolphins and a north-eastern Atlantic population (Natoli et al., 2008; Moura et al., 2013; Bearzi et al., 2021). The "outer Mediterranean" population inhabits the north-eastern Atlantic and the Alborán Sea (Cañadas and Hammond, 2008), while the "inner Mediterranean" sub-population inhabits the Mediterranean waters east of the Almerìa – Orán thermohaline front and was reassessed as 'Endangered' in 2021 (Bearzi et al., 2021)

The 'inner Mediterranean Sea' sub-population is segregated into several small groups scattered in parts of the southern Tyrrhenian Sea, Sicily Channel, and Ionian Sea (Vella, 2004; Gannier, 2005; Arcangeli et al., 2013; Aissi and Vella, 2015; Santoro et al., 2015; Pace et al., 2016; Arcangeli et al., 2017) and more regularly in the northern and eastern Aegean Sea (Frantzis et al., 2003; Milani et al., 2021). These small groups will be referred to as local populations as they inhabit different habitats who are subjected to numerous anthropogenic effects (Bearzi and Genov, 2021). Most of them present decreasing trends in abundance (Bearzi et al., 2008; Gonzalvo and Costa, 2016; Mussi et al., 2019; Genov et al., 2020; Vella et al., 2021). In the Gulf of Corinth, for example, 22 (range 16 - 32) common dolphins are observed exclusively in mixed-species groups with the striped dolphin (*Stenella coeruleoalba*) (Santostasi et al., 2016). This local population was recently declared 'Critically Endangered' and faces a high extinction risk (≥ 50%, Santostasi et al., 2018). In the north Adriatic Sea, the numbers are even lower, as the local population underwent a dramatic decline in the 1970s, and between 2009 – 2012 only four individuals were observed (Genov et al., 2020).

The small size and separation between these local populations have led to the formation of different social structures. For example, a social study conducted in the Ionian Sea between 1996 and 1999 described a small population of 47 individuals in the area who presented a fluid social structure akin to a 'fission-fusion' society composed of a single social unit divided into groups with frequently changing memberships (Bruno et al., 2004). In the Tyrrhenian Sea, however, 38 individuals presented high site fidelity and stable association patterns (Pace et al., 2009), while females in the same reproductive state maintained strong and long-lasting associations for up to five years (Mussi et al., 2019).

Each local population described above might react differently to anthropogenic effects and other environmental changes in their habitats, as observed in other cetacean species worldwide (Ansmann et al., 2012; Blasi and Boitani, 2014; Genov et al., 2019). Therefore, they may be addressed to as different management units and understanding the dynamics of each one of them, such as their abundance, trends, distribution, and social structure, is necessary to inform conservation actions and apply appropriate management actions (Notarbartolo di Sciara and Birkun, 2010).

The most eastern common dolphins in the Mediterranean inhabits the southern waters of Israel, from south Tel Aviv to the southern Israeli border (Brand et al., 2019). They are mainly observed in the coastal waters off Ashdod and Ashkelon, at water depths of 15-30 m (2-4 km from shore), frequently in the same small area. Bottlenose dolphins (*Tursiops truncatus)* also range in this area, usually in deeper waters than the common dolphins, and the two species were never observed together. Common dolphins have been observed along the Israeli coastline since 1993, mostly during occasional sightings and second-party reports, containing a mean group size of 22.2 ± 19.1 (range 1 – 75) individuals (Kerem et al., 2012). These reports are prior to the study period, and they are the earliest known encounters with common dolphins in Israel. None of them observed the common dolphins northern or western of the study area, except for two reports north of Tel Aviv in 2009 and 2011. The southern border of their habitat is less clear as reports from north Egypt do not include this species (Farrag et al., 2019), and only rare sightings and few strandings of common dolphins have been reported from the Gaza strip in the last 20 years (Abd Rabou et al., 2021). These observations might indicate the presence of another group or groups southern to this research study area but could also be the same individuals that range the southern Israeli waters.

Several individuals have been stranded on the Israeli shore over the last two decades. Their stomach content reveals that their diet is mainly composed of the Balearic eel (*Ariosoma balearicum*), Klunzinger's ponyfish (*Equulites kluzingeri*), and cephalopods (Brand et al., 2019), which are also common in the local fisheries catch. Dolphins are often observed feeding from the discards of the sorting process of the fishermen while pulling the net back to the boat.

The local population of common dolphins in Israel is subjected to many anthropogenic pressures as they inhabit a coastal area in proximity to a major port and a power station with various human activities such as fishing, sailing, and discharging of sewage. Additionally, their habitat is near the

Israeli border with the Gaza strip, which increases the presence of navy ships in the area. Part of their ranging area has been approved as the Marine Protected Area 'Evtach' (Figure 1), but it is not yet declared and enforced; therefore, it is still subject to fishing pressure. Given their delicate status in the Mediterranean Sea and their regional decrease in abundance, this study aims to provide the first insight into the dynamics of the most eastern local population of common dolphins in the Mediterranean Sea, using mark-recapture models and social network analysis. In addition, this study provides a quantitative evaluation of conservation status following the IUCN Red List criteria (IUCN, 2012a; IUCN, 2012b) framework applied to the local population of common dolphins in Israel and performs a quantitative estimate of its probability of extinction.

Methods

Sampling methods

Study area

The study area is spread along the entire 196 km of Israel's coastline of the Mediterranean Sea (Figure 1B and Supplementary Figures 1–6). The continental shelf extends to a depth of 200 m; gradually widens from its narrowest part in the north, approximately 10 km from shore, towards the widest part in the south, nearly 20 km from shore. Survey transects started from the marina's exit toward the open sea, usually between 10 - 60 m in depth with some effort in deeper waters, up to 1200 meters in depth (50 km from shore).

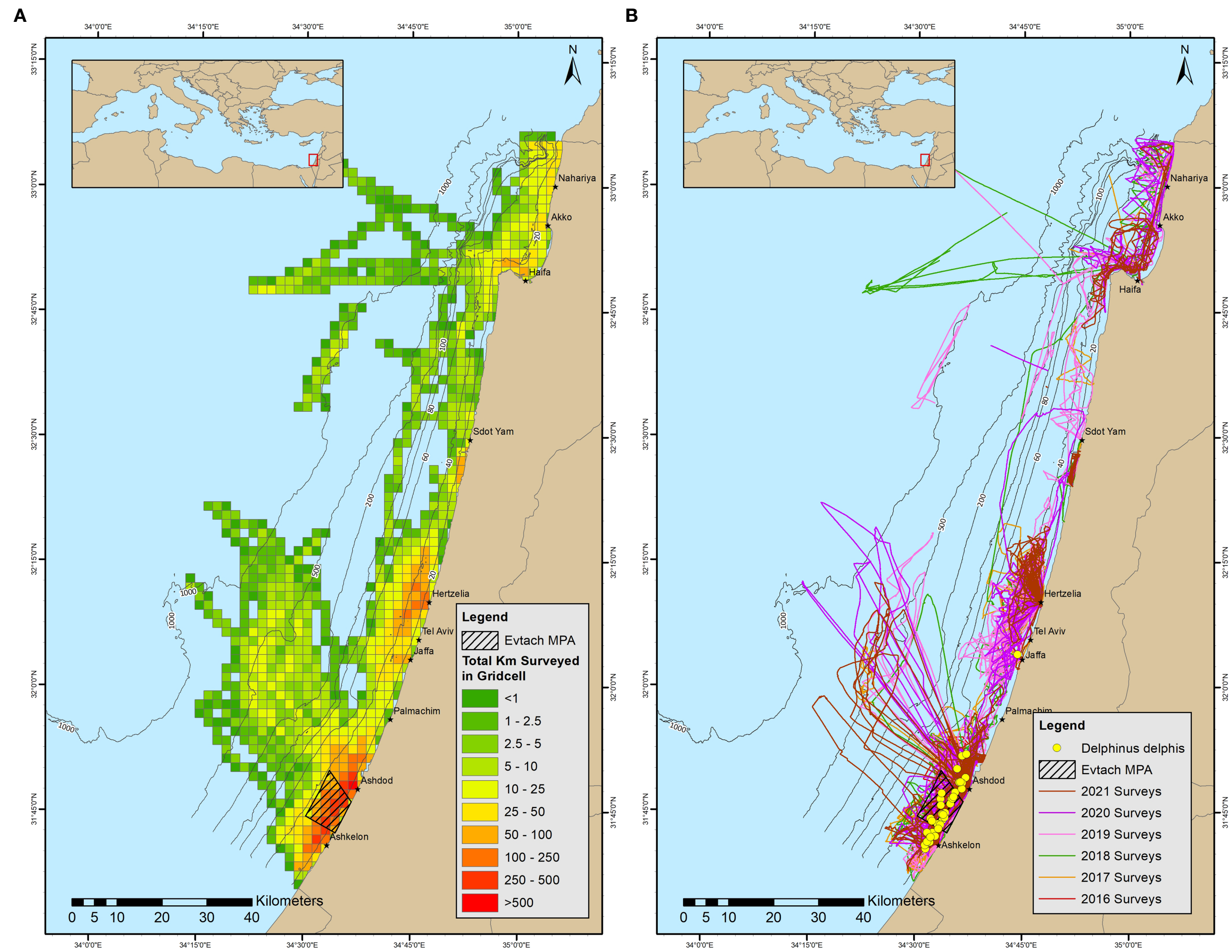

FIGURE 1

(A) Heatmap of survey effort. Colors represent effort in surveyed km in 2x2 grid cells. **(B)** Survey routs throughout the study period. Different colors represent different years. Yellow circles represent common dolphin sightings.

Data collection

Shipboard surveys were conducted year-round according to the protocol described by Scheinin (2010) as part of the long-term monitoring activity of Morris Kahn Marine Research Station (MKMRS) of the University of Haifa, in collaboration with the Israel Marine Mammal Research and Assistance Center (IMMRAC NGO), and Delphis NGO. The starting point of each survey was from one of eight locations along the coast grouped as South (Ashkelon and Ashdod), Center (Jaffa port, Tel Aviv, Herzliya, and Sdot–Yam), and North (Haifa, Akko, and Nahariya). The surveys were primarily opportunistic and dependent on collaborations with private yacht and boat owners, apart from a designated project funded by the Ministry of Energy between 2018 – 2020, which allowed regular surveys once a week from Ashdod, following the same protocol. On average, surveys were conducted two to five times a month throughout Israel's continental shelf's southern and central marine areas, while the northern area was surveyed less frequently due to fewer collaborations. The boat surveys were performed at the discretion of the research team, based on variables such as sea conditions, prior survey routes, and the locations of the last observations of dolphins. The sampling effort was distributed in an attempt to cover the entire study area equally, but due to the dependency on collaborations, that was not always the case. Areas closer to the main marinas were surveyed more frequently (Figure 1A), and during the funding time of the Ministry of Energy, there was a high concentration of effort in the south. The survey route generally followed a transverse zigzag approach between 30-60 m isobaths, parallel to the coastline, at a speed of 4-12 knots. Bottom trawlers, sailing at a speed of 3 knots, following the longshore 35-60 m isobaths, were opportunistically approached, searching for dolphins foraging nearby. Deep-water surveys were conducted aboard a commercial longline pelagic fishing boat fishing for tuna fish, traveling from Ashdod up to 50 km from shore, and deploying pelagic longlines between 800 - 1200 m isobaths.

The boat's position was recorded along the route every 20 seconds during each survey, while environmental parameters and all wildlife encounters were logged using 'Delphis', a designated data collection mobile application (Marco, 2017). Once dolphins were sighted, they were approached to photograph and collect group focal follow data, logged into the 'Delphis' application as well. Disturbances to the dolphins were reduced by following them at minimum speed from 20 - 50 meters away and avoiding sudden directional or speed changes. Close approaches, < 20 m, were only initiated by the dolphins themselves, approaching the boat from curiosity or to bow-ride. The dolphins were kept in sight until high-quality photographs of all the individuals were achieved or until vessel constraints or sea conditions forced the encounter to an end. Photographs of the left and right sided of the dorsal fin were taken using a Canon EOS 7D 18mp camera with 70-200mm f2.8 EF zoom lenses.

In addition to the data collected during this study, 11 observations of common dolphins were recorded and photographed by the marine unit of the Nature and Park Authority in Israel. These observations occurred in the same area as the study area, and the dolphins were photographed following the same protocol. To enlarge the database for this study, the locations and photographs of these 11 encounters were added to the data and were considered for the analysis, summing to a total of 43 photographed encounters between 2016 - 2021.

Three age classes were considered based on visual assessments, as suggested by Mussi et al. (2019): "calf below half of an adult length, constantly in close association with an adult, with a dorsal fin typically low and rounded, a dark, lead-grey coloration with visible fetal folds, and immature swimming style with stereotyped surfacing pattern when breathing; juvenile about two-thirds of an adult, usually swimming in association with an adult, but sometimes independently, with a coloration slightly lighter than the adult; adult approximately 2 m long". Sex was determined when photographs of the genital area were achievable (Smolker et al., 1992) or when an adult was consistently accompanied by a calf and assumed female (Shinohara et al., 1997). A group was defined according to Shane (1990), as a "group of dolphins observed in apparent association, moving in the same direction and often, but not always, engaged in the same activity". Members of the group usually remained within 100 m from each other and were assumed to all have the same probability of being detected and photographed (i.e., captured). All survey methods remained consistent throughout the study period.

Photo identification

Common dolphins were individually identified based on long-lasting markings and coloration patterns on their dorsal fins (Würsig and Würsig, 1977). Photographs were first graded by quality (Q) (Wilson et al., 1999) from 1 to 5; where 1 is assigned to photographs with no dolphins but might contain other relative information, 2 to photographs that contain dolphins but not their dorsal fins or very bad angled dorsal fins, 3 was assigned to photographs which contained less focused and tilted angled dorsal fins but with clearly visible edge, 4 to focused and good angled dorsal fins and 5 to photographs containing well lit, straight angle and focused dorsal fins. Only photographs with a Q ≥ 3 were further processed and given an additional grade by the distinctiveness of the dorsal fin (Wilson et al., 1999). Highly distinctive dorsal fins with visible and long-lasting marks or irregularly shaped ones were graded as 1. Grade 2 was assigned to the moderately marked dorsal fin, which contains, for example, only one small notch, while smooth, mark-less fins were graded as 3. Minor scratches usually heal and therefore were only used for short-term identification between observations within the same month or two and were not considered in the distinctiveness grading. The best left and right photographs of each individual from every encounter were used to create a catalog, where all uniquely identified individuals were assigned a number. Each identification was considered final when approved by the independent evaluation of at least two researchers (Berrow et al., 2012). Matches could be determined by photographs of either side of the dorsal fin, but preferably both, when photographs of both sides were available. Calves were given identification numbers in reference to their mothers for easier follow-up. Only substantially marked individuals (distinctiveness grade 1 or 2) from good-quality photographs ($Q \geq 3$) were considered in the analysis to avoid misidentification of individuals that can cause biased estimations of abundance and structure (Hammond, 2010). Five common dolphin individuals were stranded along the Israeli coastline throughout the study period: three adult males, one young male, and one young female. Their dorsal fins were checked for matches in the catalog.

Data analysis

Site fidelity

The site fidelity of the local population was estimated by calculating the mean yearly and monthly sighting rates for each individual as a proportion following the equation (Parra et al., 2006):

$$\frac{number\ \ of\ \ months/years\ \ a\ \ dolphin\ \ was\ \ sighted}{total\ \ number\ \ of\ \ surveyed\ \ months/years}$$

Abundance

The limited distribution of the local common dolphin population, the highly localized nature of their sightings, the high resighting rate of identified individuals, and the lack of new additions to the local population suggest the application of closed population mark-recapture models (Otis et al., 1978; Schwarz and Seber, 1999; Wilson et al., 1999). The output of such models includes estimations of capture (p) and recapture (c) probabilities, applied to estimate the abundance of the local population. To test the closure assumption, capture history from each of the studied years was tested for closure using 'CloseTest' software (Stanley and Richards, 2004), applying two different closure tests: the Stanley and Burnham (1999) test, which was developed under a null model allowing for time-specific variation in capture probabilities under closure, and the Otis et al. (1978) test, which was developed under a null model allowing for heterogeneity in capture probabilities under closure. A suite of closed population models was fitted to the five-year database, as listed in Table 1. These models include the following scenarios: constant capture probabilities, monthly varying capture probabilities, time changing probabilities, additive affect to capture probabilities, and behavioral response between capture and recapture. They were chosen in order to account for the dynamic environment of this local population's habitat, being so close to shore and subject to varying human activities in the area. The R (R Core Team, 2020) package RMark (Laake, 2013) to construct models for the program MARK (Cooch and White, 2014) was used to fit the models.

Each year was divided into several sampling periods, one month each, to allow mixing within the local population while maintaining the closure assumption (Seber, 1982; Thomas et al., 1986). The models estimated capture and recapture probabilities for monthly sampling occasions between April and November (Table 2). Calves were not included as their capture probability is not independent of their mothers (Hammond, 2010). The Akaike Information Criterion corrected for small sample size (AICc) (Akaike, 1973; Burnham and Anderson, 2002) was used for model selection, considering models within $\Delta AICc \leq 2$ as the most supported and using model averaging to account for uncertainty in model selection when more than one model had an $\Delta AICc$ value less than 2 (Burnham and Anderson, 2002). The estimated abundance was then divided by the mark ratio, calculated as the estimated proportion of animals with long-lasting marks in the local population (Wilson et al., 1999).

Survival

Two Cormack-Jolly-Seber (CJS) models (Cormack, 1964; Jolly, 1965; Seber, 1965) were fitted to the data to estimate the survival and capture probabilities of the local population over the years. Between two following years, the time interval was set to 1 and between 2016 and 2018 the time interval was set to 2 as data was missing from 2017. The first model was applied to the entire population without age discrimination, and the second

TABLE 1 List of models fitted to the common dolphin's data.

Model name	Parameters	Description
M_0	p(.) = c(.)	Constant p
M_t	p(t) = c(t)	Month varying p
M_T	p(T) = c(T)	p changes linearly with time
M_b	p(.), c(.)	Behavioral response
M_{tb}	p(t), c(t)	Behavioral response and month varying p
M_{t+c}	p(t+c) = c(t+c)	Month varying p with additive effect

List of closed populations models fitted to the data set of the common dolphins, p – capture probability, c – recapture probability, (.) – constant probabilities, (t) – month varying probabilities.

TABLE 2 Sampling effort used in the closed population models for the common dolphins.

Year	Sampling occasions (months)	Encounters	Marked individuals	Km surveyed
2016	2	2	20	944.52
2017	0	0	0	2036.05
2018	3	3	15	2247.46
2019	4	5	14	3897.75
2020	5	17	10	3955.5
2021	6	10	9	2450.06

included age class (i.e., calf and adult) as a group covariate. The effect of survey effort (amount of surveyed km) on capture probabilities was tested in both models. The Akaike Information Criterion corrected for small sample size (AICc) (Akaike, 1973; Burnham and Anderson, 2002) was used for model selection as for the closed population models.

Trends in abundance

A statistical power analysis was performed on the data to determine whether the model outputs can detect a trend using linear regression (Gerrodette, 1987). The power of the test is affected by the accuracy of the estimates (CV), the sample size (n), the chance for Type 1 and Type 2 errors (α and β), and the rate of change in the local population (R). The power analysis was used to measure what is the lowest rate of change (R) that can be detected with a sufficient statistical power of 0.8 (Taylor et al., 2007b) given the duration of the study (5 yearly samples of common dolphin) and the precision of the estimates. Analyses were performed using the 'fishmethods' package in "R" (Nelson, 2019; R Core Team, 2020), setting the parameters following Santostasi et al. (2016): one-tailed test, linear trend, and a ≤ 0.05 probability of Type 1 error. The overall CV of the study period was calculated as the mean of the annual CVs (Santostasi et al., 2016). A trend was considered significant when the regression of abundance estimates over the study period had a slope significantly distinct from zero (Gerrodette, 1987).

Social structure

The social network of the local population was examined over time to observe its dynamics and the strength of the relationships between individuals. Social structure analysis was conducted on the entire network (2016 – 2021) and the network of individuals remaining in the area in 2021 after the decline in the local population size. Indices based on associations within the group were used to measure the relationship between individuals. Association was defined according to the "Gambit of the Group" assumption (Whitehead and Parijs, 2010), where two individuals observed in the same group during an encounter are assumed to be associating. The Half Weight Index (HWI) (Cairns and Schwager, 1987) was used to calculate the strength of the relationship between individuals: $E_{ij} = \frac{x}{x+y_{ij}+\frac{1}{2}(y_i+y_j)}$, when x is the number of sampling periods in which the two individuals were associated, yij is the number of sampling periods in which both were observed yet not associated. Yi or yj, are the numbers of sampling periods where only one individual was observed (Whitehead, 2008a). The HWI accounts for bias from pairs more likely to be identified when separate or when not all associations can be identified (Whitehead, 2008b). A zero value of HWI indicates that the dyad was never observed together as part of the same group, while the value of 1 indicates that the dolphins were always together. The HWI is then used to create the association matrix (N x N matrix containing the association index of each dyad of dolphins within the local population), which is the primary data structure for further social network analysis (Farine and Whitehead, 2015). Calculations were made using the 'asnip' package (Farine, 2013) and 'igraph' (Csardi, 2020) in R (R Core Team, 2020).

Network diagrams were constructed to visualize the social network's social connections and complete structure. Each node describes an individual, and the associations between individuals are represented by lines (edges). The width of the line is relative to the strength of a dyad's association (Farine and Whitehead, 2015), calculated by the HWI.

Application of IUCN red list criteria

According to the guidelines for the application of IUCN Red List criteria (IUCN, 2012a), five criteria can be used to classify a subpopulation or regional/local population as Vulnerable (VU), Endangered (EN), or Critically Endangered (CE) as described in IUCN, 2012b. Moreover, when dealing with regional populations, it is essential to address the degree of their isolation, as their extinction risk might resemble that of an endemic taxon (Gärdenfors et al., 2001; IUCN, 2012a; IUCN, 2012b; Santostasi et al., 2018). Given this local population's restricted distribution and the limited data regarding the existence of neighboring populations, a precautionary approach is taken to consider this local population as isolated. Therefore, in addition to the analyses described earlier to study the local population dynamics, several other measures were calculated to assess the status of the local population of common dolphins.

Geographic range

The extent of occurrence (EOO) and area of occupancy (AOO) are two measures usually calculated to examine the distribution of a certain population. They are used to assess the distribution range of a specific population and, according to that range, how likely it is to be isolated and, therefore, at risk of extinction (IUCN, 2012a). The extent of occurrence is defined as "the area contained within the shortest continuous imaginary boundary which can be drawn to encompass all the known, inferred or projected sites of present occurrence of a taxon, excluding cases of vagrancy", and it is calculated by creating a minimum convex polygon around the plotted tracked positions of common dolphin groups observed in the study area (IUCN, 2012b; Santostasi et al., 2018). The area of occupancy is described as "the area within its extent of occurrence which is occupied by a taxon, excluding cases of vagrancy" (IUCN, 2012b). This measure takes into account the fact that a taxon will not always occur throughout the entire area of its extent of occurrence, which may contain unsuitable or unoccupied habitats (IUCN, 2001). As the southern distribution range of the Israeli common dolphins' is the Israeli border, it is not clear what is their full range of distribution. Therefore, these measures were not applied to the local population, and Criteria B was not considered in the analysis.

Population projection

Criteria E requires quantitative analysis showing the probability of extinction in the wild is at least 50% within ten years or three generations (IUCN, 2012b). The probability of extinction was calculated following the methods used in Santostasi et al. (2018) by multiplying the abundance estimated in this study by a range of biological plausible growth rates to cover a variety of scenarios caused by increasing levels of growth rate stochasticity (Morris and Doak, 2002; Currey et al., 2009). This is simulated by randomly drawing a growth rate value from a normal distribution where a higher SD represents a higher year-to-year variation of the growth rate. Scenario 1 indicates a constant growth rate (SD = 0), while scenarios 2 and 3 simulate increasing levels of stochasticity, a more realistic scenario considering the dynamic environment the local population inhabit, which is likely to have fluctuations (SD = 0.01 and SD = 0.02, respectively). The probability of extinction was then estimated under three quasi-extinction thresholds, chosen to provide a range of conservative values for extinction; two, four, and six reproductive individuals and over three-time scales; 45, 75, and 100 years (Taylor et al., 2007a). Modeling was programmed in R (R Core Team, 2020), as detailed in Santostasi et al. (2018).

Results

A total of 16,531.36 km was surveyed between 2016 –2021 (Figure 1B) during 368 boat-based surveys. Common dolphins were encountered and photographed 32 times, and together with the 11 photographed encounters from the NPA, the total number of observations is 43. No photographed encounters were obtained during 2017; therefore, this year was not considered in the analysis. Most of the observations were achieved during the warm season – between April and October, while only years 2019 and 2020 contained encounters during November and December (a total of six). In order to maintain uniformity in the surveyed months, these six encountered were not considered in the mark-recapture models (Table 2).

19,569 photographs of common dolphins were taken, containing 2,851 identifications of 42 common dolphin individuals. Of them, 25 presented substantial markings while 17 did not, including 12 calves and five adults. These five were not included in any further analysis as they could not be re-identified. Mean group size was 10.2 individuals (n = 43, range = 2 – 21, SD = 4.43). 23 individuals were sighted in multiple years, with a mean number of sightings of 11.8 (n = 37, range = 1 – 37, SD = 12.2) per individual. Of the 37 common dolphins (25 adults and 12 calves), ten were identified as females after being observed with a dependent calf, one adult was recognized as male after stranding on shore in September 2020, and one calf was recognized as male after obtaining a photograph of its genital area. All the others (n = 25) were of unknown sex. Of the five stranded dolphins, two were identifiable through photo ID and were recognized as previously observed individuals.

Of the 25 identified mature individuals, three (12%) were sighted only once during the study period, eight (32%) twice, three (12%) were identified four times, and 14 (56%) were observed seven times or more (Supplementary Figure 7). The most observed individual was 'Lavian' (number 37 in the catalog), with 37 observations during the five years study period. Mean yearly sighting rate was 0.54 ± 0.33 (range = 0.2 - 1) and mean monthly sighting rate was 0.36 ± 0.31 (range = 0.04 - 0.91). Three females were observed with multiple calves during the study period, with a mean inter-calf interval of 1.5 ± 0.58 years. The discovery curve of the common dolphins reached a clear plateau (Supplementary Figure 8), indicating a good representation of the local population (Wilson et al., 1999).

CJS models for survival estimations

The goodness of fit test for the CJS model to the data did not indicate a lack of fit ($\chi 2$ = 1.404, p = 0.924, df = 5). The best fitting model to estimate the survival probabilities for the entire population had constant survival and capture probabilities. An additional model was estimated within $\Delta AICc \leq 2$ from the best fitting model, with constant survival probability and effort variation in capture probabilities. Model averaging was applied to these two models to obtain the model-averaged parameter estimates, as shown in Table 3.

TABLE 3 Averaged parameter estimates for the two models within ΔAICc ≤ 2 in the CJS analysis to estimate survival and capture probability.

Parameter	Year	Estimate	SE	95% CI
Survival (Phi)	2016-2021	0.77	0.04	0.67 – 0.85
Detectability (p)	2018	0.87	0.10	0.54 – 0.97
	2019	0.94	0.05	0.75 – 0.99
	2020	0.94	0.05	0.75 – 0.99
	2021	0.93	0.05	0.77 – 0.98

The best fitting model to estimate adult and calf survival probabilities had constant survival and capture probability. Three other models had ΔAICc ≤ 2: 1) constant survival probability and effort variation in capture probability, 2) age varying survival and constant capture probability, 3) constant survival and group varying capture probability (Supplementary Table 1). Model averaging was applied to these four models to obtain the model-averaged parameter estimates, as shown in Table 4.

Closure test

The Otis et al. (1978) test found the local population to be closed ($P > 0.05$) in all of the study years, while the Stanley and Burnham (1999) test supports the population closure ($P > 0.05$) for the year 2016 but suggests an open population ($P < 0.05$) for years 2018 -2021.

Closed population models for abundance estimates

Closed population models were applied to each of the study years separately. Each year yielded different models within ΔAICc ≤ 2 from the best fitting model (Supplementary Table 2) that were averaged to extract the capture and recapture probabilities as shown in Table 5 and the estimated abundance as shown in Table 6.

Trends in abundance

The mean CV of the estimates was 0.017. The minimum rate of decay of the population abundance that could be detected in five years with a 0.8 statistical power was a total decrease of 10%. The local population size in 2016 was estimated to be 25 (95% CI 24 - 37) individuals, while in 2021, there were only 15 (95% CI 15 – 15). A decrease of 40% was found in linear regression, as shown in Figure 2 ($R^2 = 0.76$, $P = 0.034$, $y = 3546.14 - 1.74x$, $n = 5$), as a result of the disappearance of more than half of the local population observed at the beginning of the study period.

Population projection

Population projection analysis placed the local population under more than 50% probability of extinction in 25 of the 27 projections (Table 7 and Figure 3). Thus, meeting criteria E for Critically endangered.

Social structure

From the 625 possible associations between the 25 mature individuals, 153 (25%) were zero, meaning no association was observed between the specific dyad. 188 were 0.1 (30%), indicating a low level of association, and the rest were distributed, as shown in Figure 4A. The mean association rate over the five-year study period was 0.35 ± 0.28 (range 0.05 – 1). When examining the association patterns of the local population in 2020 - 2021 (the remaining individuals after the decline in the local population size), the mean association rate rises to 0.76 ± 0.08 (range 0.5 – 0.92), and the individuals are all part of the same highly associated social unit. The remaining individuals contained six mature females, three mature individuals of unknown sex, and five calves (one of them is a male). They were all observed together more than 25 times in 72.4% of the observations between 2020-2021 (Figure 4B).

Discussion

Geographic range and level of isolation

The local population of common dolphins was observed in the southern region of Israel all year round throughout the five years of the study. The majority of individuals (62%) were sighted over multiple years with high yearly and monthly sighting rates, indicating long-term site fidelity and residency. During the last two years of the study, 2020-2021, the same group of individuals were observed in all encounters, repeatedly in the same area, in front of Ashkelon and Ashdod, and occasionally near Palmahim. This group's small-ranging patterns and their high side fidelity

TABLE 4 Averaged parameter estimates for the four models within ΔAICc ≤ 2 from the best fitting model in the CJS analysis for calves' survival.

Group	Parameter	Year	Estimate	SE	95% CI
Adults	Survival (Phi)	2016-2021	0.78	0.05	0.68 – 0.87
	Detectability (p)	2018	0.89	0.09	0.58 – 0.98
		2019	0.93	0.05	0.76 – 0.98
		2020	0.94	0.05	0.76 – 0.98
		2021	0.93	0.04	0.77 – 0.98
Calves	Survival (Phi)	2016-2021	0.74	0.08	0.55 – 0.87
	Detectability (p)	2018	0.87	0.11	0.50 – 0.99
		2019	0.91	0.09	0.52 – 0.99
		2020	0.91	0.09	0.51 – 0.99
		2021	0.90	0.09	0.54 – 0.99

indicate year-round residency in the area. The stabilization of the discovery curve (Supplementary Figure 8) and the high frequency of observations of the same individuals strongly suggest that this group is relatively closed and isolated, yet additional information is needed from south to the Israeli border to confirm this hypothesis and understand this local population's complete distribution, and we take this opportunity to invite collaboration on this matter.

Apparent survival rates

The apparent annual survival rate was 0.77 ± 0.04 (Cl 95% 0.67-0.84), which appeared constant over the years, while capture probability rose with the increasing effort. As expected, calves had a lower survival probability and were observed in other dolphin species (Currey et al., 2009; Croft et al., 2017). Their capture probability was also low due to fewer

TABLE 5 Averaged parameters of the closed population models applied to each year.

Year	Parameter	Estimate	SE	95% CI
2016	p g1 t1	0.55	0.17	0.24 – 0.82
	p g1 t2	0.88	0.24	0.08 - 1
	c g1 t2	0.78	0.14	0.42 – 0.95
2018	p g1 t1	0.22	0.11	0.07 – 0.49
	p g1 t2	0.86	0.1	0.55 – 0.97
	p g1 t3	0.82	0.17	0.32 – 0.98
	c g1 t2	0.65	0.26	0.17 – 0.94
	c g1 t3	0.68	0.12	0.41 – 0.86
2019	p g1 t1	0.29	0.12	0.11 – 0.56
	p g1 t2	1.00	0.00	1.00 – 1
	p g1 t3	0.79	0.11	0.51 – 0.93
	p g1 t4	0.50	0.13	0.26 – 0.74
2020	p	0.82	0.11	0.5 – 0.96
	c	0.93	0.05	0.77 – 0.98
2021	p g1 t1	0.94	0.07	0.59 – 0.99
	p g1 t2	0.93	0.07	0.61 – 0.99
	p g1 t3	0.91	0.08	0.58 – 0.99
	p g1 t4	0.89	0.11	0.49 – 0.98
	p g1 t5	0.86	0.15	0.35 – 0.99
	p g1 t6	0.82	0.21	0.22 – 0.99
	c g1 t2	0.87	0.07	0.66 – 0.96
	c g1 t3	0.86	0.06	0.69 – 0.94
	c g1 t4	0.84	0.05	0.7 – 0.92
	c g1 t5	0.82	0.07	0.65 – 0.91
	c g1 t6	0.79	0.11	0.51 – 0.93

The averaged parameters for the closed population models. Estimate, real estimate of the parameters. SE, standard error; 95% CI, Confidence intervals.

TABLE 6 Yearly abundance estimates for the common dolphins.

Year	Estimated N	SE	95% CI	CV	Mark ratio	Total N
2016	20.85	1.80	20.07 – 30. 97	0.09	0.83	25.02
2018	15	0	15 - 15	0	0.83	18
2019	14	0	14 - 14	0	0.7	20
2020	10	0	10 - 10	0	0.55	18
2021	9	0	9 - 9	0	0.6	15

Abundance estimates from the closed population models applied to the common dolphin yearly data set. Estimated N, estimated abundance from the model; SE, standard error; 95% CI, confidence intervals; Mark ratio, estimated proportion of animals with long lasting marks in the local population. Total N, estimated N divided by the mark ratio; CV, coefficient of variation.

marks on their dorsal fins. These are the first survival estimates described specifically for common dolphins and their different age classes, and they appear low compared to other cetacean species worldwide and in the Mediterranean. For example, the survival rate of bottlenose dolphins in the northeastern Adriatic Sea was estimated between 0.82 and 0.93 (95% CI 0.69 – 0.98) (Fortuna, 2007). A mixed-species group of common and striped dolphins in the Gulf of Corinth presented survival probabilities of 0.94 (95% CI 0.92 – 0.96) (Santostasi et al., 2016). Even along the Israeli coastline, with the same environmental conditions, the survival of bottlenose dolphins was estimated to be higher, with a probability of 0.92 (95% CI 0.89 – 0.93) (Yaly Mevorach, unpublished data). Attempts to study the factors affecting the survival probabilities of cetaceans in the Mediterranean Sea revealed various reasons. In the Strait of Gibraltar, a local population of long-finned pilot whales appeared to be affected by epizootic episodes of morbillivirus, causing a decrease in their survival probabilities, from 0.997 ± 0.003 in the years before the epizootic episodes to 0.831 ± 0.042 after the first one and 0.649 ± 0.085 after the second one (Pons et al., 2022). Another example from the Strait of Gibraltar is the survival estimates of bottlenose dolphins, which appears to be negatively correlated with ferry traffic and are assessed between 0.918 and 0.924 (Tenan et al., 2020). The low apparent survival rate of the common dolphins in Israel could result from emigration from the study area or frequent mortality events leading to a decrease in their abundance, similar to other regions in the Mediterranean Sea.

Abundance and trends

The close tests presented controversial results as one of the tests supported the closure (Otis et al., 1978) assumption and the second one did not (Stanley and Burnham, 1999). These results should be considered carefully as close tests are usually applied to a dataset comprising many more individuals. In addition, in all the cases where the Stanley and Burnham test did not support closure, the component statistics support that there may have been population losses (not additions) which is consistent with the death or permanent emigration of individuals. If the Stanley and Burnham test is correct, the estimates for the years 2018-2021 may be an overestimation of abundance as the model

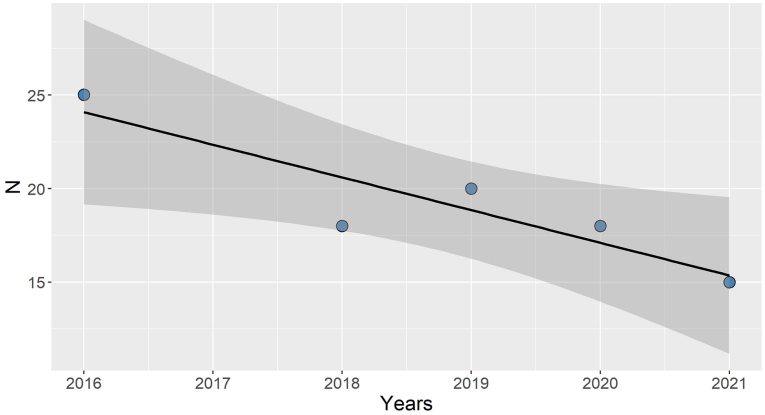

FIGURE 2
Abundance estimates for the common dolphins' local population. Estimates of abundance of the common dolphins, including 95% confidence intervals, from mark-recapture models applied every year. The presented N is the total abundance after dividing the estimated abundance bythe mark ratio. The linear model is presented on the graph.

TABLE 7 Quasi-extinction probabilities.

Quasi-extinct state	Quasi extinction probability		
	Scenario 1	Scenario 2	Scenario 3
q = 2 mature individuals			
After three generations (45 years)	0.39	0.63	0.70
After five generations (75 years)	0.50	0.67	0.75
After 100 years	0.57	0.69	0.77
q = 4 mature individuals			
After three generations (45 years)	0.88	0.84	0.88
After five generations (75 years)	0.89	0.86	0.89
After 100 years	0.89	0.86	0.89
q = 6 mature individuals			
After three generations (45 years)	0.96	0.97	0.97
After five generations (75 years)	0.96	0.97	0.97
After 100 years	0.96	0.97	0.97

Quasi-extinction probabilities after three generations (45 years), five generations (75 years) and 100 years for different thresholds for extinction (2, 4 and 6 mature individuals).

includes the present individuals (birth and immigration) but does not account for the ones that are no longer in the area (deaths or emigration). However, the characteristics of the local population, such as discovery curve, high resighting rates, high site fidelity, and no new additions, fit the ones of a closed population, and therefore these models were applied.

