Exploring the potential of environmental DNA as a conservation tool for the Mediterranean monk seal (*Monachus monachus*)

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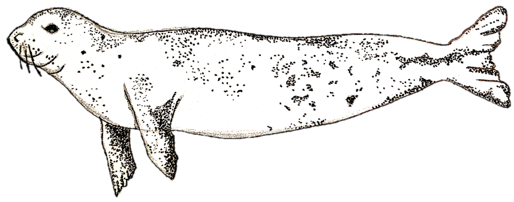

Abstract
The Mediterranean monk seal is the world's most endangered pinniped and the only one found in the Mediterranean. Anthropogenic threats have afflicted the species since prehistoric times, resulting in a steep decline of both their abundance and geographical range over time. The remaining habitat of the Mediterranean monk seal is deteriorated, destructed and highly fragmented. To restore the few remaining populations, conservation measures are necessary. In order to implement effective management and conservation strategies, monitoring programs are needed to assess the distribution and abundance of the remaining individuals. In case of rare and elusive species like Mediterranean monk seals, monitoring has proven to be a challenging, time-consuming and costly occupation. Most of the data on Mediterranean monk seal distribution relies on visual observations, which may be unreliable and insufficient. The relatively new and quickly developing technique of environmental DNA analysis may offer a solution for difficult-to-study species like the Mediterranean monk seal. eDNA assays come in different approaches, all using genetic material that organisms have shed into their environment. Although several flaws and areas for improvement remain, eDNA analysis has proven to be an effective, simple and relatively cheap way for mapping species distributions, and possibly even abundance. In this paper, the potential of applying eDNA as a tool for Mediterranean monk seal conservation is investigated. Incorporation of eDNA techniques could improve conservation strategies for elusive and rare species like the Mediterranean monk seal, perhaps by complementing traditional monitoring methods.

Introduction
Marine mammals are among the most threatened vertebrates on earth: 37% of marine mammal species are considered as endangered by the IUCN (Juhel et al., 2021). Extinction of these species can have drastic consequences for marine communities, since marine mammals are important drivers of ecosystem functioning and fulfil key ecological roles in trophic dynamics, mostly due to their role as consumers at most trophic levels and their role in nutrient cycling (Albouy et al., 2020; Kiszka et al., 2015). Increasing numbers of changes in their environment are being faced by marine mammals, the majority of which are anthropogenic threats (Clegg et al., 2021). These threats can go as far as causing population declines and extinctions (Duarte et al., 2020). These ominous developments and their far-reaching consequences for ecosystem functioning stresses the importance of appropriate conservation measures for marine mammals (Albouy et al., 2020; Kiszka et al., 2015).

Assessment of population status and anthropogenic threats to marine mammals both require monitoring programs, in order to implement effective management and conservation strategies (Suarez-Bregua et al., 2022; Székely et al., 2022; Weltz et al., 2017). Yet, monitoring marine mammals remains a challenge, most commonly due their low abundance, wide geographical range sizes, their ability to move over large distances, and their elusive behaviour (Juhel et al., 2021). For two essential parameters for population monitoring, distribution and abundance, data collection has traditionally relied on visual monitoring (Suarez-Bregua et al., 2022). Although visual data has contributed essential information to management and conservation of marine mammals, the method is costly and time-consuming, weather dependent, requires specialist expertise, may involve risk to human observers, can be invasive, and may result in large uncertainties in population estimates (Suarez-Bregua et al., 2022; Székely et al., 2022). These limitations of visual monitoring make it especially challenging to monitor cryptic, low density, or logistically difficult-to-study species (Hunter et al., 2018).

The Mediterranean monk seal, *Monachus monachus* Hermann, 1779 is the most endangered pinniped worldwide, and one of the most endangered Evolutionary Distinct and Globally Endangered (EDGE) mammals on Earth (Karamanlidis et al., 2019; Valsecchi et al., 2022). It is estimated that less than 700 seals remain in a few fragmented subpopulations (Contarinis et al., 2023). The largest population of Mediterranean monk seals lives and reproduces in the Eastern Mediterranean, mainly dispersed among islands in the Ionian and Aegean seas, and along the coasts of mainland Greece, Cyprus, and western and southern Turkey (Karamanlidis et al., 2019). Across its entire range, significant anthropogenic threats have been identified for the species, resulting in dramatic declines, both in abundance and geographical range (Karamanlidis et al., 2019). Due to its rarity, high degree of dispersal and elusive nature, Mediterranean monk seals are challenging to monitor by conducting visual surveys (Valsecchi et al., 2022).

