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Long-term monitoring for the surveillance of the conservation status of *Tursiops truncatus* in an EU Natura 2000 site in the Mediterranean Sea. A pilot study in the Tuscan Archipelago

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Abstract

A pilot study, using the dataset from the research network 'FLT Med Net', which regularly monitors transborder regions in the Mediterranean Sea using ferries as platform for systematic surveys, was undertaken to assess common bottlenose dolphin range and population trends within the Natura 2000 EU marine site "Tutela del *Tursiops truncatus*". The site was recently designated by the Tuscany Region (Italy) within the requirement of the EU Habitats Directive. In order to evaluate the conservation status of bottlenose dolphin according to the surveillance scheme of the Directive, two six-year periods (2007-2012; 2013-2018) were compared to assess trends in Distribution-occurrence (range), Sightings Per Unit of Effort and Density (population). In total, 18,146 NM were surveyed along two fixed transects, recording 90 sightings of *Tursiops truncatus* for a total of 268 specimens. Between the two periods, slight but not statistically significant differences were assessed, with decreasing trend in range and population of the species; no variation was detected in mean group sizes. Travelling was the most common behaviour, and juveniles were present in 20% of the sightings, concentrated during spring and summer. The consistent FLT Med Net dataset was found to be appropriate to evaluate important parameters for the assessment of trends in the conservation status of *Tursiops truncatus* at the Natura 2000 site scale.

Keywords: Habitats Directive; *Tursiops truncatus*; Tuscan Archipelago; Natura 2000 at sea.

Introduction

The EU Habitats Directive (92/43/CEE, HD) ensures the conservation of a wide range of rare, threatened or endemic species, and it is undoubtedly among the most important piece of legislation, at an European level, for the conservation of wildlife. In compliance with the Directive (art. 3 and 4 HD), European Union Member States designate Special Areas of Conservation (SACs) for the conservation of species included in Annex II of the HD, after being confirmed as Sites of Community Importance (SCI). Sites are designated to enable the species to be maintained or, where appropriate, restored at a favourable conservation status in their natural range. In order to identify the appropriate protection measures, it is important to have reliable information about the status of the species and the pressures to which they are exposed. Surveillance of the conservation status of species of Com-

munity interest is thus required through the assessment of four parameters: Range, Population, 'Habitat for the species' (suitable habitat), and 'Future prospects' (pressures/threats) in periods of 6-years (art. 17 HD). Reports on parameter trends are crucial for understanding which conservation/management measures have to be implemented for the conservation of the species (definitions for the HD requirements are provided in the Methods).

In 2020 after a four year process following the EU pilot 8348/16/ENVI "Completamento della designazione della Rete Natura 2000 a mare in Italia" (Completion of the designation of Natura 2000 Network at sea in Italy), the Tuscany Region (Italy) confirmed a new marine SCI ("Tutela del *Tursiops truncatus*", Deliberazione 14/01/2020, n. 2-Protection of *Tursiops truncatus*, Resolution) specifically for the protection of *Tursiops truncatus* (Montagu, 1821; common bottlenose dolphin), a species listed in Annex II of the Habitats Directive and

for whom Special Areas of Conservation are required for their conservation. In the Mediterranean Sea, the status of *Tursiops truncatus* is classified as Vulnerable by the IUCN Red List (Bearzi *et al.*, 2012); at the Italian level, the species' overall assessment status is assessed as "Favourable", according to an HD art. 17 report, while at the Mediterranean Bio-region level it is assessed as Unknown (<https://nature-art17.eionet.europa.eu/>).

