



Integrating traditional and innovative monitoring approaches to monitor the marine biodiversity in the Tyrrhenian Sea (Mediterranean sea)

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ABSTRACT

The Mediterranean Sea, a global biodiversity hotspot, is increasingly threatened by anthropogenic pressures, leading to a decline in marine biodiversity and ecosystem services. In response, effective monitoring and conservation strategies, including citizen science initiatives, are crucial for understanding and mitigating these impacts. This study presents the *Marine Adventure for Research and Education* (M.A.R.E.) initiative, which integrates public participation in marine biodiversity monitoring through visual surveys and environmental DNA (eDNA) sampling. Novel species-specific primers were developed to target key species, including Risso's dolphin, fin whale, basking shark, loggerhead sea turtle, and sperm whale. Over three months, approximately 100 participants contributed to the detection of endangered marine species, including the loggerhead sea turtle, striped dolphin, fin whale, and basking shark, in the Tyrrhenian Sea. Thus, eDNA analysis proved to be a highly sensitive and non-invasive method for detecting a wide range of species, complementing traditional visual surveys. As a matter of fact, the second most detected species with molecular analyses was the Risso's dolphin, an elusive species previously underreported in the region. Thus, this study suggests that eDNA technique might be considered a promising technique to monitor the Risso's dolphin distribution in the Mediterranean Sea.

1. Introduction

The Mediterranean Sea is one of the main biodiversity hotspots at global level, hosting approximately 8 % of all known marine biodiversity (Coll et al., 2010). However, the basin is increasingly threatened by serious anthropogenic pressures, such as habitat loss and degradation, chemical pollution, introduction of invasive species, and the over-exploitation of marine resources (Azzola et al., 2023; Barredo et al., 2016; Boldrocchi et al., 2023; Taylor and Danovaro, 2010; Tsikliras et al., 2015; Villa et al., 2024). All these factors combined contribute to biodiversity loss and diminish the capability of the ocean to provide ecosystem services (Coll et al., 2010; Taylor and Danovaro, 2010).

Currently, in the Mediterranean Sea, several keystone marine vertebrates are classified as Endangered or Data Deficient according to the IUCN Red List, and multiple studies have already highlighted the need of

increasing the research activities and current knowledge (e.g. Bargnesi et al., 2020; Casale et al., 2018; Walls and Dulvy, 2020). This is particularly true for wide-ranging and/or elusive marine vertebrates, whose scientific research often necessitates substantial financial and logistical resources, limiting extensive monitoring activities (Bargnesi et al., 2020; Tyne et al., 2016; Williams and Thomas, 2009; Rezzolla et al., 2014). Still, the increasing impact of human stressors on marine ecosystems has highlighted the urgent need to enhance conservation strategies and the sustainable management of natural resources. Central to these strategies is, not only the scientific research, but the enhancement of collective awareness about environmental and biodiversity conservation; recognized as a key objective in the European Union's Biodiversity Strategy for 2030. The EU plans promote a participatory approach, encouraging both the scientific community and the general public to become actively involved in protecting ecosystems (European

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Commission, 2020).

Incorporating citizen science into marine research provides a valuable opportunity to enhance monitoring activities across broader spatial and temporal scales, particularly for species that are challenging to study (Bargnesi et al., 2020; Boldrocchi and Storai, 2021; Giovos et al., 2019; Valsecchi et al., 2023). Moreover, the involvement of general public in research activities can provide significant social and conservation benefits, including the increase in scientific literacy (Conrad and Hilchey, 2011). The public engagement can also promote conservation initiatives, potentially favouring a shift in attitudes toward more sustainable practices (Maund et al., 2020; Theobald et al., 2015).

In the context of citizen science initiatives, the use of environmental DNA (eDNA) has emerged as a valuable and cost-effective approach for monitoring the marine biodiversity (Boldrocchi et al., 2024; Eble et al., 2020; Miya, 2022; Thomsen and Willerslev, 2015; Valsecchi et al., 2023). Indeed, one of its main advantages is the simplicity of field sampling which relies on the collection and filtration of superficial water samples to collect genetic material shed or excreted by organisms in the ocean. Both water samples and filtration methods require limited training (Thomsen and Willerslev, 2015), allowing the participation of a vast public in the sampling activities (Valsecchi and Gabbiadini, 2024). Studies have also demonstrated that the eDNA techniques employed by non-experts do not affect the quality and validity of data, when standardized protocols for field sampling are implemented (Valsecchi et al., 2021; Valsecchi and Gabbiadini, 2024). Moreover, citizen science initiatives that comprise eDNA as scientific methodology not only contribute to collecting data useful for research but also promote public engagement and environmental awareness (Valsecchi et al., 2021; Valsecchi and Gabbiadini, 2024) as well as increase social responsibility, the citizen's understanding of ecological concepts and the importance of biodiversity conservation as well as trust between scientists and citizens (Rotman et al., 2014).