The common dolphin local population along the Israeli coastline was found to be small (< 50), similar to other "inner" Mediterranean local populations (Bearzi et al., 2021; Vella et al., 2021). Given the relatively high yearly site fidelity of the common dolphins and the stabilization of their discovery curve, an assumption can be made that most of the individuals within this local population were identified during the study period, which suggests that the local population in Israel is a small group of year-round residents. Similar abundance patterns have been observed in the Mediterranean as described before (Santostasi et al., 2016; Bearzi et al., 2020) and could result from similar environmental conditions and anthropogenic pressures. The size of the local population during the study years (15 – 25) meet criteria D for a 'Critically Endangered' population as it is lower than 50 individuals (IUCN, 2012b).

An alarming decrease in the local population's size was observed in the study area during the five-year study period, with a 40% decline in size since 2016. Historical data from occasional observations describe larger groups of common dolphins (22.2 ± 19.1, range 1 – 75) ranging over a more extensive habitat, from Ashdod to Herzliya (Kerem et al., 2012). Such large groups are no longer observed along the coast, and about half of the individuals observed in 2016 have disappeared from the area during the study period. Based on this trajectory, the complete disappearance of this species from the local waters is expected by 2029. This significant trend cannot

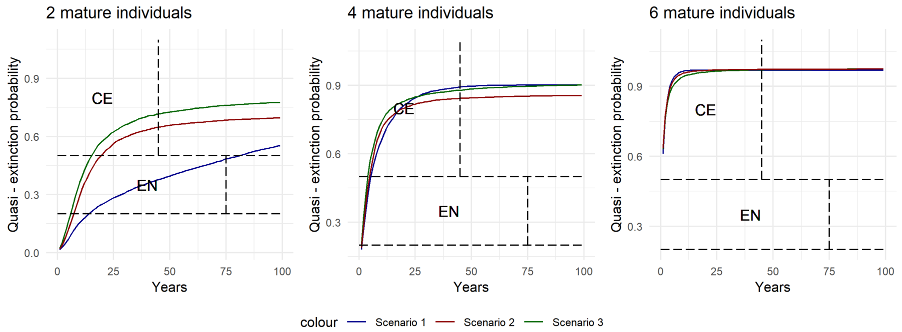

FIGURE 3
Quasi-extinction probability for the common dolphins. Quasi-extinction probability for common dolphins along the Israeli coast for 100 years. The y-axis is the quasi-extinction probability; the x-axis is years. The different scenarios represent increasing levels of growth rates randomness modeled from a normal distribution with increasing standard deviation (from 0 to 0.02).

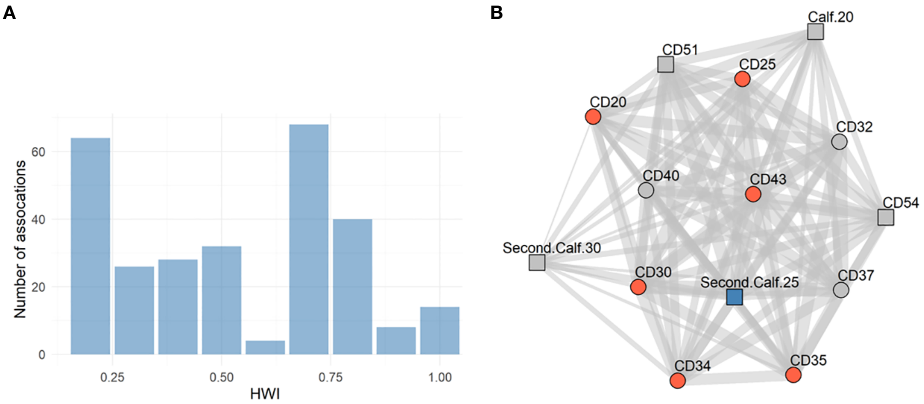

FIGURE 4
(A) Histogram of HWI values. HWI values higher than 0.1 for mature individuals (n = 25). **(B)** Social network of the common dolphins in 2021. The network structure of the local population in 2021 includes calves (n = 14). Red nodes represent females, blue represents a male, and grey represents individuals with unknown sex. Square nodes represent calves, and circles represent adults. The width of the edge represents the strength of the association between the dyad according to the HWI values.

result from decreasing effort as both effort, and capture probability increased along the study years. A similar decline was documented for the common dolphin local population in western Greece, where the local population decreased by 90% throughout 13 years of study (1995 - 2007). This decline was found to be related to prey depletion in the area, resulting from overfishing (Bearzi et al., 2008). This might also be the case for this population, but further studies regarding their prey distribution in the area is needed to test this hypothesis.

Criteria A (population size reduction over ten years or three generations) requires a decline observed, estimated, inferred, or suspected over a more extended period than the period for this study (IUCN, 2012b). The absence of previous abundance estimates for this local population and the low power of abundance estimates based on small population size (Taylor et al., 2007a; Santostasi et al., 2016) make it harder to meet this criterion adequately. Nonetheless, sharp declines in other local populations of common dolphins all over the Mediterranean Sea has led to a regional classification of this species as Endangered (Bearzi et al., 2003; Bearzi et al., 2021). These observed declines, together with the precautionary principle promoted by the IUCN Red List (Mace and Stuart, 1994), support the assumption that the decline observed in the local population in Israel (40% over five years) might be similar and even stronger than the declines observed throughout the Mediterranean Sea (50% decline over three generations; Bearzi, 2012). Criteria C (small population size and decline) requires an abundance of fewer than 250 individuals and a decline of 25% or more in three years or one generation. The observed abundance and decline meet this criterion. Therefore, the local Israeli population should be defined as 'Critically Endangered' under Red List Criteria C and as 'Endangered' under Red List criteria A.

Probability of extinction in the wild

The majority of simulated scenarios yielded a ≥ 50% probability of quasi-extinction (Table 7) for every time interval tested (45, 75, and 100 years). Furthermore, the scenario with zero temporal growth rate stochasticity also showed a ≥ 50% probability of quasi-extinction after five generations and 100 years, suggesting that the local population is at high extinction risk even in the most optimistic scenario. Therefore, the local population should be defined as Critically Endangered under criteria E (quantitative analysis).

Social structure

In light of the reduction of the local population size between 2016 and 2020, as half of the local population disappeared from the area, it is likely that the common dolphin local population's social structure changed during this time. Due to the low sample size in the early years of the study (2016 and 2018), it is hard to describe the social structure at the beginning of the study as the associations could not be measured with so few observations. In 2021, the local population was composed of nine mature individuals who maintain long-lasting and strong associations and are observed almost exclusively together in one social unit along with five calves (all above one year old).

This group of nine mature individuals and five calves were observed together more than 25 times during the last two years of the study in 72.4% of the observations. As these are the only individuals known to inhabit the area, they appear to maintain a strongly associated close social unit with high association indices. According to Vella et al. (2021), only 16 peer-reviewed studies have used photo identification to study common dolphins worldwide. From these, only three studied the social structure of a specific local population. Therefore, not much is known about common dolphin societies, and what is known varies greatly between populations. For example, in the eastern Ionian Sea, the common dolphin local population range over large areas of the neritic zone and present a fission-fusion social network with little evidence of long-term associations (Bruno et al., 2004). Similarly, the common dolphin local population in Hauraki Gulf, a semi-enclosed coastal body of temperate water located on the north-eastern coastline of the North Island, New Zealand, also presented a fluid social structure with a low mean association rate and very few long-lasting associations (Hupman, 2016). In contrast, the common dolphin local population's social structure in the Tyrrhenian Sea contains a core group of 12 females that formed long-lasting associations over five years (Pace et al., 2009; Mussi et al., 2019), indicating a highly associated closed group.

The latter presents a more similar structure to the one in Israel, comprised mostly of females. However, two males were stranded ashore in 2020. Before his death, one of these males was part of the social unit and maintained strong associations with females, indicating a mixed-sex social unit. The social structure of the local population could reflect their level of isolation as observed in the bottlenose dolphin local population in Doubtful Sound (Lusseau et al., 2003), or the utilization of a specific resource as observed in the bottlenose dolphins local population in the north Adriatic Sea (Genov et al., 2019). It could also be the consequence of extensive segregation of the common dolphins in the Mediterranean, resulting in a separation into several small local populations (Bearzi and Genov, 2021).

Threats and conservation

The proximity of the Israeli common dolphin local population to the coast puts them in a constant state of vulnerability to human pressure. Several main threats have been identified in the Mediterranean Sea as the causes of the decrease in abundance of this species: historical culling and killing, bycatch in fishing gear, prey depletion, and contamination/pollution (Bearzi and Genov, 2021; Vella et al., 2021). In Israel, there is no historical data about killing of dolphins, but it can be assumed that the massive killing in the Mediterranean led to the segregation of the Mediterranean sub-population, resulting in separation to several small local populations, such as the one in Israel (Bearzi and Genov, 2021). Throughout the study years, two common dolphins have stranded ashore, showing clear signs of entanglement and drowning, thus indicating the presence of entanglement threat to this local population as well, as several fishing methods are used in their habitat, including nets, lines, and bottom trawlers.

Common dolphins are often observed feeding around trawlers while the net is being pulled back to the boat. Stomach content analysis revealed that their diet includes several species targeted or bycaught by the trawler industry (Brand et al., 2019), such as *Ariosoma balearicum*, which was found to be among the main prey species of common dolphins in Israel (Brand et al., 2019). This species is not abundant in the common dolphins' diet in the Alboran Sea or the eastern Ionian Sea (Bearzi et al., 2006; Giménez et al., 2018), while the most abundant prey species in these areas are less common in the local population diet. The bottlenose dolphins in Israel also prey on *Ariosoma balearicum*, often from the trawler's net as well. This behavior and prey preferences of the coastal dolphin species in Israel could indicate that the trawling industry is an important food resource in their diet (Scheinin et al., 2014; Brand et al., 2019). Further research on the abundance of common prey species of common dolphins in the area, as done in western Greece (Bearzi et al., 2008) is needed in order to understand the differences from other areas in the Mediterranean.

Contamination through the food web and pollution could also pose a threat to the local population as they inhabit an area with a busy port, power station, desalination, and sewage spill but the effects of these on the dolphins in the area are still unknown.

The Marine Protected Area, 'Evtach', is approved in part of the common dolphins' habitat. Once declared, it will decrease the interaction rate between dolphins and commercial fishing boats and encourage public awareness and enforcement of conservation measures for this species. Whether it will improve the lives of the common dolphins in the area is remained to be discovered, but a change in their IUCN status will help promote the declaration of this MPA and the importance of their conservation in the area.

Conclusions

The local population of common dolphins in Israel presents a similar decline to other local populations in the inner Mediterranean Sea. Even with insufficient historical data, a clear trend is observed throughout the study years. These dolphins face a challenging environment and seem unable to adjust appropriately, resulting in almost half of them leaving the area or dying. A strong need for research collaboration with the neighboring countries arises to understand the full-ranging patterns and abundance of common dolphins in the Eastern Levantine Sea. The risk assessment provided in this study places the common dolphin local population in Israel as 'Critically Endangered' under criteria C, D, and E and as 'Endangered' under criteria A. Only one of these criteria needs to be met to reconsider the risk assessment of the local population (IUCN, 2012b). We highly recommend expediting this decision to promote the importance of this species conservation among the local influencing factors and the public. In addition, we encourage collaborations to construct innovative conservation actions to prevent the final disappearance of common dolphins from the most eastern part of the Mediterranean Sea.

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 this animal study because the study participants adhered to the current national regulations related to observational studies of this nature in Israel. As there was no physical sampling or disturbance to the dolphins during the observational surveys, no ethical permits were required.

Author contributions

AS has equal contribution. DT and AS devised the project. AS, YM and OG collected the data. YM and NS performed the analysis. OG created the maps. YM wrote 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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.916950/full#supplementary-material

References

Abd Rabou, A. F. N., Abd Rabou, M. A., Qaraman, A. F. A., Abualtayef, M. T., Abd Rabou, A. A., Khalaf, N. A., et al. (2021). Sightings and strandings of the cetacean fauna in the Mediterranean coast of the Gaza strip, Palestine. *Israa Univ. J. Appl. Sci.* 5, 152–186. doi: 10.52865/NUWV7692

Aissi, M., and Vella, A. (2015). Status and conservation of cetaceans in the Sicily Channel/Tunisian plateau. *United Nations Environment Programme/ Mediterranean Action Plan (UNEP/MAP), UNEP (DEPI)/MED WG.408/Inf.19* Tunisia: Regional Activity Centre for Specially Protected Areas (RAC/SPA). Available from: https://www.um.edu.mt/library/oar/bitstream/123456789/44073/1/Status_and_conservation_of_cetaceans_in_the_Sicily_channel_Tunisian_plateau.pdf. doi: 10.4324/9780429052347-62

Akaike, H. (1973). Information theory and an extention of the maximum likelihood principle. *Second International Symposium on Information Theory*, Eds. B. , N. Petrov and F. Csaki (Budapest: Academiai Kiado) 267–281. doi: 10.1007/978-1-4612-0919-5_38

Ansmann, I. C., Parra, G. J., Chilvers, B. L., and Lanyon, J. M. (2012). Dolphins restructure social system after reduction of commercial fisheries. *Anim. Behav.* 84, 575–581. doi: 10.1016/j.anbehav.2012.06.009

Arcangeli, A., Campana, I., and Bologna, M. A. (2017). Influence of seasonality on cetacean diversity, abundance, distribution and habitat use in the western Mediterranean Sea: Implications for conservation. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 27, 995–1010. doi: 10.1002/aqc.2758

Arcangeli, A., Marini, L., and Crosti, R. (2013). Changes in cetacean presence, relative abundance and distribution over 20 years along a trans-regional fixed line transect in the central tyrrhenian Sea. *Mar. Ecol.* 34, 112–121. doi: 10.1111/maec.12006

Bearzi, G. (2012). "Delphinus delphis," in *The IUCN red list of threatened species 2012*. e.T134817215A195829089. 8235. doi: 10.2305/IUCN.UK.2012-1.RLTS.T134817215A195829089.en

Bearzi, G., Agazzi, S., Gonzalvo, J., Costa, M., Bonizzoni, S., Politi, E., et al. (2008). Overfishing and the disappearance of short-beaked common dolphins from western Greece. *Endanger. Species Res.* 5, 1–12. doi: 10.3354/esr00103

Bearzi, G., Bonizzoni, S., and Santostasi, N. L. (2020). "Delphinus delphis (Gulf of Corinth subpopulation) (errata version published in 2021)," in *IUCN red list threat. species 2020 e.T156206333A194321818*, 8235. doi: 10.2305/IUCN.UK.2020-2.RLTS.T156206333A194321818.en

Bearzi, G., and Genov, T. (2021). Imperiled common dolphins of the Mediterranean Sea. in *Imperiled: The Encyclopedia of Conservation*. D. A. DellaSala and M. I. Goldstein (Elsevier Inc.), 837–846. doi: 10.1016/b978-0-12-821139-7.00059-3.

Bearzi, G., Genov, T., Natoli, A., Gonzalvo, J., and Pierce, G. (2021). "Delphinus delphis (Inner Mediterranean subpopulation)," in *IUCN red list threat. species 2021*, 8235. e.T41762A10557372. doi: 10.2305/IUCN.UK.2021-3.RLTS.T189865869A189865884.en%0ACopyright

Bearzi, G., Politi, E., Agazzi, S., and Azzellino, A. (2006). Prey depletion caused by overfishing and the decline of marine megafauna in eastern Ionian Sea coastal waters (central Mediterranean). *Biol. Conserv.* 127, 373–382. doi: 10.1016/j.biocon.2005.08.017

Bearzi, G., Reeves, R. R., Notarbartolo di Sciara, G., Politi, E., Canadas, A., Frantis, A., et al. (2003). Ecology, status and conservation of short-beaked common dolphins delphinus delphis in the Mediterranean Sea. *Mamm. Rev.* 33, 224–252. doi: 10.1046/j.1365-2907.2003.00032.x

Berrow, S., O'Brien, J., Groth, L., Foley, A., and Voigt, K. (2012). Abundance estimate of bottlenose dolphins (Tursiops truncatus) in the lower river Shannon candidate special area of conservation, Ireland. *Aquat. Mamm.* 38, 136–144. doi: 10.1578/AM.38.2.2012.136

Blasi, M. F., and Boitani, L. (2014). Complex social structure of an endangered population of bottlenose dolphins (Tursiops truncatus) in the aeolian archipelago (Italy). *PloS One* 9, e114849. doi: 10.1371/journal.pone.0114849

Brand, D., Edelist, D., Goffman, O., Hadar, N., Scheinin, A., and Kerem, D. (2019). Common dolphins, common in neritic waters off southern Israel, demonstrate uncommon dietary habits. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 15–21. doi: 10.1002/aqc.3165

Bruno, S., Politi, E., and Bearzi, G. (2004). "Social organization of common dolphin community in the eastern Ionian Sea: Evidence of a fluid fission-fusion society," in *European Research on cetaceans*. Eds. P. G. H. Evans and E. O'Boyle (Rome, Italy, European Cetacen Society), 49–51.

Burnham, K. P., and Anderson, D. R. (2002). *Model selection and inference: A practical information-theoretic approach* (New York: Springer).

Cairns, S. J., and Schwager, S. J. (1987). A comparison of association indices. *Anim. Behav.* 35, 1454–1469. doi: 10.1016/S0003-3472(87)80018-0

Cañadas, A., and Hammond, P. S. (2008). Abundance and habitat preferences of the short-beaked common dolphin delphinus delphis in the southwestern Mediterranean: Implications for conservation. *Endanger. Species Res.* 4, 309–331. doi: 10.3354/esr00073

Cooch, E. G., and White, G. C. (2014). *Program MARK: a gentle introduction, 13th ed.* (Fort Collins, Colorado: Colorado State University). Available at: http://www.phidot.org/software/mark/docs/book/.

Cormack, R. M. (1964). Estimates of survival from the sighting of marked animals. *Biometrika* 51, 429–438. doi: 10.1093/biomet/51.3-4.429

Croft, D. P., Johnstone, R. A., Ellis, S., Nattrass, S., Franks, D. W., Brent, L. J. N., et al. (2017). Reproductive conflict and the evolution of menopause in killer whales. *Curr. Biol.* 27, 298–304. doi: 10.1016/j.cub.2016.12.015

Csardi, G. (2020) *Igraph: Network analysis and visualization*. Available at: https://cran.r-project.org/web/packages/igraph/igraph.pdf.

Currey, R. J. C., Dawson, S. M., Slooten, E., Schneider, K., Lusseau, D., Biosseau, O. J., et al. (2009). Survival rates for a declining population of bottlenose dolphins in doubtful sound, new Zealand: an information theoretic approach to assessing the role of human impacts. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 19, 658–670. doi: 10.1002/aqc

FAO (2018). *The State of World Fisheries and Aquaculture 2018 - Meeting the sustainable development goals* (Rome, Italy).

Farine, D. R. (2013). Animal social network inference and permutations for ecologists in r using asnipe. *Methods Ecol. Evol.* 4, 1187–1194. doi: 10.1111/2041-210X.12121

Farine, D. R., and Whitehead, H. (2015). Constructing, conducting and interpreting animal social network analysis. *J. Anim. Ecol.* 84, 1144–1163. doi: 10.1111/1365-2656.12418

Farrag, M. M. S., Ahmed, H. O., TouTou, M. M. M., and Eissawi, M. M. (2019). Marine mammals on the Egyptian Mediterranean coast "Records and vulnerability." *Int. J. Ecotoxicol. Ecobiol.* 4, 8. doi: 10.11648/j.ijee.20190401.12

Fortuna, C. M. (2007). *Ecology and conservation of bottlenose dolphins (Tursiops truncatus) in the north-eastern Adriatic Sea* (University of St Andrews, Scotland). Doctoral dissertation.

Frantzis, A., Alexiadou, P., Paximadis, G., Politi, E., Gannier, A., and Corsini-Foka, M. (2003). Current knowledge of the cetacean fauna of the Greek seas. *J. Cetacean Res. Manage.* 5, 219–232.

Gannier, A. (2005). Summer distribution and relative abundance of delphinids in the Mediterranean Sea. *Rev. d'Ecologie Terre Vie* 60, 223–238.

Gärdenfors, U., Hilton-Taylor, C., Mace, G. M., and Rodriguez, J. P. (2001). The application of IUCN red list criteria at regional levels. *Conserv. Biol.* 15, 1206–1212. doi: 10.1046/j.1523-1739.2001.00112.x

Genov, T., Centrih, T., Kotnjek, P., and Hace, A. (2019). Behavioural and temporal partitioning of dolphin social groups in the northern Adriatic Sea. *Mar. Biol.* 166, 1–14. doi: 10.1007/s00227-018-3450-8

Genov, T., Kotnjek, P., and Centrih, T. (2020). Occurrence of common dolphins (Delphinus delphis) in the gulf of Trieste and the northern Adriatic Sea. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 69–75. doi: 10.1002/aqc.3407

Gero, S., Gordon, J., and Whitehead, H. (2013). Calves as social hubs: Dynamics of the social network within sperm whale units. *Proc. R. Soc B Biol. Sci.* 280. doi: 10.1098/rspb.2013.1113

Gerrodette, T. (1987). A power analysis for detecting trends. *Ecology* 68, 1364–1372. doi: 10.2307/1939220

Giménez, J., Marçalo, A., García-Polo, M., García-Barón, I., Castillo, J. J., Fernández-Maldonado, C., et al. (2018). Feeding ecology of Mediterranean common dolphins: The importance of mesopelagic fish in the diet of an endangered subpopulation. *Mar. Mammal Sci.* 34, 136–154. doi: 10.1111/mms.12442

Gonzalvo, J., and Costa, M. (2016). "Will there be any reward for common dolphin perseverance," in *Report of the 1st international workshop: Conservation and research networking on short-beaked common dolphin (Delphinus delphis) in the Mediterranean Sea*(Ischia Island, Italy, Mediterranean Common Dolphin Working Grou), 41–42. doi: 10.13140/RG.2.1.4801.3047

Hammond, P. S. (2010). "Estimating the abundance of marine mammals," in *Marine mammal ecology and conservation: a handbook of techniques*, I. L. Boyd, W. D. Bowen and S. J. Iverson (Oxford University Press Inc.: New York), 42–67.

Hammond, P. S., Bearzi, G., Bjørge, A., Forney, K., Karczmarski, L., Kasuya, T., et al. (2008). "Delphinus delphis, common dolphin," in *The IUCN Red List of Threatened Species*. 2008: e.T6336A12649851. doi: 10.2305/IUCN.UK.2008.RLTS.T6336A12649851.en

Hupman, K. (2016). *Photo-identification and its application to gregarious delphinids: Common dolphins (Delphinus sp.) in the hauraki gulf, new Zealand* (Albany, New Zeal: Massey Univ). A thesis Submitt. Partial fulfilment Requir. degree Dr. Philos. Mar. Ecol. Massey Univ.

IUCN (2001). *IUCN red list categories and criteria: Version 3.1., ed* (Gland, Switzerland and Cambridge: World Conservation Union), 30. U. K. I. +.

IUCN (2012a). *Guidelines for application of IUCN red list criteria at regional and national levels: Version 4.0* (Gland, Switzerland and Cambridge, UK: IUCN). Available at: www.iucn.org/publications.

IUCN (2012b). *IUCN red list categories and criteria: Version 3.1 second edition* (Gland, Switzerland and Cambridge, UK: IUCN).

Jolly, G. M. (1965). Explicit estimates from capture-recapture data with both death and immigration - stochastic model. *Biometrika* 52, 225–247. doi: 10.1093/biomet/52.1-2.225

Kerem, D., Hadar, N., Goffman, O., Scheinin, A., Kent, R., Boisseau, O., et al. (2012). Update on the cetacean fauna of the Mediterranean levantine basin. *Open Mar. Biol. J.* 6, 6–27. doi: 10.2174/1874450801206010009

Laake, J. L. (2013) *RMark: An r interface for analysis of capture-recapture data with MARK.* Available at: https://cran.r-project.org/web/packages/RMark/RMark.pdf.

Lusseau, D., Schneider, K., Boisseau, O. J., Haase, P., Slooten, E., and Steve, M. D. (2003). The bottlenose dolphin community of doubtful sound features a large proportion of long-lasting associations: Can geographic isolation explain this unique trait? *Behav. Ecol. Sociobiol.* 54, 396–405. doi: 10.1007/s00265-003-0651-y

Mace, G. M., and Stuart, S. N. (1994). Draft IUCN red list categories, version 2.2. *Species*, 13–24.

Marco, S. (2017) *Delphis; marine surveys platform*. Available at: https://www.shlomimarco.com/delphis.

Milani, C., Vella, A., Vidoris, P., Christidis, A., and Koutrakis, E. (2021). Abundance, distribution and diet of the common dolphin, delphinus delphis, in the northern Aegean Sea (Greece). *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 76–86. doi: 10.1002/aqc.3081

Morris, W. F., and Doak, D. F. (2002). *Quantitative conservation biology*. Sinauer, Sunderland, Massachusetts, USA

Moura, A. E., Natoli, A., Rogan, E., and Hoelzel, A. R. (2013). Atypical panmixia in a European dolphin species (Delphinus delphis): Implications for the evolution of diversity across oceanic boundaries. *J. Evol. Biol.* 26, 63–75. doi: 10.1111/jeb.12032

Mussi, B., Vivaldi, C., Zucchini, A., Miragliuolo, A., and Pace, D. S. (2019). The decline of short-beaked common dolphin (Delphinus delphis) in the waters off the island of ischia (Gulf of Naples, Italy). *Aquat. Conserv. Mar. Freshw. Ecosyst*. 31, 87–100. doi: 10.1002/aqc.3061

Natoli, A., Cañadas, A., Vaquero, C., Politi, E., Fernandez-Navarro, P., and Hoelzel, A. R. (2008). Conservation genetics of the short-beaked common dolphin (Delphinus delphis) in the Mediterranean Sea and in the eastern north Atlantic ocean. *Conserv. Genet.* 9, 1479–1487. doi: 10.1007/s10592-007-9481-1

Nelson, G. A. (2019) *Fishmethods: Fishery science methods and models in r*. Available at: https://cran.r-project.org/web/packages/fishmethods/fishmethods.pdf.

Notarbartolo di Sciara, G., and Birkun, A. J. (2010). *Conserving whales, dolphins and porpoises in the Mediterranean and black seas: an ACCOBAMS status report* (Monaco, ACCOBAMS), 212.

Otis, D. L., Burnham, K. P., White, G. C., and Anderson, D. R. (1978). Statistical inference from capture data on closed animal populations. *Wildl. Monogr.* 62, 3–135. doi: 10.1093/analys/31.6.177

Pace, D. S., Mariani, M., Miragliuolo, A., Venier, M., and Mussi, B. (2009). "Preliminary analysis of the social structure of the short-beaked common dolphin (Delphinus delphis) in the tyrrhenian Sea, Italy," in *Proceedings of the 23rd annual conference of the European cetacean society, Istanbul, Turkey*. Eds. C. Vincent, G. J. Pierce, A. A. Öztürk, P. Kotnjek, M. Siemensma and A. Tonay (Istanbul, Turkey: Türk Deniz Aras¸tırmaları Vakfı), 176–177.

Pace, D. S., Mussi, B., Vella, A., Vella, J., Frey, S., Bearzi, G., et al. (2016). *Conservation and research networking on short-beaked common dolphin (Delphinus delphi) in the Mediterranean Sea* (Ischia Island, Italy, Mediterranean Common Dolphin Working Group). 13–15 doi: 10.13140/RG.2.1.4801.3047

Parra, G. J., Corkeron, P. J., and Marsh, H. (2006). Population sizes, site fidelity and residence patterns of Australian snubfin and indo-pacific humpback dolphins: Implications for conservation. *Biol. Conserv.* 129, 167–180. doi: 10.1016/j.biocon.2005.10.031

Pons, M., De Stephanis, R., Verborgh, P., and Genovart, M. (2022). Sharp decreases in survival probabilities in the long-finned pilot whales in strait of Gibraltar. *Mar. Biol.* 169, 1–9. doi: 10.1007/s00227-022-04030-1

R Core Team (2020) *R: A language and environment for statistical computing*. Available at: http://www.r-project.org/.

Santoro, R., Sperone, E., Tringali, M., Pellegrino, G., Giglio, G., Tripepi, S., et al. (2015). Summer distribution, relative abundance and encounter rates of cetaceans in the Mediterranean waters off southern Italy (western Ionian Sea and southern tyrrhenian Sea). *Mediterr. Mar. Sci.* 16, 613–620. doi: 10.12681/mms.1007

Santostasi, N. L., Bonizzoni, S., Bearzi, G., Eddy, L., and Gimenez, O. (2016). A robust design capture-recapture analysis of abundance, survival and temporary emigration of three odontocete species in the gulf of Corinth, Greece. *PloS One* 11, e0166650. doi: 10.1371/journal.pone.0166650

Santostasi, N. L., Bonizzoni, S., Gimenez, O., Eddy, L., and Bearzi, G. (2018). Common dolphins in the gulf of Corinth are critically endangered. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 31, 101–109. doi: 10.1002/aqc.2963

Scheinin, A. P. (2010). *The population of bottlenose dolphins (Tursiops truncatus), bottom trawl catch trends and the interaction between the two along the Mediterranean continental shelf of Israel* (Israel, University of Haifa).

Scheinin, A. P., Kerem, D., Lojen, S., Liberzon, J., and Spanier, E. (2014). Resource partitioning between common bottlenose dolphin (Tursiops truncatus) and the Israeli bottom trawl fishery? assessment by stomach contents and tissue stable isotopes analysis. *J. Mar. Biol. Assoc. United Kingdom* 94, 1203–1220. doi: 10.1017/S0025315414001015

Schwarz, C. J., and Seber, G. A. F. (1999). Estimating animal abundance: Review III. *Stat. Sci.* 14, 427–456. doi: 10.1214/ss/1009212521

Seber, G. A. F. (1965). A note on the multiple-recapture census. *Biometrika* 52, 249–259. doi: 10.1093/biomet/52.1-2.249

Seber, G. A. F. (1982). *The estimation of animal abundance and related parameters*. (*2nd*) Ed. Arnold (London: Charles Griffin).

Shane, S. H. (1990). "Behavior and ecology of the bottlenose dolphin at sanibel island, Florida," in *The bottlenose dolphin*. Eds. R. R. Reeves and S. Leatherwood (San Diego: Academic Press), 245–265.

Shinohara, M., Domingo-Roura, X., and Takenaka, O. (1997). Microsatellites in the bottlenose dolphin tursiops truncatus. *Mol. Ecol.* 6, 695–696. doi: 10.1046/j.1365-294X.1997.00231.x

Smolker, R. A., Richards, A. F., Connor, R. C., and Pepper, J. W. (1992). Sex differences in patterns of association among Indian ocean bottlenose dolphins. *Behaviour* 123, 38–69. doi: 10.1163/156853992X00101

Stanley, T. R., and Richards, J. D. (2004). *CloseTest: a program for testing capture-recapture data for closure*. US Geological Survey, Fort Collins Science Center

Taylor, B. L., Chivers, S. J., Larese, J., and Perrin, W. F. (2007a). *Generation length and percent mature estimates for IUCN assessments of cetaceans*. (National Marine Fisheries Service, Southwest Fisheries Science Center)

Taylor, B. L., Martinez, M., Gerrodette, T., Barlow, J., and Hrovat, Y. N. (2007b). Lessons from monitoring trends in abundance of marine mammals. *Mar. Mammal Sci.* 23, 157–175. doi: 10.1111/j.1748-7692.2006.00092.x

Tenan, S., Hernández, N., Fearnbach, H., de Stephanis, R., Verborgh, P., and Oro, D. (2020). Impact of maritime traffic and whale-watching on apparent survival of bottlenose dolphins in the strait of Gibraltar. *Aquat. Conserv. Mar. Freshw. Ecosyst.* 30, 949–958. doi: 10.1002/aqc.3292

Thomas, J. A., Fisher, S. R., Ferm, L. M., and Holt, R. S. (1986). "Behaviour of whales in relation to management," in *Reports of the international whaling commission (Special issue)*. Ed. G.P. Donovan (International Whaling Commission The Red House, Cambridge, U.K.), 139–148.

Vella, A. (2004). *Common dolphins (Delphinus delphis) status in the central and southern Mediterranean around the Maltese Islands*. in 18th Annual Conference on Common Dolphins : Current Research, Threats and Issues (Kolmarden: European Cetacen Society), 4–12.

Vella, A., Murphy, S., Giménez, J., de Stephanis, R., Mussi, B., Vella, J. G., et al. (2021). The conservation of the endangered Mediterranean common dolphin (Delphinus delphis): Current knowledge and research priorities. *Aquat. Conserv. Mar. Freshw. Ecosyst.*, 31, 1–27. doi: 10.1002/aqc.3538

Whitehead, H. (2008a). *Analyzing animal societies: Quantitative methods for vertebrate social analysis* (Chicago; University of Chicago Press). doi: 10.7208/9780226895246

Whitehead, H. (2008b). Precision and power in the analysis of social structure using associations. *Anim. Behav.* 75, 1093–1099. doi: 10.1016/j.anbehav.2007.08.022

Whitehead, H., and Parijs, S. V. (2010). "Studying marine mammal social systems," in *Marine mammal ecology and conservation: a handbook of techniques*. Eds. I. L. Boyd, W. D. Bowen and S. J. Iverson (Oxford: Oxford University Press), 263–282.

Wilson, B., Hammond, P. S., and Thompson, P. M. (1999). Estimating size and assessing trends in a coastal bottlenose dolphin population. *Ecol. Appl.* 9, 288–300. doi: 10.1890/1051-0761(1999)009[0288:ESAATI]2.0.CO;2

Würsig, B., and Würsig, M. (1977). The photographic determination of group size, composition, and stability of coastal porpoises (Tursiops truncatus). *Sci. (80-.).* 198, 755–756. doi: 10.1126/science.198.4318.755

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Morganella morganii septicemia and concurrent renal crassicaudiasis in a Cuvier's beaked whale (*Ziphius cavirostris*) stranded in Italy

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Information regarding bacterial diseases in Cuvier's beaked whale (CBW, *Ziphius cavirostris*) is scattered and mostly incomplete. This report describes a case of septicemia by *Morganella morganii* in a juvenile male CBW with concurrent renal crassicaudiasis. The animal stranded along the Ligurian coastline (Italy) and underwent a systematic post-mortem examination to determine the cause of death. Histopathology showed lesions consistent with a septicemic infection, severe meningoencephalitis, and renal crassicaudiasis. An *M. morganii* alpha-hemolytic strain was isolated in pure culture from liver, lung, prescapular lymph node, spleen, hepatic and renal abscesses, and central nervous system (CNS). The antimicrobial susceptibility profile of the strain was evaluated with the minimum inhibitory concentrations (MICs) method and reduced susceptibility to Trimethoprim-Sulfamethoxazole is reported. Crassicauda sp. nematodes were retrieved from both kidneys. No other pathogens were detected by immunohistochemistry, serology, or biomolecular analyses. Toxicological investigations detected high concentrations of immunosuppressant pollutants in the blubber. The chronic parasitic infestation and the toxic effects of xenobiotics likely compromised the animal's health, predisposing it to an opportunistic bacterial infection. To our knowledge, this is the first description of *M. morganii* septicemia with CNS involvement in a wild cetacean.

KEYWORDS

Cuvier's beaked whale, *Morganella morganii*, *Crassicauda*, antimicrobial resistance, septicemia, pollutants(environmental)

Introduction

The Ligurian sea is listed as a high-density area for Cuvier's beaked whales CBW (*Ziphius cavirostris)*, the only commonly-observed beaked whale in the Mediterranean Sea (Podestà et al., 2006). CBW's Mediterranean sub-population is listed as "Vulnerable" on the International Union for the Conservation of Nature (IUCN) Red List (Cañadas and Notarbartolo di Sciara, 2018). Being an elusive, deep diver predator, this odontocete is difficult to study in the field and most of the available biological data derive from strandings (Podestà et al., 2006; Heyning and Mead, 2009; Podestà et al., 2016; Carlucci et al., 2020).

Infectious diseases reported in stranded CBW include viral infection by alpha herpesvirus (Arbelo et al., 2010) or morbillivirus (Centelleghe et al., 2017; Felipe-Jiménez et al., 2022), crassicaudiasis (Díaz-Delgado et al., 2016; Febronio et al., 2021), and bacterial diseases (Alstrup et al., 2021; Febronio et al., 2021), including septicemia by *Citrobacter freundii* (Fernández et al., 2011).

Parasitic disease due to *Crassicauda* spp. has been increasingly reported as a significant cause of death in beaked whales (Díaz-Delgado et al., 2016; Febronio et al., 2021; Jerdy et al., 2022) and other cetaceans (Balbuena and Simpkin, 2014) worldwide. For most of the 14 species of the genus, life cycle and transmission are still unclear. Since other marine spirurids usually require intermediate hosts (Anderson, 1988), an indirect cycle involving crustaceans, cephalopods and fishes has been speculated (Lambertsen, 1986; Marcer et al., 2019). However direct transmission cannot be discarded as larvae and/or eggs have been reported in milk (Geraci et al., 2011) and urine (Lambertsen, 1986; Febronio et al., 2021) and transplacental infection has been documented as well (Lambertsen, 1986; Suárez-Santana et al., 2018). These nematodes show different tissue tropisms (urogenital, vascular, integumentary, respiratory) and pathogenic potential, playing a regulatory role in some marine mammal populations (Balbuena and Simpkin, 2014). *Crassicauda boopis* causes severe renal lesions in baleen whales (Lambertsen, 1986; Marcer et al., 2019), *C. grampicola* is associated with moderate to severe sinusitis in Risso's dolphins (*G. griseus*) (Cuvertoret-Sanz et al., 2020) whereas *C. anthonyi* and *C. magna* migration produces verminous arteritis and chronic renal disease in beaked whales (Díaz-Delgado et al., 2016; Febronio et al., 2021; Jerdy et al., 2022). Moreover, several species of crassicaudid nematodes, including *C. grampicola* (Geraci et al., 2011) and C. *fuelleborni* (Kot et al., 2022) produce parasitic mastitis impacting the reproductive success of endangered cetaceans.

Furthermore, a chronic parasitic infestation may favor infection by opportunistic bacteria, such as members of the family *Enterobacteriaceae* (Paterson and Mathers, 2020), disrupting tissue integrity, translocating pathogens, and/or modulating the immune system of the host (Ashour and Othman, 2020).