In order to investigate cetaceans' status and related pressures in the Mediterranean Sea, since 2007, a research network (Fixed Line Transect Mediterranean monitoring Network, FLT Med Net) coordinated by ISPRA started continuously monitoring the presence and distribution of cetaceans and their main threats along several inter-regional fixed line transects using ferries/large ships as platforms of observation for systematic surveys (Arcangeli *et al.*, 2019). At present, the surveys are regularly scheduled in the Western Mediterranean and ADRION (Adriatic and Ionian) Regions. In European sea water, ferries/cargos are regularly used to systematically monitor cetaceans and are also used in different sea regions in the North Sea, Atlantic, and Macaronesia sea area (Correia *et al.*, 2015; Robbins *et al.*, 2020). Monitoring cetaceans from ferries in the Mediterranean Sea provided new insights on cetacean trends, feeding grounds, disturbance from maritime traffic, and risks of exposure to plastic (e.g. Arcangeli *et al.*, 2013; Arcangeli *et al.*, 2014; Campana *et al.*, 2015; Campana *et al.*, 2018). Within the Tuscan Archipelago, two routes have been continuously monitored since 2008 to assess changes in years and seasons.

The aim of this study was to analyse the capacity of the dataset collected from the research network in the area of the Site of Community Importance ("Tutela del *Tursiops truncatus*") during two HD reporting periods (2007-2012

and 2013-2018) to investigate short term trends for the "range" and "population" of *Tursiops truncatus*.

Materials and Methods

The investigated area was the new designated Natura 2000 site 'Tutela del *Tursiops truncatus*' IT5160021 located in the Western Mediterranean Sea Region in the Tyrrhenian Sea just off the Tuscany coast (town of Livorno). The site is included within the SPAMI area of the "Pelagos Sanctuary", partially coinciding with the Tuscan Archipelago National Park and including other Natura 2000 terrestrial and marine sites, such as the Secche della Meloria (IT5160018), Isola di Gorgona (IT5160002), and Isola di Capraia (IT5160006) (Fig. 1). The investigated site has a surface area of 3,719 km² all inside the continental shelf, characterized by shallow waters and shoal; between the islands of Capraia and Gorgona, the platform is cut by the Elba canyon which descends deeply towards the North-West. Within the Pelagos Sanctuary, the bottlenose dolphin is regularly sighted with a continuous distribution over the continental shelf, tends to form local units, and displays a philopatric behaviour with maximum displacements of about 50 km on average (Gnone *et al.*, 2011).

Data were collected along sampling transects following a systematic protocol for monitoring marine mega and macro fauna, maritime traffic, and marine litter from ferries/large vessels (ISPRA, 2015; Arcangeli *et al.*, 2019); the protocol sets the conditions in order to maintain equal probability of detecting species in the 'on effort' state. The 'on effort' state was considered only in good weather conditions (Beaufort ≤ 3 ; good visibility).