From a scientific perspective, eDNA analyses have demonstrated high sensitivity in detecting both common and rare or elusive species, especially in regions difficult to study using more traditional methods (Bohmann et al., 2014; Ferretti et al., 2024; Valsecchi et al., 2023; Valsecchi and Gabbiadini, 2024). Thus, a vast number of citizen scientists can collect water samples from various locations at the same time, significantly increasing the spatial coverage of biodiversity monitoring projects and allowing for the detection of species that might otherwise go unnoticed (Lahoz-Monfort and Tingley, 2018).

However, while environmental DNA has been established as a reliable tool for species detection, it can provide biased information about the exact distribution of species. Environmental DNA gradually degrades in seawater, but this degradation can vary from hours up to several weeks (Collins et al., 2018; Salter, 2018; McCartin et al., 2022) due to abiotic factors, such as water temperature (Caza-Aillard et al., 2022; McCartin et al., 2022; Strickler et al., 2015) or salinity (Collins et al., 2018) and sunlight (Andruszkiewicz et al., 2017). Moreover, DNA traces can be transported by water flow quite far from the original release position. Thus, it is impossible to determine whether the animals were recently in a precise spot, if the eDNA came from another location and was carried by sea currents, or if it originated from the remains of a long-deceased animal (Haile et al., 2009). Still, the technique has been proven to be the most non-invasive and cost-effective method for detecting endangered and invasive species over large areas (e.g. Boldrocchi et al., 2024; Valsecchi et al., 2023). Indeed, recent literature has provided evidence that also RNA biomolecules (eRNA) released by organisms into the surrounding environment exhibits detectability and high release rates in aquatic ecosystems (Guardiola et al., 2016; Laroche et al., 2017). Environmental RNA stability in marine water is low (i.e. up to a few hours; Wood et al., 2020), but sufficient to be potentially detected by molecular analysis, so that the search for eRNA has been proposed as a suitable alternative to eDNA, when a precise geographical position of species records is of primary importance (Stevens and Parsley, 2023). However, the advantage of eRNA in providing precise

localization may be less relevant for species like cetaceans or certain species of elasmobranchs, which travel large distances during daily or seasonal migrations (Stalder et al., 2020; Vighi et al., 2016), where core movement areas are more informative than pinpointing single locations. In such cases, eDNA's longer persistence may offer more valuable information, yielding more positive detections. Given these considerations, eDNA remains a preferred approach for tracking the presence and distribution of large marine species, especially when used as complementary approach to traditional monitoring, such as visual surveys. Indeed, several studies showed that the combination of both approaches enhances the sensitivity and contextual understanding of species presence, as well as increases the detection of species presence, especially for pelagic marine vertebrates which often occur in low densities or vast areas, (Afonso et al., 2024; Bohmann et al., 2014; Burns et al., 2024). While eDNA increases spatial coverage and may lower costs and reduce disturbance, it does not replace the need for visual surveys which remain essential for providing real-time observations of species behavior and ecological data, which eDNA cannot capture (Afonso et al., 2024; Gold et al., 2021; Valdivia-Carrillo et al., 2021).

In light of these considerations, in 2022, the *Marine Adventure for Research and Education* (M.A.R.E.) initiative was developed to integrate marine biodiversity monitoring with active public participation; throughout visual survey monitoring coupled with the collection of water samples for eDNA analyses to increase the detectability of the target species, i.e. the loggerhead sea turtle (*Caretta caretta*), the striped dolphin (*Stenella coeruleoalba*), the fin whale (*Balaenoptera physalus*), the sperm whale (*Physeter macrocephalus*), Risso's dolphin (*Grampus griseus*), the basking shark (*Cetorhinus maximus*), the Atlantic blue crab (*Callinectes sapidus*). Within this framework, this study provides information on the distribution of several endangered vertebrates in the Tyrrhenian Sea (Mediterranean Sea).

2. Material and methods

2.1. Field survey

Sampling was conducted in the North-Western Mediterranean Sea, mainly in the Tyrrhenian Sea, but including also three samples collected in the Ligurian Sea and eight in the Sardinian Sea (Fig. 1). Sampling was carried out onboard a 14 m catamaran, that travelled approximately 1500 miles starting in Sardinia, in La Maddalena Island, on last week of April 2022 and ended in the same location on the third week of July 2022. The catamaran, once left the La Maddalena Island, travelled all the Sardinia coasts, and headed to Egadi Islands, in Sicily. From that area, the sailing vessel moved northward through the regions of Calabria, Campania, Lazio, Tuscany, Liguria and back to Sardinia (Fig. 1). During the sampling campaign, which consisted of 12 sailing trips, each lasting one-week, multiple guests alternated onboard each week. By the end of the project, a total of approximately 100 guests participated to the field sampling campaign, including influencers of brands related to the fashion industries. Each participant onboard attended workshops and seminars on the Mediterranean marine biodiversity, including the main characteristics and life-histories of the most commonly present vertebrate species. Participants were also trained for species recognition so that, during each sailing crossing, they were able to note the following information for each sighting: date, geographical coordinates, identified species, and number of specimens. A researcher was always on board to oversee the sampling and certify the sightings. Moreover, during the M. A.R.E. initiative, scientists trained volunteers to sample and filter surface water for environmental DNA analyses, following the protocols implemented by Valsecchi et al. (2021). Specifically, sampling of superficial water was performed at 36 different stations along the route (Fig. 1) by collecting 12 L of seawater with a manual pump and a refill dispenser storage bag. For each 12 L sample, two aliquots of 6 L were then filtered on a 0.45 µm cellulose nitrate filter. Both aliquots were then stored at -18 °C for DNA preservation, until laboratory analyses