Morganella morganii is a gram-negative bacillus, belonging to the *Enterobacteriaceae* family, found in the environment and the digestive tract of humans and animals. It is considered an unusual opportunistic pathogen in animals and nosocomial infections, frequently isolated from the urinary tract or skin wounds (Liu et al., 2016). Its zoonotic potential should not be underestimated, especially in hosts with compromised health status, considering the relatively high mortality rate in human hospitals and the emergence of virulent antimicrobial resistance (AMR) strains.

M. morganii has been identified as a pathogen in ocular lesions in pinnipeds (Thornton et al., 1998), and in two bottlenose dolphins (*Tursiops truncatus*) under human care (Elfadl et al., 2017; Sánchez Contreras and Biancani, 2021). In both cases, the strain exhibited resistance against several antibiotic classes, including new-generation cephalosporins, and in one case was linked to a fatal infection, causing fibrino-hemorrhagic bronchopneumonia and septicemia.

In cetaceans, higher concentrations of *M. morganii* were cultured from diseased or stranded animals compared with the relatively low numbers isolated from their free-ranging counterparts (Martineau et al., 2003; Buck et al., 2006). Moreover, the presence of high numbers of opportunistic pathogens, including *M. morganii* has been linked to immunosuppression in beluga whales (*Delphinapterus leucas*) living in the polluted waters of the Saint Lawrence Estuary (SLE) in Canada (Martineau et al., 2003).

Here we describe the findings of the post-mortem examination and the advanced diagnostic investigations performed on a juvenile male CBW stranded in Italy to determine the cause of death. A case of septicemia caused by *M. morganii* with concurrent renal crassicaudiasis is reported in a threatened cetacean inhabiting the highly polluted waters of the Mediterranean Sea. This study provides valuable information for the conservation of this species and strengthens the role of marine mammals as sentinels for human and ecosystem health (Bossart, 2011).

Materials and methods

Post mortem examination

On October, 3rd 2020, a CBW (IZS number 70726/20) stranded in Sanremo (Italy), along the Ligurian coastline of the Pelagos Sanctuary. A complete field post-mortem examination was performed according to standard protocols (Geraci and Lounsbury, 2005). The animal was a juvenile-subadult male of 498 cm (total length, TL) in good nutritional status, and in moderate decomposition (code 3 – Figure 1A). Blubber thickness was 6 cm. This animal did not display any evidence of interaction with fishing activities, and the stomach content was scarce, consisting of a few, highly-digested cephalopod beaks and lenses. Photo-identification (ID) analysis resulted in a positive match of an individual that was photo-identified in the Ligurian Sea in 2017 and 2018 (CIMA Foundation database, ID: 31705302). Age at first identification was estimated to be 3 or 4yo (weaned, totally brown colored, poorly marked). Photo-ID life history data and natural markings (from marking gain rate; Rosso et al., 2011) suggested an estimated age at death of about seven years.

The necropsy was performed in the field under high environmental temperatures and, due to logistic issues, there was a significant delay between carcass retrieval, visual evaluation of the organs, and sampling. The tissue samples of all the major organs and lesions were collected and split into aliquots for subsequent analyses as previously described (Giorda et al., 2021). Blood serum, aqueous humor, and cerebrospinal fluid (CSF) were collected and kept frozen at -20°C for serological investigations. Parasites were collected in 70% ethanol and morphologically identified according to taxonomic criteria proposed by Anderson et al., 2009.

Histopathology and immunohistochemistry

Representative tissues (brain, tonsils, lung, prescapular and tracheobronchial lymph nodes, heart, liver, spleen, pancreas, intestine, skeletal muscle, skin, kidney, urinary bladder, adrenal gland, mesenteric artery, and reproductive system) were collected and fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned at 4 ± 2 µm, stained with hematoxylin and eosin (H&E) and examined through a light microscope.

Immunohistochemistry (IHC) for *Morbillivirus* was performed on tissue sections using a monoclonal anti-*Canine Distemper Virus* (CDV) antibody (VMRD) (Di Guardo et al., 2010). *Toxoplasma gondii* IHC was carried out on the brain tissues, using a polyclonal serum of caprine origin (VMRD) (Di Guardo et al., 2010).

Microbiology

Tissue samples including brain, lung, lymph nodes, liver, and spleen were processed for standard aerobic, anaerobic, and microaerobic (5% CO2) bacterial culture and identification, by biochemical analyses (VITEK® MS, bioMérieux SA, Marcy l'Etoile, France) and matrix-assisted laser desorption ionization-time-of-flight mass spectrometry (MALDI-TOF MS, Bruker Daltonics, Bremen,

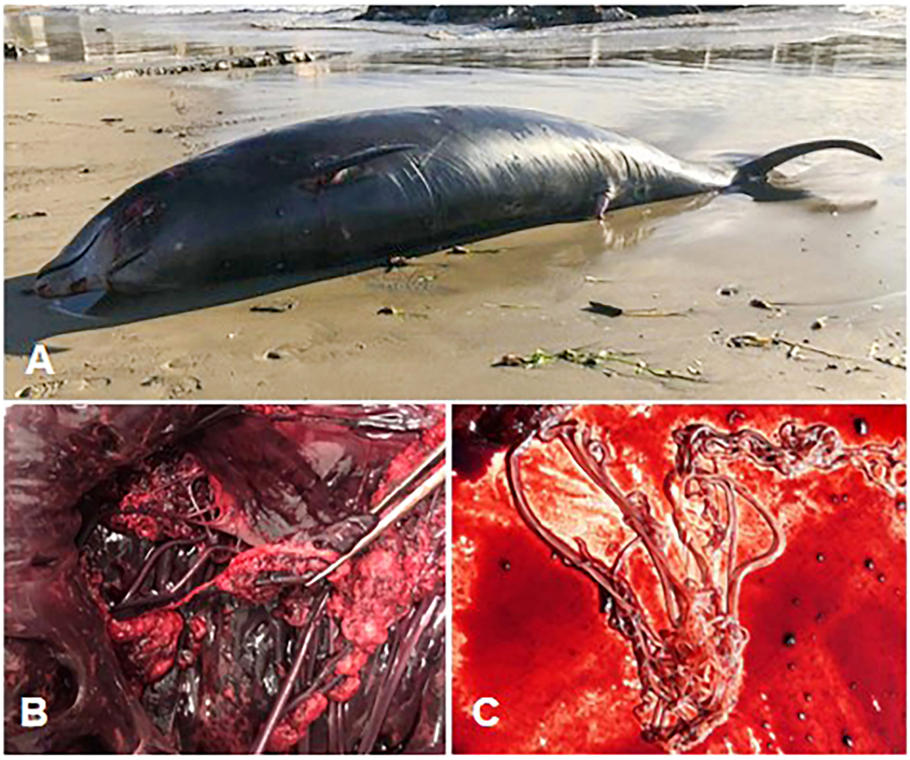

FIGURE 1
(A) Cuvier's beaked whale carcass before retrieval. **(B)** Right atrioventricular valve - fibrinous valvular endocarditis. **(C)** Adult *Crassicauda* spp. nematodes retrieved from the renal parenchyma for parasitological analysis.

Germany). Following international recommendations (World Organisation for Animal Health (WOAH), 2018), samples from target tissues underwent specific bacteriological procedures to screen for *Salmonella* spp., *Listeria* spp., and *Brucella* spp.

Urease test on *M. morganii* grown on Blood Agar Base (Liofilchem srl, Roseto degli Abruzzi, Teramo, Italy) was performed according to the manufacturer's instruction (Microbiol srl, UTA, Cagliari, Italy). Intermediate checks of the vials occurred at 30 minutes, 60 minutes, and 4 hours.

To detect and evaluate biofilm formation by *M.morganii*, the Safranin Staining (SS) was performed as previously described (Ceri et al., 2001; Olson et al., 2002). Briefly, the bacteria were grown into Tryptone Soy Broth (Microbiol srl, UTA, Cagliari, Italy) added with 2% Glucose (Microbiol srl, UTA, Cagliari, Italy) aerobically at 37°C overnight, then resuspended in the same medium at a bacterial concentration of 10^6 CFU/ml and 2ml were dispensed in a 24-wells plate. The plate was incubated aerobically at 37°C overnight. The wells were washed three times with Phosphate Buffered Saline (PBS, Sigma-Aldrich Merck KGaA, Darmstadt, Germany) and then stained with 2 ml of Safranin 1% (Merck KGaA, Darmstadt, Germany) for 1 minute, due to its affinity to biofilm matrix components (polysaccharides, proteins, lipids, and nucleic acids). After overnight incubation aerobically at 37°C, the wells were washed with Acetic Acid 30% (CH3COOH, Sigma-Aldrich Merck KGaA, Darmstadt, Germany) to remove biofilm and 200 µL of well content were dispensed in triplicate into 96 wells-microplate and read spectrophotometrically at a wavelength of 492 nm. *Pseudomonas aeruginosa* ATCC27853 and *Escherichia coli* ATCC 25922 were used as positive control (strong biofilm former) and negative control (no biofilm former), respectively.

Antibiotic susceptibility testing

Antibiotic susceptibility of the cryopreserved *M. morganii* strain was tested using the minimum inhibitory concentrations (MICs) method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2018b). Quality controls of the plates used for MIC were performed according to Table 5 of the CLSI VET01S supplement (CLSI, 2020). MIC breakpoints (expressed in µg/mL) were evaluated and interpretative criteria were retrieved from both human (CLSI, 2018b; CLSI, 2018a) and veterinary CLSI Standards (CLSI, 2020) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (The European Committee on Antimicrobial Susceptibility Testing, 2022b).

M. morganii strain was tested with cefotaxime for evaluating the synergistic effects when combined with clavulanic acid, which inhibits ESBL β-lactamases, and cloxacillin, which inhibits AmpC β-lactamases. The Combination Disc Tests (Liofilchem srl, Roseto degli Abruzzi, Italy) are carried out using a 10mm paper disk containing cefotaxime alone or in combination with clavulanic acid, cloxacillin, or both of these inhibitors. The inhibition zone around the cefotaxime disc combined with inhibitors is compared with the zone around the disc without the inhibitors according to EUCAST guidelines (The European Committee on Antimicrobial Susceptibility Testing, 2017).

MIC values were also compared with EUCAST MIC distributions based on collated data from an increasing total of more than 30 000 MIC distributions from worldwide sources (The European Committee on Antimicrobial Susceptibility Testing, 2022a).

When available on the EUCAST website, Epidemiological Cut-off values (ECOFFs) that distinguish microorganisms without (wild type) and with (non-wild type) phenotypically detectable acquired resistance mechanisms to a specific pharmacological active substance, were reported and compared with the values obtained from *M. morganii* MIC tests.

Toxicology

Polychlorinated biphenyls (PCBs), hexachlorobenzene (HCB), and dichlorodiphenyltrichloroethanes (DDTs) were measured in blubber. Data were expressed in mg/kg dry weight (d.w) or mg/kg lipid weight (l.w) if the Extracted Organic Material percentage (MOE%) was considered. Measurements were made according to the Environmental Protection Agency method 8081/8082, with modifications (Marsili and Focardi, 1997). To assess the potential toxicological impact of these POP concentrations in the specimen, two different threshold limits proposed in the literature for PCBs were considered. Kannan et al. (2000) and Jepson et al. (2005) proposed the value of 17.0 mg/kg l.w. of ΣPCB in blubber as the tolerance threshold for no deleterious effects in marine mammals; Helle et al. (1976) and Jepson et al. (2016) prefer a much higher value (41 mg/kg l.w. ΣPCB in blubber) as a toxicity threshold for reproductive impairment in Baltic ringed seals (*Pusa hispida*).

Molecular and serological investigations

Molecular detection of *Dolphin Morbillivirus* (DMV) (Verna et al., 2017), *Herpesvirus* (HV) (VanDevanter et al., 1996), *T. gondii* (Vitale et al., 2013), *Brucella* spp. (Baily et al., 1992) and *Photobacterium damselae sub. damselae* (Osorio et al., 2000) was routinely performed on target tissues.

Serological investigations to screen for the presence of specific antibodies against DMV, *Brucella* spp. and *T. gondii* (Di Guardo et al., 2010) were performed on serum, CSF, and aqueous humor.

To corroborate bacterial ID obtained by MALDI-TOF MS, the MicroSEQ™ 500 16S rDNA PCR Kit (Thermo Fisher Scientific Inc., Waltham, USA) was used for the amplification of the first 500 base pairs(bp) of the 16S ribosomal RNA gene (rDNA) of the *M. morganii* isolate. DNA extracts were made from pure cultures of *M. morganii* by thermal lyses in PrepMan™ Ultra solution (Thermo Fisher Scientific Inc., Waltham, USA) following protocol for gram-negative bacteria described by the manufacturer. Positive samples were subjected to Sanger sequencing reaction using the MicroSEQ™ 500 16S rDNA Sequencing Kit (Thermo Fisher Scientific Inc., Waltham, USA). Sequencing products were analyzed on the Applied Biosystems® Sanger Sequencing 3500 Series Genetic Analyzers (Thermo Fisher Scientific Inc., Waltham, USA). Electropherograms were processed with the Bioedit 7.2.5 software (Hall, T.A. 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98/NT. Nucl. Acids. Symp. Ser. 41:95-98.) and the sequences (forward and reverse) were aligned to obtain a *consensus* sequence. The *consensus* sequence was uploaded to the GenBank® server and compared with available sequences retrieved from the National Center for Biotechnology Information (NCBI) database through the Basic Local Alignment Search Tool (BLAST, http://blast.ncbi.nlm.nih.gov/Blast.cgi, accessed on 10/08/2022)

Results

Post-mortem findings

The most significant gross findings at necropsy were observed in the vascular system and the kidneys. The vascular wall of the mesenteric arteries and their branches was hard, thickened with endothelial intraluminal pale-yellow plaques of fibrinous-purulent material, partly calcified. Greyish-yellow fibrinous deposits were attached to the right atrioventricular valve leaflets (fibrinous valvular endocarditis - Figure 1B). Light discoloration of the left myocardium was also observed. Multifocal pyogranulomatous lesions were observed in both kidneys, heavily parasitized by *Crassicauda* sp. nematodes. Multifocal abscess-like lesions of approximately 3 cm in diameter were observed in the perisplenic and perihepatic areas. Red discoloration of the blubber was reported in the cranio-dorsal region and in the melon, alongside multiple, light yellow, abscess lesions of about 0.5 cm in diameter. Upon the opening of the skull, meningeal fibrosis and hemorrhagic CSF were observed.

Renal nematodes were identified as *Crassicauda* sp. according to the morphometrics of the cephalic and terminal portions of adult parasites (Figure 1C).

Although specific investigations could not be performed, there was no evidence of gas emboli in the tissues examined, as a consequence of gas and fat embolic syndrome, known to affect this species in particular circumstances, such as military exercises (Fernández et al., 2005).

Histopathology and immunohistochemistry

Histologically, a mixed inflammatory infiltrate associated with foci of necrosis, partly mineralized, was observed mostly in the tunica media of arterial vessels. In the SNC, severe pyogranulomatous encephalitis associated with mild non-suppurative meningitis was diagnosed (Figure 2D). In all SNC areas, perivascular cuffings and vasculitis were observed (Figures 2A, B). Microabscesses, granulomas, and a mixed inflammatory infiltrate invaded the cerebral parenchyma (Figure 2C).

Multiple hemorrhages were scattered in the pulmonary parenchyma. The bronchial submucosa was filled with a mixed inflammatory infiltrate and the associated blood vessels were congested. In the mucosa of the first gastric chamber, foci of pyogranulomatous inflammation (gastritis) were observed multifocally. In the spleen and lymph nodes, lymphoid follicles were depleted and surrounded by multifocal hemorrhages. In all

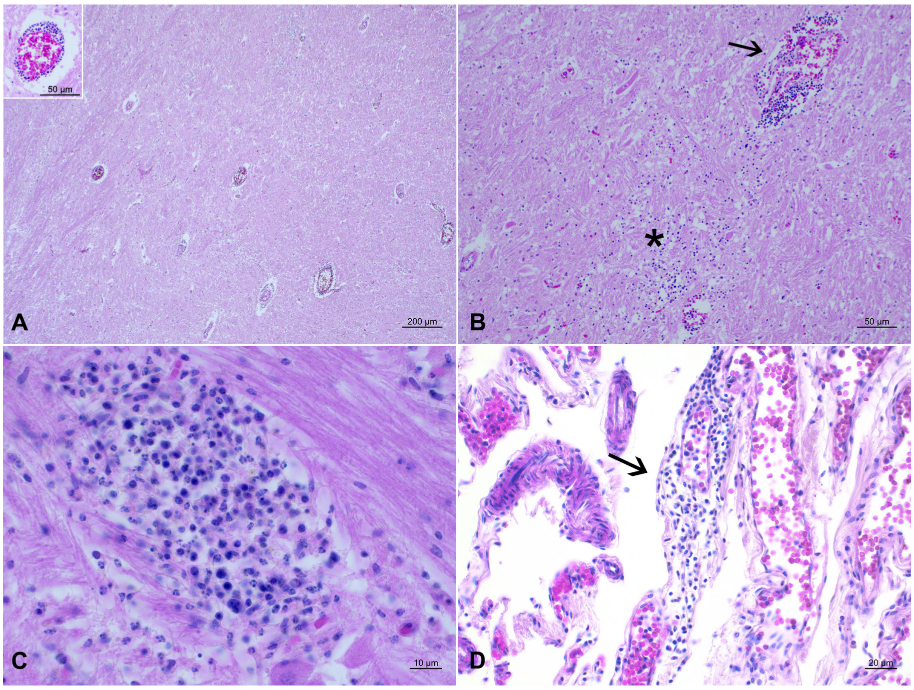

FIGURE 2
Neuropathological lesions observed in the stranded Cuvier's beaked whale. HE. **(A)** Thalamus. Severe encephalitis with several perivascular mononuclear cuffings. Inset: perivascular cuffing characterized by mononuclear cells infiltration. **(B)** Pons. Vasculitis with damage to the vessel wall causing leakage of red blood cells with formation of perivascular hemorrhages and presence of mononuclear inflammatory infiltration around it (arrow) and invading the underlying neuroparenchyma (asterisk). **(C)** Pons. Small granuloma, as an outcome of a microabscess, constituted of macrophages, lymphocytes, and plasma cells. **(D)** Parietal cortex. Focal and mild non-suppurative meningitis (arrow).

these organs, lesions were described alongside changes compatible with mild to moderate autolysis.

Several other internal organs presented with a severe grade of autolysis due to the delay in sampling and unfavorable environmental conditions. Heart, liver, kidney, and urinary bladder could not be evaluated due to advanced autolysis.

Immunohistochemical investigations against DMV and *T. gondii* were negative in all tissues examined.

Microbiology

M. morganii alpha-hemolytic strain was retrieved in pure culture from liver, lung, prescapular lymph node, spleen, hepatic and renal abscesses, and CNS. *Pasteurella canis* was also isolated from the lungs. There was no evidence of growth for *Salmonella* sp., *Listeria* spp., and *Brucella* spp.

Urease test performed on freshly cultured *M. morganii* strains gave positive results in less than 30 minutes. SS test revealed that the isolated *M. morganii* strain was a weak biofilm former (*M. morganii* OD Mean = 0.082; Negative control OD Mean = 0.057; Positive control OD Mean = 0.273).

Antibiotic susceptibility testing

The results of the MIC test performed on the *M. morganii* isolate, including both CLSI and EUCAST interpretative criteria (clinical breakpoints), are shown in Table SM1. The obtained MIC values were compared to the ECOFFs (and/or TECOFFs) available on the EUCAST site ("European Committee on Antimicrobial Susceptibility Testing. Data from the EUCAST MIC distribution website, last accessed 11/09/2022". http://www.eucast.org).

The isolate showed natural resistance to the fixed-dose association Ampicillin-Sulbactam and Amoxicillin-Clavulanic Acid, Oxacillin, Ampicillin, Amoxicillin, most of the first- and second-generation Cephalosporins (Cefazolin, Cefalothin, Cefalexin), Macrolides, Lincosamides, Tetracycline, Nitrofurantoin and Colistin as reported in Morganellaceae. Combination Disc Tests for evaluation of Extended-spectrum β-lactamases (ESBLs) and/or AmpC β-lactamase producers gave negative results.

Based on ECOFF and TECOFF records on the EUCAST website, the strain exhibited MIC values suggestive of a non-wild type *M. morganii* for Trimethoprim-Sulfamethoxazole.

Biomolecular and serological investigations

The biomolecular analysis did not detect any DVM, *Herpesvirus*, *T. gondii*, and PDD DNA in target organs. Serological screening for the presence of DMV, *Brucella* spp, and *T. gondii*-specific antibodies retrieved negative results.

Amplification of the 16S rDNA region yielded an amplicon of approximately 530 bp. Sequence alignment with the BLAST tool allowed the identification of *M. morganii* species with homology greater than 99% (99,25%) with the *M. morganii* strain DG56-16 chromosome (accession number CP032295.1). The new sequence was deposited in GenBank® with the accession number OQ096688.

Toxicology

Blubber concentrations of organochlorine (OC) pollutants are reported in Table 1. MOE% was 92,9%. The blubber levels of organochlorine contaminants, in particularly polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethane and related compounds (DDTs), were extremely high reaching concentrations of 77.831 mg/kg l.w and 68.279 mg/kg l.w respectively,

Discussion

M. morganii septicemia

We diagnosed the stranded cetacean with a septicemic infection by *M. morganii*. Our diagnosis is supported by the systemic isolation of the bacteria in all major organs and by both macroscopic and microscopic lesions suggestive of septicemia such as multifocal hemorrhages, multifocal abscesses and granulomas, severe pyogranulomatous encephalitis, and endocarditis.

In human medicine, bacteremia and sepsis are quite common features (Bandy, 2020) of *M. morganii* infections whereas CNS involvement is rare. When the pathogen succeeds in trespassing the hematoencephalic barrier, meningitis and parenchymal abscesses are the main lesions observed (Abdalla et al., 2006). In animals, to date, this bacterium has been mostly associated with respiratory pathologies (Elfadl et al., 2017) and there are no reports of CNS involvement, unlike in this CBW that suffered from both meningitis and encephalitis.

All of these pathological conditions are reported more frequently in patients with underlying diseases and/or immunosuppression (Bandy, 2020). Urinary tract infections (UTIs) are frequently the original foci of the bacteremia, as biofilm formation and bacterial growth in the urinary tract are enhanced by the urease activity of this organism. Other typical sources of bacteremia in humans are soft tissues and hepatobiliary tract infections (Liu et al., 2016).

In this CBW, dermatological disease or deep wounds were not observed. Gross hepatobiliary abnormalities were not reported either, even though autolysis prevented exhaustive histopathological examination. Therefore, we hypothesize that renal crassicaudiasis and a high burden of organic pollutants have been the most likely predisposing factors for an infection by *M. morganii*.

TABLE 1 OCs levels in the blubber of the stranded CBW.

	mg/kg d.w	mg/kg l.w.
HCB	0.396	0.426
DDTs	63.431	68.279
PCBs	72.305	77.831
Total OCs	136.133	146.538

A parasitized organ represents an optimal pabulum for bacterial growth. For instance, Suárez-Santana et al. (2018) described septic prostatitis and ascending cystitis in two spotted dolphins (*Stenella frontalis*) with heavy prostatic and urethral *Crassicauda* infestation, speculating that nematode parasitism might have favored bacterial colonization. Furthermore, our isolate was phenotypically capable of urease and biofilm production, and alpha-hemolytic strains produce a potent hemolysin with hemolytic and leucocidal properties similar to *E.coli* ones (Eberspacher et al., 1990). However, since autolysis did not allow to confirm or exclude a UTI, it is difficult to determine whether *M. morganii* retrogradely colonized the kidney *via* the lower urinary tract or if the pathogen could have been translocated during the parasitic migration from the intestine.

Interestingly, another opportunistic pathogen, *P. canis*, was isolated from the lung. This bacterium is also part of the microbiota of the oral and upper respiratory tract mucosa of animals, especially carnivores (biovar 1) and ruminants (biovar 2), and it has been rarely reported as a cause of pneumonia in patients with lung disease or immunodeficiency (Arun et al., 2019). *P. canis* could have overgrown on existing pulmonary lesions observed in this CBW, worsening the respiratory competence of the animal.

The health status of the animal was also compromised by a high burden of organic pollutants with demonstrated detrimental effects on the immune system (Marsili et al., 2004; Marsili et al., 2019; Centelleghe et al., 2019). Blubber concentrations of organochlorine compounds (OCs), particularly DDTs and PCBs, were very high both compared to the values found in other CBWs sampled in the Mediterranean basin (Hatzianestis et al., 1998; Baini et al., 2020) and compared to those measured in BWs from other parts of the world (Knap and Jickells, 1983; Bachman et al., 2014). The blubber PCBs concentrations in the studied specimen of Cuvier's beaked (77.8 mg/kg l.w.) largely exceeded the PCBs toxicity thresholds reported (Kannan et al., 2000; Jepson et al., 2005; Jepson et al., 2016).

A chronic parasitic infestation and the toxic effects of immunosuppressant xenobiotics represented for this CWB a lethal combination that made the animal poorly immunocompetent, as confirmed histologically by the severe lymphoid depletion, and prone to succumb to a non-negligent opportunistic pathogen as *M. morganii* (Liu et al., 2016).

Antimicrobial susceptibility of the *M. morganii* isolate

Since the isolation of antibacterial-resistant (ABR) strains is also increasing in marine animals (Blasi et al., 2020), we performed additional testing to evaluate the antimicrobial susceptibility profile of our strain.

The isolate was confirmed to be sensitive to the antibiotics normally used to treat *M. morganii* infection: aminoglycosides, third- and fourth-generation cephalosporins, carbapenems, quinolones, chloramphenicol, sulfonamides and their association with trimethoprim.

Differently from our case, resistance to third- and fourth-generation cephalosporins has been previously observed in two *M. morganii* strains cultured from captive cetaceans lesions (Park et al., 2020; Sánchez Contreras and Biancani, 2021). However, ESBLs production was not demonstrated in these cases whereas is increasingly reported in strains related to nosocomial infections. ESBLs and AmpC production has been observed in most *Enterobacteriaceae* species, particularly *E. coli* and *Klebsiella pnuemoniae* (Sheng et al., 2013), and can be transferred to other bacterial species by horizontal gene transfer. Furthermore, apart from being plasmid-mediated, AmpC production can derive from the deregulation of natural genetically-encoded β-lactamase enzymes in response to antibiotic exposure (Mizrahi et al., 2020). As expected in a free-ranging wildlife species, our strain did not show any AmpC enzyme production since it is unlikely for a wild CBW to be directly exposed to β-lactam antibiotics.

Nevertheless, the natural wide distribution of *M. morganii* could be the direct consequence of its adaptability to the environment. Several mechanisms are involved in acquiring antibiotic resistance, even if, often, adaptive resistance is a reversible reply of the bacteria to gradual antibiotic increases (Liu et al., 2016).

On the other hand, the MIC value (1mg/L) that was observed for Trimetroprim-Sulfamethoxazole exceeded the (T)ECOFF value proposed by EUCAST of 0,5 mg/L to distinguish between wild and non-wild type microorganisms.

As occurred for *Acinetobacter* species exhibiting the highest abundance of sulphonamide-resistant strains (Xiong et al., 2015) or for bacteria isolated from fluvial sediment samples in India and Spain (Kristiansson et al., 2011; Marti et al., 2013), also for *M. morganii*, the relatively low but constant environmental concentrations of sulpha antibiotics could explain the elevation of the MIC of our strains versus Sulfamethoxazole-Trimethoprim and leading to classify it as a "non-Wild Type" strain.

Wastewaters, frequently insufficiently treated, produced in livestock and poultry breeding, aquaculture, and hospitals (Zhou et al., 2022) are one of the main sources of subinhibitory concentrations of antibiotics, favoring the rise and spreading of ABR bacteria and resistance genes (Lépesová et al., 2019).

Moreover, sulphonamides, apart from being widely used in human and veterinary medicine, including aquatic farmed species, exhibit strong hydrophilicity and easily persist in freshwater (Pruden et al., 2012; Danner et al., 2019) and marine ecosystems where they can exert acute or chronic toxic effects to a wide range of organisms, from algae to fishes (Zhou et al., 2022).

Sulfonamides toxicity has been proven in marine fishes (Zhou et al., 2022) but no ecotoxicological studies have been conducted on marine mammals regarding these antimicrobials. Moreover, the health status of marine species is not impacted just by chronic exposure to environmental levels of antibiotics. Still, it is threatened by other contaminants such as heavy metals, microplastics, OCs, and other chemicals.

In this scenario, the data reported in this study may contribute to the understanding of the combined effects of toxic marine pollutants and pathogens on the health status of an endangered species, such as the CWB, that is challenging to study at sea.

Conclusion

Information regarding bacterial infections in CBW is scattered and mostly incomplete, with no antimicrobial susceptibility testing reported before (Fernández et al., 2011; Alstrup et al., 2021; Febronio et al., 2021). Moreover, detailed and conclusive necropsies are rare for this species in the Mediterranean region (Podestà et al., 2016). In this report, we present the results of systematic post-mortem investigations stressing their importance to fill knowledge gaps and identify both non-anthropogenic and anthropogenic threats for cetaceans, especially for the elusive ones such as the beaked whales. This study expands information on infectious diseases and chemical contaminants affecting marine mammal health and gives new insights into the interaction between pathogens, host, and environment, whose understanding is still scarce to date (Di Guardo et al., 2018) and contributes to better conservation strategies for marine animals.

To our knowledge, this is the first description of *M. morganii* septicemia with CNS involvement in a wild cetacean. *M. morganii* can represent a threat to marine mammals, especially when they are immunocompromised and inhabit highly polluted environments, such as CBWs in the Mediterranean Sea (Baini et al., 2020). As occurred in terrestrial animals (Franzoni et al., 2022), further research is advised to investigate the cumulative and synergic effect of antibiotics and other contaminants on aquatic species. Although the isolate did not show any antibiotic resistance "*sensu stricto*", a higher threshold of sensitivity for Trimethoprim-Sulfamethoxazole is reported in this study consisting of the first report of a non-wild *M. morganii* strain in a Mediterranean Cuvier's beaked whale.

In addition, since adaptive resistance has been well established in *Enterobacteriaceae* (*E. coli, S. enterica*), while further investigation for *M. morganii* is suggested (Liu et al., 2016), this report should be the door opener for these studies both in human as well as in veterinary medicine in a One Health approach.

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 authors. The sequence generated in the present study was submitted to the GenBank® database with the accession number OQ096688.

Ethics statement

Ethical review and approval was not required for the animal study because the investigations were performed on a dead stranded cetacean. No live animals were involved.

Author contributions

Conceptualization, UR-C, CG, FG. Acquisition of data, VM, TA, LM, KV, BI, CM, RZ, RB, AD, FGa, EB, AP, MR, DA. Methodology, UR-C, SZ, TA, FG. Data curation, UR-C, SZ, CG, FG. Software, FG. Supervision, CG, FG. Manuscript drafting, UR-C, SZ. Funding acquisition, CC. Writing review and editing, CC, FG All authors reviewed and agreed on the current version of the manuscript.

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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.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2022.1058724/full#supplementary-material

SUPPLEMENTARY TABLE 1

Results of the MIC test performed on the *M. morganii* isolate, including CLSI and EUCAST interpretative criteria (clinical breakpoints). When ECOFF (and TECOFF) were available, they were inserted and compared with the MIC values of the tested strain.

References

Abdalla, J., Saad, M., Samnani, I., Lee, P., and Moorman, J. (2006). Central nervous system infection caused by morganella morganii. *Am. J. Med. Sci.* 331, 44–47. doi: 10.1097/00000441-200601000-00013

Alstrup, A. K. O., Thostesen, C. B., Madsen, P. T., Petersen, H. H., Jensen, T. K., Olsen, M. T., et al. (2021). First stranding of cuvier's beaked whale (Ziphius cavirostris) on the Danish north Sea coast. *Aquat. Mamm.* 47, 303–310. doi: 10.1578/AM.47.3.2021.303

Anderson, R. C. (1988). Nematode transmission patterns. *J. Parasitol.* 30–45 (16 pages). doi: 10.2307/3282477

Anderson, R. C., Chabaud, A. G., and Willmott, S. (2009). *Keys to the nematode parasites of vertebrates: archival volume*. Eds. R. C. Anderson, A. G. Chabaud and S. Willmott (Wallingford (UK: CABI). doi: 10.1079/9781845935726.0000

Arbelo, M., Sierra, E., Esperón, F., Watanabe, T. T. N. N., Bellière, E. N., De Los Monteros, A. E., et al. (2010). Herpesvirus infection with severe lymphoid necrosis affecting a beaked whale stranded in the canary islands. *Dis. Aquat. Organ.* 89, 261–264. doi: 10.3354/dao02208

Arun, M., Bhat, G., Acharya, V., and Shenoy, S. (2019). Varied clinical spectrum of a rare entity pneumonia-pasteurella canis: Case series and review of the literature. *J. Clin. Diagn. Res.* 13, 1–4. doi: 10.7860/jcdr/2019/40234.12656

Ashour, D. S., and Othman, A. A. (2020). Parasite–bacteria interrelationship. *Parasitol. Res. 2020. 11910.* 119, 3145–3164. doi: 10.1007/S00436-020-06804-2

Bachman, M. J., Keller, J. M., West, K. L., and Jensen, B. A. (2014). Persistent organic pollutant concentrations in blubber of 16 species of cetaceans stranded in the pacific islands from 1997 through 2011. *Sci. Total. Environ.* 488–489, 115–123. doi: 10.1016/j.scitotenv.2014.04.073

Baily, G. G., Krahn, J. B., Drasar, B. S., and Stoker, N. G. (1992). Detection of brucella melitensis and brucella abortus by DNA amplification. *J. Trop. Med. Hyg.* 95, 271–275.

Baini, M., Panti, C., Fossi, M. C., Tepsich, P., Jiménez, B., Coomber, F., et al. (2020). First assessment of POPs and cytochrome P450 expression in cuvier's beaked whales (Ziphius cavirostris) skin biopsies from the Mediterranean Sea *Sci. Rep.* 10, 21891. doi: 10.1038/s41598-020-78962-3

Balbuena, J. A., and Simpkin, A. (2014). Role of crassicauda sp. in natural mortality of pantropical spotted dolphins stenella attenuata: a reassessment. *Dis. Aquat. Organ.* 108, 83–89. doi: 10.3354/DAO02694

Bandy, A. (2020). Ringing bells: Morganella morganii fights for recognition. *Public Health* 182, 45–50. doi: 10.1016/j.puhe.2020.01.016

Blasi, M. F., Migliore, L., Mattei, D., Rotini, A., Thaller, M. C., and Alduina, R. (2020). Antibiotic resistance of gram-negative bacteria from wild captured loggerhead Sea turtles. *Antibiot.* 9, 162. doi: 10.3390/ANTIBIOTICS9040162

Bossart, G. D. (2011). Marine mammals as sentinel species for oceans and human health. *Vet. Pathol.* 48, 676–690. doi: 10.1177/0300985810388525

Buck, J. D., Wells, R. S., Rhinehart, H. L., and Hansen, L. J. (2006). Aerobic microorganisms associated with free-ranging bottlenose dolphins in coastal gulf of Mexico and Atlantic ocean waters. *J. Wildl. Dis.* 42, 536–544. doi: 10.7589/0090-3558-42.3.536

Cañadas, A., and Notarbartolo di Sciara, G. (2018) *Ziphius cavirostris Mediterranean subpopulation (Cuvier's beaked whale). IUCN red list threat. species*. Available at: https://dx.doi.org/10.2305/IUCN.UK.2018-2.RLTS.T16381144A199549199.en (Accessed September 27, 2022).

Carlucci, R., Cipriano, G., Santacesaria, F. C., Ricci, P., Maglietta, R., Petrella, A., et al. (2020). Exploring data from an individual stranding of a cuvier's beaked whale in the gulf of taranto (Northern Ionian Sea, central-eastern Mediterranean Sea). *J. Exp. Mar. Bio. Ecol.* 533, 151473. doi: 10.1016/J.JEMBE.2020.151473

Centelleghe, C., Beffagna, G., Palmisano, G., Franzo, G., Casalone, C., Pautasso, A., et al. (2017). Dolphin morbillivirus in a cuvier's beaked whale (Ziphius cavirostris), Italy. *Front. Microbiol.* 8. doi: 10.3389/FMICB.2017.00111

Centelleghe, C., Da Dalt, L., Marsili, L., Zanetti, R., Fernandez, A., Arbelo, M., et al. (2019). Insights into dolphins' immunology: Immuno-phenotypic study on mediterranean and atlantic stranded cetaceans. *Front. Immunol.* 10. doi: 10.3389/FIMMU.2019.00888/BIBTEX

Ceri, H., Olson, M., Morck, D., Storey, D., Read, R., Buret, A., et al. (2001). The MBEC assay system: multiple equivalent biofilms for antibiotic and biocide susceptibility testing. *Methods Enzymol.* 337, 377–385. doi: 10.1016/S0076-6879(01)37026-X

CLSI (2018a). *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically* (USA: CLSI Standard M07).

CLSI (2018b). *Performance standards for antimicrobial disk susceptibility Tests.CLSI standard M02. 13th ed* (USA: Clinical and Laboratory Standards Institute (CLSI).

CLSI (2020). *Performance standards for antimicrobial disk and dilution susceptibility tests for bacteria isolated from Animals.Supplement VET01S. 5th ed* (USA: Clinical and Laboratory Standards Institute (CLSI).