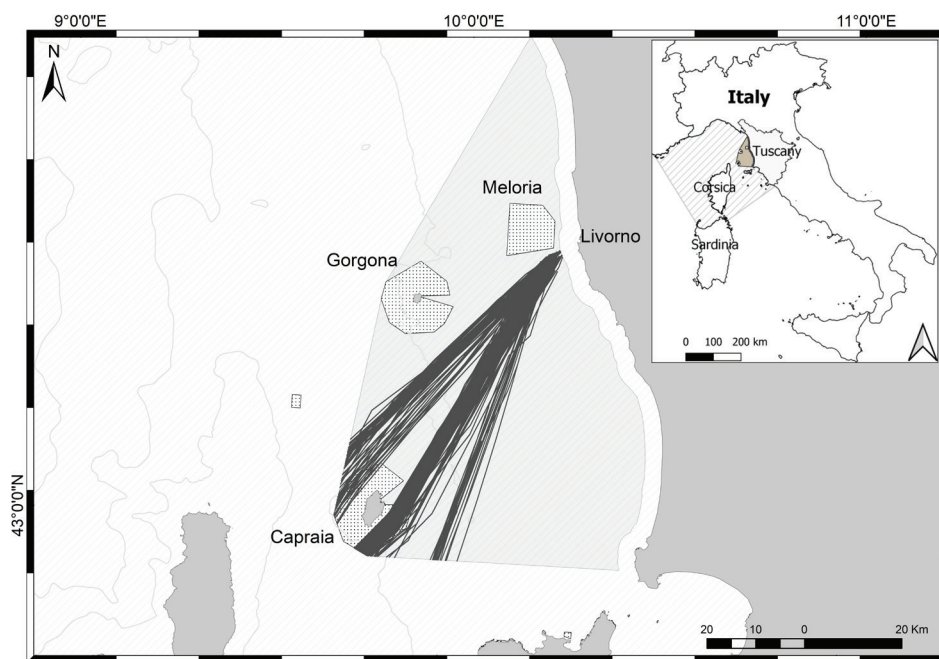


Fig. 1: Study area (grey area) coinciding with the Natura 2000 site "Tutela del *Tursiops truncatus*" IT5160021, other Natura 2000 areas (Meloria, Gorgona, Capraia, dotted areas) and the Pelagos Sanctuary (grey lines). Monitored fixed transects are in black lines (effort).

The effort tracklines were automatically recorded by a dedicated handheld GPS. Each sighting was marked on the GPS and data were annotated on a standard datasheet with information about the time, ship's position, species, distance and angle from the ship, number of individuals, presence of juveniles, direction of swimming, and surface behaviour. Dedicated and experienced marine mammal observers were located on both sides of the ship's command bridge which continuously scanned, during daylight, a 270° arc forward by naked eye and binoculars; the latter and photographs were used to confirm species identification and group size. During surveys, a group was defined as more than one individual seen at the same time within a few body lengths of another and engaged in the same activity (as in Arcangeli *et al.*, 2013).

Surveys were performed continuously during the year with a frequency of at least three surveys per season/year (winter: January–March; spring: April–June; summer: July–September; autumn: October–December). Two different Corsica-Sardinia line ferries (with slight differences in features, such as in command bridge height and speed) were used as observation platforms.

The two investigated routes ran across the studied Site, and specifically from Livorno (Tuscany-IT) to Bastia (Corsica-FR) and to Golfo Aranci (Sardinia-IT). Data were pooled for the two investigated periods, 2007-2012 and 2013-2018, corresponding to the 3rd and 4th HD reporting periods/cycles.

According to the HD art. 17 guidelines (DG Environment, 2017) “trend” is a (measure of a) directional change of a parameter over time and is a decisive information for conservation status assessment. The Habitats Directive reporting period is six years, but estimates of trends are more likely to be statistically robust over longer time periods; it is therefore recommended to estimate at least short-term trends over two reporting cycles, i.e. 12 years. “Range” is defined as ‘the outer limits of the overall area in which a species is found at present, and it can be considered as an envelope within which areas actually occupied occur’: for this study, range was calculated as distribution-occurrence as from the guidelines, but given the scale of the study area, at finer resolution (5x5 km grid cells). In order to monitor “population” trends, relative units, such as abundance, density, or number of records per unit of effort, are often used by Member States.

In this study, “range trend values” were estimated through spatial analyses performed in a GIS environment (ArcGis): I) distribution-occurrence was analysed as the number of 5x5 km grid cells with bottlenose dolphin sightings within the cells crossed by the transect per each investigated period and calculated as the percentage of the Number of occupied cells/Number of cells with effort; percentage differences in distribution between the two investigated periods were then computed as: [(occurrence period 2 – occurrence period 1) / occurrence period 1]. II) Core areas of presence (spatial generalization of the distribution) along the transects were investigated with the Kernel smoother (Hengl *et al.*, 2009) to map areas of higher sighting densities. Isopleths of 75% of sightings of the two periods were then overlapped and

visually inspected to investigate potential differences in core area locations.