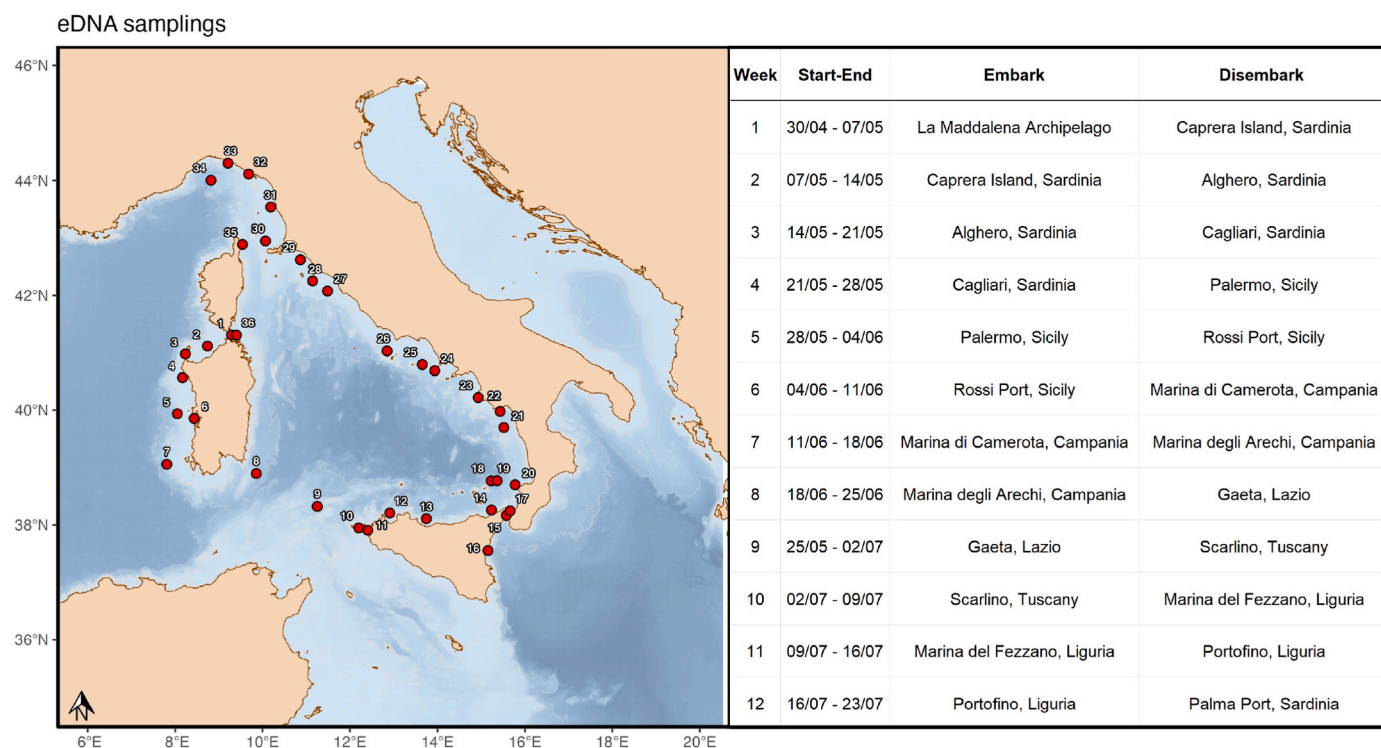


Fig. 1. Environmental DNA sampling points conducted in the Western Mediterranean Sea (Tyrrhenian Sea, Ligurian Sea, and Sardinian Sea) as part of the M.A.R.E. Project between April and July 2022.

performed at IRSA-CNR (Brugherio, Italy).