Cuvertoret-Sanz, M., López-Figueroa, C., O'Byrne, A., Canturri, A., Martí-Garcia, B., Pintado, E., et al. (2020). Causes of cetacean stranding and death on the catalonian coast (western Mediterranean Sea), 2012-2019. *Dis. Aquat. Organ.* 142, 239–253. doi: 10.3354/DAO03550

Danner, M. C., Robertson, A., Behrends, V., and Reiss, J. (2019). Antibiotic pollution in surface fresh waters: Occurrence and effects. *Sci. Total. Environ.* 664, 793–804. doi: 10.1016/j.scitotenv.2019.01.406

Díaz-Delgado, J., Fernández, A., Xuriach, A., Sierra, E., Bernaldo de Quirós, Y., Mompeo, B., et al. (2016). Verminous arteritis due to crassicauda sp. in cuvier's beaked whales (Ziphius cavirostris). *Vet. Pathol.* 53, 1233–1240. doi: 10.1177/0300985816642228

Di Guardo, G., Centelleghe, C., and Mazzariol, S. (2018). Cetacean host-pathogen interaction(s): Critical knowledge gaps. *Front. Immunol.* 92815. doi: 10.3389/FIMMU.2018.02815

Di Guardo, G., Proietto, U., Di Francesco, C. E., Marsilio, F., Zaccaroni, A., Scaravelli, D., et al. (2010). Cerebral toxoplasmosis in striped dolphins (Stenella coeruleoalba) stranded along the ligurian Sea coast of Italy. *Vet. Pathol.* 47, 245–253. doi: 10.1177/0300985809358036

Eberspacher, B., Hugo, F., Pohl, M., and Bhakdi, S. (1990). Functional similarity between the haemolysins of escherichia coli and morganella morganii. *J. Med. Microbiol.* 33, 165–170. doi: 10.1099/00222615-33-3-165

Elfadl, A. K., Lee, S. W., Kim, J. H., Lee, K. L., Ullah, H. M. A., Chung, M. J., et al. (2017). Fatal fibrino-hemorrhagic bronchopneumonia associated with morganella morganii in a bottlenose dolphin: A case report. *Dis. Aquat. Organ.* 127, 41–47. doi: 10.3354/dao03184

Febronio, A. M. B., Boos, G. S., Batista, R. L. G., Amorim, D. B., Guimarães, J. P., Bianchi, M. V., et al. (2021). Crassicaudiasis in three geographically and chronologically distant cuvier's beaked whales (Ziphius cavirostris) stranded off Brazil. *Int. J. Parasitol. Parasites Wildl.* 16, 262–269. doi: 10.1016/j.ijppaw.2021.10.010

Felipe-Jiménez, I., Fernández, A., Arbelo, M., Segura-Göthlin, S., Colom-Rivero, A., Suárez-Santana, C. M., et al. (2022). Molecular diagnosis of cetacean morbillivirus in beaked whales stranded in the canary island (1999–2017). *Vet. Sci.* 9 (3), p.121. doi: 10.3390/VETSCI9030121

Fernández, A., Edwards, J. F., Rodríguez, F., Espinosa De Los Monteros, A., Herráez, P., Castro, P., et al. (2005). "'Gas and fat embolic syndrome'" involving a mass stranding of beaked whales (Family ziphiidae) exposed to anthropogenic sonar signals. *Vet. Pathol.* 42, 446–457. doi: 10.1354/vp.42-4-446

Fernández, A., Vela, A. I., Andrada, M., Herraez, P., Díaz-Delgado, J., Domínguez, L., et al. (2011). Citrobacter freundii septicemia in a stranded newborn cuvier's beaked whale (Ziphius cavirostris). *J. Wildl. Dis.* 47, 1043–1046. doi: 10.7589/0090-3558-47.4.1043

Franzoni, G., Ciccotelli, V., Masiello, L., De Ciucis, C. G., Anfossi, A. G., Vivaldi, B., et al. (2022). Cadmium and wild boar: Environmental exposure and immunological impact on macrophages. *Toxicol. Rep.* 9, 171–180. doi: 10.1016/J.TOXREP.2022.01.009

Geraci, J. R., Dailey, M. D., and Aubin, St. D.J. (2011). Parasitic mastitis in the Atlantic white-sided dolphin, lagenorhynchus acutus, as a probable factor in herd productivity. *Journal of the Fisheries Board of Canada* 35, 1350–1355. doi: 10.1139/F78-210

Geraci, J. R., and Lounsbury, V. J. (2005). *Marine mammals ashore: A field guide for strandings. second., ed* (Baltimore,MD: National Aquarium in Baltimore).

Giorda, F., Romani-Cremaschi, U., Marsh, A. E., Grattarola, C., Iulini, B., Pautasso, A., et al. (2021). Evidence for unknown sarcocystis-like infection in stranded striped dolphins (Stenella coeruleoalba) from the ligurian Sea, Italy. *Animals* 11 (5), 1201. doi: 10.3390/ani11051201

Hatzianestis, J., Georgakopoulou-Gregoriadou, E., and Frantzis, A. (1998). Organochlorine levels in cuvier's beaked whales from the Ionian Sea, hellas. *Fresenius. Environ. Bull.* 7, 345–350.

Helle, E., Olsson, M., and Jensen, S. (1976). DDT And PCB levels and reproduction in ringed seal from the bothnian bay. *Ambio* 5, 188–189.

Heyning, J. E., and Mead, J. G. (2009). ""Cuvier's beaked whale: Ziphius cavirostris,"," in *Encyclopedia of marine mammals*. Eds. W. Perrin, B. Wursig and J. Thewissen (San Diego: Academic Press), 294–295. doi: 10.1016/B978-0-12-373553-9.00069-9

Jepson, P. D., Bennett, P. M., Deaville, R., Allchin, C. R., Baker, J. R., and Law, R. J. (2005). Relationships between polychlorinated biphenyls and health status in harbor porpoises (Phocoena phocoena) stranded in the united kingdom. *Environ. Toxicol. Chem.* 24(1), 238–248. doi: 10.1897/03-663.1

Jepson, P. D., Deaville, R., Barber, J. L., Aguilar, À., Borrell, A., Murphy, S., et al. (2016). PCB Pollution continues to impact populations of orcas and other dolphins in European waters. *Sci. Rep.* 6. doi: 10.1038/SREP18573

Jerdy, H., Werneck, M., Barbosa, L., Hauser-Davis, R. A., De-Oliveira-Nogueira, C. H., and da Silveira, L. S. (2022). First report on phyllobothrium delphini infection and crassicauda sp. parasitism resulting in osseous metaplasia in a cuvier's beaked whale (Ziphius cavirostris) from the Brazilian region. *Int. J. Parasitol. Parasites Wildl.* 17, 60–64. doi: 10.1016/J.IJPPAW.2021.12.005

Kannan, K., Blankenship, A. L., Jones, P. D., and Giesy, J. P. (2000). Toxicity reference values for the toxic effects of polychlorinated biphenyls to aquatic mammals. *Hum. Ecol. Risk Assess. Int. J.* 6, 181–201. doi: 10.1080/10807030091124491

Knap, A. H., and Jickells, T. D. (1983). Trace metals and organochlorines in the goosebeaked whale. *Mar. pollut. Bull.* 14, 271–274. doi: 10.1016/0025-326X(83)90172-8

Kot, B. C. W., Ho, H. H. N., Leung, E. K. C., Chung, T. Y. T., and Tsui, H. C. L. (2022). Characterisation of crassicauda fuelleborni nematode infection in indo-pacific finless porpoises (Neophocaena phocaenoides) using postmortem computed tomography. *Int. J. Parasitol. Parasites Wildl.* 18, 68–75. doi: 10.1016/J.IJPPAW.2022.04.005

Kristiansson, E., Fick, J., Janzon, A., Grabic, R., Rutgersson, C., Weijdegård, B., et al. (2011). Pyrosequencing of antibiotic-contaminated river sediments reveals high levels of resistance and gene transfer elements. *PloS One* 6, e17038. doi: 10.1371/JOURNAL.PONE.0017038

Lambertsen, R. H. (1986). Disease of the common fin whale (Balaenoptera physalus): crassicaudiosis of the urinary system. *J. Mammal.* 67, 353–366. doi: 10.2307/1380889

Lépesová, K., Olejníková, P., Mackuľak, T., Tichý, J., and Birošová, L. (2019). Annual changes in the occurrence of antibiotic-resistant coliform bacteria and enterococci in municipal wastewater. *Environ. Sci. pollut. Res.* 26, 18470–18483. doi: 10.1007/s11356-019-05240-9

Liu, H., Zhu, J., Hu, Q., and Rao, X. (2016). Morganella morganii, a non-negligent opportunistic pathogen. *Int. J. Infect. Dis.* 50, 10–17. doi: 10.1016/j.ijid.2016.07.006

Marcer, F., Marchiori, E., Centelleghe, C., Ajzenberg, D., Gustinelli, A., Meroni, V., et al. (2019). Parasitological and pathological findings in fin whales balaenoptera physalus stranded along Italian coastlines. *Dis. Aquat. Organ.* 133, 25–37. doi: 10.3354/dao03327

Marsili, L., D'Agostino, A., Bucalossi, D., Malatesta, T., and Fossi, M. C. (2004). Theoretical models to evaluate hazard due to organochlorine compounds (OCs) in Mediterranean striped dolphin (Stenella coeruleoalba). *Chemosphere* 56, 791–801. doi: 10.1016/j.chemosphere.2004.03.014

Marsili, L., Di Guardo, G., Mazzariol, S., and Casini, S. (2019). Insights into cetacean immunology: Do ecological and biological factors make the difference? *Front. Immunol.* 10. doi: 10.3389/FIMMU.2019.01219/XML/NLM

Marsili, L., and Focardi, S. (1997). Chlorinated hydrocarbon (HCB, DDTs and PCBs) levels in cetaceans stranded along the Italian coasts: An overview. *Environ. Monit. Assess.* 45, 129–180. doi: 10.1023/A:1005786627533

Marti, E., Jofre, J., and Balcazar, J. L. (2013). Prevalence of antibiotic resistance genes and bacterial community composition in a river influenced by a wastewater treatment plant. *PloS One* 8, e78906. doi: 10.1371/JOURNAL.PONE.0078906

Martineau, D., Mikaelian, I., Lapointe, J.-M., Labelle, P., and Higgins, R. (2003). ""Pathology of cetaceans. a case study: Beluga from the st. Lawrence estuary,"," in *Toxicology of marine mammals* (London: Taylor & Francis), 333–380. Available at: https://www.taylorfrancis.com/chapters/edit/10.1201/9780203165577-18/pathology-cetaceans-case-study-beluga-st-lawrence-estuary-daniel-martineau-igor-mikaelian-jean-martin-lapointe-philippe-labelle-robert-higgins.

Mizrahi, A., Delerue, T., Morel, H., Le Monnier, A., Carbonnelle, E., Pilmis, B., et al. (2020). Infections caused by naturally AmpC-producing enterobacteriaceae: Can we use third-generation cephalosporins? a narrative review. *Int. J. Antimicrob. Agents* 55. doi: 10.1016/J.IJANTIMICAG.2019.10.015

Olson, M. E., Ceri, H., Morck, D. W., Buret, A. G., and Read, R. R. (2002). Biofilm bacteria: formation and comparative susceptibility to antibiotics. *Can. J. Vet. Res.* 66, 86–92.

Osorio, C. R., Toranzo, A. E., Romalde, J. L., and Barja, J. L. (2000). Multiplex PCR assay for ureC and 16S rRNA genes clearly discriminates between both subspecies of photobacterium damselae. *Dis. Aquat. Organ.* 40, 177–183. doi: 10.3354/DAO040177

Park, S. Y., Lee, K., Cho, Y., Lim, S. R., Kwon, H., Han, J. E., et al. (2020). Emergence of third-generation cephalosporin-resistant morganella morganii in a captive breeding dolphin in south Korea. *Animals* 10, 1–7. doi: 10.3390/ani10112052

Paterson, D. L., and Mathers, A. J. (2020). ""Infections due to other members of the enterobacteriaceae, including management of multidrug-resistant strains - ClinicalKey,"," in *Goldman-Cecil Medicine*. Eds. L. Goldman and A. Schafer (USA: Elsevier), 1927–1930.

Podestà, M., Azzellino, A., Cañadas, A., Frantzis, A., Moulins, A., Rosso, M., et al. (2016). Cuvier's beaked whale, ziphius cavirostris, distribution and occurrence in the Mediterranean Sea: High-use areas and conservation threats. *Adv. Mar. Biol.* 75, 103–140. doi: 10.1016/BS.AMB.2016.07.007

Podestà, M., D'Amico, A., Pavan, G., Drougas, A., Komnenou, A., and Portunato, N. (2006). A review of cuvier's beaked whale strandings in the Mediterranean Sea. *J. Cetacean. Res. Manage.* 7, 251–261.

Pruden, A., Arabi, M., and Storteboom, H. N. (2012). Correlation between upstream human activities and riverine antibiotic resistance genes. *Environ. Sci. Technol.* 46, 11541–11549. doi: 10.1021/es302657r

Rosso, M., Ballardini, M., Moulins, A., Würtz, M., et al. (2011). Natural markings of Cuvier's beaked whale Ziphius cavirostris in the Mediterranean Sea. *African Journal of Marine Science* 33 (1), 45–57. doi: 10.2989/1814232X.2011.572336

Sánchez Contreras, G. J., and Biancani, B. (2021). Third- and fourth-generation cephalosporin resistant morganella morganii associated to an abscess on the perineum of a Male bottlenose dolphin (Tursiops truncatus). *Aquat. Mamm.* 47, 530–539. doi: 10.1578/am.47.6.2021.530

Sheng, W. H., Badal, R. E., and Hsueh, P. R. (2013). Distribution of extended-spectrum β-lactamases, AmpC β-lactamases, and carbapenemases among enterobacteriaceae isolates causing intra-abdominal infections in the Asia-pacific region: results of the study for monitoring antimicrobial resistance trends (SMART). *Antimicrob. Agents Chemother.* 57, 2981–2988. doi: 10.1128/AAC.00971-12

Suárez-Santana, C. M., Sierra, E., Díaz-Delgado, J., Zucca, D., de Quirós, Y. B., Puig-Lozano, R., et al. (2018). Prostatic Lesions in Odontocete Cetaceans. *Veterinary Pathology* 55 (3), 466–472. doi: 10.1177/0300985818755252

The European Committee on Antimicrobial Susceptibility Testing (2017). *EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, version 2.0.* (EUCAST).

The European Committee on Antimicrobial Susceptibility Testing (2022a) *Antimicrobial wild type distributions of microorganisms. MIC distributions for morganella morganii*. Available at: https://mic.eucast.org/search/?search%5Bmethod%5D=mic&search%5Bantibiotic%5D=-1&search%5Bspecies%5D=324&search%5Bdisk_content%5D=-1&search%5Blimit%5D=50 (Accessed September 12, 2022).

The European Committee on Antimicrobial Susceptibility Testing (2022b) *Breakpoint tables for interpretation of MICs and zone diameters, version 12.0*. Available at: http://www.eucast.org/clinical_breakpoints/ (Accessed August 8, 2022).

Thornton, S. M., Nolan, S., and Gulland, F. M. D. (1998). Bacterial isolates from California Sea lions (Zalophus californianus), harbor seals (Phoca vitulina), and northern elephant seals (Mirounga angustirostris) admitted to a rehabilitation center along the central California coas-1995. *J. Zoo Wildl. Med.* 29, 171–176.

VanDevanter, D. R., Warrener, P., Bennett, L., Schultz, E. R., Coulter, S., Garber, R. L., et al. (1996). Detection and analysis of diverse herpesviral species by consensus primer PCR. *J. Clin. Microbiol.* 34, 1666–1671. doi: 10.1128/JCM.34.7.1666-1671.1996

Verna, F., Giorda, F., Miceli, I., Rizzo, G., Pautasso, A., Romano, A., et al. (2017). Detection of morbillivirus infection by RT-PCR RFLP analysis in cetaceans and carnivores. *J. Virol. Methods* 247, 22–27. doi: 10.1016/j.jviromet.2017.05.009

Vitale, M., Galluzzo, P., Currò, V., Gozdzik, K., Schillaci, D., and Di Marco Lo Presti, V. (2013). A high sensitive nested PCR for toxoplasma gondii detection in animal and food samples. *J. Microb. Biochem. Technol.* 05, 39–41. doi: 10.4172/1948-5948.1000097

World Organisation for Animal Health (WOAH) (2018). *Manual of diagnostic tests and vaccines for terrestrial animals (Terrestrial manual). 8th ed.* (Paris, France: WOAH).

Xiong, W., Sun, Y., Zhang, T., Ding, X., Li, Y., Wang, M., et al. (2015). Antibiotics, antibiotic resistance genes, and bacterial community composition in fresh water aquaculture environment in China. *Microb. Ecol.* 70, 425–432. doi: 10.1007/S00248-015-0583-X

Zhou, J., Yun, X., Wang, J., Li, Q., and Wang, Y. (2022). A review on the ecotoxicological effect of sulphonamides on aquatic organisms. *Toxicol. Rep.* 9, 534–540. doi: 10.1016/J.TOXREP.2022.03.034

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Mitochondrial DNA diversity and genetic structure of striped dolphin *Stenella coeruleoalba* in the Northern Ionian Sea

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In the framework of global and EU policies focused on stopping the loss of biodiversity process, deepening the genetic variability, especially of populations species identified as threatened, is crucial for defining conservation units and developing appropriate conservation strategies. This is more urgent for cetacean species in the Mediterranean because they assume a key ecological role in the marine food web and are severely affected by numerous and different anthropogenic pressures. This study aims to increase information on the genetic variability of striped dolphin in the Northern Ionian Sea by investigating the population structure, phylogenetic relationships and phylogeographic patterns using two mtDNA markers. From October 2020 to August 2021, a total of 88 skin tissue samples were collected from free-ranging dolphins in the Gulf of Taranto by applying the non-invasive technique of skin swabbing. An acceptable amount of DNA was extracted from 86 samples and used for subsequent genetic analysis conducted on the partial sequences of 421 and 704 bp in length of the cytb gene and D-loop control region, respectively. In addition, the sequences of the two mtDNA markers were joined together to compose a mtDNA concatenated sequence of 1125 bp for each sampled dolphin in order to investigate the genetic variability of the species population in the study area. Genetic analysis highlighted a low nucleotide diversity and high haplotypic diversity of the striped dolphin of the Gulf of Taranto, suggesting a population in rapid expansion after a period of reduction in size and diversity of the initial population. The phylogenetic analyses revealed the presence of at least two different lineages of *Stenella coeruleoalba* in the Mediterranean Sea, one specific to the Northern Ionian Sea and one shared with the Mediterranean population, confirming results already obtained for the local unit in the Gulf of Taranto. The results point out a potential problem of hybridization between striped and common dolphins which needs to be further investigated. Therefore, increasing the analysis of several markers may increase understanding of the genetic diversity of the population in the Ionian Sea and represent a useful tool to support the implementation of future effective conservation measures.

KEYWORDS

striped dolphin, genetic variability, cytochrome b, D-*loop* control region, genetic markers

1 Introduction

Biological diversity and richness are decreasing globally because of the massive impact of anthropogenic activities responsible for habitat destruction and fragmentation, pollution, exotic invasions, and climate change (Féral, 2002; Duffy and Stachowicz, 2006; Storch et al., 2022). This trend, if not quickly stopped or slowed down, will continue to negatively affect biodiversity which is strongly linked to human well-being as highlighted by the New Global Framework for Managing Nature Through 2030 from the Convention on Biological Diversity (CBD) (https://www.cbd.int/article/draft-1-global-biodiversity-framework), the Intergovernmental Science-Policy Platform on Biodiversity and Ecosystem Services (IPBES) with the Global assessment report on biodiversity and ecosystem services (IPBES, 2019) and the United Nations Sustainable Development Goals (Stange et al., 2021). Therefore, a quantitative evaluation of the status and trends of intraspecific and interspecific genetic diversity and that at the community level is necessary to point out possible adaptations to environmental changes in ecosystem services, resilience capacity and stability of functions (Prieto et al., 2015; Hunter et al., 2018; Hoban et al., 2020). This type of investigation can be carried out thanks to novel molecular and advanced genomic tools developed within conservation genetics, a discipline that helps us to resolve taxonomic uncertainties, to define evolutionarily diverged units such as subpopulations within the same species (de los Angeles Bayas-Rea et al., 2018) and to obtain information important for species conservation (sex, population structure, gene flow, inbreeding and outbreeding rates). It also allows us to integrate genetics with demographic and environmental variables to predict extinction risks and find proper conservation measures in order to slow biodiversity erosion (Allendorf et al., 2007; Frankham, 2010; Coker, 2017). More specifically, analyzing genetic variation over time [genetic diversity, differentiation, and distance (Taylor et al., 2010)], differentiations in specific local populations with a defined population genetic structure could be revealed as a result of low gene flow due, for example, to a recent geographic isolation or a divergence (Gaspari et al., 2019). This phenomenon is highly challenging for biodiversity conservation and mostly for that of highly mobile species such as cetaceans assuming a key ecological role in the marine food web (Roman and McCarthy, 2010; Ricci et al., 2019; Carlucci et al., 2021a) even as sentinel species for human and ecosystem health (Bossart, 2011) and a broad geographic range of distribution. Moreover, cetacean species in the Mediterranean Sea are subject to multiple stressors such as habitat fragmentation and loss, alterations to distribution and availability of resources, climate change, chemical and noise pollution as well as several other threats (Coll et al., 2012; Pace et al., 2015; Notarbartolo di Sciara, 2016).

Although genetic variability in some cetacean species has already been investigated, such as for the killer whale in the Northern Pacific Ocean (Barrett-Lennard and Ellis, 2001), the blue whale in southern Australian waters (Attard et al., 2015), the common bottlenose dolphin in the southern Pacific Ocean (de los Angeles Bayas-Rea et al., 2018), the striped dolphin (Bourret et al., 2007; Gkafas et al., 2017; Ciccarese et al., 2019; Gaspari et al., 2019) and the short-backed common dolphin in different areas of the Mediterranean Sea (Santostasi et al., 2021), increasing these studies in the Mediterranean Sea is very urgent given that genetic diversity generally underpins population resilience and persistence, thus determining the success and long term survival rate of a species in threatened and changing environments (Pace et al., 2015). Indeed, in-depth investigations on genetic variability of cetacean species might help recognize subpopulations or metapopulations, clarify possible connectivity between putative populations and prevent the decline of a species due to environmental and genetic threats such as inbreeding or hybridization as demonstrated for the striped dolphin and common dolphin in the Greek seas (Antoniou et al., 2018; Johnson et al., 2022). Moreover, further studies on this theme could provide more reliable information for the assessment of the extinction risk of species listed in the IUCN Red List even if, genetic information is currently used indirectly to assess the conservation status of species according to different criteria considered (IUCN, 2012; Garner et al., 2020).

In the Mediterranean Sea, studies on genetic variability of cetacean species have demonstrated, for all species regularly occurring in the basin, that the Mediterranean populations are differentiated from the Atlantic ones (ACCOBAMS, 2021). Within the Mediterranean, there is currently only evidence of genetic differences between groups living in the same area for the striped dolphin, *Stenella coeruleoalba*. In the Ligurian Sea, little differentiation is detected between offshore and inshore groups probably linked to different environmental factors and/or prey resources that reflect on group behaviour (Gaspari et al., 2007). In the Gulf of Taranto, the existence of an independent lineage was suggested by studies carried out on genetic variability of the *cytb* gene sequence (Ciccarese et al., 2019; Linguiti et al., 2021). However, a recent study comparing genetic information from both nuclear and mitochondrial samples from Gibraltar to Israel has revealed that the population of striped dolphins in the Mediterranean is structured with low levels of gene flow across the region (Gaspari et al., 2019).

This state of knowledge and the recent change in the conservation status of the species from Vulnerable to Least Concern (ACCOBAMS, 2021; Lauriano, 2021) in any case do not solve the question about the possible occurrence of subpopulations, as recently identified for the Gulf of Corinth (Bearzi et al., 2022), or metapopulations across the Mediterranean regions. Thus, further genetic investigations are necessary. Therefore, this study aimed to deepen understanding of the genetic variability of individuals of striped dolphin in the Gulf of Taranto through investigations of mtDNA sequences of two markers, the cytochrome b (*cytb*) gene and the D-*loop* control region (CR). The *cytb* gene was chosen as a coding marker of the mtDNA to compare the results with those of a previous study conducted in the same study area (Ciccarese et al., 2019). Moreover, improvements in the sampling activity and in laboratory protocols allowed us to analyze also the D-*loop* as mtDNA not coding region. This choice was also guided by the large number of *cytb* and D-*loop* sequences, both of the striped dolphin and of other Delphinidae species, available in the GenBank database.

2 Materials and methods

2.1 Study area

The Gulf of Taranto is in the northernmost part of the Northern Ionian Sea (Central Mediterranean Sea) encompassing an area of about 14000 km^2 from Santa Maria di Leuca to Punta Alice (Figure 1). It is characterized by a narrow continental shelf with a steep slope and several channels in the western sector and by descending terraces toward the submarine canyon known as the "Taranto Valley" in the eastern one. The complex morphology of the area together with the circulation of water masses involve the occurrence of seasonal and decadal upwelling currents (Civitarese et al., 2010; Matarrese et al., 2011; Carlucci et al., 2014; Pinardi et al., 2016) playing a significant role in sustaining productivity (Capezzuto et al., 2010; Maiorano et al., 2010; Carlucci et al., 2018; Ricci et al., 2019) and favouring the occurrence of valuable habitats from the conservation perspective such as the Santa Maria di Leuca cold-water coral province (D'Onghia et al., 2016; Vassallo et al., 2017) and those inhabited by several species of cetaceans (Bellomo et al., 2019; 2020c; Carlucci et al., 2018b; Carlucci et al., 2018c; Carlucci et al., 2020a; Carlucci et al., 2020b; 2021a; Santacesaria et al., 2019; Cipriano et al., 2022). Unfortunately, the basin is potentially affected by several human pressures or threats resulting in possible direct and indirect impacts on cetaceans (Carlucci et al., 2021; Ricci et al., 2021).

2.2 Sample collection

Skin tissue samples were collected during standardized vessel-based surveys carried out while investigating an area of 960 km^2 in the northernmost portion of the Northern Ionian Sea from October 2020 to August 2021. Surveys were carried out only in favourable sea-weather conditions (Douglas scale ≤ 3 and Beaufort scale ≤ 4) applying an effort of approximately 5 h per day along 35 nautical miles and adopting a zig-zag line transect sampling (Buckland et al., 2004; Thomas et al., 2010). Genetic sampling was carried out under authorizations provided by Ministry of the Environment and the Protection of the Territory and Sea, under Authorization 367-REG-1570798753503, Prot. n. 28525, applying the non-invasive technique of skin swabbing (Harlin et al., 1999; Cosentino et al., 2015; Ciccarese et al., 2019; Linguiti et al., 2021). This method consists of applying moderate friction using a 4 x 4 cm synthetic fibre scrub pad on the dorsal-lateral region of an individual to collect the superficial layer of the skin, taking advantage of the moment at which, it approaches the boat during sightings and then came to the surface to breath. The scrub pad was attached with plastic fasteners to the tip of a 130 cm long telescopic aluminium stick covered with a soft sponge-like tissue to prevent hurting the animals. Although striped dolphins can react to the skin swab by swimming, jumping, or diving sometimes they came back close to the boat confirming that the sampling method did not cause any damage or irreversible stress. Individuals sampled are

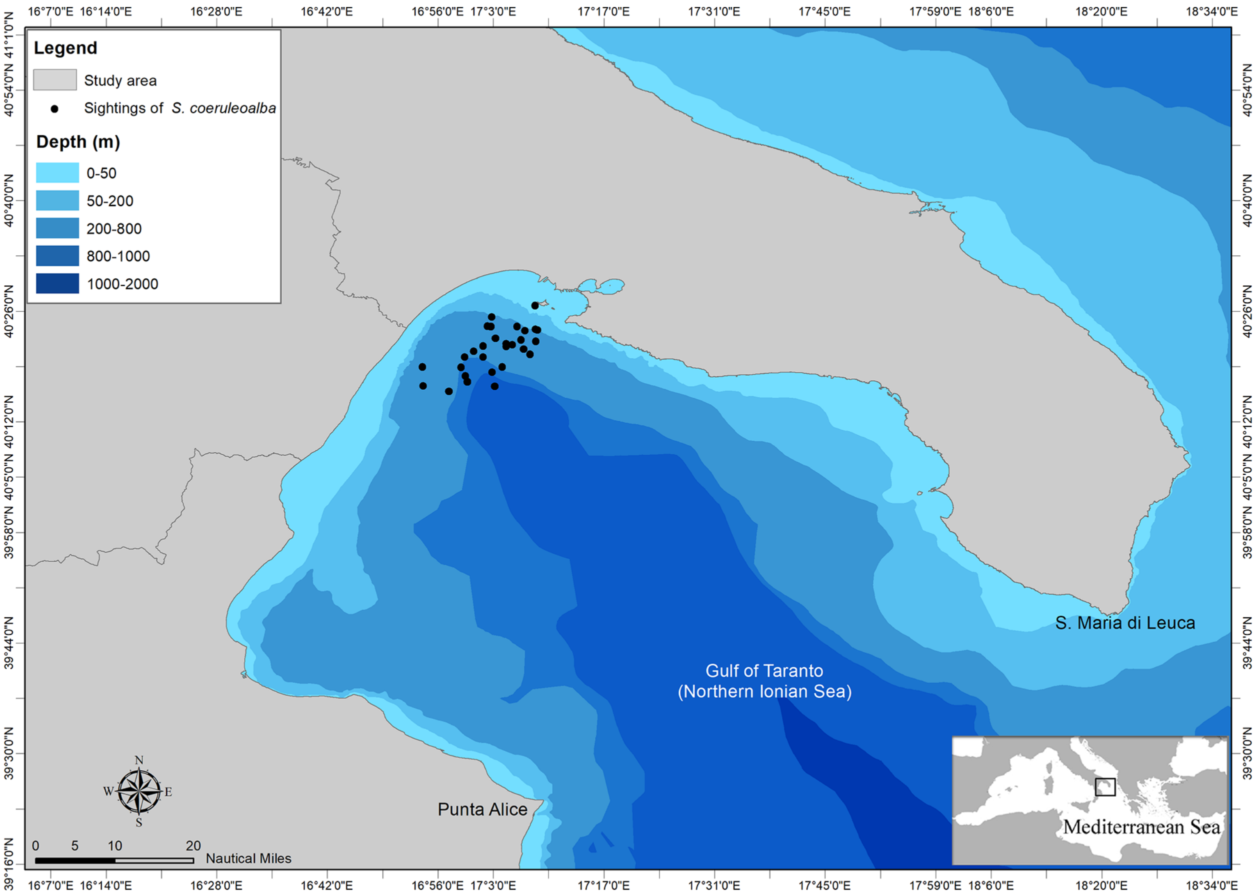

FIGURE 1
Map of the study area with indication of sightings of striped dolphin from which genetic samples were collected.

always recognizable by the light skin scratches caused by the scrub pad, preventing unwanted resampling of the same dolphin.

The skin tissue samples collected were then immediately removed with sterilized forceps from the scrub pad and transferred to a flask containing a 70% alcohol solution, labelled, and preserved at 4°C. At the same time as the genetic samples were taken, information about sighting date, geographic coordinates, depth (m), group size (number of individuals) and predominant activity state was collected. In particular, the collection of data concerning the predominant activity states of groups of striped dolphins encountered was carried out applying the focal-group protocol with instantaneous scan sampling (Mann, 1999; Neumann, 2001).

The sampling activities were performed with complete respect for the animals, respecting their space and trying not to interfere with their natural behaviour or their activities (also taking into account their attitude towards the research boats and the researchers on board).

Together with the skin tissue samples collected from live individuals, two samples were collected from two stranded striped dolphins found on 2nd March 2021 (on the Marina di Ginosa coast, west of Taranto) and on 27th April 2021 (on the Leporano Marina coast, east off Taranto), respectively. Sampling was carried out by directly taking the skin from the bodies using a sterile dermal biopsy punch curette and preserving the samples at 4°C in labelled flasks containing 70% alcohol (ACCOBAMS-MOP7/2019/Doc 33, 2019).

2.3 DNA extraction

Total genomic DNA from skin samples was extracted using a Chelex-100 (Sigma) resin suspension in Tris-EDTA (10 mM Tris HCl, 1 mM EDTA, pH 8.0). Chelex solution (500μL at 15%) was added to each tube containing the sloughed skin sample previously preserved at 4°C. Due to the fast sedimentation of the Chelex solution, it was crucial for it to be frequently stirred prior to pipetting and putting it in the tube. The tubes were vortexed and incubated at 100˚C for 20 minutes, and then placed on ice for 2 minutes. The skin samples were then centrifuged at 13000 rpm for 5 minutes, and the supernatant was transferred to new tubes. The DNA was purified using the standard phenol/chloroform method, quantified, and the quality was checked with a Nanodrop 1000 spectrophotometer from Thermo Scientific.

2.4 Sex determination

A protocol for sex determination was applied as an additional tool, to photography of sampled individuals, to ensure the uniqueness of the samples from individuals sharing the same haplotype within the same sampling group.

The sex of free-ranging and stranded dolphins was identified with a duplex PCR amplification of the striped dolphin ZFX/ZFY and SRY gene fragments.

A set of three oligonucleotide primers for multiplex PCR amplification of the ZFX and ZFY partial sequences was designed: a forward-orientated oligonucleotide primer designed to anneal to the ZFY, as well as the ZFX sequence (ZFYX0582F, 5'-ATAGGTCTGCAGACTCTTCTA-3'), and two reverse-orientated oligonucleotide primers placed within a polymorphic position between the ZFX (ZFX0923R 5'-AGAATATGGCGACTTAGAACG-3') and ZFY sequences (ZFY00767R 5'-TTTGTGTGAACTGAAATTACA-3'). PCR amplification reactions were carried out in a 25ml reaction mixture containing 500 ng of sample DNA, 5X PCR buffer (with 15 mM $MgCl_2$ and 5 mM dNTPs), 10 μM of each primer, and 1 U of Taq enzyme – yourSIALc HiFi Polymerase (S.I.A.L.). PCR thermo-cycling conditions consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 60 s, annealing at 56°C for 30 s, and extension at 72°C for 30 s with a final extension step at 72°C for 5 min.

Gender was determined by the banding pattern on a 2% agarose gel, stained with 0.5 μg/ml ethidium bromide, and visualized under ultraviolet light. The expected product size was a 382 bp single band, as determined by electrophoresis, for females and two bands (382 bp and 226 bp long) for males.

To verify the male gender, another primer set (SRYF 5'-GAGAATCCCCAAATGCAAAACTCAGA-3', SRYR 5'-GGAATTGAGTTGCAAATGGCAGCAA-3') was used to amplify a 418 bp fragment of the SRY gene. The PCR conditions were those described above.

2.5 mtDNA amplification and sequencing

2-mtDNA regions were amplified for all samples: the *cytochrome b* (*cytb*) gene and the mtDNA CR (D-*loop*), designed based on the striped dolphin mitochondrial genome.

A fragment of the *cytb* gene was amplified by a PCR reaction, in a volume of 50 µl, using Taq polymerase - Platinum (Life Technology). The primer set (F1*cytb* 5'-TAACAGTCATGGCCACTGCATT-3' and R2*cytb* 5'-TGGTTTGATGTGTGCAGGGGTG-3') was used under the following conditions: 500 ng of each DNA sample, 10 mM dNTP, 50 mM $MgCl_2$, 1 U Taq, 10 μM of each primer, and 10X PCR buffer. PCR thermo-cycling conditions consisted of an initial denaturation step at 94°C for 4 min, followed by 35 cycles of denaturation at 94°C for 15 s, annealing at 60°C for 30 s, and extension at 72°C for 30 s, with a final extension step at 72°C for 5 min.

The PCR products were purified and fully-sequenced, in both directions, by a sequencing commercial service, using another forward primer (F2*cytb* 5'-CCAACCTCTTATCAGCAATC-3') along with another reverse primer (R1*cytb* 5'-AGGGTGGAATGGAATTATGTCT-3'). The forward primer (F2*cytb*) and the reverse one (R1*cytb*) were drawn respectively downstream of the forward and reverse primers used in the PCR reaction. The sequences acquired were used to assemble and edit the sequence of 421 bp of the *cytb* fragment for each sample.

A different primer set was used to amplify a fragment of the mtDNA CR (D-*loop*). A forward primer dLp1.5L (5'-CACCCAAAGCTGRARTTCTA-3') and a reverse primer dLp8scR (5'-TAGGGACGAAGCACTGTAGG-3') were used under the same PCR conditions as above. The PCR program consisted of an initial denaturation step at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 60°C for 60 s, and extension at 72°C for 60 s with a final extension step at 72°C for 10 min. The PCR products were purified and fully-sequenced, in both directions, using a forward primer (dLpF2 5'-CAACATCAC AGTACTACGTC-3') along with another reverse primer (dLp5H 5'-CCATCGWGATGTCTTATTTAAGRGGAA-3'), designed downstream of the primers used in the PCR reaction. The sequences acquired were used to assemble and edit the sequence of 704 bp of the mtDNA CR (D-*loop*) fragment for each sample.

All *cytb* and D-*loop* haplotype sequences of individuals of striped dolphin sampled are available from GenBank database (Accession numbers ON959814 - ON959831).

2.6 Genetic, phylogenetic and phylogeographic analyses

The *cytb* and D-*loop* mtDNA sequences obtained from striped dolphins sampled in the Gulf of Taranto were aligned using Clustal Omega software (EBI). The genetic analysis was conducted on the partial sequences of 421 and 704 bp in length of the *cytb* gene and D-*loop*, respectively. The number of haplotypes and polymorphic sites as well as the type (single variable and parsimony information sites; transitions and transversions; synonymous and replacement changes, only for *cytb* coding sequence) of single nucleotide polymorphisms were assessed using Arlequin v. 3.5.2.2 (Excoffier and Lischer, 2010). Moreover, considering all samples from the Gulf of Taranto as incorporated into a single unit, the sample genetic diversity was estimated by calculating the haplotype diversity (h) and nucleotide diversity (π) with the same software package.

The Fu's FS (Fu, 1996) and Tajima's D neutrality (Tajima, 1989) tests, implemented in the program Arlequin v. 3.5.2.2 (Excoffier and Lischer, 2010), was performed in order to test past population expansion. A negative value of FS and Tajima's D is considered evidence for excess of rare haplotypes over what would be expected under neutrality, as it would be expected from a recent population expansion or from genetic hitchhiking.

Pair-wise F_{ST} values among the considered taxonomic groups were also calculated.

The evolutionary relationships was investigated by building up phylogenetic trees based on the *cytb* and D-*loop* nucleotide sequences including samples of striped dolphin from the Gulf of Taranto (obtained in this work plus those reported in Ciccarese et al., 2019) and samples, retrieved from GenBank, of the same species from other geographic areas and of other phylogenetically comparable species such as *Stenella clymene*, *S. longirostris*, *S. frontalis*, *S. attenuata*, *Delphinus delphis*, *Tursiops truncatus* and *T. aduncus*. The corresponding *cytb* (GenBank ID: LC630882) and D-*loop* (GenBank ID: NC_012062) sequences from *Grampus griseus* were used as outgroup. Multiple alignments were carried out with the MUSCLE program (Edgar, 2004) both for the *cytb* and D-*loop* sequences. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000), and the units are in the number of base differences per site. Phylogenetic trees were built using the neighbor-joining (NJ) (Saitou and Nei, 1987) method implemented in MEGA X (Kumar et al., 2018; Stecher et al., 2020) and the grouping in the tree was supported by high bootstrap probability values. To support the phylogenetic inference additional phylogenetic analyses such as maximum likelihood (ML) and Bayesian Inference (BI) based on JModelTest (Posada, 2008) were carried out.