“Population trend values” were estimated through: III) a relative unit, such as the abundance index SPUE (Sightings Per Unit of Effort), and was calculated as the number of sightings/length of transect travelled on effort x 100 km (as in Weir *et al.*, 2007), considering each single transect/survey as a unit of effort, and investigating the two six-year periods and seasonal variations. IV) Population density (D) applying distance sampling analysis (Thomas *et al.*, 2010) was estimated by assessing Effective Strip Width (ESW), and then computing D as the total number of sighted animals/total length of transects x 2ESW in km² (as in Tepsich *et al.*, 2020). Considering the slight differences in the type of ferries used, which could influence detection capability, two different ESWs were computed for Type I and Type II ferries as in Cominelli *et al.* (2016) and Tepsich *et al.* (2020); radial distances and angles between sightings and ferry heading were used to compute perpendicular distances. For each type of ferry, three different detection functions were tested, with zero or one adjustment: Half normal, Uniform and Hazard rate. In order to choose the optimal detection function, the AIC (Akaike information criterion, Akaike, 1974) was used to compare models' performance, and the best model was chosen according to lowest AIC value. Only transects longer than 10 km were considered for the density investigation.

Two Sample Mann-Whitney (MW) test was used to investigate differences between the two investigated periods and between/among seasons. All statistical analyses were performed using Past software (Hammer *et al.*, 2001) and line-transect data were analysed using Rdistance.

Surface behaviour and the presence of juveniles were also investigated. General behaviour categories (travelling, resting, playing, feeding wild, feeding net, mating, unknown) were deducted when possible from five objective class categories such as: superficial behaviour (e.g. half, full leap, dorsal fin, surfing etc), speed (slow, fast, porpoising, floating), progress (e.g. straight, irregular, none), direction of the group (same, different circle), group association, association with fishing related gears.

Within the total investigation period (2007-2018), sightings at sea of the other cetacean species of annex IV of the HD recorded during the monitoring were also reported.

Results

During the entire investigated period, 18,146 NM were surveyed on effort in good weather conditions within 573 transects/surveys (mean length of transect on effort 59.6 ± 0.7 km). 90 bottlenose dolphin sightings were recorded totalling 268 specimens (Table 1).

Of the 171 grid cells included in the SCI, 73 cells were continuously surveyed during the whole study period (effort cells, 43% of the total area). The range trend was slightly decreasing by 16% during the 2013-2018 period (Fig. 2, Table 1).

Table 1. Numbers of *Tursiops truncatus* sightings, for the first (2007-2012) and second period (2013-2018) considered. On effort: Km surveyed and number of transects. Sightings: number of sightings, number of 5x5 km cells crossed by the transects; mean group size; mean SPUE (number of sightings on length of transect x 100 km) and density for the entire period (number of specimens on km²).

Period	Km effort	N. of surveys	Cells effort	N of sightings	Cells with sightings	Group size	SPUE± SE	Density
2007-2012	18,050	293	69	52	35	2.8	0.284 ± 0.04	0.0099
2013-2018	15,550	280	56	38	24	2.9	0.234 ± 0.07	0.0084