Over the last decade, environmental DNA (eDNA) has emerged as an important and powerful tool to detect elusive, rare and endangered species in the wild (Bonfil et al., 2021). For this technique, genetic material is extracted from different kinds of environmental samples, although water samples are most commonly used (Hunter et al., 2018). One way of using eDNA, is analysing water samples to make inferences about the presence/absence of a targeted species. eDNA potentially allows for relatively cheap, sensitive and non-invasive species monitoring, overcoming some of the challenges of labour-intensive traditional surveys, like reaching difficult-to-study areas, observers' bias, costly monitoring equipment and damage to the target species or its habitat (Beng & Corlett, 2020; Suarez-Bregua et al., 2022). Despite the significance of questions eDNA can possibly answer for conservation purposes, aspects like collection, detection, analysis, and interpretation propose challenges for application of eDNA in marine mammal science (Beng & Corlett, 2020; Székely et al., 2022). In this study, the potential of eDNA for Mediterranean monk seal conservation is explored, by reviewing previous applications of eDNA in marine vertebrates and taking into account limitations and challenges of the technique.

Mediterranean monk seal

The Mediterranean monk seal *Monachus monachus* is the only species of the genus *Monachus*. The first observations of the Mediterranean monk seal date back to 2500 years ago (Mihnovets, 2017). At that time, the species occupied a continuous range from the Black and Mediterranean seas to the eastern Atlantic Ocean. Although Aristotle was the first to describe the species in antiquity, Johann Hermann named the species *Phoca monachus* in 1779 (Karamanlidis et al., 2016). In Hermann's understanding, the name 'moine' (monk) was already in use for seals of the same species in Mediterranean France, and the head of the seal reminded him of a hooded monk (Karamanlidis et al., 2016).

Anthropogenic exploitation of the Mediterranean monk seal has been recorded as early as prehistoric times (Johnson & Lavigne, 1999). By using computational methods, Salmona et al. (2022) found scenarios that suggest that early human densification around the Mediterranean basin and the development of seafaring techniques were the main drivers of the decline of Mediterranean monk seals in Antiquity. Although exact numbers are unknown, historical sources from antiquity support this finding by mentioning Mediterranean monk seals in relation to hunting, meat consumption, oil and skin use, conflict with fisherman, use in circus shows and use of body parts to produce medicines

(Salmona et al., 2022). In addition to intense exploitation of Mediterranean monk seals during the Roman era, certain areas were targets of major exploitation events in the Middle Ages, such as the Madeira and Canary Islands and the Bay of Dhakla in the Western Sahara (Karamanlidis et al., 2016). During the 20th century, the species remained to be heavily persecuted by fishermen, resulting in a steep decline of its former home range (Karamanlidis et al., 2016). The deliberate killing of Mediterranean monk seals by fishermen is a recurring phenomenon that poses a threat for Mediterranean monk seals from the Roman era up to today, due to alleged losses of catch and fishing gear damage (Hale, 2011; Mihnovets, 2017).

Currently, the total abundance of the Mediterranean monk seal is estimated at 600 to 700 individuals, making it the most endangered pinniped species in the world (Karamanlidis et al., 2019; NOAA Fisheries, 2022). The geographical range of the species is highly fragmented and the three or four remaining subpopulations are found in the Mediterranean Sea and the eastern Atlantic Ocean along the coast of Northwest Africa (Fig. 1) (Karamanlidis et al., 2019; NOAA Fisheries, 2022). In the North Atlantic, two subpopulations are present: one at Cabo Blanco at the border of Mauritania and Western Sahara, and one at the archipelago of Madeira. The largest aggregation of Mediterranean monk seals is found in the subpopulation at Capo Blanco: more than 300 individuals in total (Fernández de Larrinoa et al., 2021). The subpopulation at Madeira is estimated at 30 to 35 adult seals (Hale, 2011). More than 4000 km from Capo blanco, in the eastern Mediterranean, a population of approximately 350 to 400 individuals live (Fernández de Larrinoa et al., 2021; Pastor et al., 2007). In contrast to the Capo Blanco population, the individuals of this population are highly dispersed, although most of them were observed among Greek and Turkish islands and mainland coast (Fernández de Larrinoa et al., 2021; Pastor et al., 2007). According to the IUCN Red List of Threatened Species, the Mediterranean monk seal subpopulation in the eastern Mediterranean qualifies for the status ‘endangered’ (Karamanlidis et al., 2019).