Phylogeographic networks were constructed using the sequences of *cytb* and D-*loop* of *S. coeruleoalba*. Networks were constructed adopting the median-joining algorithm implemented in the package Network 10.2.0.0 (Copyright 2004-2022 Fluxus Technology Ltd.). Haplotype frequency distribution in the considered major geographic areas were visualized through pie charts using different color codes. For better visualization of the network topology, branch lengths were not maintained proportional to the number of mutations.

Finally, to deepen knowledge of the genetic structure of the population of striped dolphin in the Gulf of Taranto the sequences of the two mitochondrial DNA-fragments, *cytb* and D-*loop*, were joined together to compose a mtDNA concatenated sequence of 1125 bp for each sampled dolphin. This allowed identification of the haplotypes and investigation of their variability.

3 Results

3.1 Sampling and sex determination

During the sampling period from October 2020 to August 2021, the number of individuals sampled during each survey varied between 1 and 9 for a total of 88 striped dolphins sampled. An acceptable amount of DNA for subsequent analysis, ranging from 18 to 120 µg, was only extracted from 86 samples from free-ranging individuals (Supplementary Table 1). Sex was determined for 56 individuals, showing a sampling bias in favor of males (42) over females (14) (sex ratio 3:1).

3.2 Genetic diversity and phylogenetic analysis

3.2.1 mtDNA *cytb* gene

The mtDNA *cytb* gene fragment of 421 bp was successfully amplified and sequenced in 85 out of the 86 samples extracted (99%) (Table 1). The sequences aligned were compared with those obtained in the previous work of Ciccarese et al. (2019) to verifying the possible match with haplotypes already identified. New haplotypes have been labelled with the abbreviation "Hap" and a progressive number. From this study, six *cytb* haplotypes were obtained (Table 1). Hap10 was identified in 80 of the 85 striped dolphin individuals (94%), confirming it to be the most common in the Gulf of Taranto as assessed in the previous work (Ciccarese et al., 2019). Haplotypes Hap-3, Hap-14, Hap-29, Hap-31 and Hap-32 were found in single individuals. While the Hap-3 had already been found in the Gulf of Taranto (Ciccarese et al., 2019), the other haplotypes were new findings in the area. In addition, Hap-31 and Hap-32 are new haplotypes also at the global level as they are not present in any database.

The haplotype analysis indicated 23 polymorphic loci, including 6 single variable sites (34, 280, 286, 295, 388 397 bp) and 17 parsimony information sites (91, 94, 109, 130, 178, 181, 184, 217, 244, 250, 259, 304, 310, 329, 341, 350, 394 bp) (Tables 2, 3). Within the observed substitutions, 11 are transition changes and only one is a transversion (ratio 11:1) (Tables 2, 3). The ratio between transition and transversion is in line with ratios observed in mammalian mtDNA

TABLE 1 Sampling code and haplotypes of *cytb*, D-*loop* and concatenated identified from individuals of *S. coeruleoalba* sampled in the Gulf of Taranto.

ID	Sampling code	*Cytb* haplotype	D-loop haplotype	Concatenated haplotype
1	1-SS18/10	Hap10	Hap 1	Hap A
2	2-SS24/10	Hap10	Hap 2	Hap B
3a	3-SS1	Hap10	Hap 2	Hap B
3b	SS2 SS3	Hap10 Hap10	Hap 3 Hap 3	Hap C Hap C
4a	SS1 grp1 SS3 grp1	Hap10 Hap10	Hap 3 Hap 3	Hap C Hap C
4b	8-SS2 grp2 9-SS4 grp2 10-SS5 grp2	Hap10 Hap10 Hap29	Hap 3 Hap 3 Hap 3	Hap C Hap C Hap D
5a	11-SS1 grp1 12-SS2 grp1 13-SS3 grp1 14-SS4 grp1 15-SS5 grp1 16-SS6 grp1	Hap10 Hap10 Hap10 Hap10 Hap10 Hap10	Hap 3 Hap 3 Hap 3 Hap 3 Hap 3 Hap 4	Hap C Hap C Hap C Hap C Hap C Hap E
5b	17-SS7 grp2 18-SS8 grp2 19-SS9 grp2	Hap10 Hap10 Hap31 Hap14	Hap 5 Hap 6 Hap 3 Hap 3	Hap F Hap G Hap H Hap I
	SS10 grp2 SS11 grp2 SS12 grp2	Hap10 Hap10	Hap 3 Hap 3	Hap C Hap C
6	23-SS1	Hap10	Hap 7	Hap L
7	24-SS4	Hap10	Hap 8	Hap M
8	SS1 SS2 SS3 SS4	Hap10 Hap10 Hap10 Hap10	Hap 2 Hap 2 Hap 2 Hap 8	Hap B Hap B Hap B Hap M
9	SS1 SS2 SS3 SS4 SS5	Hap10 Hap10 Hap10 Hap10 Hap10	Hap 2 Hap 2 Hap 2 Hap 2 Hap 2	Hap B Hap B Hap B Hap B Hap B
10	SS1 SS2	Hap10 Hap10	Hap 2 Hap 2	Hap B Hap B
11	37-SS1	Hap10	Hap 2	Hap B
12	SS1 SS2	Hap10 Hap10	Hap 9 Hap 2	Hap N Hap B
13	40-SS1	Hap10	Hap 2	Hap B
14	SS1 SS2	Hap10 Hap10	Hap 3 Hap 2	Hap C Hap B
15	43-SS1	Hap10	Hap 2	Hap B
16	44-SS1	Hap10	Hap 8	Hap M
17	SS1 SS2 SS3 SS4	Hap10 Hap10 Hap10 Hap10	Hap 2 Hap 2 Hap 2 Hap 2	Hap B Hap B Hap B Hap B
18	49-SS1	Hap10	Hap 7	Hap L
19a	SC2 SC3	Hap10 nd	Hap 9 nd	Hap N nd

(Continued)

TABLE 1 Continued

ID	Sampling code	*Cytb* haplotype	D-loop haplotype	Concatenated haplotype
	SC4 SC5	Hap10 Hap10	Hap 2 Hap 2	Hap B Hap B
19b	SS1 SS2 SS3	Hap10 Hap10 Hap10	Hap 2 Hap 2 Hap 2	Hap B Hap B Hap B
20	57-SC2	Hap10	Hap 2	Hap B
21	SS1 SS2 SS3 61-SS4(1)	Hap3 Hap10 Hap10 Hap10	Hap 12 Hap 2 Hap 8 Hap 2	Hap R Hap B Hap M Hap B
22	SC1 SC2	Hap10 Hap10	Hap 2 Hap 3	Hap B Hap C
23	SC1 SC2 SC3	Hap10 Hap10 Hap10	Hap 2 Hap 2 Hap 2	Hap B Hap B Hap B
	SC4 SC5 SC6 SC7 SC8 SC9	Hap10 Hap10 Hap10 Hap10 Hap10 Hap10	Hap 4 nd Hap 12 Hap 2 Hap 2 Hap 2	Hap E nd Hap Q Hap B Hap B Hap B
24	74-SS1	Hap10	Hap 3	Hap C
25	75-SS1	Hap10	Hap 2	Hap B
26	SS1 SS2	Hap10 Hap10	Hap 3 Hap 5	Hap C Hap F
27	SS1 SS2 SS3 SS4 SS5 SS6 SS7	Hap10 Hap10 Hap10 Hap10 Hap10 Hap10 Hap10	Hap 10 Hap 3 Hap 2 Hap 3 Hap 3 Hap 3 Hap 2	Hap O Hap C Hap B Hap C Hap C Hap C Hap B
28	SS1 SS2 SS3 SS4	Hap32 Hap10 Hap10 Hap10	Hap 11 Hap 3 Hap 2 Hap 3	Hap P Hap C Hap B Hap C

Nd, not detectable.

ranging from 10:1 to 20:1 (or more) (Irvine et al., 1981) and with previous data on the same species (Ciccarese et al., 2019). Substitutions are observed chiefly at third codon positions except for the transitions at the first codon position of 329, 341 and 350 polymorphic sites. All changes represent silent substitutions (Table 2). The analysis aimed to identify the genetic diversity of the Gulf of Taranto dolphin population showed a mean value of nucleotide diversity (π) equal to 0.000885 ± 0.000946 and a mean value of haplotype diversity (*h*) equal to 0.1148 ± 0.0475, which are both lower than the values observed in the previous study (Ciccarese et al., 2019). The combined group, i.e. haplotypes found in this (group 1) plus those identified in the previous study by Ciccarese et al. (2019), (group 2), containing all the samples of *S. coeruleoalba* from the Gulf of Taranto still gives low levels of both nucleotide and haplotype diversity (Table 3).

The evolutionary relationship of the *cytb* haplotypes was investigated by comparing the sequences of *S. coeruleoalba* from the Gulf of Taranto with sequences, retrieved from the GenBank dataset, of the same species from other geographic areas, and of other phylogenetically comparable species, i.e. *S. clymene, S. longirostris, S. frontalis, S. attenuata, D. delphis, T. truncatus* and *T. aduncus* (Supplementary Table 2). The corresponding *cytb* sequence from *G. griseus* was used as an outgroup. In particular, the following selection criterion was adopted. Only one gene sequence for each haplotype of each species was included in the analysis. All sequences were combined in the same alignment to build a phylogenetic tree using the NJ method (Figure 2). Since the different methods applied gave overlapping results (data not shown for ML and BI), the NJ tree has been preferred in order to be in line with previous evolutionary analysis performed by Ciccarese et al. (2019), of which this represents

TABLE 2 Haplotypes identified in the 421bp mitochondrial *cytb* gene sequences, along with sample size.

Substitution sites	34	91	94	109	130	178	181	184	217	244	250	259	280	286	295	304	310	329	341	350	388	394	397	No. of sequences
	GG-	TA-	AT-	GT-	TT-	AT-	CT-	CC-	CT-	AA-	CC-	AT-	AT-	TT-	TA-	AT-	GA-	-TA	-TA	-TA	GA-	TA-	AC-	
aa	**G**	**Y**	**I**	**V**	**F**	**I**	**L**	**P**	**L**	**N**	**P**	**I**	**I**	**F**	**Y**	**I**	**D**	**L**	**L**	**L**	**D**	**Y**	**T**	
Hap1	A	C	C	C	T	T	T	A	A	C	C	C	C	C	T	C	C	T	T	T	T	C	C	1*
Hap2	-	-	-	A	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	-	-	T	-	1*
Hap3	G	T	T	-	C	C	C	G	G	T	A	T	-	-	-	T	T	C	C	C	-	T	-	1 + 1*
Hap4	-	T	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2*
Hap5	-	T	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	4*
Hap6	-	-	-	A	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1*
Hap7	-	T	-	-	-	C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1*
Hap8	-	T	-	-	-	-	-	-	G	-	-	-	-	-	-	-	-	-	-	C	-	T	-	1*
Hap9	-	T	-	-	-	C	C	-	G	T	-	-	-	-	-	-	-	-	-	C	-	T	T	1*
Hap10	-	T	T	-	C	C	C	G	G	T	A	T	-	-	-	T	T	C	-	C	-	T	-	80 + 11*
Hap11	-	T	T	-	C	C	C	G	G	T	-	T	T	T	C	-	T	C	C	C	C	T	-	1*
Hap14	-	T	T	-	C	C	C	G	G	T	-	-	-	-	-	-	T	C	C	-	-	T	T	1
Hap29	-	T	T	-	C	C	C	G	G	T	A	T	-	-	-	T	T	C	C	C	-	T	-	1
Hap31	-	T	T	-	C	C	C	G	G	T	A	T	-	-	-	T	T	C	C	C	-	T	T	1
Hap32	-	T	T	-	C	C	C	G	G	-	A	T	-	-	-	T	T	C	-	C	-	T	-	1

The position in the sequence where the substitution occurred is numbered in the header. The position of polymorphic sites within codons and the encoded ammino acids are also reported. The haplotypes described by Ciccarese et al. (2019) are also inserted and the sample size labelled with a "*".

an updated version. The grouping in the tree was supported by high bootstrap probability values. The addition of new haplotype sequences reinforces without modifying the paraphyletic distribution of *S. coeruleoalba*. As a matter of a fact, it is possible to recognize five different groups (Figures 2A–E) divided into two principal branches as indicated by an arrow. In the upper branch, group A is the largest and most represented, with five haplotypes (Hap-3, Hap-10, Hap-29, Hap-31 and Hap-32) identified in the Gulf of Taranto intermingled with sequences derived from other geographic areas such as the Mediterranean Sea, Northeast Atlantic, Pacific Ocean, Indian Ocean, Eastern and Northern Pacific. Among these, Hap-10 and Hap-29 are the only ones to be shared between the dolphins of the Gulf of Taranto and those of the other seas. As previously described (Ciccarese et al., 2019), other haplotypes exclusive to the Gulf of Taranto, Hap-1, 2, 4, 5, 6, 7, 8, and 9, form a separate group in the tree (Figure 2E), suggesting the possible existence of a distinct Ionian (sub)population. Moreover, these haplotypes are closer to *Tursiops truncatus* sequences than to other sequences of congeneric species. Instead, *S. coeruleoalba* haplotypes distributed in the other clades are closer to *D. delphis* haplotypes, with Hap-12 (Figure 2C) clustering with *D. delphis* haplotypes, and Hap14 apart (Figure 2B) and tightly related to *S. clymene* as Hap-11 and Hap-13 are (Figure 2D). Moreover, the results of the analysis show the expected species-specific clustering of the *cytb* gene sequences of *S. longirostris, S. frontalis, S. attenuata, D. delphis, T. aduncus* and *T. truncatus*, together with the polyphyletic distribution of the *S. clymene*, already described (Amaral et al., 2014). *S. clymene* seems to be the result of an ancient speciation by a natural hybridization between two other species of dolphin closely related to each other, *S. coeruleoalba* and *S. longirostris*. This conclusion is in line with our findings. In fact, the sequences of the haplotypes of *S. clymene* in the tree form monophyletic groups, with the corresponding sequences of *S. coeruleoalba* and *S. longirostris*.

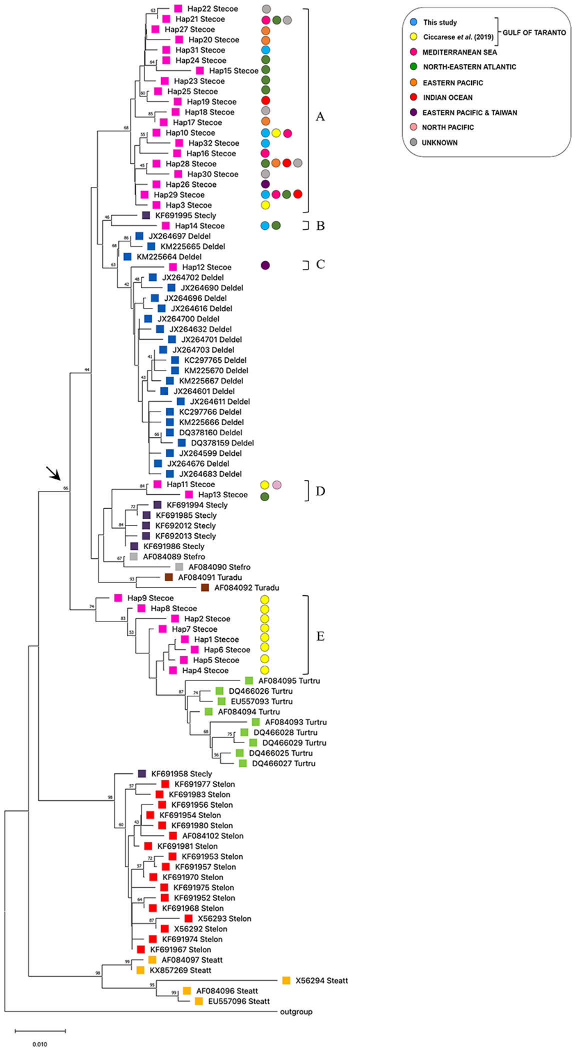

FIGURE 2

NJ tree inferred from Delphinidae *cytb* sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2018). The optimal tree, with the sum of branch length = 0.52128464 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 98 nucleotide sequences. Codon positions included were 1st+2nd+3rd+Noncoding. All ambiguous positions were removed for each sequence pair. There was a total of 421 positions in the final dataset. The colored circles indicate the geographic origin of the samples of the haplotypes of *S. coeruleoalba*. Every species is highlighted with a different colored square to enhance the distribution on the phylogenetic tree. The arrow indicates the paraphyletic branches containing the Stenella coeruleoalba haplotypes. Letters from **(A–E)** indicate striped dolphin clades.

The *cytb* haplotypes were further investigated to evaluate the genetic distances between the two paraphyletic groups of *S. coeruleoalba*, one exclusive of the Gulf of Taranto (Figure 2E), and one shared with other marine sites. Pair-wise F_{ST} comparisons confirmed significant differences between the two groups of *S. coeruleoalba* individuals. The observed F_{ST} value (0.67, Supplementary Table 3) was lower than most of the interspecific comparisons, while being higher than the pair-wise F_{ST} distances observed between *T. aduncus* and *S. attenuata*, *S. frontalis* and *S. attenuata*, as well as between *T. aduncus* and *S. frontalis*.

3.2.2 mtDNA *D-loop*

The mtDNA *D-loop* fragment of 704 bp was successfully amplified and sequenced in 84 out of the 86 samples extracted (approximately 98%). The sequence analysis revealed 12 distinct haplotypes from the study area (Table 1). Hap-2 and Hap-3 were the most frequent, being represented in 40 (48%) and 26 (31%) of samples respectively, followed by Hap-8 identified in 4 samples (5%). Hap-4, Hap-5, Hap-7, Hap-9 and Hap-12 were each found in two individuals and the remaining four haplotypes (Hap-1, Hap-6, Hap-10, Hap-11) were found in single individuals.

The haplotype analysis indicated 21 polymorphic loci, including 3 single variable sites (positions 50, 54 and 215) and 18 parsimony information sites (positions 81, 95, 101, 133, 245, 262, 280, 289, 299, 366, 387, 390, 447, 453, 500, 521, 552 and 600*)* (Tables 3, 4). The frequency of substitution sites along the D-*loop* is lower (21 sites/704 bp, one polymorphism every 33 nucleotides) than the *cytb* (23 sites/421 bp, a polymorphism every 18.3 nucleotides) sequence portion. Within the observed substitutions, 19 are transition changes and three are transversions.

The overall nucleotide diversity and haplotype diversity were 0.002908 ± 0.001826 and 0.6799 ± 0.0384, respectively, for the 84 D-*loop* sequences analyzed.

TABLE 3 Nucleotide and haplotype diversity in mtDNA *cytb* and D-*loop* sequences of individuals of *S. coeruleoalba* sampled in the Gulf of Taranto.

mDNA marker	N° of sequences	Nucleotide diversity (π)	Singleton variable sites	Parsimony-sites	N° of haplotypes	Haplotype diversity (h)	Transitions	Transversions	Neutrality Test	
									Fu's Fs*	Tajima's D**
Cytb Group 1 (this study)	85	0.000885 ± 0.000946	6	17	6	0.1148 ± 0.0475	11	1	–	–
Cytb Group 2 (Ciccarese et al., 2019)	25	0.01990 ± 0.01062			11	0.793 ± 0.075	21	2	–	–
cytb Combined groups	110	0.007520 ± 0.004343			15	0.3156 ± 0.0585	25	2	-24.73188	-0.71928 ns
D-loop Group 1 (this study)	84	0.002908 ± 0.001826	4	12	12	0.6799 ± 0.0384	19	3	-6.33445	0.15404 ns
cytb + *Dloop* Group 1 (this study)	84	0.002259 ± 0.001359	12	22	16	0.7008 ± 0.0408	31	5	–	–

ns, no significant; *p < 0.02; **p < 0.10.

The evolutionary relationship of D-*loop* haplotypes occurring between samples of striped dolphin collected in the Gulf of Taranto and those of other geographic areas is shown in the phylogenetic tree reported in Figure 3. The accession numbers of all D-*loop* sequences used for this phylogenetic analysis are listed in Supplementary Table 4. This dataset was created by querying the GenBank database. Only one sequence for each haplotype was included in the analysis. Since most of the sequences available in the database covered 88% of the D-*loop* sequence used for the query, it was decided to shorten all sequences by 78 bp in order to compare them. It should be emphasized that the 78 nucleotides cut at 5' of each sequence did not contain any polymorphic sites and therefore were not informative for the analysis. Thus, the D-*loop* region used in the phylogenetic analysis was 626 bp long instead of 704.

D-*loop* haplotype sequences of the Gulf of Taranto dolphins were distributed in two distinct groupings that separate at node A. One group contains only Hap-10 and Hap-12, distributed among haplotypes of striped dolphins sampled in the Mediterranean and in other seas, such as the Atlantic and Pacific Oceans. This confirms the high motility and extensive migratory patterns of this species through different and distant geographical areas between the Mediterranean, the Atlantic Ocean and the Pacific Ocean up to the Sea of China, also passing through the Gulf of Taranto. Node B forms a second monophyletic group containing most of the striped dolphin sequences sampled in the Gulf of Taranto (from Hap-1 to Hap-9 and Hap 11) intermingled with sequences of striped dolphins sampled exclusively in the Mediterranean Sea, confirming the idea of the distinct evolution of the Mediterranean dolphin population.

A second phylogenetic tree was built to identify evolutionary relationship between D-*loop* haplotypes of striped dolphins occurring in the Gulf of Taranto and those of different species belonging to the Delphinidae family (Figure 4). The D-*loop* sequences used for the phylogenetic tree retrieved from Genbank are reported in Supplementary Table 5. The phylogenetic distribution of the D-*loop* sequences between and within the different Delphinidae species is broadly in agreement with the previous *cytb* evolutionary analysis (Figure 2). The tree shows the predicted species-specific clustering of *S. longirostris, S. attenuata*, and *T. truncatus* sequences, along with the only two *S. clymene* sequences available in the database intermingled between *S. coeruleoalba* sequences. The sequences of the haplotypes of *T. aduncus* also are distributed in two different clades and they both form a monophyletic group with the *S. coeruleoalba* sequences. In fact, molecular evidence supports *T. aduncus* as a species indistinct from but more closely related to *S. coeruleoalba* than to *T. truncatus* (LeDuc et al., 1999; Charlton et al., 2006; Nishida et al., 2007; Möller et al., 2008; Kingston et al., 2009; Xiong et al., 2009; Vilstrup et al., 2011). As in the previous tree, the sequences of the haplotypes of *S. coeruleoalba* show a paraphyletic grouping that separates sequences of striped dolphins sampled exclusively in the Mediterranean Sea from sequences of individuals also sampled in other sites. In addition, the greater phylogenetic affinity of *S. coeruleoalba* with *D. delphis* than other species indicates the possible cross-breeding between these two dolphin species as already reported in Greek seas (Antoniou et al., 2018).

TABLE 4 Haplotypes identified in the 704bp mitochondrial D-*loop* sequences, along with sample size.

Haplotype	No. of sequences	50	54	81	95	101	133	215	245	262	280	289	299	366	387	390	447	453	500	521	552	600
Hap-0	–	T	A	G	T	C	G	C	A	A	T	T	G	T	T	C	T	T	T	T	T	A
Hap-1	1	–	G	A	C	T	–	–	–	–	–	C	T	–	–	–	C	C	–	–	C	–
Hap-2	40	–	–	A	C	T	–	–	–	G	C	C	T	–	–	–	C	C	C	–	C	–
Hap-3	26	–	–	A	C	T	–	–	–	G	C	C	T	–	–	–	C	C	C	–	–	–
Hap-4	2	–	–	A	C	T	–	–	–	G	C	C	T	–	–	–	C	–	C	–	–	–
Hap-5	2	–	–	A	C	T	–	–	–	G	C	C	T	–	–	–	–	C	C	–	–	–
Hap-6	1	A	T	A	C	T	–	–	–	G	C	C	T	–	–	–	C	C	C	–	–	–
Hap-7	2	–	–	A	C	T	–	–	–	G	C	C	T	–	–	–	C	C	–	–	C	–
Hap-8	4	–	–	A	C	T	–	–	–	G	–	C	T	–	–	–	C	C	C	–	C	–
Hap-9	2	–	–	A	C	T	–	T	–	G	–	C	T	–	–	–	–	C	C	–	C	–
Hap-10	1	–	–	–	–	–	A	–	G	G	C	–	–	C	C	T	–	–	–	C	–	G
Hap-11	1	–	–	A	C	T	–	–	–	G	–	C	T	–	–	–	C	C	C	–	–	–
Hap-12	2	–	–	–	–	–	A	–	G	–	C	–	–	C	C	T	–	–	–	C	–	G

The position in the sequence where the substitution occurred is numbered in the header. Hap-0 represents the reference sequence retrieved from the database (Acc. N° NC_012053).

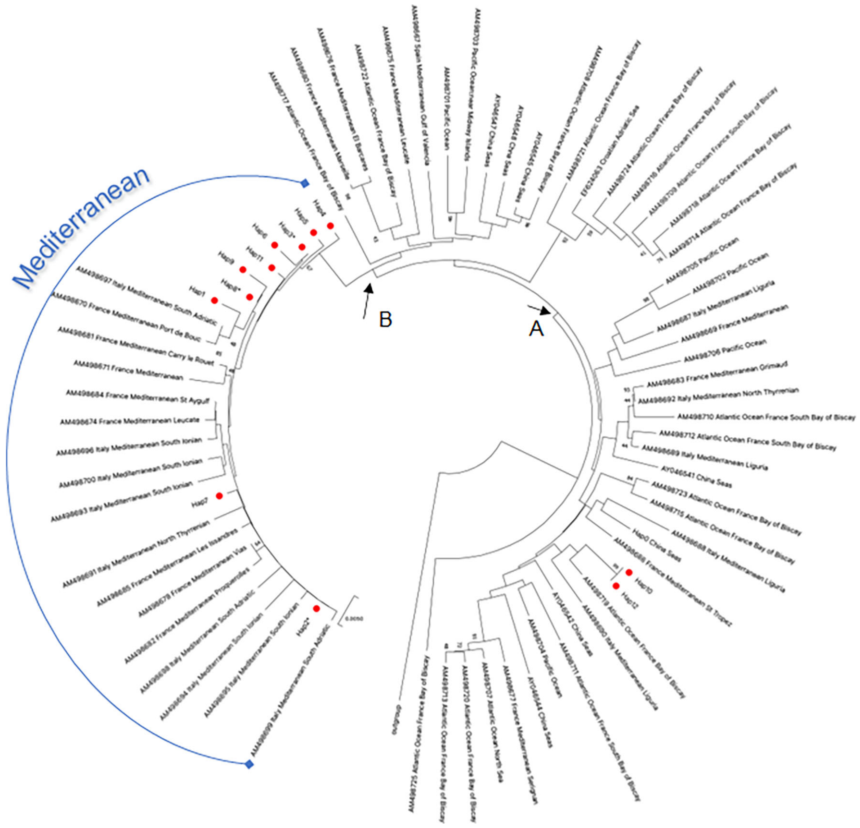

FIGURE 3
NJ tree inferred from *S. coeruleoalba* D-*loop* sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2020). The optimal tree, with the sum of branch length = 1.27736443 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 281 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 638 positions in the final dataset. The red circles indicated the haplotypes of *S. coeruleoalba* identified in the study area. An asterisk indicates haplotypes in the Gulf of Taranto also identified in other geographic areas (Supplementary Table 2). **(A, B)** nodes indicate the distribution of the striped dolphin haplotypes from the study area as discussed in the text.

3.2.3 Concatenated haplotype analysis

A total of 84 concatenated sequences were obtained and analyzed to identify two-marker mtDNA haplotypes. The sequence analysis revealed 16 distinct haplotypes named with the acronym "Hap" followed by an alphabetical letter (Table 1 and Supplementary Table 6). The number of concatenated haplotypes is higher than that found for both haplotypes that compose it (6 for *cytb* and 12 for D-*loop*), suggesting a remarkable heterogeneity that characterizes the striped dolphins in the study area. Of these, Hap-B (40/84, approximately 48%) and Hap-C (23/84, 27%) are the most represented followed by Hap-M identified in 4 individuals (approximately 4.8%); Hap-E, Hap-F and Hap-L identified in 2 individuals each; and the remaining haplotypes identified in single individuals. Analyzing the combinations in detail, the most frequent *cytb* haplotype, Hap-10, contributes mainly to the constitution of the two-marker haplotypes (11/16) and it is in combination with all D-*loop* haplotypes except one (Hap-11). On the other hand, the most represented D-*loop* haplotype, Hap-2, is always associated with *cytb* Hap-10 to form Hap-B, which is the most represented concatenated haplotype (Table 1 and Supplementary Table 6).

Hap-3 is also a more represented D-*loop* haplotype and in most samples (23) it is combined with *cytb* Hap-10, but it has also been found in association with unique *cytb* haplotypes (Hap-14, Hap-29 and Hap-31) (Table 1 and Supplementary Table 6). Moreover, the D-*loop* Hap-12 besides constituting the concatenated Hap-Q haplotype in combination with *cytb* Hap-10, determines the Hap-R haplotype when associated with *cytb* Hap-3. Finally, Hap-P is the only combined haplotype determined by the union of two unique haplotypes (*cytb* Hap-32 and D-*loop* Hap11) (Table 1 and Supplementary Table 6).

The analysis of concatenated haplotypes indicated 35 polymorphic loci, including 12 single variable sites and 22 parsimony information sites (Table 3). Within the observed substitutions, 31 are transition changes and five are transversions. The nucleotide diversity is 0.002259 ± 0.001359, and the haplotype diversity is 0.7008 ± 0.0408 (Table 3).

4 Discussion

The need to increase information about the genetic variability of cetaceans occurring in a semi-closed basin such as the Mediterranean Sea is a crucial point to implement effective measures for the conservation of putative populations or local units in this basin. This study, increasing samples and improving the methodology applied in a previous work (Ciccarese et al., 2019), has allowed us to better understand how wide the genetic variability of this species is

FIGURE 4
NJ tree inferred from Delphinidae D-*loop* sequences. Evolutionary analyses were conducted in MEGA X (Kumar et al., 2020). The optimal tree, with the sum of branch length = 0.44828118 is shown. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the p-distance method (Nei and Kumar, 2000) and are in the units of the number of base differences per site. This analysis involved 76 nucleotide sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). There was a total of 632 positions in the final dataset. Every species is highlighted with a different colored circle to enhance the distribution on the phylogenetic tree. S. coeruleoalba of the Gulf of Taranto and of other areas were highlighted with different shape but the same color. The haplotype sequences identified in the Gulf of Taranto are highlighted with a red circle. Some of them (Hap-2, Hap-3 e Hap-8) are marked with an asterisk to indicate that their presence has also been documented in other areas.

at the local and Mediterranean scale. Furthermore, it contributes to broaden the available data source that can be used in the future evaluation phase of the health status of the Mediterranean subpopulation, shedding light on the possible presence of at least two different lineages of *S. coeruleoalba* in the Mediterranean Sea.

The ability to extract a good quantity (efficiency of extraction of 98%) of high quality of DNA from 88 samples collected during the study period was achieved thanks to improvements in the skill of the on-board team and in the DNA extraction protocol. Great attention was paid to not letting the Chelex solution sediment before being adequately mixed with the skin sample. To prevent fast sedimentation, it was enough to frequently stir the Chelex solution before pipetting and putting it in the sample. Moreover, the time of incubation of the sample with the Chelex resin was prolonged to 20 minutes, 5 minutes longer than the protocol adopted in Ciccarese et al. (2019) because a better yield was observed.

The choice of analyzing the nucleotide sequences of the mitochondrial markers *cytb* and D-*loop* was driven by their different evolutionary mutational rate. *Cytb* changes its amino acid sequence more slowly than any other mitochondrial gene (Simmons & Weller 2001). The protein function limits the nucleotide changes of the gene, as confirmed by our analysis that showed how all nucleotide changes were silent variations. Conversely, D-*loop*, a non-coding region, tends to be widely used as a marker due to its higher variation than the remaining regions of the mitogenome (Cann et al., 1984; Wang et al., 2019) and thus, has been frequently used for phylogenetic studies of closely related groups, especially for determining intra-specific phylogenies. Both these mitochondrial markers have been widely and successfully used for population differentiation analysis in different species (Imsiridou et al., 2019) as well as in different species among different areas (Giantsis et al., 2014; Turan et al., 2015; Šegvić-Bubić et al., 2016).

Although the π value of D-*loop* sequences is a little higher (just over three times) than those calculated for the *cytb* gene sequences of the same samples, both π values of the 2 markers indicate a low level of nucleotide diversity (<0.5%, as suggested by Grant and Bowen, 1998) and, consequently, little genetic divergence of the striped dolphin population in the Ionian Sea. On the contrary, the h value of D-*loop* sequences is to be considered clearly higher (about six times) than those calculated for the *cytb* sequences in the same sample group as well as in the combined group. Moreover, the h value calculated for the D-*loop* marker is >0.5, suggesting a large haplotype diversity (Grant and Bowen, 1998). This condition of high h and low π is attributed to a population in rapid expansion after a period of low effective population size as already suggested by Gaspari et al. (2019). The rapid growth of a population, in fact, enhances the retention of new mutations. The signature of the striped dolphin population expansion in the Ionian Sea was supported by the negative and significant (p<0.02) values of the neutrality Fu's FS test statistics (Table 3).

The same trend about nucleotide (<0.5%) and haplotypes (>0.5) diversity values was observed for concatenated haplotypes confirming the hypothesis that striped dolphins in the Gulf of Taranto represent an evolving population. In detail, our results provide evidence of an increase in variability starting from prevalent haplotypes, represented by Hap-10 for *cytb* (94%) and Hap-2 for the D-*loop* (48%), along with groups of minor haplotypes that often derive from the founder haplotype after accumulating one or a few mutations.

A phylogeographic analysis reinforced this idea. Two median joining networks were constructed using, respectively, the *32* different haplotypes of striped dolphin (Table 2) based on the 421 bp *cytb* target region, and the 71 different haplotypes (Supplementary Table 7) based on the 626 bp *D-loop* target region.

From the point of view of phylogeographic analysis of the *cytb* sequences, Hap-10 and Hap-29 proved to be central haplotypes closely related to each other as indicated by only one mutational step that separates them (Figure 5). Hap-10, largely consisting of samples from the Gulf of Taranto, represents the Mediterranean haplotype, whereas Hap-29 is shared between individuals from the Mediterranean and other seas. Due to its co-presence in the Atlantic Ocean, Mediterranean Sea and Indian Ocean, Hap-29 could represent a junction point in the evolution between haplotypes of different areas and, due to the geographic continuity between the North-Eastern Atlantic and the Mediterranean Sea, it could have entered the Mediterranean evolving into Hap-10, the most represented haplotype in this area. The identification of Hap-29 in the Gulf of Taranto would confirm this hypothesis. The subsequent evolution of Hap-10 would have generated the grouping of the eight haplotypes (Hap-1, 2, 4, 5, 6, 7, 8 and 9) only observed in the Gulf of Taranto and significantly diverging from all the other haplotypes (Ciccarese et al., 2019). Pair-wise F_{ST} comparisons performed with mtDNA *cytb* sequences seems to support this hypothesis by showing a genetic differentiation between the *S. coeruleoalba* haplotypes exclusive from the Northern Ionian Sea and those found also in other areas, comparable to interspecific and even intergeneric distances observed in our study.

The unique Hap-32 may also have been generated directly from Hap-10. In contrast, the other unique haplotype identified in the Gulf of Taranto, Hap-31, is related to Hap-29, as is the case with Hap-3. Whatever the evolutionary steps, our results suggest and confirm the presence of at least two different lineages of *S. coeruleoalba* in the Mediterranean Sea.

In line with those reported for the *cytb* gene, the phylogeographic analysis of D-*loop* haplotypes also revealed evidence of a genetic divergence between the Mediterranean population of striped dolphin and those occurring in other seas (Figure 6). The network topology distinguishes two main haplotype groups. The right group consists of individuals almost exclusively sampled in the Mediterranean Sea, confirming the existence of a Mediterranean lineage; while, the left part of the network, typically reticulated, shows the relationships and connections between different marine sites including the Mediterranean Sea. All haplotypes of the D-*loop* gene identified in the Gulf of Taranto (this study), except for Hap-10 and Hap-12, are present among Mediterranean haplotypes. Hap-2, which is the most represented among samples, occupies a central position. Its star-like appearance suggests the hypothesis of a Mediterranean population expanding from it, as in the case of Hap-10 of the *cytb* gene. Specifically, since Hap-2 is always associated with *cytb* Hap-10, resulting in the concatenated haplotype Hap-B, it might represent the founder of the Mediterranean population as well as of the putative Ionian metapopulation.

Furthermore, another interesting result is the possibility of highlighting the presence of hybridization phenomena through phylogenetic analyzes. In effect, this type of analysis is useful to highlight phenomena of natural and anthropogenic hybridization

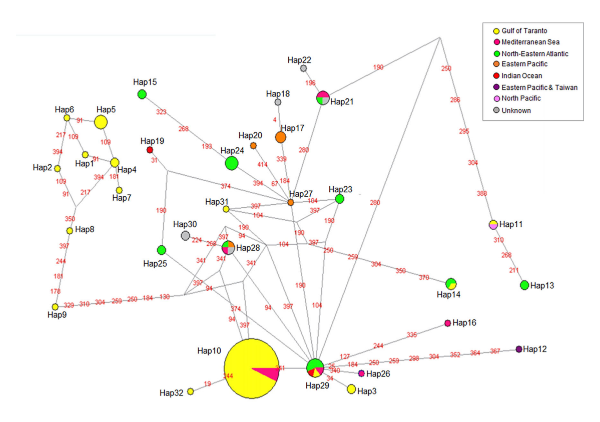

FIGURE 5
Median-joining network of *cytb* haplotypes of *S. coeruleoalba*. Haplotype frequency distribution in the considered geographic areas is visualized through pie charts using different color codes (see the graphic legend). For better visualization of the network topology, branch lengths were not maintained proportional to the number of mutations. The latter are indicated by red labels describing the mutated nucleotide position with respect to the first sequence in the dataset (Hap-1).