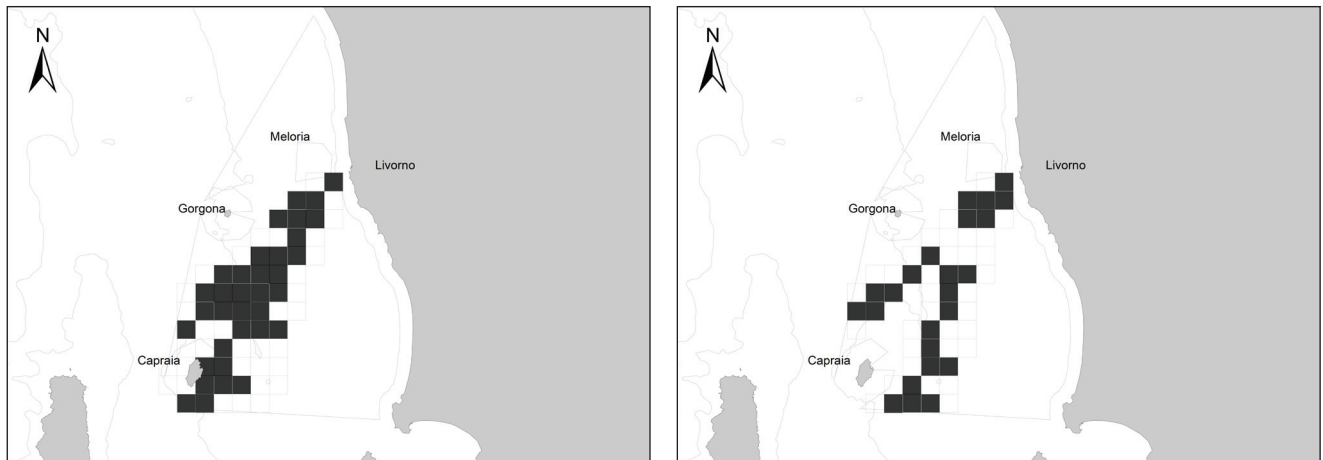


Fig. 2: Distribution of cells with occurrences of bottlenose dolphin during the first (left) and second (right) investigated period.

The bottlenose dolphin's core areas, revealed by the Kernel analysis, were consistent between the two periods and were concentrated mainly out of the Livorno harbour and south-east of Capraia Island (Fig. 3).

No significant statistical differences were recorded in the SPUEs between the two periods, even if a higher variance was detected during the 2007-2012 period, as well as an overall decrease of the index by 18% (Table 1).

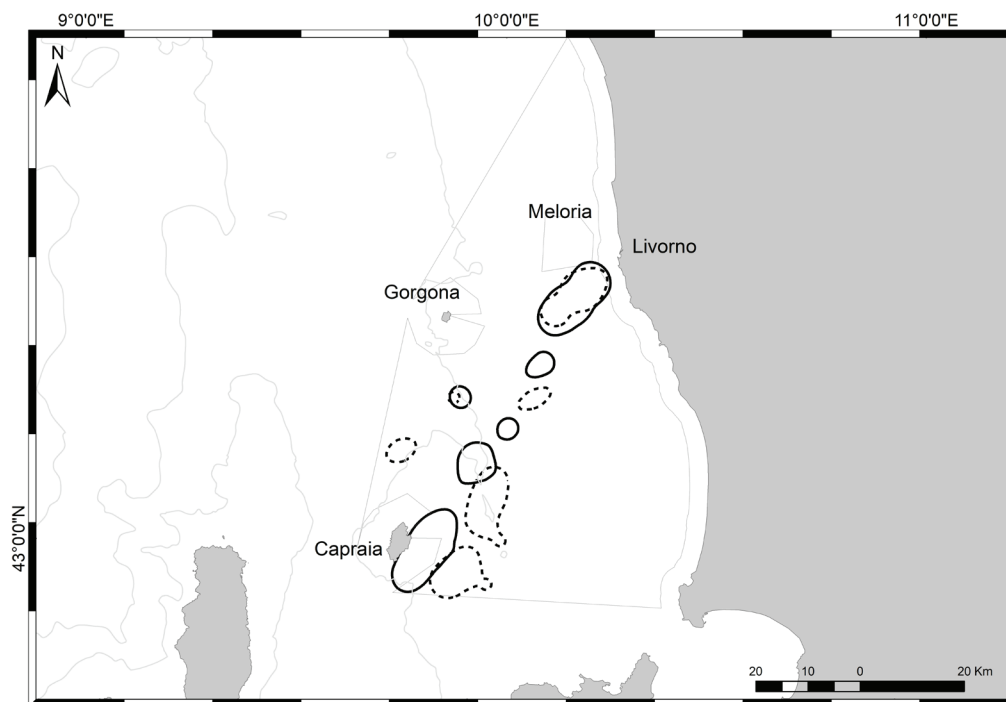


Fig. 3: Kernel density estimates to assess species trends in distribution: 75% isopleths of the first (black line) and second investigated period (dotted line).