2.2. Target species

To determine that eDNA monitoring is an effective method to be applied to a citizen science project, we decided to monitor the presence of two commonly found species in the Mediterranean Sea: the loggerhead sea turtle (*Caretta caretta*) and the striped dolphin (*Stenella coeruleoalba*). Both species were used as “control species” for the environmental DNA detection due to their abundance in the Tyrrhenian Sea. With regards to the other species, we focused on those that are considered threatened at Mediterranean level, such as the fin whale (*Balaenoptera physalus*); sperm whale (*Physeter macrocephalus*); basking shark (*Cetorhinus maximus*); and Risso’s dolphin (*Grampus griseus*), all classified as Endangered by the IUCN Red List of Threatened Species (Lanfredi et al., 2022; Panigada et al., 2021; Pirota et al., 2021; Sims et al., 2016). The Atlantic blue crab (*Callinectes sapidus*) was chosen as a representant of a commonly found invasive alien species (Nehring, 2011) to evaluate if the environmental DNA technique can be useful to detect also benthic species.

2.3. Creation of species-specific primers

While sampling was conducted along the Tyrrhenian Sea, six species-specific primers were developed in the laboratory for the target species, besides for the Atlantic blue crab, whose pair of primers were already available (Knudsen and Møller, 2020). The new set of species-specific primers was based on an alignment of the complete mitochondrial DNA of the target species and of other phylogenetically related taxa of the Mediterranean Sea (NCBI Genbank, at least two genomes per species were included). The loggerhead turtle species were aligned together after the same process. Refer to the Supplementary Materials for a comprehensive description of the methodologies employed to assess the specificity of the new primers, utilizing both *in silico* and *in vitro* approaches.

2.4. Molecular analyses

Once in the laboratory, each filter collected from the Tyrrhenian Sea was processed to extract the total genomic DNA, which included genetic material of all organisms collected in each sample. For this analysis, the DNeasy Blood and Tissue Kit (Qiagen) was used (further details in Supplementary Materials).

A quantitative real-time PCR (qPCR) amplification was performed in triplicate for each sample, also including a quantification standard curve (six 10-fold dilutions). In each analysis, two control samples, one positive and one negative, were included to check for false amplifications or failures. The positive control sample was derived from tissues taken from an individual of the target species, processed as for the filters until amplification. Specificity of the obtained amplifications was tested by melting curve analysis, using the positive control sample as reference, or by Sanger sequencing. Limit of detection (LOD) of the specific protocols were also estimated (see Supplementary Materials).

3. Results and discussion

After receiving the necessary training, the approximately 100 participants played an active role in spreading knowledge about the research initiative, but also about the Mediterranean marine biodiversity, and the key human pressures affecting marine ecosystems. They leveraged their social media platforms contributing to raise awareness in Italy.

The inclusion of such wide number of citizens in visual survey activities allowed to detect multiple species over the Tyrrhenian Sea, that might otherwise go unnoticed. A total of 24 sightings have been detected during the project, with bottlenose dolphins the most sighted species (54.2 %), followed by striped dolphins (29.2 %), loggerhead turtle (8.3 %) and devil rays (*Mobula mobular*) (8.3 %) (Table 1). While the bottlenose dolphin was the most sighted species, the striped dolphin was the most numerous in term of individuals with sightings of more than 30 specimens (Table 1).

Table 1
Sightings recorded during visual survey monitoring in April–July 2022 in the Tyrrhenian Sea.

Date	GPS		Location	Species	N of individuals
May 10, 2022	41° 14.813' N	9° 8.366' E	Cape Testa, Sardinia	<i>T. truncatus</i>	10
May 11, 2022	41° 3.786' N	8° 45.069' E	Castelsardo Canyon, Sardinia	<i>T. truncatus</i>	3+
May 12, 2022	40° 56.645' N	8° 26.420' E	Stintino, Sardinia	<i>T. truncatus</i>	2
May 12, 2022	40° 56.584' N	8° 14.680' E	Stintino, Sardinia	<i>T. truncatus</i>	3
May 13, 2022	40° 56.258' N	8° 10.263' E	Punta Scoglietti, Sardinia	<i>T. truncatus</i>	2
May 13, 2022	40° 33.953' N	8° 11.977' E	Port Conte, Sardinia	<i>T. truncatus</i>	2
May 16, 2022	39° 29.365' N	8° 23.816' E	Piscinas, Sardinia	<i>T. truncatus</i>	3
May 19, 2022	39° 5.914' N	8° 18.474' E	Carloforte, Sardinia	<i>T. truncatus</i>	3
May 19, 2022	39° 3.738' N	8° 16.988' E	Carloforte, Sardinia	<i>T. truncatus</i>	3
May 22, 2022	38° 57.039' N	9° 42.282' E	Cagliari-Palermo	<i>M. mobular</i>	1
May 22, 2022	38° 53.879' N	9° 51.358' E	Cagliari-Palermo	<i>C. caretta</i>	1
May 23, 2022	38° 11.941' N	11° 29.004' E	Cagliari-Palermo	<i>S. coeruleoalba</i>	2
May 23, 2022	38° 17.331' N	11° 20.750' E	Cagliari-Palermo	<i>S. coeruleoalba</i>	1
June 01, 2022	38° 11.527' N	14° 51.286' E	Cape d'Orlando, Sicily	<i>S. coeruleoalba</i>	9
June 02, 2022	37° 50.392' N	15° 22.280' E	Taormina, Sicily	<i>S. coeruleoalba</i>	50
June 12, 2022	38° 55.888' N	15° 50.340' E	Tropea-Camerota	<i>M. mobular</i>	1
June 12, 2022	39° 26.090' N	15° 38.823' E	Tropea-Camerota	<i>C. caretta</i>	2
June 22, 2022	40° 44.579' N	14° 3.071' E	Island of Procida	<i>T. truncatus</i>	4
June 29, 2022	42° 4.465' N	11° 29.161' E	Ostia-Giglio Island	<i>T. truncatus</i>	2
July 19, 2022	44° 6.659' N	8° 44.537' E	Genoa, Liguria	<i>T. truncatus</i>	4
July 19, 2022	43° 33.390' N	9° 9.520' E	Pelagos Sanctuary	<i>T. truncatus</i>	4
July 19, 2022	43° 32.016' N	9° 10.445' E	Pelagos Sanctuary	<i>S. coeruleoalba</i>	5
July 19, 2022	43° 18.382' N	9° 18.358' E	Pelagos Sanctuary	<i>S. coeruleoalba</i>	10
July 19, 2022	43° 16.532' N	9° 19.388' E	Pelagos Sanctuary	<i>S. coeruleoalba</i>	30