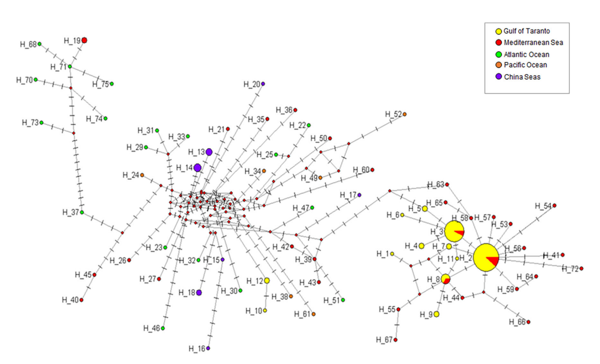

FIGURE 6
Median-joining network of D-*loop* haplotypes of *S. coeruleoalba*. The network was constructed using 71 different sequences (Supplementary Table 7). Haplotype frequency distribution in the considered geographic areas is visualized through pie charts using different color codes. For better visualization of the network topology, branch lengths were not maintained proportional to the number of mutations. The latter are indicated by lines parallel to branch lines, each position line describing a mutated nucleotide position with respect to the first sequence in the dataset (Hap-1). Red circles indicate missing intermediate haplotypes.

(driven by anthropogenic disruption of biological genetic patterns) representing a crucial point for the implementation of effective conservation measures (Faria et al., 2022). In effect, understanding anthropogenic hybridization dynamics can help identify effective and timely management actions for threatened species avoiding genomic extinction potentially led by the presence of admixed individuals and by human disturbances that cause hybridization (Santostasi et al., 2020). In this light, to overcome limits of this study further future analysis on a wider number of mitochondrial and/or nuclear genetic markers could help to better understand and investigate genetic diversity of this species and, consequently, to assist in delineating conservation strategies of local units or putative metapopulations occurring in different regions of the Mediterranean Sea. However, it should be kept in mind that to collect larger amounts of nuclear DNA for genetic analysis it is necessary to apply other sampling methods such as biopsies which require specific permits.

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 in the article/Supplementary Material.

Ethics statement

The animal study was reviewed and approved by Ministry of the Environment and the Protection of the Territory and Sea.

Author contributions

RC, RA, SC, Conceptualization. RA, SC, GL, CF, RC, methodology and sampling design. RA, GL, FP, CS, EC, formal analysis. RA, RC, GC, GL, writing—original draft preparation. RA, RC, GC, GL, FP, CS, CF, EC, SC, writing—review and editing. RC, RA supervision. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2023.1088598/full#supplementary-material

References

ACCOBAMS (2021). *Conserving whales, dolphins and porpoises in the Mediterranean Sea, black Sea and adjacent areas: an ACCOBAMS status report, (2021).* Eds. G. Notarbartolo di Sciara and A. M. Tonay (Monaco: ACCOBAMS).

ACCOBAMS-MOP7/2019/Doc 33 (2019). "Best practice on cetacean post mortem investigation and tissue sampling," in *Joint ACCOBAMS and ASCOBANS document* (Istanbul).

Allendorf, F. W., Luikart, G., and Aitken, S. N. (2007). Conservation and the genetics of populations. *Mammalia* 2007, 189–197. doi: 10.1515/MAMM.2007.038

Amaral, A. R., Lovewell, G., Coelho, M. M., Amato, G., and Rosenbaum, H. C. (2014). Hybrid speciation in a marine mammal: The clymene dolphin (Stenella clymene). *Plos One*. 9(1):e83654.

Antoniou, A., Frantzis, A., Alexiadou, P., Paschou, N., and Poulakakis, N. (2018). Evidence of introgressive hybridization between *Stenella coeruleoalba* and *Delphinus delphis* in the Greek seas. *Mol. Phylogenet. Evol.* 129, 325–337. doi: 10.1016/j.ympev.2018.09.007

Attard, A., Brander, R. W., and Shaw, W. S. (2015). Rescues conducted by surfers on Australian beaches. *Accident Analysis & Prevention*. 82, 70–78.

Barrett-Lennard, L. G., and Ellis, G. (2001). Population structure and genetic variability in northeastern pacific killer whales: Towards an assessment of population viability. *Anim. Behav.* 51, 553–565. doi: 10.1006/anbe.1996.0059

Bearzi, G., Bonizzoni, S., and Santostasi, N. L. (2022). Striped dolphin Stenella coeruleoalba (Gulf of Corinth subpopulation). In book: *The IUCN Red List of Threatened Species* IUCN International Union for Conservation of Nature. doi: 10.2305/IUCN.UK.2022-1.RLTS.T210188066A210188619.en

Bellomo, S., Santacesaria, F. C., Fanizza, C., Cipriano, G., Renò, V., Carlucci, R., et al. (2019). Photo-identification of Physeter macrocephalus in the gulf of Taranto (northern Ionian Sea, central-eastern Mediterranean Sea). In: *Proceedings IEEE Metrology for the Sea 2019 IMEKO TC-19*. International Workshop on Metrology for the Sea, Genoa, Italy. 2019, pp. 33–37.

Bossart, G. D. (2011). Marine mammals as sentinel species for oceans and human health. *Vet. Pathol.* 48, 676–690, 2011. doi: 10.1177/0300985810388525

Bourret, V. J., Macé, M. R., and Crouau-Roy, B. (2007). Genetic variation and population structure of western Mediterranean and northern Atlantic stenella coeruleoalba populations inferred from microsatellite data. *J. Mar. Biol. Assoc. United Kingdom* 87 (1), 265–269. doi: 10.1017/S0025315407054859

Buckland, S. T., Anderson, D. R., Burnham, K. P., Laake, J. L., Borchers, D. L., Thomas, L., et al (Eds.) (2004). *Advanced Distance Sampling: Estimating Abundance of Biological Populations*; OUP: Oxford, UK.

Cann, R. L., Brown, W. M., and Wilson, A. C. (1984). Polymorphic sites and the mechanism of evolution in human mitochondrial DNA. *Genetics* 106, 479–499. doi: 10.1093/genetics/106.3.479

Capezzuto, F., Carlucci, R., Maiorano, P., Sion, L., Battista, D., Giove, A., et al. (2010). The bathyal benthopelagic fauna in the northwestern Ionian Sea: Structure, patterns and interactions. *Chem. Ecol.* 26, 199–217. doi: 10.1080/02757541003639188

Carlucci, R., Bandelj, V., Ricci, P., Capezzuto, F., Sion, L., Maiorano, P., et al. (2018a). Exploring spatio-temporal changes in the demersal and benthopelagic assemblages of the northwestern Ionian Sea (Central Mediterranean Sea). *Mar. Ecol. Prog. Ser.* 598, 1–19. 79. doi: 10.3354/meps12613

Carlucci, R., Cipriano, G., Santacesaria, F. C., Ricci, P., Maglietta, R., Petrella, A., et al. (2020b). Exploring data from an individual stranding of a Cuvier's beaked whale in the gulf of Taranto (northern Ionian Sea, central-eastern Mediterranean Sea). *J. Exp. Mar. Biol. Ecol.* 553, 151473. doi: 10.1016/j.jembe.2020.151473.

Carlucci, R., Battista, D., Capezzuto, F., Serena, F., and Sion, L. (2014). Occurrence of the basking shark *Cetorhinus maximus* (Gunnerus 1765) in the central-Eastern Mediterranean Sea. *Ital. J. Zool* 81, 280–286. doi: 10.1080/11250003.2014.910275

Carlucci, R., Baş, A. A., Liebig, P., Renò, V., Santacesaria, F. C., Bellomo, S., et al. (2020a). Residency patterns and site fidelity of Grampus griseus (Cuvier, 1812) in the Gulf of Taranto (Northern Ionian Sea, central-eastern Mediterranean Sea). *Mammal Res.* 65, 445–455.

Carlucci, R., Capezzuto, F., Cipriano, G., D'onghia, G., Fanizza, C., Libralato, S., et al. (2021a). Assessment of cetacean–fishery interactions in the marine food web of the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea). *Rev. Fish Biol. Fish* 31, 135–156. doi: 10.1007/s11160-020-09623-x

Carlucci, R., Cipriano, G., Paoli, C., Ricci, P., Fanizza, C., and Capezzuto, F.. (2018c). Random Forest population modelling of striped and common-bottlenose dolphins in the Gulf of Taranto (Northern Ionian Sea, Central-eastern Mediterranean Sea). *Estuar. Coast. Shelf Sci.* 204, 177–192.

Carlucci, R., Fanizza, C., Cipriano, G., Paoli, C., Russo, T., and Vassallo, P. (2016). Modelling the spatial distribution of the striped dolphin (*Stenella coeruleoalba*) and common bottlenose dolphin (*Tursiops truncatus*) in the gulf of taranto (Northern Ionian Sea, central-eastern Mediterranean Sea). *Ecol. Indic.* 69, 707–721. doi: 10.1016/j.ecolind.2016.05.035

Carlucci, R., Manea, E., Ricci, P., Cipriano, G., Fanizza, C., Maglietta, R., et al. (2021). Managing multiple pressures for cetaceans' conservation with an ecosystem-based marine spatial planning approach. *J. Environ. Manag.* 287, 112240. doi: 10.1016/j.jenvman.2021.112240

Carlucci, R., Ricci, P., Cipriano, G., and Fanizza, C. (2018b). Abundance, activity and critical habitat of the striped dolphin stenella coeruleoalba in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea). *Aquat. Conservation: Mar. Freshw. Ecosyst.* 28 (2), 324–336. doi: 10.1002/aqc.2867

Charlton, K., Taylor, A. C., and McKechnie, S. W. (2006). A note on divergent mtDNA lineages of bottlenose dolphins from coastal waters of southern Australia. *Journal of Cetacean Research & Management* 8:173–179.

Ciccarese, S., Carlucci, R., Ciani, E., Corcella, E., Cosentino, A., Fanizza, C., et al. (2019). Cytochrome b marker reveals an independent lineage of *Stenella coeruleoalba* in the gulf of taranto. *PloS One* 14 (3), 0213826. doi: 10.1371/journal.pone.0213826

Cipriano, G., Carlucci, R., Bellomo, S., Santacesaria, F. C., Fanizza, C., Ricci, P., et al. (2022). Behavioral Pattern of Risso's Dolphin(Grampus griseus) in the Gulf of Taranto (Northern Ionian Sea, Central-Eastern Mediterranean Sea). *J. Mar. Sci. Eng.* 10, 175.

Civitarese, G., Gačić, M., Lipizer, M., and Eusebi Borzelli, G. L. (2010). On the impact of the bimodal oscillating system (BiOS) on the biogeochemistry and biology of the Adriatic and Ionian seas (eastern Mediterranean). *Biogeosciences* 7, 3987–3997. doi: 10.5194/bg-7-3987-2010

Coker, O. M. (2017). Importance of genetics in conservation of biodiversity. *J. Wildl. Manage* 1, 11–18.

Coll, M., Piroddi, C., Albouy, C., Lasram, F. B.R., Cheung, W. W.L., Christensen, V., et al. (2012). The Mediterranean Sea under siege: spatial overlap between marine biodiversity: cumulative threats and marine reserves. *Glob. Ecol. Biogeogr* 21, 465–480.

Cosentino, A., Fanizza, C., Corcella, M. E., Pala, R., Carlucci, R., Sartori, S. M., et al. (2015). Genetic-molecular protocols for the study of the social structure in Stenella Coeruleoalba in the Gulf of Taranto. *Biol Mar Mediterr.* 22(1):271–272.

de los Angeles Bayas-Rea, R., Félix, F., and Montufar, R. (2018). Genetic divergence and fine scale population structure of the common bottlenose dolphin (*Tursiops truncatus*, montagu) found in the gulf of guayaquil, Ecuador. *PeerJ* 6, 4589. doi: 10.7717/peerj.4589

D'Onghia, G., Calculli, E., Capezzuto, F., Carlucci, R., Carluccio, A., Maiorano, P., et al. (2016). New records of cold-water coral sites and fish fauna characterization of a potential network existing in the Mediterranean Sea. *Mar. Ecol.* 37, 1398–1422. doi: 10.1111/maec.12356

Duffy, J. E., and Stachowicz, J. J. (2006). Why biodiversity is important to oceanography: potential roles of genetic, species, and trophic diversity in pelagic ecosystem processes. *Mar. Ecol. Prog. Ser.* 311, 179–189. doi: 10.3354/meps311179

Edgar, R. C. (2004). MUSCLE: a multiple sequence alignment method with reduced time and space complexity. *BMC Bioinf.* 5, 113. doi: 10.1186/1471-2105-5-113 PMID:15318951 doi: 10.1186/1471-2105-5-113

Excoffier, L., and Lischer, H. E. L. (2010). Arlequin suite ver 3.5: A new series of programs to perform population genetics analyses under Linux and windows. *Mol. Ecol. Resour.* 10, 564–567. doi: 10.1111/j.1755-0998.2010.02847.x

Faria, D. M., Steel, D., Baker, C. S., da Silva, J. M., de Meirelles, A. C. O., Souto, L. R. A., et al. (2022). Mitochondrial diversity and inter-specific phylogeny among dolphins of the genus stenella in the southwest Atlantic ocean. *PloS One* 17 (7), e0270690. doi: 10.1371/journal.pone.0270690

Féral, J. P. (2002). How useful are the genetic markers in attempts to understand and manage marine biodiversity? *J. Exp. Mar. Biol. Ecol.* 268 (2), 121–145. doi: 10.1016/S0022-0981(01)00382-3

Frankham, R. (2010). Challenges and opportunities of genetic approaches to biological conservation. *Biol. Conserv.* 143 (9), 1919–1927. doi: 10.1016/j.biocon.2010.05.011

Fu, Y. X. (1996) "New statistical tests of neutrality for DNA samples from a population". *Genetics* 143, 557–570. doi: 10.1093/genetics/143.1.557

Garner, B. A., Hoban, S., and Luikart, G. (2020). IUCN red list and the value of integrating genetics. *Conserv. Genet.* 21, 795–801. doi: 10.1007/s10592-020-01301-6

Gaspari, S., Azzellino, A., Airoldi, S., and Hoelzel, A. R. (2007). Social kin associations and genetic structuring of striped dolphin populations (*Stenella coeruleoalba*) in the Mediterranean Sea. *Mol. Ecol.* 16 (14), 2922–2933. doi: 10.1111/j.1365-294X.2007.03295.x

Gaspari, S., Marsili, L., Natali, C., Airoldi, S., Lanfredi, C., Deeming, C., et al. (2019). Spatio-temporal patterns of genetic diversity in the Mediterranean striped dolphin (*Stenella coeruleoalba*). *J. Zoological Systematics Evolutionary Res.* 57 (3), 721–734. doi: 10.1111/jzs.12265

Giantsis, I. A., Abatzopoulos, T. J., Angelidis, P., and Apostolidis, A. P. (2014) "Mitochondrial control region variability in *Mytilus galloprovincialis* populations from the central-Eastern Mediterranean sea." *Int. J. Mol. Sci.* 15, 11614–11625. doi: 10.3390/ijms150711614

Gkafas, G. A., Exadactylos, A., Rogan, E., Raga, J. A., Reid, R., and Hoelzel, A. R. (2017). Biogeography and temporal progression during the evolution of striped dolphin population structure in European waters. *J. biogeography* 44 (12), 2681–2691. doi: 10.1111/jbi.13079

Harlin, A. D., Würsig, B., Baker, C. S., and Markowitz, T. M. (1999). Skin swabbing for genetic analysis: application to dusky dolphins (Lagenorhynchus obscurus). *Marine Mammal Science* 15(2), 409–425.

Hoban, S., Bruford, M., Jackson, J. D. U., Lopes-Fernandes, M., Heuertz, M., Hohenlohe, P. A., et al. (2020). Genetic diversity targets and indicators in the CBD post-2020 global biodiversity framework must be improved. *Biol. Conserv.* 248, 108654. doi: 10.1016/j.biocon.2020.108654

Hunter, M. E., Hoban, S. M., Bruford, M. W., Segelbacher, G., and Bernatchez, L. (2018) Next-generation conservation genetics and biodiversity monitoring." *Evolutionary Appl.* 11 (7), 1029–1034. doi: 10.1111/eva.12661

Irvine, A. B., Scott, M. D., Wells, R. S., and Kaufmann, J. H. (1981). Movements and activities of the Atlantic bottlenose dolphin, Tursiops truncatus, near Sarasota, Florida. *Fish. Bull.* 79, 671–688.

Imsiridou, A., Papapetrou, M., Tilikidis, A., Loukovitis, D., Minos, G., Gouva, E., et al. (2019). Can the population structure of three Greek marine species (*Sardina pilchardus*, *Penaeus kerathurus*, *Mullus barbatus*) become a tool for their future characterization as PGI products. *J. Nutr. Food Lipid Sci.* 2019, 1901–1908.

IPBES (2019). *Global assessment report on biodiversity and ecosystem services of the intergovernmental science-policy platform on biodiversity and ecosystem services.* Eds. E. S. Brondizio, J. Settele, S. Díaz and H. T. Ngo (Bonn, Germany: IPBES secretariat), 1148 pages.

IUCN. (2012). *IUCN Red List Categories and Criteria: Version 3.1*; IUCN: Gland, Switzerland; Cambridge, UK.

Johnson, C., Reisinger, R., Palacios, D., Friedlaender, A., Zerbini, A., Willson, A., et al. (2022). *Protecting blue corridors, challenges and solutions for migratory whales navigating international and national seas* (Switzerland: WWF, Oregon State University, University of California, Santa Cruz, Publisher: WWF International).

Kingston, S. E., Adams, L. D., and Rosel, P. E. (2009). Testing mitochondrial sequences and anonymous nuclear markers for phylogeny reconstruction in a rapidly radiating group: molecular systematics of the Delphininae (Cetacea: Odontoceti: Delphinidae). *BMC Evol. Biol.* 9, 245.

Kumar, S., Stecher, G., Li, M., Knyaz, C., and Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549. doi: 10.1093/molbev/msy096

Lauriano, G. (2021). Striped dolphin, Stenella coeruleoalba, Mediterranean subpopulation. *The IUCN Red List of Threatened Species* 2021.

LeDuc, R. G., Perrin, W. F., and Dizon, A. E. (1999). Phylogenetic relationships among the delphinid cetaceans based on full cytochrome b sequences. *Marine mammal science* 15 (3), 619–648.

Linguiti, G., Fanizza, C., Ciani, E., Bellomo, S., Cipriano, G., Santacesaria, F. C., et al. (2021). "October. "Assessment of genetic diversity of the striped dolphin population in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea)," in *2021* *international workshop on metrology for the sea; learning to measure Sea health parameters (MetroSea)* (Reggio Calabria: IEEE), 144–147.

Maiorano, P., Sion, L., Carlucci, R., Capezzuto, F., Giove, A., Costantino, G., et al. (2010). The demersal faunal assemblage of the NW Ionian Sea (Central mediterranean): Current knowledge and perspectives. *Chem. Ecol.* 26, 219–240. doi: 10.1080/02757541003693987

Mann, J. (1999). Behavioral sampling methods for cetaceans: A review and critique. *Mar. Mamm. Sci.* 15, 102–122. doi: 10.1111/j.1748-7692.1999.tb00784.x

Matarrese, R., Chiaradia, M. T., Tijani, K., Morea, A., and Carlucci, R. (2011). Chlorophyll a'multi-temporal analysis in coastal waters with MODIS data. *Ital. J. Remote Sens.* 43, 39–48. doi: 10.5721/ItJRS20114333

Möller, L. M., Bilgmann, K., Charlton-Robb, K., and Beheregaray, L. (2008). Multi-gene evidence for a new bottlenose dolphin species in southern Australia. *Molecular Phylogenetics and Evolution.* 49(2):674–681. doi: 10.1016/j.ympev.2008.08.011

Nei, M., and Kumar, S. (2000). *Molecular evolution and phylogenetics* (New York: Oxford University Press).

Neumann, D. R. (2001). Activity budget of free-ranging common dolphins (Delphinus delphis) in the northwestern bay of plenty, new Zealand. *Aquat. Mamm.* 27, 121–136.

Nishida, S, Goto, M, Pastene, LA, Kanda, N, and Koike, H (2007). Phylogenetic relationships among cetaceans revealed by Y-chromosome sequences. *Zool Sci* 7, 723–732.

Notarbartolo di Sciara, G. (2016). Chapter One - Marine Mammals in the Mediterranean Sea: An Overview. *InAdvances in Marine Biology*, G. Notarbartolo di Sciara, M. Podestà and B. E. Curry, Eds. volume 75, 2–248.

Pace, D. S., Tizzi, R., and Mussi, B. (2015). Cetaceans value and conservation in the Mediterranean Sea. *J. Biodiversity Endangered Species* S1:(004). doi: 10.4172/2332-2543.S1.004

Pinardi, N., Lyubartsev, V., Cardellicchio, N., Caporale, C., Ciliberti, S., Coppini, G., et al. (2016). Marine rapid environmental assessment in the gulf of taranto: A multiscale approach. *Nat. Hazards Earth Syst. Sci.* 16, 2623–2639. doi: 10.5194/nhess-16-2623-2016

Posada, D. (2008). JModelTest: Phylogenetic model averaging. *Mol. Biol. Evol.* 25 (7), 1253–1256. doi: 10.1093/molbev/msn083

Prieto, I., Violle, C., Barre, P., Durand, J. L., Ghesquiere, M., and Litrico, I. (2015). Complementary effects of species and genetic diversity on productivity and stability of sown grasslands. *Nat. Plants* 1 (4), 1–5. doi: 10.1038/nplants.2015.33

Ricci, P., Libralato, S., Capezzuto, F., D'Onghia, G., Maiorano, P., Sion, L., et al. (2019). Ecosystem functioning of two marine food webs in the north-Western Ionian Sea (Central Mediterranean Sea). *Ecol. Evol.* 9, 10198–10212. doi: 10.1002/ece3.5527

Ricci, P., Manea, E., Cipriano, G., Cascione, D., D'Onghia, G., Ingrosso, M., et al. (2021). Addressing cetacean–fishery interactions to inform a deep-Sea ecosystem-based management in the gulf of taranto (Northern Ionian Sea, central Mediterranean Sea). *J. Mar. Sci. Eng.* 9, 872. doi: 10.3390/jmse9080872

Roman, J., and McCarthy, J. J. (2010). The whale pump: marine mammals enhance primary productivity in a coastal basin. *PloS one* 5(10), e13255.

Saitou, N., and Nei, M. (1987). The neighbor-joining method: A new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425. doi: 10.1093/oxfordjournals.molbev.a040454

Santacesaria, F. C., Bellomo, S., Fanizza, C., Maglietta, R., Renò, V., Cipriano, G., et al. (2019). Long-term residency of Tursiops truncatus in the Gulf of Taranto (Northern Ionian Sea, Central-eastern Mediterranean Sea). In *Proceedings of the IMEKO Metrology for the Sea*, Genova, Italy, 3–5 October 2019.

Santostasi, N. L., Bonizzoni, S., Gimenez, O., Eddy, L., and Bearzi, G. (2021). Common dolphins in the gulf of Corinth are critically endangered. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 31, 101–109. doi: 10.1002/aqc.2963

Santostasi, N. L., Ciucci, P., Bearzi, G., Bonizzoni, S., and Gimenez, O. (2020). Assessing the dynamics of hybridization through a matrix modelling approach. *Ecological Modelling* 431, 109120

Šegvić-Bubić, T., Marrone, F., Grubišić, L., Izquierdo-Gomez, D., Katavić, I., Arculeo, M., et al. (2016). Two seas, two lineages: How genetic diversity is structured in Atlantic and Mediterranean greater amberjack *Seriola dumerili* riss(Perciformes, carangidae). *Fisheries Res.* 179, 271–279. doi: 10.1016/j.fishres.2016.03.018

Stange, M., Barrett, R. D., and Hendry, A. P. (2021). The importance of genomic variation for biodiversity, ecosystems and people. *Nat. Rev. Genet.* 22 (2), 89–105. doi: 10.1038/s41576-020-00288-7

Stecher, G., Tamura, K., and Kumar, S. (2020). Molecular evolutionary genetics analysis (MEGA) for macOS. *Mol. Biol. Evol.* doi: 10.1093/molbev/msz312

Storch, D., Šímová, I., Smyčka, J., Bohdalková, E., Toszogyova, A., and Okie, J. G. (2022). Biodiversity dynamics in the anthropocene: how human activities change equilibria of species richness. *Ecography* 2022 (4). doi: 10.1111/ecog.05778

Tajima, F. (1989). Statistical method for testing the neutral mutation hypothesis by DNA polymorphism. *Genetics* 123, 585–595. doi: 10.1093/genetics/123.3.585

Taylor, B. L., Martien, K., and Morin, P. (2010). "Identifying units to conserve using genetic data," in *Marine mammal ecology and conservation–a handbook of techniques* (Oxford: Oxford University Press), 306–344.

Thomas, L., Buckland, S. T., Rexstad, E. A., Laake, J. L., Strindberg, S., Hedley, S. L., et al. Distance software: Design and analysis of distance sampling surveys for estimating population size. *J. Appl. Ecol.* (2010) 47, 5–14.

Turan, C., Gürlek, M., Ergüden, D., Yağıoğlu, D., Reyhaniye, A., Özbalcılar, B., et al. (2015). "Population genetic analysis of Atlantic bonito sarda sarda (Bloc) using sequence analysis of mtDNA d-loop region," in *Fresenius environmental bulletin*, vol. 24. Parlar Scientific Publications. Available at: https://hdl.handle.net/20.500.12462/9277.

Vassallo, P., D'Onghia, G., Fabiano, M., Maiorano, P., Lionetti, A., Paoli, C., et al. (2017). A trophic model of the benthopelagic fauna distributed in the Santa maria di leuca cold-water coral province (Mediterranean Sea). *Energy Ecol. Environ.* 2, 114–124. doi: 10.1007/s40974-016-0047-2

Vilstrup, J. T., Ho, S. Y., Foote, A. D., Morin, P. A., Kreb, D., Krützen, M., et al. (2011). Mitogenomic phylogenetic analyses of the Delphinidae with an emphasis on the Globicephalinae. *BMC Evol Biol.* 11, 65. doi: 10.1186/1471-2148-11-65.

Wang, C., Chen, Y.-s., Han, J.-l., Mo, D.-l., Li, X.-j., and Liu, X.-h. (2019). Mitochondrial DNA diversity and origin of indigenous pigs in south China and their contribution to Western modern pig breeds. *J. Integr. Agric.* 18, 2338–2350. doi: 10.1016/S2095-3119(19)62731-0

Xiong, Y., Brandley, M. C., Xu, S., Zhou, K., and Yang, G. (2009). Seven new dolphin mitochondrial genomes and a time-calibrated phylogeny of whales. *BMC Evol Biol.* 9, 20. doi: 10.1186/1471-2148-9-20.

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Submarine canyons as key habitats to preserve Risso's dolphin (*Grampus griseus*) populations in the northwestern Mediterranean Sea

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This paper summarises the occurrence of Risso's dolphin (*Grampus griseus*) over 12 years (2009 to 2021) in the northwestern Mediterranean. The study was conducted off the central coast of Catalonia (NE Spain) in an area covering 8,026 km^2 and featuring a system of submarine canyons. The habitat is suitable for a wide diversity of species, including deep-diving cetaceans. In addition, a different dataset from other projects was included to compare distances to the coast from different periods. A visual effort of 8,756 km was carried out with the recording of 17 Risso's dolphin sightings. The relative mean density of Risso's dolphin was 0.0078 individuals/km^2 (SD 0.1, n = 17), and the densities were higher in the southern canyons than in the northern canyons. Furthermore, the distance to the coast and the depth of any sighting were compared by gathering data from different periods and surveys (first period 1985–2014; second period 2016–2021) with 34 sightings analysed. Significant differences were found related to the distance to the coast, showing a displacement of the animals to more pelagic areas between periods. The spatial distribution of Risso's dolphins was investigated by applying a generalized additive model based on sighting data collected during standardized vessel surveys. Four predictive variables were considered, taking into account the local physiographical features. The model showed that the spatial distribution of Risso's dolphin in the area was related to the slope and slope variation. The species showed preferences for offshore areas as significant differences were obtained in terms of the distance of sightings to the coast. This paper provides new insights into the distribution of Risso's dolphin in the central western Mediterranean Sea and identifies the submarine canyons of northern Catalonia as an essential habitat for the species.

KEYWORDS

conservation, Risso's dolphin, submarine canyons, marine protected area, distribution

1 Introduction

Risso's dolphin, *Grampus griseus* (Cuvier, 1812), is a cosmopolitan cetacean species that occurs in temperate and tropical waters and that has a heterogeneous distribution in the Mediterranean Sea (Azzellino et al., 2008; Boisseau, 2010; Bearzi et al., 2011; Gaspari and Natoli, 2012; Azzellino et al., 2016). The species' area of occurrence in the Mediterranean includes the Alboran Sea (Cañadas et al., 2002; Cañadas et al., 2005; Gannier, 2005), the Ligurian Sea (Di Sciara et al., 1993; Gannier, 2005; Azzellino et al., 2008; Moulins et al., 2008; Azzellino and Lanfredi, 2015; Azzellino et al., 2016), the Sardinian-Balearic Basin (Gómez de Segura et al., 2008; Arcangeli et al., 2018), Chicote et al., 2015 the Strait of Sicily (Corrias et al., 2021), the Tyrrhenian Sea (Raga and Pantoja et al., 2004; Arcangeli et al., 2012; Campana et al., 2015), the Adriatic Sea (UNEP MAP-RAC/SPA, 2014), and the Ionian (Frantzis and Herzing, 2002; Dimatteo et al., 2011; Carlucci et al., 2020; Menniti and Vella., 2022) and Aegean basins (Frantzis and Herzing, 2002). Very little is known about the waters of Levantine and North Africa (Kerem et al., 2012), although some efforts were done in 2018 in North Africa (ACCOBAMS, 2021).

The group size for the species has been described to vary between regions: six to 12 individuals around the British Isles (Evans et al., 2003; Evans, 2008); groups up to 20 individuals (modal six to 10), averaging 12.3 (1 to 55; $N = 74$) in the Azores; Pereira and Nuno (2008); 10–25 individuals in the Spanish Mediterranean (Cañadas et al., 2005; Gómez de Segura et al., 2008); 10–40 individuals in the Ligurian Sea (Airoldi et al., 2005; Azzellino et al., 2008); and the group size ranged between two and 42 Risso's dolphin with a mean value of 19 ± 9 individuals in the Golf of Taranto (central-eastern Mediterranean Sea) (Cipriano et al., 2022). Association patterns occur in pairs and in numbers of three to 12 individuals and are defined as long-term, stable units of a stratified social organisation based on age and sex classes (Hartman et al., 2008).

Abundance estimates of Risso's dolphin have been conducted at the local, regional, and basin scales. In the western Ligurian Sea (Northwestern Mediterranean), a long-term study of mark–recapture (1990–2014) resulted in a local population estimate of 100 individuals (95% CI of 60–220 individuals) (Azzellino et al., 2016). In the same study, the authors reported a significant decrease in the average population of Risso's dolphin from 120 to 150 individuals (2000 to 2005) to 70 to 100 individuals (2010–2014). Also Airoldi et al. (2015) reported a decrease in the abundance of the population in the Ligurian Sea. Aerial seasonal surveys covering an area of 181,400 km^2 of the northwestern Mediterranean Sea were conducted during the winter 2011–2012 and winter 2019 to provide estimates of abundance and distribution patterns for cetacean species, including Risso's dolphin (Laran et al., 2021). The total estimated abundance of Risso's dolphin was 2,000 individuals (95% CI: 700–5,900) in winter and 1,400 individuals (95% CI: 500–3,700) in summer. In the Spanish Mediterranean Sea, an abundance estimate based on line transect method was conducted in an area of 32,270 km^2, where aerial surveys in 2001–2003 yielded an estimate of 493 individuals (CV = 60.6%; Gómez de Segura et al., 2006). In the Alboran Sea, 864 individuals (CV = 15.65) were estimated between 2009 and 2012, based on modelling data from ship surveys, in an area covering approximately 45,000 km^2 in the Alboran Sea (INDEMARES, 2013). In summer 2018, a large-scale Mediterranean Sea survey was conducted to estimate marine megafauna within the framework of the ACCOBAMS Survey Initiative (ASI project) framework. The abundance estimate for the Risso's dolphins resulted in 24,106 individuals (95% CI = 13,986–41,548) (ACCOBAMS, 2021). The conservation status in the ACCOBAMS area for the Mediterranean population has recently been updated to endangered by the International Union for Conservation of Nature (IUCN) (Lanfredi et al., 2021).

The Mediterranean population of this species favours waters over steep slopes, submarine canyons, and seamounts (Cañadas et al., 2002; Azzellino et al., 2008; Bearzi et al., 2011; Azzellino et al., 2012; Azzellino et al., 2016) and prefers areas with depths of more than 500–2,500 m (Cañadas et al., 2002; Gómez de Segura et al., 2008). The range distance from the 200-m isobaths is around 5–30 km (Mangion and Gannier, 2002) and 14 km from the coast (Di Sciara et al., 1993). The depth range preferred by the species is explained by the trophic requirements of a mainly teuthophagus species occasionally foraging on fish and thaliaceans (Sekiguchi et al., 1992; Blanco et al., 2006; Luna et al., 2021). The adaptability of habitat use and evidence for genetic differentiation suggest the existence of various geographical units of the species within the Mediterranean (Gaspari, 2004; Jefferson et al., 2014).

A high degree of residency and site fidelity has been reported in different study areas from the Mediterranean Sea to the Atlantic Ocean based on the ecology and behaviour of the species and the availability of food resources (Hartman et al., 2008; De Boer et al., 2013; Remonato et al., 2013; Hartman et al., 2015; Maglietta et al., 2018). Studies based on photo-identification in West Provence (Golf of Lyon-Mediterranean Sea) for a part of the population called "resident" showed short movements; 63% of the individuals recaptured were within 50 km (Labach et al., 2015), sometimes recaptured after up to 18 years. However, the wide-range movements for "transient" animals, up to 493 km, were also recorded (Casacci and Gannier, 2000; Miragliuolo et al., 2004; Airoldi et al., 2005; Polo et al., 2009; Remonato et al., 2018). These long-distance movements, from offshore locations to the continental slope habitat, suggest that inter-regional movements are also possible (Delrocq and Gannier, 2016).

The range of species distribution in the Mediterranean extends from the eastern to the western end of the Mediterranean Sea, with a higher number of sightings in the western and northwest areas of the basin (Bearzi et al., 2011). Seasonal movements of the species have been monitored in the Ligurian Sea (northwestern Mediterranean Sea), where groups seem to follow a preferential route to the west, frequenting the same sites from year to year (Azzellino et al., 2008).

In this study, we analyse a long-term monitoring program on Risso's dolphins from the central area of the northwestern Mediterranean to improve the knowledge on the ecology of the species by highlighting the relevance of the proximal areas of the submarine canyons as crucial habitats for the preservation of the species.

2 Methodology

2.1 Study area

The study area (8,026 km^2) is located in the Catalano-Balearic basin in the northwestern Mediterranean Sea, off the coast of Catalonia (NE Spain). The area extends from the continental shelf to 20 NM from the coast. It includes three different systems of submarine canyons: Creus, Palamós and Maresme, which reach depths of up to 2,000 m. The study area also encompassed several Spanish Marine Protected Areas (MPA) (Figure 1).

The Creus canyon is located at the western part of the Gulf of Lion continental margin and drags waters from the Rhône River. The head of the Creus canyon is located 5 km from the coast and reaches 6 km in width and almost 2,000 m in depth. The large amounts of organic material transported along the canyon play an essential role in maintaining biodiversity rates and its associated deep-sea ecosystems (Canals et al., 2009; Orejas et al., 2009).

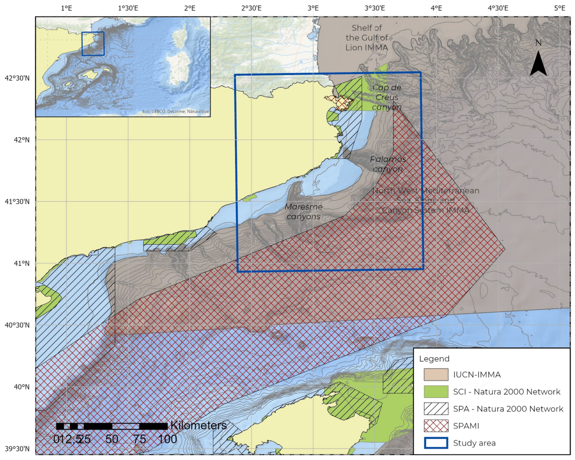

FIGURE 1
Map of the study area and nearby marine protected areas.

The Palamós canyon is one of the most prominent topographical features of the Catalan Sea. This canyon is located 20 km south of Creus canyon and has a total length of 40 km and a maximum depth of 2,200 meters. The Palamós canyon is one of the most extensive and deep canyons in the northwestern Mediterranean and transports sediments from the coastal shelf to the open sea (Martín et al., 2006; Palanques et al., 2006; Palanques and Puig, 2007). This submarine canyon constitutes a notable "hot spot" for suspended and downward sediment flows in this margin (Martín, 2005; Martín et al., 2006).

The submarine canyon system of Maresme includes three different canyons, with one of them being the most relevant as it cuts deeply into the continental slope in a non-usual north–south direction (Díaz and Maldonado, 1990). The canyon's width increases with depth, reaching up to 2,000 m with a width of 20 km (Canals et al., 2004).

2.2 Data collection

Data was collected from 2009 to 2021 in specific sighting surveys using sailing vessels 12–15 m in length. The research team included two observers at different heights, using a crow's nest and an angle meter to calculate the perpendicular distance. Planned transects were designed as triangles to cross-depth contours as perpendicularly as possible (Figure 2A) and to cover as much of the area as possible (Figure 2B). The sighting effort was measured as the number of kilometres travelled with adequate sighting conditions (up to Beaufort Sea state 3) and observers at the lookout posts. The effort was recorded with a GPS navigation system, using Logger, the IFAW Data Logging Software (NMEA data automatically recorded every minute in a database), which continuously recorded the geographic position of the ship. Data on time, species, number of individuals, behaviour, presence of calves, movement to the vessel, and specific observations were also recorded. The group size was estimated by visual counts defined as the minimum number of individuals sighted at the same time (min), the maximum number of individuals sighted at the same time (max), and the most agreed number from the observers (best

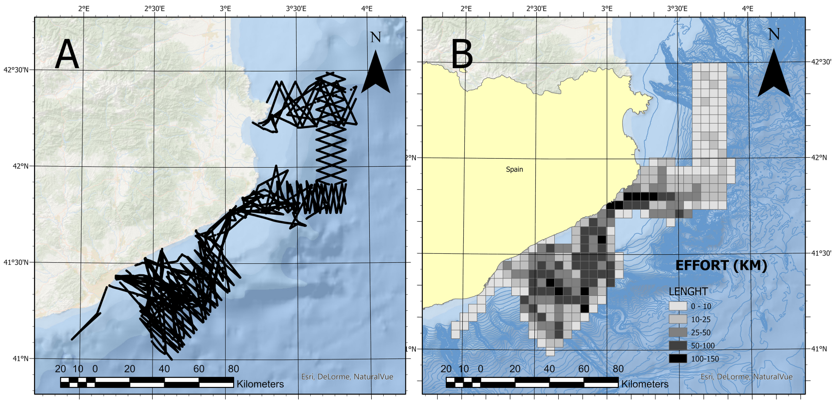

FIGURE 2
(A) Map with on-effort track legs during the sampling period. **(B)** Effort distribution calculated on a 5 × 5-km grid shape file (EPSG:25831).

estimate). A group was defined as all the individuals that interacted socially and/or showed coordinated behaviour with a distance of less than five body lengths from the others (Whitehead, 2003).