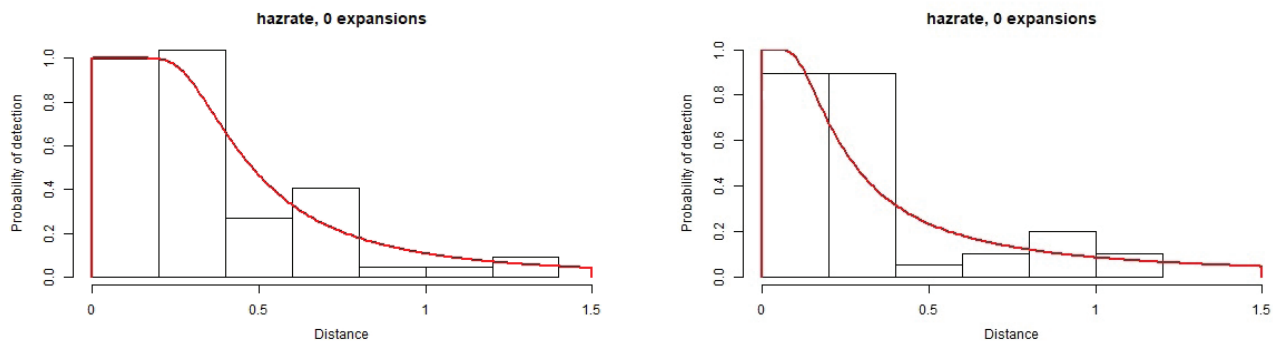


Fig. 4: Probability function of *Tursiops truncatus* within the study: type I ferry (left) and type II ferry (right).

For both periods, the seasonal SPUE showed higher values during spring/summer compared to winter/autumn, even if only spring and autumn of the 2007-2012 period were statistically different from each other (MW test, $P < 0.05$). No additional differences were detected among inter- and intra-period seasonal SPUEs.

The ESWs were similar for both ferry types (570 m for type I ferry and 520 m for type II ferry). Based on AIC values, for the two groups of ferries a Hazard rate model with no adjustment terms was chosen as the final detection function (Fig. 4). ESW output should be considered site related considering features such as coastal habitat, presence of islands, mean group size.

Bottlenose dolphin density, computed with distance sampling analysis, showed a slight decrease by 15% during the 2013-2018 period compared to the previous one (Table 1).

Mean group size was 2.8 in the first period and 2.9 in the second one. Behaviour was recorded in 55 sightings (61% of the total sightings). Behavioural analysis showed that “travel” was the most common behaviour (76% of recorded behaviour), followed by “feeding on fishing net” which was however recorded mainly during the first period, with a mean group size significantly greater ($= 4.9$; MW test, $P < 0.05$) than those adopting other behaviours. Juveniles were present in 20% of the sightings, mainly during the spring and summer seasons.

The other species sighted were *Stenella coeruleoalba* ($N = 57$) and *Balaenoptera physalus* ($N = 27$). *Physeter macrocephalus* and *Delphinus delphis*, were only occasionally recorded.

Discussion

Several studies on cetaceans were undertaken in the extended area of the new designated marine Natura 2000 site “Tutela del *Tursiops truncatus*” (e.g. Nuti *et al.*, 2007; Gnone *et al.*, 2011; Regione Toscana, 2012; Carnabuci *et al.*, 2016). The use of a consistent systematic research protocol for long term monitoring, as the one used in this study, provided a dataset specifically useful for the detection, within the site, of short-term trends in range and population abundance of *Tursiops truncatus*, as is required for surveillance under art. 17 of the Habitats Directive.

Bottlenose dolphins were regularly detected in the study area throughout the investigated periods and during all the seasons. A slightly decreasing trend, not statistically significant, between the third and fourth Habitats Directive recording periods was recognised by the study both in the range criteria and in the abundance indicators (Distribution-occurrence, SPUE and Density). No differences were recorded in the mean group sizes between periods, as well as in habitat preference, as the core sighting areas out of Livorno harbour and south-east of Capraia emerged in both the investigated periods.