All the novel species-specific markers, designed in the present study, correctly amplified DNA deriving each target species tissue: amplification yielding the expected length fragment (for each species, see Table S1, Supplementary Materials) resulted in a single band product, which showed a distinct peak in melting curve analysis. The same melting curve profile was obtained for all positives (including standard curve dilutions) controls, and in some filters' DNA. Low frequent nonspecific products, which presented poorly defined melting profiles, were sequenced and no reliable electropherograms were obtained, likely due to primer-dimers artefacts. Thus, excluding those aspecific amplifications, a total of 25 out of 36 samples collected in the Tyrrhenian Sea provided positive amplifications of the targeted species eDNA (Fig. 2).

Specifically, this study successfully monitored the presence of 6 out of the 7 target species using DNA traces: 19 samples resulted positive for DNA of striped dolphins, 13 of Risso's dolphin, 10 samples for fin whales, 4 for the loggerhead turtle, 3 for the basking shark, and 2

samples for the Atlantic blue crab (Fig. 3). Results from this study are consistent with previous findings demonstrating the utility of eDNA as complement method for detecting a wide range of marine species (Foote et al., 2012). Specifically, eDNA has been shown to be highly sensitive in detecting both marine mammals, elasmobranch and sea turtle species (Baker et al., 2018; Gargan et al., 2017; Lafferty et al., 2018; Davy et al., 2015). The same approach was adopted by Faure et al. (2023), who designed a primer pair and a probe to successfully detect angel sharks, belonging to the *Squatina* genus in Corsican waters. While Pichot et al. (2024), used eDNA metabarcoding to detect a variety of elasmobranchs around Corsica and up north on the French and Spanish coastlines.

Thus, the present research is based on an established and reliable method, advancing our knowledge of eDNA-based detection of marine species and offering novel species-specific markers.

As expected, the most common species recorded in this study was the striped dolphin, both for what concerns the eDNA sampling and the visual monitoring (Table S3, Supplementary Materials). As a matter of fact, available data indicated that this cetacean is the most abundant within the Mediterranean Sea, especially in the western part of the basin, distributed both inshore and offshore (Aguilar, 2000; Forcada and Hammond, 1998; Laran and Drouot-Dulau, 2007). The striped dolphin is normally found in groups of approximately 20–50 individuals, although large groups of up to 500 individuals as well as solitary specimens can be recorded as well (Cañadas and Sagarminaga, 1994; Canales-Cáceres et al., 2023). Consistently, in this study, both solitary and gregarious sightings have been documented during visual survey activity, with a mean of 15.3 ± 18.1 individuals per group. Considering results from both monitoring techniques combined, the majority of sightings were recorded in two areas: 1) in the Ligurian Sea; and 2) along the north coast of Sicily between Egadi Islands up to the Calabrian coasts at the border with Campania (Table 1 and Fig. 3A). Consistently, multiple studies have documented the presence of this species in both the southern Tyrrhenian Sea and the Ligurian Sea, reporting the striped dolphin as one of the most encountered cetacean species (e.g. Azzellino et al., 2008; Notarbartolo di Sciara et al., 1993; Gannier, 2005).