The authors had access to the Proyecto Mediterráneo dataset (Raga and Pantoja, 2004) and the ACCOBAMS Survey Initiative dataset (ASI 2018), which included 17 sightings of Risso's dolphin in the study area since 1985. These datasets were added to the authors' dataset, resulting in 34 Risso's dolphin sightings that were used for the depth and distance-to-the-coast analysis.

2.3 Data analysis

2.3.1 Relative density

The study area was divided into a grid of 338 squares with a cell resolution of 5 × 5 km each, and the effort was calculated for each grid cell. The effort was evaluated in terms of kilometres of track lines (Figure 2A) per cell unit. Only the effort in "favourable conditions" (*i.e.*, wind not exceeding 3 on the Beaufort scale) was considered. The tracks corresponding to the time spent with the same group of animals were also excluded. The relative density [density per unit of effort (animals/km)] was calculated as the number of individuals per kilometre of effort within each cell unit. The encounter rate (ER) was also calculated as sightings per kilometre for the different years. Geospatial analysis was performed with ArcMap 10.6.1. The 31N UTM Transverse Mercator projection was used for all GIS analyses (EPSG:25831).

2.3.2 General additive model

Presence–absence habitat models are suitable to relate species occurrence with information on the spatial characteristics of locations where the species was found (Elith and Leathwick, 2009). In this case, we used a generalized additive model (GAM) with binomial distribution and a logit link, using as a covariate of response the presence (1) and absence (0) of Risso's dolphin in each grid cell. The benefit of additive modelling resides in its flexibility in capturing non-linear species–habitat relationships. When the data is related to certain variables but the relationships fail to be simply linear, GAM uses a link function to establish a relationship between the mean of the response variable and the smooth function of the explanatory variables. Consequently, the association between response and explanatory variables derives from the data itself and not from the model because no kind of parametric assumption is made (Hastie and Tibshirani, 1990; Yee and Mitchell, 1991). In this study, the GAM approach was applied to determine whether the selected variables affect the distribution of *Grampus griseus* in the study area. Zero-inflated models (Poisson regression and negative binomial) are used to model count data that has an excess of zero counts. Our data responds to binary data (1/0) and not to count data with an excess of zeros. Therefore, binary logistic regression (family = binomial), with a logit function, was applied.

We start by fitting a linear model. Figure 3 represents the relationship between each possible pairing of environmental variables: depth, distance to coast, slope, and the variation of the slope. The relationship between variables (depth and distance to the coast) showed a covariance between them, tested through a Pearson correlation coefficient, so only one was included in the model. The selection of the model was based on the Akaike information criterion and the explanation of the model's deviance. In all models, the significance of the deviance was tested with a χ^2 test, and a visual inspection of the residuals was made, especially to look for trends.

The general structure of the selected model was as follows:

$$E(p_i) = \frac{\mathbf{exp}\ [\beta_0 + \sum_i f_i\ (z_{ij})]}{1 + \mathbf{exp}[\beta_0 + \sum_i f_i\ (z_{ij})]}$$

where p_i is the proportion of positive observations in grid i, β_0 is the intercept, f_i is smooth functions of the predictor covariates, and z_{ij} is

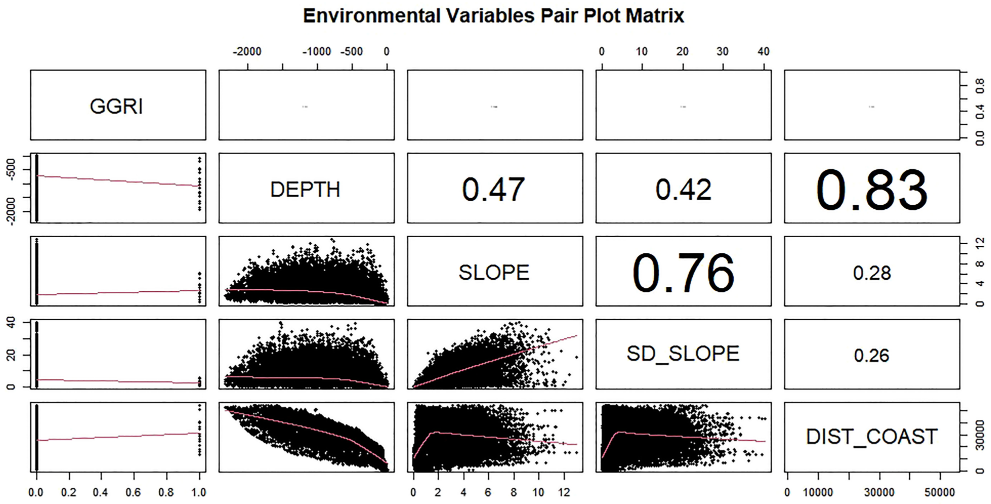

FIGURE 3
Linear correlation between environmental variables (DEPTH_MASK = depth, SLOPE = slope, SD_SLOPE = standard deviation slope, DIST COASt = distance coast).

the value of the predictor covariate k in grid i. The models were fitted using the "mgcv" package version 1.7-26 for R version 3.0.2 (Wood and Wood, 2015), performing the manual selection.

A raster data layer of 1 km/1 km cell was created, combining effort and sighting in the study area. The cell value was indicated with 1 or 0 for the presence of Risso's dolphin. The cell size was chosen since other studies in the Mediterranean indicate the size as appropriate for the spatial variation in environmental variables.

Due to data variation, only physiographical variables were measured for describing the presence/absence of Risso's dolphin: depth, slope, slope variation, and distance to the coast. An environmental raster data layer of 1 km/1 km was created using a geographical information system (QGIS 3.14, QGIS Desktop 2.8.3). Physiographical variables were calculated for each cell, calculating the mean cell centroid coordinates. Depth was calculated from bathymetric data available at GEBCO (https://www.emodnet.eu/, 2020), using the GIS tool surf.contour, by calculating the difference between water isolines and the raster depth while avoiding the land parts of the raster. The slope was calculated with the Terrain analysis tool (Grass Package QGIS Desktop 2.8.4) and expressed in degrees. The variation in the slope was also calculated as the standard deviation of the slope. The distance to the coast was calculated with the r.neighbours tool (Grass Package 7.8.3 for QGIS).

2.4 Depth and distance to the coast

The depth and the distance to the coast of each sighting were compared to determine changes in Risso's dolphin habitat distribution preference in the area. No data on Risso's dolphin was registered in 2015. We established 2015 as the breaking point for the first and second study periods. Thus, data was clustered into two periods, according to the year they were recorded: first period 1985–2014 (n = 24) and second period 2016–2021 (n = 10) (Table 1).

A parametric test (t-test) was applied to test the difference for central values with depth and distances to the coast during the two different periods since the data was found not to differ significantly from a normal distribution (depth: Shapiro–Wilk test, W = 0.96545, p-value = 0.3479, n = 34; distance to coast: Shapiro–Wilk test, W = 0.96545, p-value = 0.3479).

3 Results

From 2009 to 2021, a total of 8,756.68 km of effort was surveyed within the study area, and data from 17 sightings of Risso's dolphins was registered. The overall ER was 0.0019 sightings/km, although this number changed between years. Table 2 show a summary of the total effort and ER for the different years. The average group size was 5.7 individuals ± 3.8 SD.

The mean relative density was 0.078 individuals/km^2 (SD 0.1, n = 17) (Figure 4). The sightings were generally located in the southern and central submarine canyons, and no sightings were registered in the northern canyon.

The sightings occurred in a depth ranging between 100 and 1,900 m, with a mean depth of 1,754 m (SD 567, n = 17). The mean distance from the coast was 31.7 km (Table 3).

TABLE 1 Summary of the sightings for the different projects and years clustered into different periods.

Year	Data	GGRI school sightings	Period
1985	Proyecto Mediterraneo	1	First period
1987	Proyecto Mediterraneo	1	First period
1991	Proyecto Mediterraneo	1	First period
1994	Proyecto Mediterraneo	3	First period
1998	Proyecto Mediterraneo	1	First period
1999	Proyecto Mediterraneo	1	First period
2001	Proyecto Mediterraneo	5	First period
2009	SUBMON	4	First period
2010	SUBMON	3	First period
2011	SUBMON	1	First period
2013	SUBMON	2	First period
2014	SUBMON	1	First period
2016	SUBMON	1	Second period
2018	ASI	3	Second period
2020	SUBMON	2	Second period
2021	SUBMON	4	Second period
1985–2021	Total	34	

TABLE 2 Summary of the vessel surveys carried out in the study area showing the effort (km) and the number of schools of Risso's dolphin (GGRI = *Grampus griseus*) observed and the encounter rate (ER = sightings/eff).

Year	Number of surveys (days)	Effort (km)	GGRI school sightings	ER (sightings/eff)
2009	8	513.275	4	0.00779309
2010	15	1,026.768	3	0.00292179
2011	7	521.345	1	0.00191812
2013	5	228.754	2	0.00874302
2014	7	2,102.749	1	0.00047557
2015	13	2,683.139	0	0
2016	1	63.548	1	0.01573614
2020	16	679.928	2	0.00294149
2021	12	937.174	3	0.00320111
Total	72	8,756.68	17	0.00194138

Figure 5 represents the physiographical conditions in which the species was present (1) and absent (0), showing that Risso's dolphins were present in deep zones, although they could be observed in shallower areas. The presence of Risso's dolphin only occurred in offshore waters (>12 km perpendicular distance in the area), coincident with the presence of the submarine canyons in the area. The presence of Risso's dolphin was also related to steep slopes.

3.1 GAM results

GAM developed for Risso's dolphin reached 19.8% of explained deviance. Table 3 shows the results of the final model selected for the presence of Risso's dolphin. The final occurrence probability model retained three covariates: distance to the coast, slope, and slope variation.

The spatial distribution of Risso's dolphin was strongly related to the slope and to the slope variation (SD_SLOPE) (Table 3). These two variables are associated with the geomorphological features of submarine canyons within the study area, characterized by having steep slopes.

Figure 6 shows that the relationship with the distance to the coast was significant, indicating a direct relationship between the presence of Risso's dolphin and the distance to the coast. The GAM identified that the habitat for Risso's dolphin was an offshore one, with distances from the coast greater than 12 km and their presence increasing after 47 km from the coast. Medium slopes and slope variance, corresponding to the head and centre parts of the submarine canyons, also characterized it and were related to the presence of Risso's dolphin in the model.

3.2 Depth and distance to the coast

The distance to the coast of Risso's dolphin sightings differed significantly between the two study periods (*t*-test: t = -2.9302, df = 14.14, *p*-value = 0.01087). While in the first period (1985–2014) the average distance to the coast was 30.77 km (SD =12.6 km), in the second period (2016–2021) the average distance was 47 km (SD = 10.7 km). The mean depth of Risso's dolphin sightings showed no significant differences between the two study periods, although in the second period the sightings occurred in deeper waters [1,366.6 m (SD = 441 m)] than in the first study period [918 m (SD = 372 m)] (*t*-test: t = -2.1458, df = 12.298, *p*-value = 0.0525). Figure 7 shows the localization of the sighting during the two study periods. Table 4 shows the descriptive statistics of Risso's dolphin total sightings related to the physiographic variables.

4 Discussion

The ER of Risso's dolphin in the study area obtained in this study is consistent with the results obtained in the West Mediterranean Basin (Gómez de Segura et al., 2006; Laran et al., 2021) and Gulf of Taranto (Central-eastern Mediterranean Sea) (Carlucci et al., 2020) and higher than the ER from aerial surveys in a closer area (Gómez de Segura et al., 2008; ACCOBAMS, 2021).

TABLE 3 Results of the final model selected for the Risso's dolphin.

	Edf	Ref. df	Chi-square	*p*-value	
s(SLOPE)	2.334	2.861	17.75	0.000511	***
s(SD_SLOPE)	1.001	1.002	14.07	0.000176	***
s(DIST_COAST)	4.897	5.561	15.40	0.010109*	*

Variables: distance to coast, slope, and SD of slope. R-sq.(adj) = 0.017. Deviance explained = 19.8%. Akaike information criterion = 252.4452. Significance codes: 0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1.

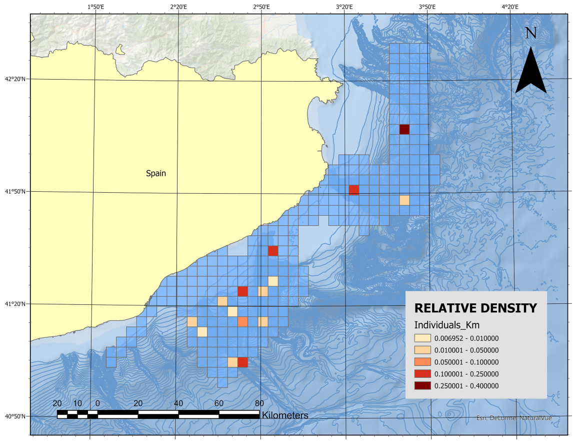

FIGURE 4
Map of the relative density of Risso's dolphin.

TABLE 4 Descriptive statistics of the distribution by depth and slope (degrees), standard deviation slope (Std. Dev., slope), and the distance to the coast (Dist. coast) for Risso's dolphin sightings.

	Depth (m)	Slope	Std. Dev., slope	Dist. coast (m)
Mean	1,074	10.3	2.8	31,748.40
Standard deviation	567	8.4	1.76	12,675.50
Max	1,950	26.5	6	54,203.30
Min	100	1.5	0.38	13,341.70
Median	1,143	6	2.7	28,635.60

However, the ER value was lower than in the south-central Mediterranean Sea (Corrias et al., 2021).

On the contrary, the results from the ACCOBAMS report (ACCOBAMS, 2021) showed a density of 0.548 Risso's dolphin/km^2 for the Levantine Balearic area and 0.344 Risso's dolphin/km^2 for the Alboran Sea and northern Algeria. This data can be contrasted with the last estimate available for an area similar to these blocks, which was carried out by Gómez de Segura et al. (2006) with a result of 0.041 Risso's dolphin/km^2. These two values differ substantially, and given that the estimate using data from the ASI Project is based on information from only three sightings, this later density should be considered cautiously.

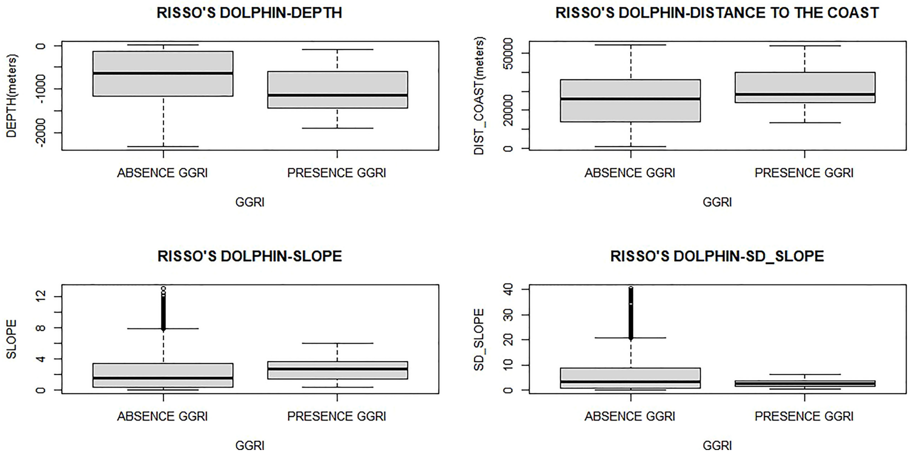

FIGURE 5
Physiographical conditions in which the species *Grampus griseus* is present (1) and absent (0).

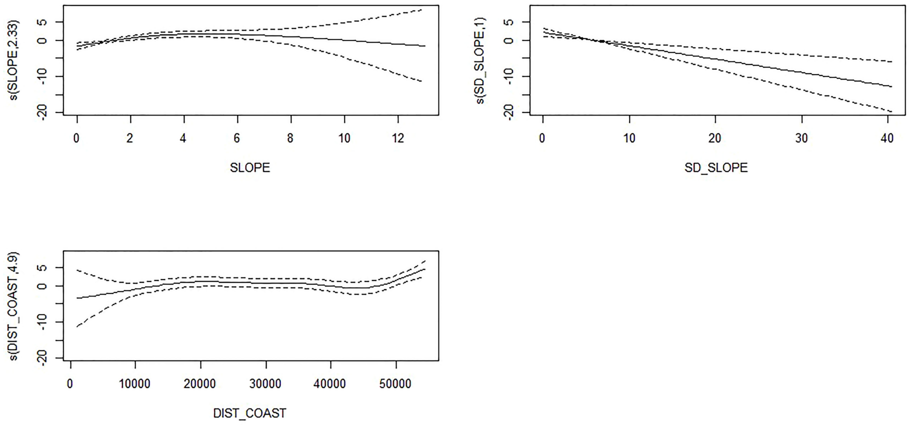

FIGURE 6
Predicted smooth splines of the response variable presence/absence of *Grampus griseus* as a function of the validated explanatory variables. The degrees of freedom for non-linear fits are in parentheses on the y-axis. Tick marks above the x-axis indicate the distribution of sightings. Dotted lines represent the 95% confidence intervals of the smooth spline functions.

The relative density from our study shows that the highest values in the study area occurred in the southern and central submarine canyons (Maresme and Palamós canyons) (Figure 4), thus overlapping with the less protected area from the zone. These two submarine canyons have been included in the IMMAs proposal, but no specific binding protected figure has been established yet. Additionally, the SPAMI—Cetacean Migration Corridor, an MPA declared in 2018, is also farther away, only including a small area of the submarine canyon system.

The model explained a strong relationship between the presence of Risso's dolphins with slope and slope variation, showing a preference for the steeper areas that, in the case of the study area, corresponds to the underwater canyons' geomorphological features. Our findings are consistent with previous studies on Risso's dolphin in the Mediterranean (Di Sciara et al., 1993; Cañadas et al., 2002; Gannier, 2005; Bearzi et al., 2011), which indicated a predilection for the continental slope with depths ranging from 500 to 1,500 m (Azzellino et al., 2012).

According to our model results, the distribution of Risso's dolphins was also correlated with the distance to the coast as it was also found in the waters of Valencia and Murcia Regions (central Spanish Mediterranean) located southern to our study area (Gómez de Segura et al., 2008) but was not correlated with depth as it was in Gómez de Segura et al. (2006). This can be explained because distance from the coast is not related to depth in our area as it is more related into the southern area since the underwater canyons are quite close to the coast. Therefore, at the same depths, distances to the coast can vary substantially.

Our model explained 19.8% of the deviance; therefore, other factors could be related to the distribution of Risso's dolphin in the area. Gómez de Segura et al. (2008) found that the second-best model for Risso's dolphin incorporated the temporal variability of SST. Environmental variations could not be included in our model because of the big-scale temporal variability of the data. Moreover, data scarcity could explain the low adjustment of R^2 and therefore the low deviance, thus producing a less accurate model.

Data analyzed from the different datasets (1985 to 2021) confirm that the species' sightings in coastal areas and over the continental shelf have decreased while remaining stable in pelagic areas of the Western Mediterranean Sea ((Azzellino et al., 2016; ACCOBAMS, 2021). Data on the second period of the study (2016–2021) confirm that Risso's dolphin sightings were made at a substantially greater distance from the coast (50 km, SD = 1.7 km) than those made in the first period of the study (1985–2014) (31.2 km, SD = 12.6 km), supporting the results from Azzellino et al. (2016) that suggest a displacement of the species to offshore areas.

The role of submarine canyon systems as a relevant habitat for the species is also supported by its feeding ecology. The species is considered mainly teutophagous: in the analyses of stomach content from individuals stranded in the northwestern Mediterranean conducted by Blanco et al. (2006), there was reported predation on cephalopod species from the middle slope, and more recently, Luna et al. (2021) identified

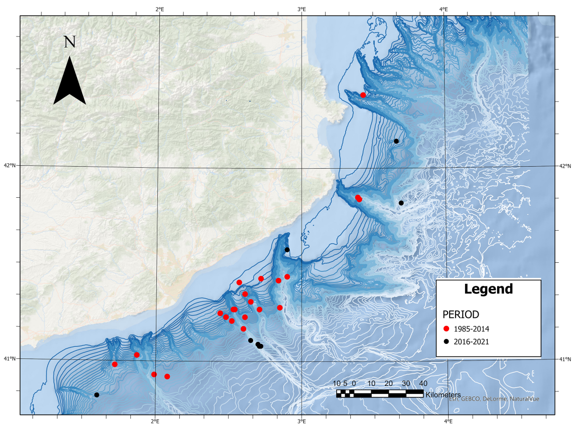

FIGURE 7
Map of the sightings in the different periods (1985–2014 and 2016–2021).

cephalopod species associated to deeper waters of the continental shelf from stranded individuals along the study site. Considering trophic ecology, Borrell et al. (2021) conducted a study of stable isotopic niches of carbon, nitrogen, and sulphur for five species of cetaceans inhabiting the northwestern Mediterranean Sea, and the results placed the Risso's dolphin in the highest trophic value, together with two deep-diver cetaceans—the long-finned pilot whale (*Globicephala melas*) and the Cuvier's beaked whales (*Ziphius cavirostris*). Thus, similarities between the feeding strategies of the three species arise. The same study showed, for the species, a narrow range of isotopic sulphur values compared with the other deep-diver species, suggesting that Risso's dolphin may occupy a relatively narrow range of the offshore habitat, primarily associated with submarine canyons, as has been suggested in distribution studies based on visual or acoustic data (Praca and Gannier, 2008; David and Di-Meglio, 2012).

Submarine canyon systems are marine ecosystems that support high levels of biodiversity (Fernandez et al., 2017; Santora et al., 2018). In the northwestern Mediterranean Sea, the submarine canyon systems and upwellings are two geomorphological and oceanographic features that encourage levels of productivity of outstanding biological and ecological relevance for the area. The interplay between the canyon topography and the oceanic currents has profound consequences for the high diversity that they support, affecting not only benthic communities but pelagic ones as well. In this sense, our results on the distribution of Risso's dolphins show how relevant this deep habitat is for the species.

The results and data provided in this study will be incorporated into the conservation and management plans for the Risso's dolphin in Spanish Mediterranean waters within the framework of the Marine Strategy Framework Directive. In a wider scope, our results will also contribute to establish the Important Marine Mammal Area of the North-West Mediterranean Sea, Slope, and Canyon System IMMA proposed by the International Committee on Marine Mammal Protected Areas, the IUCN, and World Commission on Protected Areas.

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 were not required for the animal study because the study was carried out without any invasive technique.

Author contributions

CC, NA, and MG contributed to conception and design of the study and also to the data collection. CC and NA organized the database, and CC performed the statistical analysis. CC wrote the first draft of the manuscript. All authors contributed to manuscript revision, read, and approved the submitted version. NA is author of the picture from the cover. MG contributed to the final structure of the paper. 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.

Publisher's note

References

ACCOBAMS (2021). Absolute abundance of Risso's dolphin (*Grampus griseus*) in the north western part of the Pelagos Sanctuary. *Pelagos Sanctuary Convention No. 03/2014*, 59 pages. Istituto Tethys /EcoOcean Institut/GECEM/Fondazione CIMA/GIS3M.

Airoldi, S., Azzellino, A., David, L., Dhermain, F., Di-Meglio, N., Jimenez, V., et al. (2015). *Final report "Absolute abundance of risso's dolphin (Grampus griseus) in the northwestern part of the pelagos sanctuary " Pelagos sanctuary convention no.* 59.

Airoldi, S., Bendinoni, F., Azzellini, A., Fadda, V., and Profice, A. (2005). "Abundance estimates of risso's dolphins (*Grampus griseus*) in the western ligurian Sea through photographic mark-recapture," in *19th Conference of the European Cetacean Society*, La Rochelle, France.

Arcangeli, A., Aissi, M., Atzori, F., Azzolin, M., Buoninsegni, J., Campana, L., et al. (2018). "Improving knowledge and conservation of the Mediterranean population of risso's dolphins through effective partnerships," in *Preliminary report of the Mediterranean grampus project 2.0.* Eds. C. Lanfredi, E. Remonato and S. Airoldi(La Spezia, Italy), 50.

Arcangeli, A., Marini, L., and Crosti, R. (2012). Changes in cetacean presence, relative abundance and distribution over 20 years along a trans-regional fixed line transect in the central tyrrhenian Sea. *Mar. Ecol.* 34. doi: 10.1111/maec.12006.

Azzellino, A., Airoldi, S., Gaspari, S., Lanfredi, C., Moulins, A., Podestà, M., et al. (2016). "Risso's dolphin, grampus griseus, in the Western ligurian Sea: trends in population size and habitat use," in *Advances in marine biology*, vol. 75 . Eds. G. N. Di Sciara, M. Podestà and B. E. Curry (Oxford: Academic Press), 205–232.

Azzellino, A., Gaspari, S., Airoldi, S., and Nani, B. (2008). Habitat use and preferences of cetaceans along the continental slope and the adjacent pelagic waters in the western ligurian Sea. *Deep Sea Res. Part I: Oceanographic Res. Papers.* 55 (3), 296–323. doi: 10.1016/j.dsr.2007.11.006

Azzellino, A., and Lanfredi, C. (2015). *Analisi di distribuzione e abbondanza assoluta e relativa delle specie capodoglio (Physeter macrocephalus), grampo (Grampus griseus) e zifio (Ziphius cavirostris) nel santuario pelagos alla luce dei cambiamenti ambientali e dei fattori di pressione antropica*. ACCOBAMS-SC14/2021/Doc16

Azzellino, A., Panigada, S., Lanfredi, C., Zanardelli, M., Airoldi, S., and Notarbartolo di Sciara, G. (2012). Predictive habitat models for managing marine areas: spatial and temporal distribution of marine mammals within the pelagos sanctuary (Northwestern Mediterranean sea). *Ocean Coast. Manage.* 67, 63e74. doi: 10.1016/j.ocecoaman.2012.05.024

Bearzi, G., Reeves, R. R., Remonato, E., Pierantonio, N., and Airoldi, S. (2011). Risso's dolphin grampus griseus in the Mediterranean Sea. *Mamm. Biol.* 76, 385–400. doi: 10.1016/j.mambio.2010.06.003

Blanco, C., Raduan, M. A., and Raga, J. A. (2006). Diet of risso's dolphin (*Grampus griseus*) in the western Mediterranean Sea. *Scientia Marina* 70, 407–411. doi: 10.3989/scimar.2006.70n3407

Boisseau, O., Lacey, C., Lewis, T., Moscrop, A., Danbolt, M., and McLanaghan, R. (2010). Encounter rates of cetaceans in the Mediterranean Sea and contiguous Atlantic area. *J. Mar. Biol. Assoc. U. K.*. 90, 1589–1599. doi: 10.1017/S0025315410000342

Borrell, A., Gazo, M., Aguilar, A., Raga, J. A., Degollada, E., Gozalbes, P., et al. (2021). Niche partitioning amongst northwestern Mediterranean cetaceans using stable isotopes. *Prog. Oceanography* 193, 102559. doi: 10.1016/j.pocean.2021.102559

Campana, I., Crosti, R., Angeletti, D., Carosso, L., David, L., Di-Méglio, N., et al. (2015). Cetacean response to summer maritime traffic in the Western Mediterranean Sea. *Mar. Environ. Res.* 109, 1–8. doi: 10.1016/j.marenvres.2015.05.009

Cañadas, A., Sagarminaga, R., De Stephanis, R., Urquiola, E., and Hammond, P. S. (2005). Habitat selection models as a conservation tool: proposal of marine protected areas for cetaceans in southern Spain. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 15, 495–521. doi: 10.1002/aqc.689

Cañadas, A., Sagarminaga, R., and Garcıa-Tiscar, S. (2002). Cetacean distribution related with depth and slope in the Mediterranean waters off southern Spain. *Deep Sea Res. Part I: Oceanographic Res. Papers* 49 (11), 2053–2073. doi: 10.1016/S0967-0637(02)00123-1

Canals, M., Casamor, J., Lastras, G., Monaco, A., Acosta, J., Berné, S., et al. (2004). The role of canyons in strata formation. *Oceanography* 17 (4), 80–91. doi: 10.5670/oceanog.2004.06

Canals, M., Danovaro, R., Heussner, S., Lykousis, V., Puig, P., Trincardi, P., et al. (2009). Cascades in Mediterranean submarine grand canyons. *Oceanography* 22, 26–43. doi: 10.5670/oceanog.2009.03

Carlucci, R., Baş, A. A., Liebig, P., Renò, V., Santacesaria, F., Bellomo, S., et al. (2020). Residency patterns and site fidelity of grampus griseus (Cuvier 1812) in the gulf of taranto (Northern Ionian Sea, central-Eastern Mediterranean Sea). *Mamm Res.* 65, 445–455. doi: 10.1007/s13364-020-00485-z

Casacci, C., and Gannier, A. (2000). Habitat variability and site fidelity of the risso's dolphin in the northwestern Mediterranean: defining a home range for a nomad. *Eur. Res. Cetacean.* 14, 19–22.

Chicote, C. A., Gazo, M., Cañadas, A., Pauner, O., Saiz, L., Pastor, C., et al. (2015). Proyecto grampus: estudio y seguimiento de la población de calderón gris asociada a cañones submarinos en la costa catalana. *Tech. report-Fundación Biodiversidad*.

Cipriano, G., Carlucci, R., Bellomo, S., Santacesaria, F. C., Fanizza, C., Ricci, P., et al. (2022). Behavioral pattern of risso's dolphin (*Grampus griseus*) in the gulf of taranto (Northern Ionian Sea, central-Eastern Mediterranean Sea). *J. Mar. Sci. Eng.* 10, 175. doi: 10.3390/jmse10020175

Corrias, V., Filiciotto, F., and Giardina, F. (2021). Sightings of risso's dolphin (*Grampus griseus*) off the southern coast of linosa island (south-central Mediterranean Sea). *Mediterr. Mar. Sci.* 22 (2), 387–392. doi: 10.12681/mms.23648

David, L., and Di-Meglio, N. (2012). "Role and importance of submarine canyons for cetaceansand seabirds in the north-westen Mediterranean Sea," in *Mediterranean Submarine canyons: ecology and governance*. Ed. M. Würtz (Switzerland and M íalaga, Spain: IUCN Gland), 113–122.

De Boer, M., Clark, J., Leopold, M., Simmonds, M., and Reijnders, P. (2013). Photo-identification methods reveal seasonal and long-term site-fidelity of risso's dolphins (*Grampus griseus*) in shallow waters (Cardigan bay, Wales). *Open J. Mar. Science.* 03, 66–75. doi: 10.4236/ojms.2013.32A007

Delrocq, S., and Gannier, A. (2016). Contribution a l'etude du dauphin de risso en mediterranee occidentale: nouveaux resultats obtenus par photo-identification. *Rev. d'Ecologie-la Terre la Vie* 71 (2), 192–201. doi: 10.3406/revec.2016.1840

Díaz, J., and Maldonado, A. (1990). Transgressive sand bodies on the maresme continental shelf, western Mediterranean Sea. *Mar. Geology* 91 (1-2), 53–72. doi: 10.1016/0025-3227(90)90132-4

Dimatteo, S., Siniscalchi, M., Esposito, L., Prunella, V., Bondanese, P., Bearzi, G., et al. (2011). Encounters with pelagic and continental slope cetacean species near the northern shore of the gulf of taranto, Italy. *Ital. J. Zoology* 78 (1), 130–132. doi: 10.1080/11250003.2010.532161

Di Sciara, N., Venturino, M. C., Zanardelli, M., Bearzi, G., and Borsani, J. B. (1993). Cetaceans in the central Mediterranean Sea: distribution and sighting frequencies. *Bollettino di Zoologia* 60, 131–138. doi: 10.1080/11250009309355800

Elith, J., and Leathwick, J. R. (2009). Species distribution models: ecological explanation and prediction across space and time. *Annu. Rev. ecology evolution systematics* 40, 677–697. doi: 10.1146/annurev.ecolsys.110308.120159

Evans, P. G. H. (2008). "Risso's dolphin grampus griseus," in *Mammals of the British isles*Handbook, *4th Edition*. Eds. S. Harris and D. W. Yalden. (Southampton: The Mammal Society), 740–743.

Evans, P. G. H., Anderwald, P., and Baines, M. E. (2003). UK Cetacean Status Review. *Report to English Nature and the Countryside Council for Wales*. SeaWatch Foundation, Oxford. 160pp.

Fernandez, U., Ramirez, E., Aguzzi, J., Allcock, A. L., Davies, J. S., Dissanayake, A., et al. (2017). Ecological role of submarine canyons and need for canyon conservation: a review. *Front. Mar. Sci.* 4. doi: 10.3389/fmars.2017.00005

Frantzis, A., and Herzing, D. L. (2002). Mixed-species associations of striped dolphins (*Stenella coeruleoalba*), short-beaked common dolphins (Delphinus delphis), and risso's dolphins (*Grampus griseus*) in the gulf of Corinth (Greece, Mediterranean Sea). *Aquat. Mammals.* 28, 188–197.

Gannier, A. (2005). Summer distribution and relative abundance of delphinids in the Mediterranean Sea. *Rev. D'Ecologie* 60, 223–238. doi: 10.3406/revec.2005.1267

Gaspari, S. (2004). *Social and population structure of striped and risso's dolphins in the Mediterranean Sea* (Durham, UK: University of Durham).

Gaspari, S., and Natoli, A. (2012). *Grampus griseus (Mediterranean subpopulation)* (The IUCN).

Gómez de Segura, A., Crespo, E. A., Pedraza, S. N., Hammond, P. S., and Raga, J. A. (2006). Abundance of small cetaceans in the waters of the central Spanish Mediterranean. *Mar. Biol.* 150, 149–160. doi: 10.1007/s00227-006-0334-0

Gómez de Segura, A., Hammond, P. S., and Raga, J. A. (2008). Influence of environmental factors on small cetacean distribution in the Spanish Mediterranean. *J. Mar. Biol. Assoc. U. K.*. 8, 1185–1192. doi: 10.1017/S0025315408000386.

Hartman, K. L., Fernandez, M., Wittich, A., and Azevedo, J. M. N. (2015). Sex differences in residency patterns of risso's dolphins (*Grampus griseus*) in the Azores: causes and management implications. *Mar. Mammal Sci.* 31, 1153–1167. doi: 10.1111/mms.12209

Hartman, K., Visser, F., and Hendriks, A. (2008). Social structure of risso's dolphins (*Grampus griseus*) at the Azores: a stratified community based on highly associated social units. *Can. J. Zoology.* 86, 294–306. doi: 10.1139/Z07-138

Hastie, T. J., and Tibshirani, R. J. (1990). *Generalized additive models* (Boca Raton, Florida, USA: Chapman and Hall/CRC).

INDEMARES (2013). *Informe final técnico*. Available at: https://www.indemares.es/sites/default/files/informe_final_tecnico_alnitak.pdf.

Jefferson, T., Weir, C., Anderson, R., Ballance, L., Kenney, R., and Kiszka, J. (2014). Global distribution of risso's dolphin (*Grampus griseus*): a review and critical evaluation. *Mammal Rev.* doi: 10.1111/mam.12008

Kerem, D., Hadar, N., Goffman, O., Scheinin, A., Kent, R., Boisseau, O., et al. (2012). Update on the cetacean fauna of the Mediterranean levantine basin. *Open Mar. Biol. J.* 6, 6–27. doi: 10.2174/1874450801206010006

Labach, H., Dhermain, F., Bompar, J.-M., Dupraz, F., Couvat, J., David, L., et al. (2015). Analysis of 23 years of risso's dolphins photo-identification in the north-Western Mediterranean Sea, first results on movements and site fidelity sci. *Rep. Port-Cros Natl. Park* 29, 263–266.

Lanfredi, C., Arcangeli, A., David, L., Holcer, D., Rosso, M., and Natoli, A. (2021). *Grampus griseus (Mediterranean subpopulation)* (The IUCN Red List of Threatened Species 2021: e.T16378423A190737150).

Laran, S., Nivière, M., Genu, M., Dorémus, G., Serre, S., Spitz, J., et al. (2021). *Distribution et abondance de la mégafaune marine lors des campagnes SAMM cycle I et II en méditerranée* (La Rochelle Université: Rapport final. Observatoire Pelagis), 76.

Luna, A., Sánchez, P., Chicote, C., and Gazo, M. (2021). Cephalopods in the diet of risso's dolphin (*Grampus griseus*) from the Mediterranean Sea: a review. *Mar. Mammal Sci.* 38 (2). doi: 10.1111/mms.12869

Maglietta, R., Renò, V., Cipriano, G., Fanizza, C., Milella Stella, E., Carlucci, R., et al. (2018). DolFin: an innovative digital platform for studying. risso's dolphins in the northern Ionian Sea (North-eastern central Mediterranean). *Sci. Rep.* 8 (1), 1–11.

Mangion, P., and Gannier, A. (2002). Improving the comparative distribution picture for Risso's dolphin and long-finned pilot whale in the Mediterranean Sea. *Eur. Res. Cet.* 16, 68–72.

Martín, J. (2005). *Sedimentary dynamics in the palamós submarine canyon* (Universitat Politècnica de Catalunya), 198.

Martín, J., Palanques, A., and Puig, P. (2006). Composition and variability of downward particulate matter fluxes in the palamós submarine canyon (NW Mediterranean). *J. Mar. Syst.* 60 (1-2), 75–97. doi: 10.1016/j.jmarsys.2005.09.010

Menniti, M. A., and Vella, A. (2022). Sighting of risso's dolphin (*Grampus griseus*) during scientific research of the calabrian southern Ionian Sea (Central Eastern Mediterranean). *Natural Eng. Sci.* 7 (3), 248–259. doi: 10.28978/nesciences.1206056

Miragliuolo, A., Mussi, B., and Bearzi, G. (2004). Risso's dolphin harassment by pleasure boaters off the island of ischia, central Mediterranean Sea. *Eur. Res. Cetaceans* 15.