Even though a philopatric behaviour was detected for the species in the region (Gnone *et al.*, 2011), according to Carnabuci *et al.* (2016) there is a strong connectivity between dolphins occurring in the Gulf of La Spezia, the coasts of Tuscany, and the Tuscany Archipelago, all within a continuous habitat extension characterized by shallow waters with an extended continental shelf. Dolphins in the Tuscany coastal part of the Sanctuary are preferentially sighted around 10 km from the coast, in shallow waters, and to a certain distance from the 100 m isobaths in the Northern part (coinciding in our study area), while individuals, just south, display a preference for relatively deep waters even if only within a short distance from the coast (alpha and beta social clusters in Vassallo *et al.*, 2020). Given that, the vagrant behaviour of the species could likely be an explanation for the decreased short-term trend in range and population of *Tursiops truncatus*.

Density values were of same magnitude as those found by Lauriano *et al.* (2014) who assessed bottlenose dolphin density in the area of the “Central Tyrrhenian Sea, the Ligurian Sea and portions of the Seas of Corsica and Sardinia Sea” through 13 sightings during cetacean aerial surveys, being 0.0051 ind/km². In the Tuscany Natura 2000 site, however, density values were twice those detected by Lauriano, highlighting the relative importance of the area for the presence of the species. Nevertheless, compared to Gómez de Segura *et al.* (2006) in the central Spanish Mediterranean waters, Fortuna *et al.* (2018) in the Adriatic Sea, and Carlucci *et al.* (2018) in the Gulf of Taranto (Northern Ionian Sea), density estimates are between one and two orders of magnitude higher than those recorded in the Tuscany site. These differences could be due to the fact that, for the investigation of trends between reporting cycles, data from all the seasons were pooled together, while other studies refer to the summer season only.

Notwithstanding the fact that behaviour analysis is not the purpose of the methodological approach, still the recorded behaviours further confirm the relationship between bottlenose dolphins and fishery activity reported from a large amount of studies (e.g. Pennino *et al.*, 2016; Carlucci *et al.*, 2018; Genov *et al.*, 2019), as the species was often recorded in close proximity to fishing nets. While from one side this result confirms the need for mitigation and regulatory measures limiting the potential negative interaction with this human activity, from the other side, the absence of records of this behaviour during the latter period is a signal that requires further investigation.

Juveniles were recorded in both investigated periods, confirming what was stated by Rossi *et al.* (2017) that associations of adults and youngs are common in the area, endorsing the importance of the site for the protection of the species at a more vulnerable stage, and the need of specific protection measures especially during the spring and summer seasons. Indeed, these seasons also correspond to the periods of most intense anthropogenic activities at sea, represented by shipping, fishing, and leisure boats that can produce disturbing effects and potential physical risk for the animals (Campana *et al.*, 2017).

The at sea monitoring from the ferry confirmed the presence also of striped dolphin and fin whale in the study area, as well as the occasional presence of sperm whale and the rare common dolphin. These sightings further confirm the importance of the Natura 2000 site for the preservation of cetacean species of Annex IV of the Habitats Directive, and the need of including consideration on these species too in planning conservation and mitigation measures in the site.

The dataset used in the present study was found to be reliable and appropriate both in space and time scales as it detected trends in both range and population of *Tursiops truncatus* in the Natura 2000 site, while also providing information on seasonal variations and behaviour, and on other cetacean protected species. In addition, the use of such platforms allows feasible surveys, repeatability, and coverage of offshore areas, which are needed to assess the complete habitat use of these animals and adjust the conservation strategies accordingly (Robbins *et al.*, 2020). The same approach was successfully used to compare cetacean distribution, habitat use, and abundance in the central Tyrrhenian Sea over a 20 year time span (Arcangeli *et al.*, 2013, 2016). Other studies also showed the ability of this type of long-term monitoring to assess the suitability of an area to be designated as a SAC or IMMA (Leeney, 2007; Matear *et al.*, 2019). The monitoring will continue for the next 6-year period 2019-2024, and further analyses could be included in the near future to provide integrative information also on the trends of parameters like Habitat Suitability and 'Future prospects' such as the potential impact of marine macro-plastic, shipping lanes, and by-catch (Campana *et al.*, 2017; Crosti *et al.*, 2017; Poeta *et al.*, 2017), in order to provide evidence-based indications when assessing the conservation status of the bottlenose dolphin and evaluating the most appropriate mitigation and conservation measures.

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