Interestingly, the second most detected species with molecular analyses was the Risso's dolphin. This cetacean was listed as Data Deficient by the IUCN Red List until recently, when it was updated to the status of Endangered species (Lanfredi et al., 2022). It is a cosmopolitan cetacean, whose occurrence in the Tyrrhenian Sea has been largely described (e.g. Azzellino et al., 2008, 2016; Notarbartolo di Sciara et al., 1993; Moullins

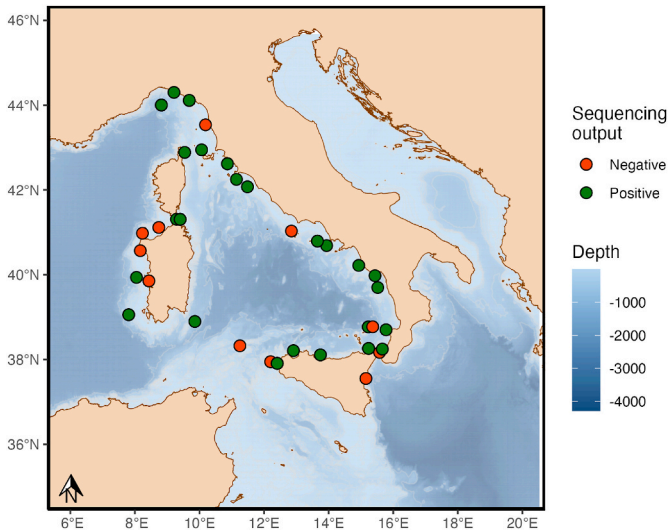


Fig. 2. Positive (green dots) and negative (red dots) signals of DNA traces for one or more species from sampling carried in the Western Mediterranean Sea (Tyrrhenian Sea, Ligurian Sea, and Sardinian Sea) as part of the M.A.R.E. Project between April and July 2022.