Moulins, A., Rosso, M., Ballardini, M., and Wurtz, M. (2008). Partitioning of the pelagos sanctuary (northwestern Mediterranean Sea) into hotspots and coldspots of cetacean distributions. *J. Mar. Biol. Assoc. U. K.* 88, 1273–1281. doi: 10.1017/S0025315408000763

Orejas, C., Gori, A., Lo Iacono, C., Puig, P., Gili, J. M., and Dale, M. R. T. (2009). Cold-water corals in the cap de creus canyon, northwestern Mediterranean: spatial distribution, density and anthropogenic impact. *Mar. Ecol. Prog. Ser.* 397, 37–51. doi: 10.3354/meps08314

Palanques, A., Martín, P., Puig, J., Guillén, J., Company,, and Sarda, F. (2006). Evidence of sediment gravity flows induced by trawling in the palamo´s (Fonera) submarine canyon (northwestern Mediterranean). *Deep-Sea Res. I* 53, 201–214. doi: 10.1016/j.dsr.2005.10.003

Palanques, A., and Puig, P. (2007). Near-bottom horizontal transfer of particulate matter in the palamós submarine canyon (NW Mediterranean). *J. Mar. Res.* 65 (2), 193–218.

Pereira, J., and Nuno, D. S. G. (2008). Field notes on risso's dolphin (*Grampus griseus*) distribution, social ecology, behaviour, and occurrence in the Azores. *Aquat. Mammals* 34 (4), 426–435. doi: 10.1578/AM.34.4.2008.426

Polo, L., David, L., Di-Meglio, N., and Rosso, M. (2009). First analysis of long-term association of risso's dolphin (*Grampus griseus*) population in the liguro – provençal basin and gulf of lion. *Eur. Res. Cetacean* 23.

Praca, E., and Gannier, A. (2008). Ecological niches of three teuthophageous odontocetes in the northwestern Mediterranean Sea. *Ocean Sci.* 4 (1), 49–59. doi: 10.5194/os-4-49-2008

Raga, J. A., and Pantoja, J. (2004). *Proyecto mediterráneo: zonas de especial interés para la conservación de los cetáceos en el mediterráneo español* (Organismo Autónomo Parques Nacionales).

Remonato, E., Aimi, A., and Airoldi, S. (2013). "Study of residency patterns, home ranges and movements of risso's dolphins (*Grampus griseus*, cuvier 1812) in the Western ligurian Sea," in *Grampus griseus 200th anniversary: Risso's dolphins in the contemporary world". Report from the European Cetacean Society Conference Workshop*, , 25 March 2012. 54–68.

Remonato, E., Airoldi, S., Rosso, M., Lanfredi, C., Bittau, L., Renata, M., et al. (2018). "Where are the risso's dolphins (*Grampus griseus*) of the north Western Mediterranean Sea? study of movements within and outside the pelagos sanctuary," in *32nd annual European Cetacean Society conference*, La Spezia (Italy, 8-10 April, 2018.

Santora, J. A., Zeno, R., Dorman, J. G., and Syderman, W. (2018). Submarine canyons represent an essential habitat network for krill hotspots in a Large marine ecosystem. *Sci. Rep.* 8, 7579. doi: 10.1038/s41598-018-25742-9

Sekiguchi, K., Klages, N. T. W., and Best, P. B. (1992). Comparative- analysis of the diets of smaller odontocete cetaceans along the coast of southern Africa. *South Afr. J. Mar. Sci.* 12, 843–861. doi: 10.2989/02577619209504746

UNEP MAP-RAC/SPA (2014). *Status and Conservation of Cetaceans in the Adriatic Sea*. By D. Holcer, C. M. Fortuna and P. C. Mackelworth. Draft internal report for the purposes of the Mediterranean Regional Workshop to Facilitate the Description of Ecologically or Biologically Significant Marine Areas, Malaga, Spain, 7–11 April 2014.

Whitehead, H. (2003). *Sperm whales: social evolution in the ocean* (Chicago: University of Chicago Press).

Wood, S., and Wood, M. S. (2015). "Package 'mgcv'," in *R package version*, 1–7.

Yee, T. W., and Mitchell, N. D. (1991). Generalized additive models in plant ecology. *J. Veg. Sci.* 2, 587–602. doi: 10.2307/3236170

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

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

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

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

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

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

KEYWORDS

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

1 Introduction

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

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

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

1.2 Overview of approaches for assessing range and habitat trends

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

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

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

1.3 Aim of the study

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

2 Material and methods

2.1 Study area

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

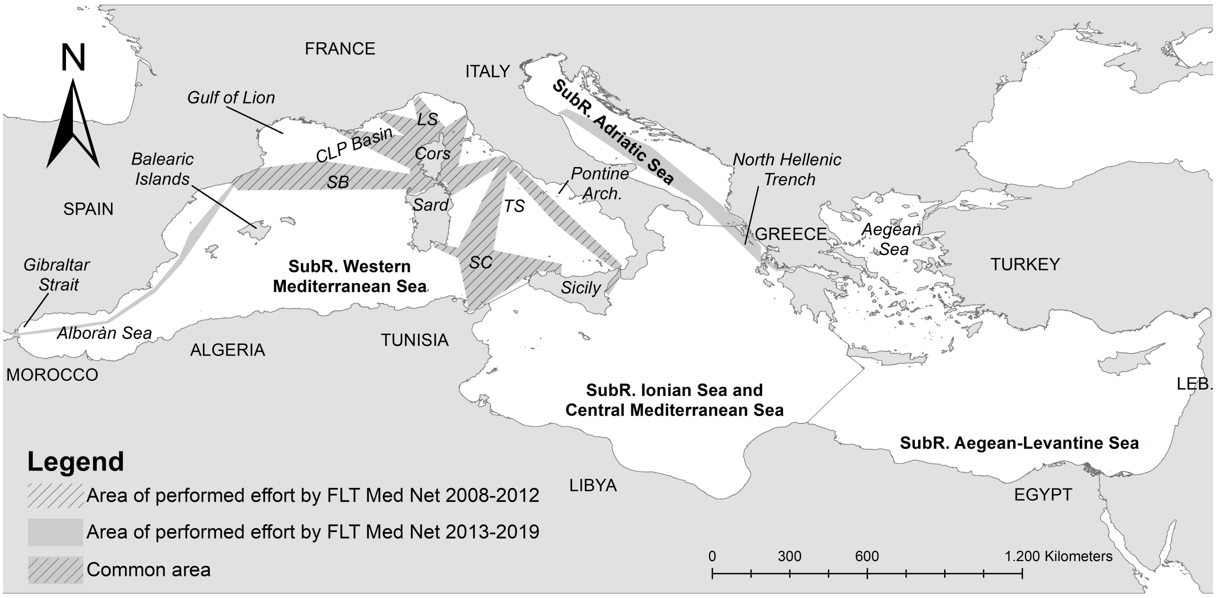

FIGURE 1
Study Area with the survey effort performed by the FLT Med Net during 2008-2012 (I baseline period, gridded grey only) and 2013-2019 (II period, plain grey). The four Mediterranean MSFD Subregions are shown in the figure: Western-Mediterranean (WMED), central-Mediterranean (Central MED), Adriatic,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

2.2 Data collection

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

2.3 Data analysis

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

2.3.1 Observed distributional range, ODR

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

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

2.3.2 Ecological potential range, EPR

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

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

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

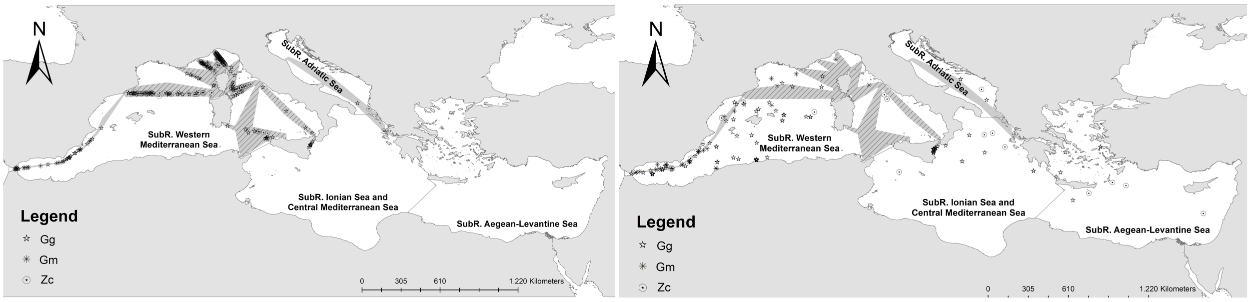

FIGURE 2
Dataset used for model building (left) and independent dataset used for validation (right).

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

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

2.3.3 Range pattern

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

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

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

3 Results

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

3.1 Observed distributional range, ODR

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

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

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

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

NA, Not Available.

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

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

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

3.2 Ecological potential range, EPR

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

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

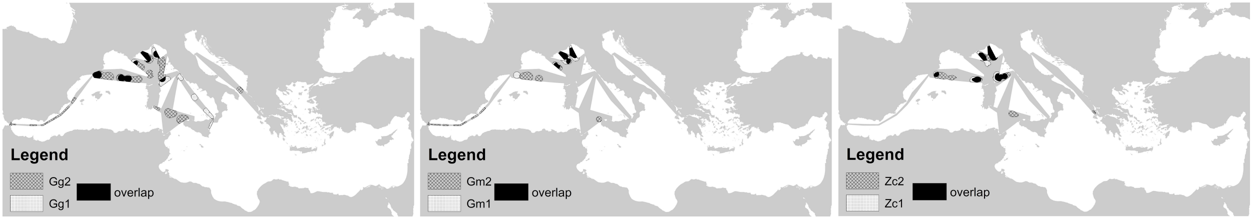

FIGURE 3
Core areas highlighted by the 95% KDE isopleth within the area covered on effort during the two periods (in grey), used to define the Observed Distributional Range, ODR (Gg left, Gm centre, Zc right).

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

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

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

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

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

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

3.3 Range pattern

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

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

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

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

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

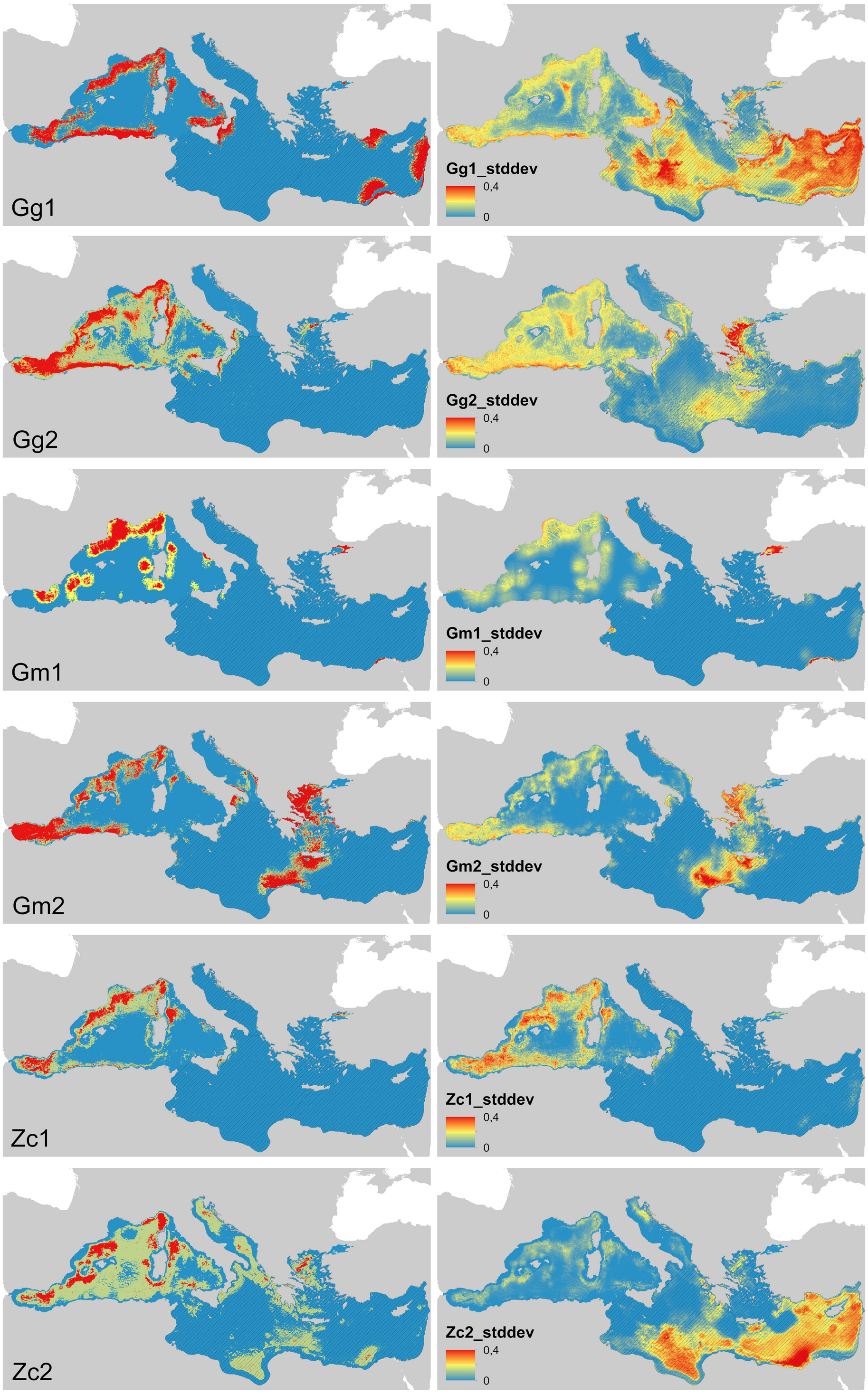

FIGURE 4
Output of the Suitable Habitats predicted based on 2008-2012 (Gg_1, Gm_1, Zc_1) and 2013-2019 (Gg_2, Gm_2, Zc_2) FLT Med Net data (left) with the relative Standard Deviation (right). The partition of suitable habitat is shown under three threshold scenarios defined by: 'Equal training sensitivity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

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

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

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

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

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

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

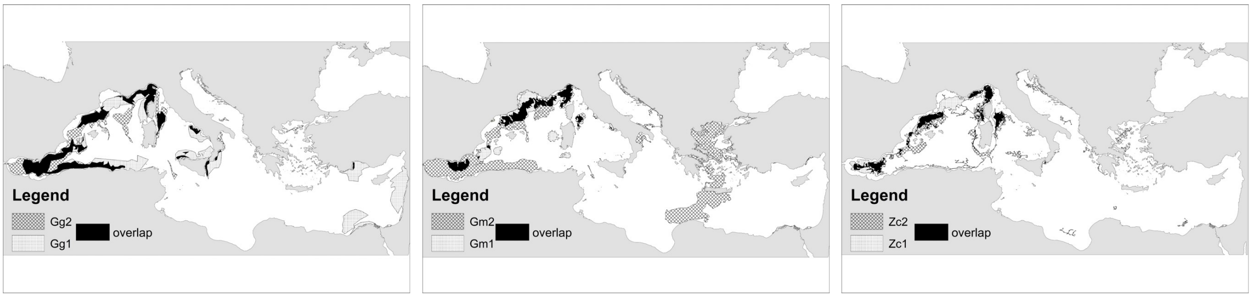

FIGURE 5
Overlap of EPRs over the two periods. Points EPR of the first period, strips EPR second period, and in black the overlapping areas.

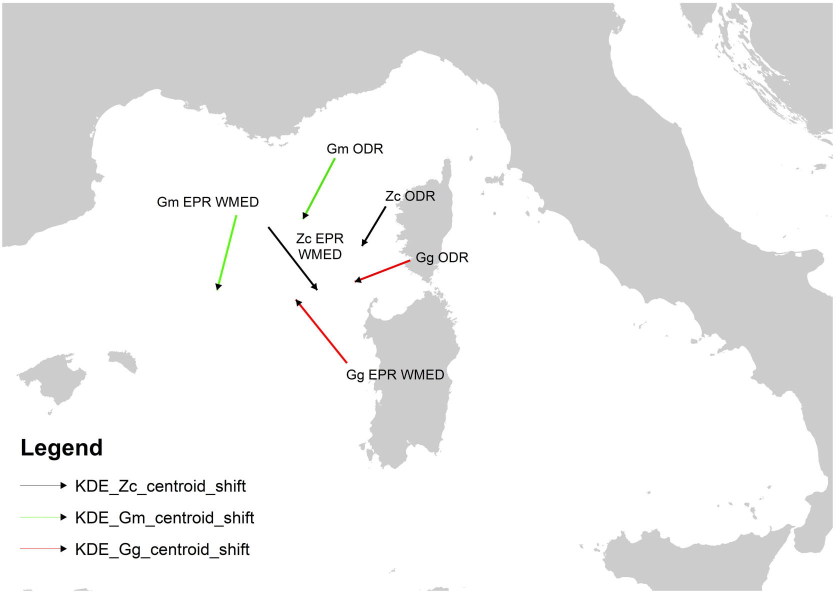

FIGURE 6
Direction and magnitude shift of the centroids of the distributional area respectively of ODR, and EPR WMED. Gg red, Gm green, Zc black lines.

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

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

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

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

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

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

4 Discussion

4.1 Sampling strategy

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

4.2 Main findings on species distributional range and habitat

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

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

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

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

4.3 Interpretation of trends

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

4.4 Methodological approach and indicators

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

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

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

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

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

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

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

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

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

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

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

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

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

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

5 Conclusions

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

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

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

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

Data availability statement

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

Ethics statement

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

Author contributions

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

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Conflict of interest

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

Publisher's note

Supplementary material

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

References

ACCOBAMS (2021). *Estimates of abundance and distribution of cetaceans, marine mega-fauna and marine litter in the Mediterranean Sea from 2018-2019 surveys*. Eds. S. Panigada, O. Boisseau, A. Canadas, C. Lambert, S. Laran, R. McLanaghan and A. Moscrop (Monaco: ACCOBAMS - ACCOBAMS Survey Initiative Project), 177.

Arcangeli, A., Aissi, M., Atzori, F., Azzolin, M., Campana, I., Carosso, L., et al. (2019). Fixed line transect mediterranean monitoring network (FLT med net), an international collaboration for long term monitoring of macro-mega fauna and main threats fixed line transect mediterranean monitoring network. *Biol. Mar. Mediterr* 26 (1), 400–401.

Arcangeli, A., Campana, I., and Bologna, M. A. (2017). Influence of seasonality on cetacean diversity, abundance, distribution and habitat use in the western Mediterranean Sea: implications for conservation. *Aquat. Conserv: Mar. Freshw. Ecosyst.* 27 (5), 995–1010. doi: 10.1002/aqc.2758

Arcangeli, A., Campana, I., Marini, L., and MacLeod, C. D. (2016). Long-term presence and habitat use of cuvier's beaked whale (Ziphius cavirostris) in the central tyrrhenian Sea. *Mar. Ecol.* 37 (2), 269–282. doi: 10.1111/maec.12272

Arcangeli, A., Marini, L., and Crosti, R. (2013). Changes in cetacean presence, relative abundance and distribution over 20 years along a trans-regional fixed line transect in the central tyrrhenian Sea. *Mar. Ecol.* 34, 112–121. doi: 10.1111/maec.12006

Attia El Hili, H., Cozzi, B., Salah, C. B., Podestà, M., Ayari, W., Amor, N. B., et al. (2010). A survey of cetaceans stranded along the northern coast of Tunisia: recent findings (2005–2008) and a short review of the literature. *J. Coast. Res.* 26 (5), 982–985. doi: 10.2112/JCOASTRES-D-09-00010.1

Azzellino, A., Airoldi, S., Gaspari, S., Lanfredi, C., Moulins, A., Podestà, M., et al. (2016). "Risso's dolphin, grampus griseus, in the Western ligurian Sea: trends in population size and habitat use," in *Advances in marine biology*, vol. 75 . Eds. G.N. Di Sciara, M. Podestà and B. E. Curry (London, United Kingdom: Academic Press), 205–223.

Azzellino, A., Gaspari, S., Airoldi, S., and Nani, B. (2008). Habitat use and preferences of cetaceans along the continental slope and the adjacent pelagic waters in the western ligurian Sea. *Deep-Sea Res. Part I* 55, 296–323. doi: 10.1016/j.dsr.2007.11.006

Bayed, A. (1996). "First data on the distribution of cetaceans along the Moroccan coasts," in *European Research on cetaceans-10. proceedings of the X annual conference of the European cetacean society*. Ed. P. G. H. Evans (Lisbon, Portugal: European Research on Cetaceans) 10, 106.

Bearzi, G., Reeves, R. R., Remonato, E., Pierantonio, N., and Airoldi, S. (2011). Risso's dolphin grampus griseus in the Mediterranean Sea. *Mamm. Biol.* 76 (4), 385–400. doi: 10.1016/j.mambio.2010.06.003

Bouslah, Y. (2012). *Bilan actuel des échouages de cétacés sur le littoral occidental algérien* (Oran, Algeria: Université d'Oran).

Boutiba, Z. (1994). Bilan de nos connaissances sur la présence des cétacés le long des côtes algériennes. *Mammalia* 58 (4), 613–622. doi: 10.1515/mamm.1994.58.4.613

Boyse, E., Beger, M., Valsecchi, E., and Goodman, S. J. (2023). Sampling from commercial vessel routes can capture marine biodiversity distributions effectively. *Ecol. Evol.* 13 (2), e9810. doi: 10.1002/ece3.9810

Breen, P., Pirotta, E., Allcock, L., Bennison, A., Boisseau, O., Bouch, P., et al. (2020). Insights into the habitat of deep diving odontocetes around a canyon system in the northeast Atlantic ocean from a short multidisciplinary survey. *Deep-Sea Res. Part I: Oceanographic Res. Papers* 159 (February), 103236. doi: 10.1016/j.dsr.2020.103236

Cañadas, A., Sagarminaga, R., and Garcıa-Tiscar, S. (2002). Cetacean distribution related with depth and slope in the mediterranean waters off southern spain. *Deep Sea Res. Part I Oceanogr.* 49 (11), 2053–2073. doi: 10.1016/S0967-0637(02)00123-1

Cañadas, A., Sagarminaga, R., De Stephanis, R., Urquiola, E., and Hammond, P. S. (2005). Habitat preference modelling as a conservation tool: proposals for marine protected areas for cetaceans in southern spanish waters. *Aquat. Conserv.* 15 (5), 495–521. doi: 10.1002/aqc.689

Cañadas, A., De Soto, N. A., Aissi, M., Arcangeli, A., Azzolin, M., B-Nagy, A., et al. (2018). The challenge of habitat modelling for threatened low density species using heterogeneous data: the case of cuvier's beaked whales in the Mediterranean. *Ecol. Indic.* 85, 128–136. doi: 10.1016/j.ecolind.2017.10.021

Cañadas, A., and Notarbartolo di Sciara, G. (2018). *Ziphius cavirostris (Mediterranean subpopulation)* Vol. 2018 (The IUCN Red List of Threatened Species).

Cañadas, A., and Vázquez, J. A. (2014). Conserving cuvier's beaked whales in the alboran Sea (SW mediterranean): identification of high density areas to be avoided by intense man-made sound. *Biol. Conserv.* 178, 155–162. doi: 10.1016/j.biocon.2014.07.018

Carlucci, R., Baş, A. A., Liebig, P., Renò, V., Santacesaria, F. C., Bellomo, S., et al. (2020a). Residency patterns and site fidelity of grampus griseus (Cuvier 1812) in the gulf of taranto (northern Ionian Sea, central-eastern Mediterranean Sea). *Mammal Res.* 65 (3), 445–455. doi: 10.1007/s13364-020-00485-z

Carlucci, R., Cipriano, G., Santacesaria, F. C., Ricci, P., Maglietta, R., Petrella, A., et al. (2020b). Exploring data from an individual stranding of a cuvier's beaked whale in the gulf of taranto (Northern Ionian Sea, central-eastern Mediterranean Sea). *J. Exp. Mar. Biol. Ecol.* 533, 151473. doi: 10.1016/j.jembe.2020.151473

Cipriano, G., Carlucci, R., Bellomo, S., Santacesaria, F. C., Fanizzi, C., Ricci, P., et al. (2022). Behavioral pattern of risso's dolphin (*Grampus griseus*) in the gulf of taranto (Northern Ionian Sea, central-Eastern Mediterranean Sea). *J. Mar. Sci. Eng.* 10, 175. doi: 10.3390/jmse10020175

Dede, A., Öztürk, A. A., Tonay, A. M., Uğur, Ö., Gönülal, O., and Öztürk, B. (2022). Cetacean sightings in the finike seamounts area and adjacent waters during the surveys in 2021. *J. Black Sea Mediterr. Environ.* 28 (2), 221–239.

Dede, A., Tonay, A. M., Bayar, H., and Öztürk, A. A. (2013). First stranding record of a risso's dolphin (*Grampus griseus*) in the marmara Sea, Turkey. *J. Black Sea/Mediterranean Environ.* 19 (1), 121–126.

De Stephanis, R., Verborgh, P., Pérez, S., Esteban, R., Minvielle-Sebastia, L., and Guinet, C. (2008). Long-term social structure of long-finned pilot whales (*Globicephala melas*) in the strait of Gibraltar. *Acta Ethologica* 11, 81–94. doi: 10.1007/s10211-008-0045-2

DG Environment (2017). Reporting under article 17 of the habitats directive: explanatory notes and guidelines for the period 2013-2018. *Brussels*, 188.

Efron, B., and Tibshirani, R. (1997). Improvements on cross-validation: the 632+ bootstrap method. *J. Am. Stat. Assoc.* 92 (438), 548–560. doi: 10.1080/01621459.1997.10474007

Franklin, J. (2010). *Mapping species distributions: spatial inference and prediction* (UK: Cambridge University Press).

Frantzis, A., Alexiadou, P., Paximadis, G., Politi, E., Gannier, A., and Corsini-Foka, M. (2003). Current knowledge of the cetacean fauna of the Greek seas. *J. Cetacean Res. Manage.* 5 (3), 219–232.

Frantzis, A., and Herzing, D. L. (2002). Mixed-species associations of striped dolphins (*Stenella coeruleoalba*), short-beaked common dolphins (*Delphinus delphis*), and risso's dolphins (*Grampus griseus*) in the gulf of Corinth (Greece, Mediterranean Sea). *Aquat. Mammals* 28 (2), 188–197.

Fullard, K. J., Early, G., Heide-JØrgensen, M. P., Bloch, D., Rosing-Asvid, A., and Amos, W. (2000). Population structure of long-finned pilot whales in the north Atlantic: a correlation with sea surface temperature? *Mol. Ecol.* 9 (7), 949–958. doi: 10.1046/j.1365-294X.2000.00957.x

Gannier, A. (2015). Cuvier's beaked whale (*Ziphius cavirostris*) diving behavior as obtained by visual observation methods and consequences in terms of visual detection during surveys. *Sci. Rep. Port-Cros Natl. Park* 29, 127–134.

Gannier, A., and Epinat, J. (2008). Cuvier's beaked whale distribution in the Mediterranean Sea: results from small boat surveys 1996–2007. *J. Mar. Biol. Assoc. United Kingdom* 88 (6), 1245–1251. doi: 10.1017/S0025315408000428

Gauffier, P., and Verborgh, P. (2021). *Globicephala melas (Inner Mediterranean subpopulation)* Vol. 2021 (The IUCN Red List of Threatened Species). doi: 10.2305/IUCN.UK.2021-3.RLTS.T198785664A198787672.en

GEBCO Bathymetric Compilation Group (2020). *The GEBCO_2020 grid - a continuous terrain model of the global oceans and land* (UK: British Oceanographic Data Centre, National Oceanography Centre, NERC). doi: 10.5285/a29c5465-b138-234d-e053-6c86abc040b9

Girard, F., Girard, A., Monsinjon, J., Arcangeli, A., Belda, E. J., Cardona, L., et al. (2022). Toward a common approach for assessing the conservation status of marine turtle species within the European marine strategy framework directive. *Front. Mar. Sci.* 9, 1–22. doi: 10.3389/fmars.2022.790733

Gómez de Segura, A., Crespo, E. A., Pedraza, S. N., Hammond, P. S., and Raga, J. A. (2006). Abundance of small cetaceans in waters of the central Spanish Mediterranean. *Mar. Biol.* 150 (1), 149–160. doi: 10.1007/s00227-006-0334-0

ISPRA (2015). *Fixed line transect using ferries as platform of observation-monitoring protocol. technical annex I of the agreement for FLT med monitoring net*. Available at: https://www.isprambiente.gov.it/files2021/progetti/technical-annex-i_monitoring-protocol_2015.pdf.

IUCN (2001). *IUCN red list categories and criteria: version 3.1* (Gland, Switzerland and Cambridge, U.K: IUCN Species Survival Commission. IUCN).

IUCN Standards and Petitions Committee (2022). *Guidelines for using the IUCN red list categories and criteria. version 15. prepared by the standards and petitions committee*. Available at: https://www.iucnredlist.org/documents/RedListGuidelines.pdf.

Karaa, S., Bradai, M. N., Jribi, I., Hili, H. A. E., and Bouain, A. (2012). Status of cetaceans in Tunisia through analysis of stranding data from 1937 to 2009. *DeG* 76 (1), 21–29. doi: 10.1515/mamm.2011.100

Kerem, D., Hadar, N., Goffman, O., Scheinin, A., Kent, R., Boisseau, O., et al. (2012). Update on the cetacean fauna of the Mediterranean levantine basin. *Open Mar. Biol. J.* 6 (1), 6–27. doi: 10.2174/1874450801206010006

Labach, H., Dhermain, F., Bompar, J. M., Dupraz, F., Couvat, J., David, L., et al. (2015). Analysis of 23 years of risso's dolphins photo-identification in the north-Western Mediterranean Sea, first results on movements and site fidelity. *Sci. Rep. Port-Cros Natl. Park* 29, 263–266.

Lanfredi, C., Arcangeli, A., David, L., Holcer, D., Rosso, M., and Natoli, A. (2021). *Grampus griseus (Mediterranean subpopulation)* (The IUCN Red List of Threatened Species) 2021. doi: 10.2305/IUCN.UK.2021-.RLTS.T16378423A190737150.en

Laran, S., Joiris, C., Gannier, A., and Kenney, R. D. (2010). Seasonal estimates of densities and predation rates of cetaceans in the ligurian Sea, northwestern Mediterranean Sea: an initial examination. *J. Cetacean Res. Manage* 11 (1), 31–40.

Laran, S., Pettex, E., Authier, M., Blanck, A., David, L., Dorémus, G., et al. (2017). Seasonal distribution and abundance of cetaceans within French waters-part I: the north-Western Mediterranean, including the pelagos sanctuary. *Deep Sea Res. Part II: Topical Stud. Oceanography* 141, 20–30. doi: 10.1016/j.dsr2.2016.12.011

Maiorano, L., Chiaverini, L., Falco, M., and Ciucci, P. (2019). Combining multi-state species distribution models, mortality estimates, and landscape connectivity to model potential species distribution for endangered species in human dominated landscapes. *Biol. Conserv.* 237, 19–27. doi: 10.1016/j.biocon.2019.06.014

Masski, H., and De Stephanis, R. (2018). Cetaceans of the Moroccan coast: information from a reconstructed strandings database. *J. Mar. Biol. Assoc. United Kingdom* 98 (5), 1029–1037. doi: 10.1017/S0025315415001563

Merow, C., Smith, M. J., and Silander, J. A.Jr. (2013). A practical guide to MaxEnt for modeling species' distributions: what it does, and why inputs and settings matter. *Ecography* 36 (10), 1058–1069. doi: 10.1111/j.1600-0587.2013.07872.x

Monaco, C., Ibáñez, J. M., Carrión, F., and Tringali, L. M. (2016). Cetacean behavioral responses to noise exposure generated by seismic surveys: how to mitigate better? *Ann. Geophysics* 59 (4), S0436–S0436. doi: 10.4401/ag-7089

Moors-Murphy, H. B. (2014). Submarine canyons as important habitat for cetaceans, with special reference to the gully: a review. *Deep-Sea Res. Part II: Topical Stud. Oceanography* 104, 6–19. doi: 10.1016/j.dsr2.2013.12.016

Moulins, A., Rosso, M., Nani, B., and Würtz, M. (2007). Aspects of the distribution of cuvier's beaked whale (*Ziphius cavirostris*) in relation to topographic features in the pelagos sanctuary (north-western Mediterranean Sea). *J. Mar. Biol. Assoc. United Kingdom* 87 (1), 177–186. doi: 10.1017/S0025315407055002

Mussi, B., Miragliuolo, A., and Dıaz Lopez, B. (2000). "Social structure and male parental care in a long finned pilot whale (Globicephala melas) pod off ventotene island (southern tyrrhenian Sea, Italy)," in *European Research on cetaceans 14: fourteenth annual conference of the European cetacean society*. Eds. P. G. H. Evans, R. Pitt-Aiken and E. Rogan (Cork: European Cetacean Society), 141–145.

Öztürk, A. A., Tonay, A. M., and Dede, A. (2011). Strandings of the beaked whales, risso's dolphins, and a minke whale on the Turkish coast of the Eastern Mediterranean Sea. *J. Black Sea/Mediterr. Environ.* 17, 269–274.

Palialexis, A., Connor, D., Damalas, D., Gonzalvo, J., Micu, D., Mitchel, I., et al. (2019). *Indicators for status assessment of species, relevant to MSFD biodiversity descriptor* (Luxembourg: Publications Office of the European Union).

Pearson, R. G., Raxworthy, C. J., Nakamura, M., and Townsend Peterson, A. (2007). Predicting species distributions from small numbers of occurrence records: a test case using cryptic geckos in Madagascar. *J. biogeography* 34 (1), 102–117. doi: 10.1111/j.1365-2699.2006.01594.x

Phillips, S. J., and Dudík, M. (2008). Modelling of species distributions with maxent: new extensions and a comprehensive evaluation. *Ecography* 31, 161–175. doi: 10.1111/j.0906-7590.2008.5203.x

Phillips, S., Dudik, M., and Schapire, R. (2006) *Una breve guía didáctica sobre MaxEnt*. Available at: http://www.cs.princeton.edu/~schapire/maxent/tutorial/tutorial-in-spanish.doc (Accessed 4 June 2008).

Podestà, M., Azzellino, A., Cañadas, A., Frantzis, A., Moulins, A., Rosso, M., et al. (2016). "Cuvier's beaked whale, ziphius cavirostris, distribution and occurrence in the Mediterranean Sea: high-use areas and conservation threats," in *Advances in marine biology*, vol. 75. (London, United Kingdom: Academic Press), 103–140.

Praca, E., and Gannier, A. (2008). Ecological niches of three teuthophageous odontocetes in the northwestern Mediterranean Sea. *Ocean Sci.* 4 (1), 49–59. doi: 10.5194/os-4-49-2008

Raga, J. A., and Pantoja, J. (2004). *Proyecto mediterráneo. zonas de especial interés para la conservación de los cetáceos en el mediterráneo español* (Madrid: Ministerio de Medio Ambiente. Organismo Autónomo Parques Nacionales).

Tepsich, P., Rosso, M., Halpin, P. N., and Moulins, A. (2014). Habitat preferences of two deep-diving cetacean species in the northern ligurian Sea. *Mar. Ecol. Prog. Ser.* 508, 247–260. doi: 10.3354/meps10851

Thorne, L. H., Johnston, D. W., Urban, D. L., Tyne, J., Bejder, L., Baird, R. W., et al. (2012). Predictive modeling of spinner dolphin (Stenella longirostris) resting habitat in the main hawaiian islands. *PloS One* 7 (8), e43167. doi: 10.1371/journal.pone.0043167

Verborgh, P., and Gauffier, P. (2021). *Globicephala melas (Strait of Gibraltar subpopulation)* Vol. 2021 (The IUCN Red List of Threatened Species). doi: 10.2305/IUCN.UK.2021-3.RLTS.T198787290A198788152.en

Verborgh, P., Gauffier, P., Esteban, R., Giménez, J., Cañadas, A., Salazar-Sierra, J. M., et al. (2016). Conservation status of long-finned pilot whales, globicephala melas, in the Mediterranean Sea. *Adv. Mar. Biol.* 75, 173–203. doi: 10.1016/bs.amb.2016.07.004

Würtz, M., and Rovere, M. (2015). *Atlas of the Mediterranean seamounts and seamount-like structures* (Gland, Switzerland: IUCN), 276.

Zampollo, A., Arcangeli, A., Costantino, M., Mancino, C., Crosti, R., Pietroluongo, P., et al. (2022). Seasonal niche and spatial distribution modelling of the loggerhead (Caretta caretta) in the Adriatic and Ionian seas. *Aquat. Conservation: Mar. Freshw. Ecosyst.* 32 (7), 1141–1155. doi: 10.1002/AQC.3815

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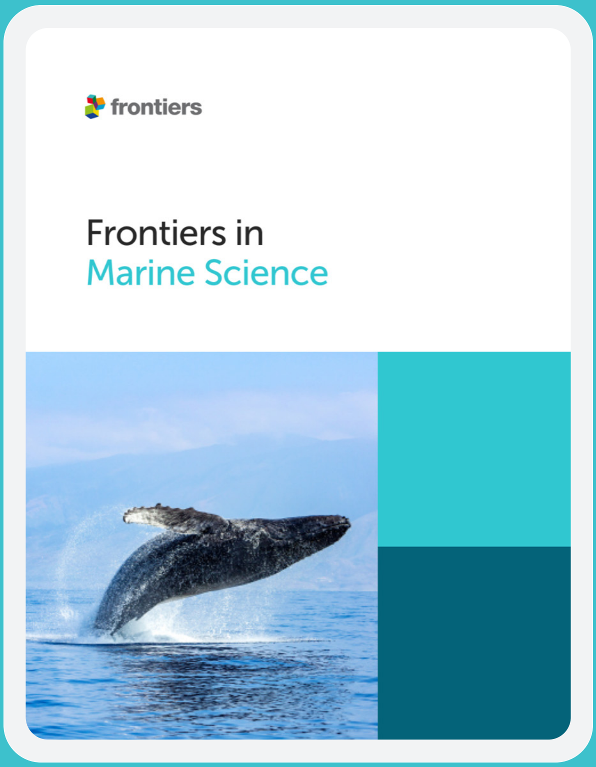
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