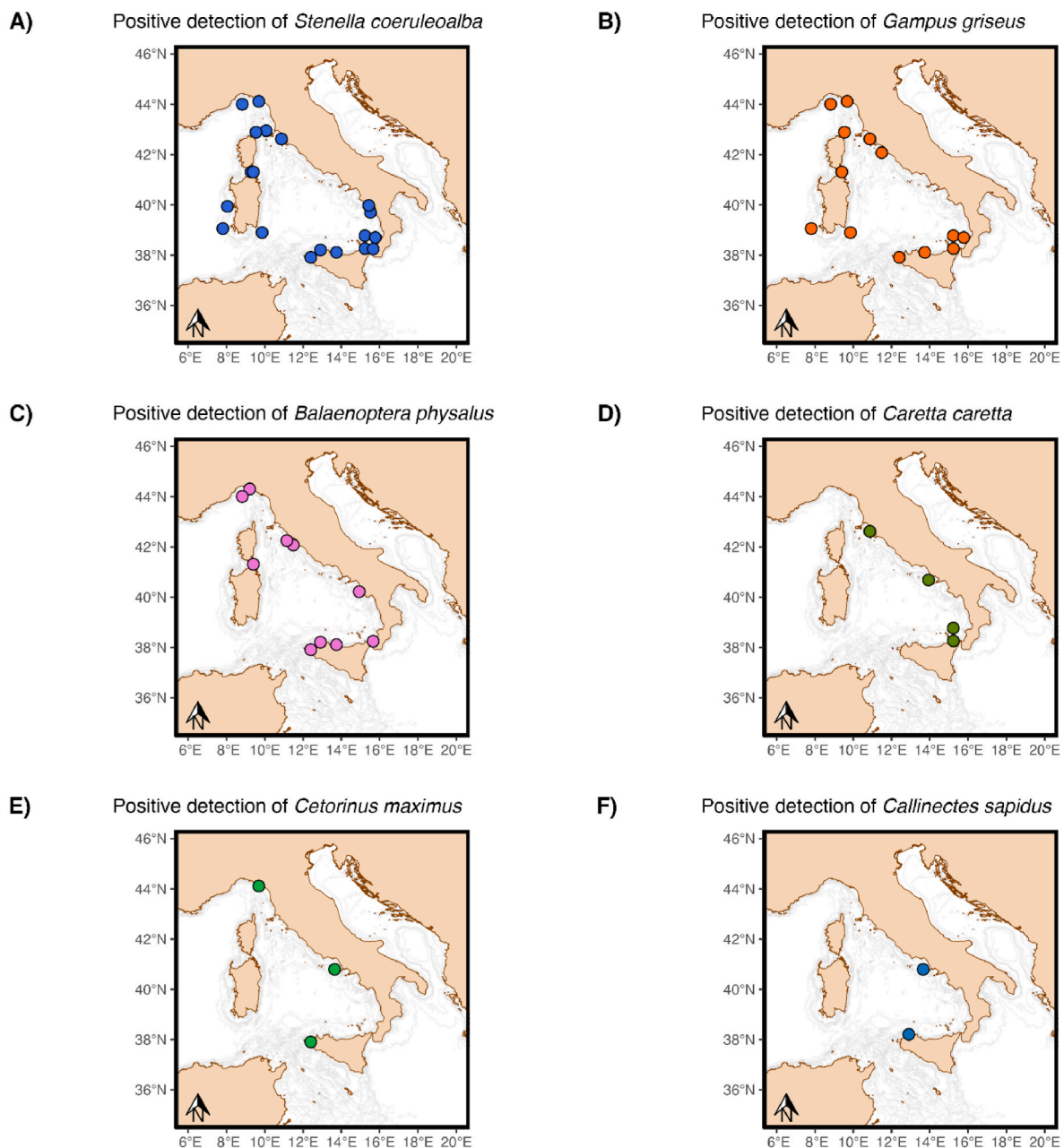


Fig. 3. Positive detection of eDNA traces of the following target species: A) striped dolphin; B) Risso's dolphin; C) fin whale; D) loggerhead turtle; E) basking shark; and F) the Atlantic blue crab. Samples were collected in April–July 2022.

et al., 2008). However, this species shows a more scattered distribution, often restricted to the western basin (Gnone et al., 2023), with a preference for the continental slope and submarine canyons (Azzellino et al., 2008, 2016; Bearzi et al., 2011; Gnone et al., 2023). Moreover, in the Mediterranean Sea Risso's dolphins are not abundant and their occurrence can be unpredictable, possibly due to wide-ranging movements (Bearzi et al., 2011). Even within the few Mediterranean areas where Risso's dolphins are known to be consistently present, limited information has been obtained; while in large parts of the region its presence is still poorly documented due to a lack of sightings (Bearzi et al., 2011; Corrias et al., 2021). For instance, no sightings of Risso's dolphins were recorded during three ship-based surveys in 2002–2003 within an area of approximately 13,000 km² off northern Sicily (Fortuna et al., 2007). Similarly, limited information is reported from the northern area of the Strait of Sicily (Papale et al., 2020). For the first time at global and Mediterranean levels, this study detected the presence of Risso's dolphin

throughout the eDNA technique in multiple areas: in the Ligurian Sea, in the northern and southern Tyrrhenian Sea, particularly in the northern Sicily and Calabria coasts (Fig. 3B). It is interesting to note that in all Mediterranean areas with suitable habitat that have been surveyed with aerial and ship surveys, encounter rates for Risso's dolphins have been low compared with rates for other more common delphinids (Bearzi et al., 2011), but not in this study with molecular detection. As a matter of fact, this species was the second most detected after the striped dolphins (36 % of samples) suggesting that eDNA technique might be considered a promising technique to monitor the Risso's dolphin distribution in the Mediterranean Sea.

The third most detected species using eDNA technique was the fin whale. This species, in the Mediterranean Sea, is classified as Endangered by the IUCN Red List with a declining trend in abundance (Panigada et al., 2021). In the western Mediterranean Sea, it is regularly found in highly productive areas along frontal zones and submarine

canyons, especially in the Ligurian and southern Tyrrhenian Seas (Aïssi et al., 2008; Arcangeli et al., 2014; Notarbartolo di Sciarra et al., 2003; Panigada et al., 2021). During spring, upwelling and other oceanographic processes enhance primary production, leading to an increase of zooplankton biomass, which in turn attracts fin whales to these feeding areas (Notarbartolo di Sciarra et al., 2003). In this study, genetic traces of fin whales were detected mainly in the Pelagos Sanctuary area (Fig. 3C), where fin whales tend to concentrate to forage during spring and summer time in frontal systems and upwelling phenomena (Cotté et al., 2009; Panigada et al., 2008). Another area where fin whales were detected is the northern Sicily, including the Strait of Messina, another renowned feeding area (Aïssi et al., 2008; Druon et al., 2012).

With regards to the loggerhead turtle, which is considered the most widespread sea turtle species in the Mediterranean Sea (Almpanidou et al., 2022; Mancino et al., 2022), both visual and molecular surveys detected this species in the Tyrrhenian Sea. Specifically, visual survey activities documented 3 individuals on surface offshore Sardinia and Sicily and offshore Calabria coasts, while the primer developed for this species detected genetic traces in the northern Sicily, around Aeolian islands, but also offshore Campania and Tuscany coasts (Figure D).

The most commonly way to study loggerhead turtles relies on accidentally or deliberately caught individuals, that are released soon after capture (Barceló et al., 2013; Howell et al., 2010) or after a period spent in captivity for rehabilitation (Cardona et al., 2012; Dalleau et al., 2014). Satellite tracking is also used to track the large movements, although it is often difficult to equip non-nesting turtles, with tracking instruments, since they do not come ashore at any time and cannot be easily accessed or captured (Schofield et al., 2013; Van Dam et al., 2008). Thus, the approach used in this study might contribute to further investigate whether turtles use oceanic foraging areas and to identify the location and extension of the most frequented sites.

With regards to the basking shark, very few studies have been conducted in the Mediterranean Sea (Boldrocchi et al., 2022; Carlucci et al., 2014; de Sabata and Clò, 2010; Mancusi et al., 2005), although this species is considered Endangered both at global and Mediterranean level (Rigby et al., 2021; Sims et al., 2016). Thus, the presence, distribution, movements and habitat use of this species remain poorly understood in the Mediterranean Sea. Existing information, however, suggests a potential preference for certain areas characterized by upwelling and high productivity (Carlucci et al., 2014; Mancusi et al., 2005). Indeed, the sporadic sightings of this species tend to increase during late winter and spring in areas such as the east coasts of Sardinia (De Sabata et al., 2013), characterized by seasonal zooplankton blooms. Despite never detected during visual survey activities, molecular analyses showed 3 positive eDNA detections (8 %) off the coasts of Liguria, Campania, and Sicily, respectively (Fig. 3E), confirming the utility of eDNA analysis as a valuable tool for monitoring basking shark presence in the Mediterranean basin.

The Atlantic blue crab is a species native to the western Atlantic that has spread to the Mediterranean Sea via ballast waters of ships (Mancinelli et al., 2017; Nehring, 2011). Despite the known presence in some areas of the Tyrrhenian Sea, the results of this study detected positivity only in two samples, one in Sicily and the other one in Campania (Fig. 3F). The paucity of positive detection is probably linked to the fact that the Atlantic blue crab is benthic and thus its DNA traces are likely not found in surface waters where sampling has been carried out. Thus, considering the widespread presence of this invasive species and the poor results obtained with eDNA monitoring, the use of the Local Ecological Knowledge represents a better valuable source of data for mapping the Atlantic blue crab in the Mediterranean Sea (Azzurro and Cerri, 2021).

The only species that was never detected either with visual survey activities or molecular analyses was the sperm whale, despite both the Ligurian and Tyrrhenian Sea are regularly frequented by this species. Several studies, indeed, indicated that the area of the Pelagos Sanctuary is considered a hotspot for sperm whales (Azzellino et al., 2008), as well

as the waters around the Pontine Islands and the Aeolian Archipelago in the Tyrrhenian Sea, where regular sightings have been reported (Pirodda et al., 2021), and the seamounts off southern Sardinia and the submarine canyons near the Tuscan Archipelago (Claro et al., 2020; Virgili et al., 2019).

In this study, the absence of detection could be attributed to several factors. First, sperm whales are highly mobile and can exhibit seasonal or sporadic use of feeding grounds, potentially reducing the likelihood of detecting eDNA during certain periods (Pirodda et al., 2020). Additionally, the oceanographic conditions at the time of sampling, including water currents and vertical mixing, might have dispersed or diluted eDNA, lowering its detectability. The depth at which sperm whales typically feed, often exceeding 500 m, combined with their pelagic nature, further complicates the detection of eDNA in surface waters, where our sampling was concentrated. Finally, the timing of our sampling, which was primarily conducted in spring, may not have coincided with peak periods of sperm whale activity in these areas, as the species may migrate or shift their feeding patterns based on prey availability and environmental conditions.

4. Conclusions

The results from the M.A.R.E. initiative highlight the significant potential of integrating citizen science with traditional and innovative techniques to monitor marine biodiversity in the Mediterranean and raise public awareness about marine biodiversity. By combining visual monitoring with eDNA sampling, the project provided valuable insights into species distribution and abundance in the Tyrrhenian Sea. Both methods identified a diverse range of species, with striped dolphins being the most frequently detected, followed by Risso's dolphins and fin whales. Environmental DNA, in particular, proved effective in detecting also Risso's dolphins and basking sharks, both Endangered species that are difficult to monitor through traditional methods, highlighting eDNA's potential for tracking species with poorly known distributions. Moreover, this study confirmed that eDNA is a reliable tool for detecting species even when visual sightings are limited, like the basking shark, despite its sporadic sightings from the Mediterranean Sea.

CRedit authorship contribution statement

E. Fasola: Writing – review & editing, Writing – original draft, Investigation, Formal analysis, Conceptualization. **C. Santolini:** Writing – review & editing, Writing – original draft, Visualization, Investigation, Conceptualization. **B. Villa:** Writing – review & editing, Writing – original draft, Investigation, Conceptualization. **A. Zanoletti:** Writing – review & editing, Investigation. **G. Magni:** Writing – review & editing, Investigation. **J. Pachner:** Writing – review & editing, Investigation. **F. Stefani:** Writing – review & editing, Validation, Supervision, Methodology, Investigation, Formal analysis. **G. Boldrocchi:** Writing – review & editing, Writing – original draft, Visualization, Validation, Supervision, Methodology, Investigation, Formal analysis, Conceptualization. **R. Bettinetti:** Writing – review & editing, Validation, Resources, Methodology.

Declaration of competing interest

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

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.marenvres.2025.107160>.

Data availability

Data will be made available on request.

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