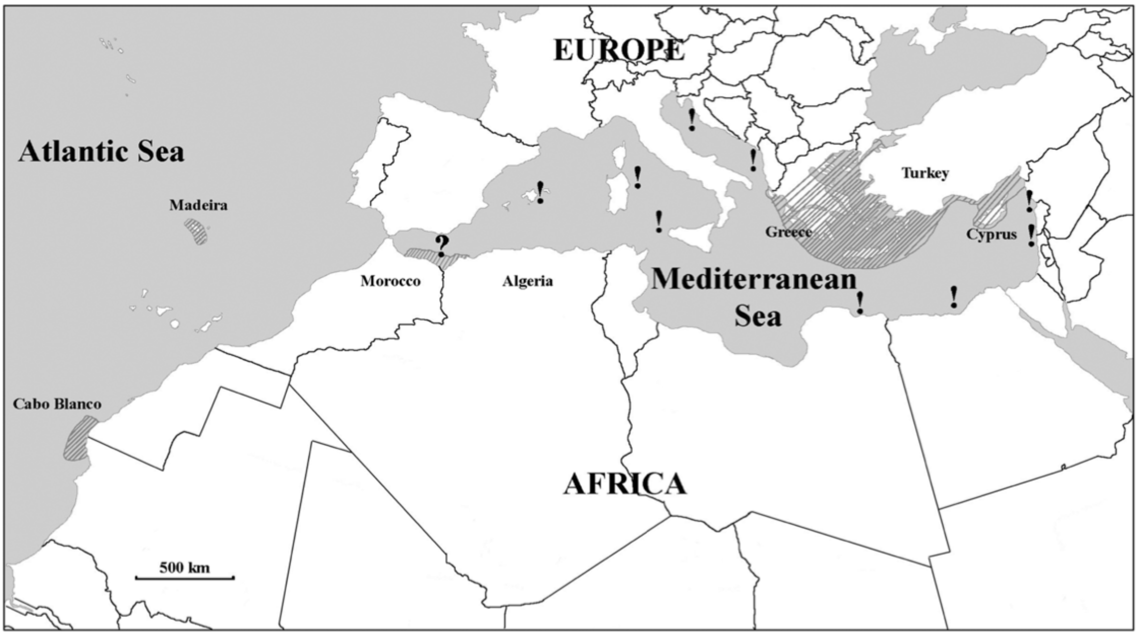

Fig. 1. Map of the Mediterranean Sea and North Africa. The striped areas indicate the currently known geographical range of the three remaining populations of Mediterranean monk seals, as mentioned in the text. The explanation marks indicate observations of Mediterranean monk seals outside their current range and the question marks indicates an area where the fate of the population is unknown (Karamanlidis et al., 2016).

An assessment from 2015 mentions evidence of small increases in all known subpopulations of Mediterranean monk seals after introduction of conservation measures in the past 30 years (Karamanlidis & Dendrinos, 2015). However, the species remains listed as endangered under the Endangered species act and depleted under the Marine mammal Protection Act (NOAA Fisheries, 2022). There is regional variation in intensity and importance of the various threats that Mediterranean monk seals face, and the threats are often interrelated (Karamanlidis et al., 2016). Apart from the previously mentioned exploitation for human consumption and deliberate killing by fisherman, current human-related pressures involve destruction of shoreline habitat, fishery related reduction of prey availability and pollution (Pastor et al., 2007). Stochastic and unusual events and food scarcity propose a risk of mortality for Mediterranean monk seals, although they are not generally considered as serious threats to the survival of the species (Karamanlidis et al., 2016). Examples of stochastic and unusual events of the past are toxic algal blooms, virus outbreaks, rock slides and cave collapses, and abnormally low sea temperatures.

Habitat deterioration, destruction, and fragmentation have played a significant role in the decline of the Mediterranean monk seal subpopulation in the eastern Mediterranean (Karamanlidis et al., 2019). These threats have also caused collateral damage: once Mediterranean monk seals lived on open beaches, but nowadays they are forced to occupy inaccessible marine caves to find refuge from human persecution. Low pup survival rates have been related to the occupation of such habitat: waves that surge into the caves cause the newborn pups to be at risk of drowning or starving by being separated from their mothers (Karamanlidis et al., 2016).

As a consequence of the substantial decline in Mediterranean monk seals and fragmentation of their habitat due to anthropogenic threats, all remaining populations of Mediterranean monk seals show high levels of genetic drift and inbreeding (A. A. Karamanlidis et al., 2021; Salmona et al., 2022). Genetic drift and inbreeding lower the effective population size, which means that the populations are more prone to decreases in fitness and evolutionary potential, putting the Mediterranean monk seal at increased risk of extinction (A. A. Karamanlidis et al., 2021; Ryman et al., 2019). Therefore, conservation measures are required to increase effective population size, in order to improve the long-term prospect of survival for the Mediterranean monk seal (A. A. Karamanlidis et al., 2021).

Current challenges for conservation

The status of the Mediterranean monk seal in the eastern Mediterranean region remains compromised, despite encouraging signs of recent recovery of the subpopulation (Karamanlidis et al., 2021). In the eastern Mediterranean, the Mediterranean monk is legally protected by enforcement of numerous laws, regional and international treaties and European Union (EU) regulations (Karamanlidis et al., 2019). In both Greece and Turkey, legislative measures and research, management, and conservation actions to protect Mediterranean monk seals are implemented in several areas, like the National Marine Park of Alonissos and the Northern Sporades Island in Greece, and the Cilician coasts and Bodrum Peninsula in Turkey (Karamanlidis et al., 2019).

Conservation surveys, such as monitoring the subpopulations at large spatial scales and identifying habitats of high value to species of conservation concern, can benefit greatly from the presence/absence information (Beng & Corlett, 2020). However, the current distribution of the Mediterranean monk seal is still only partially defined. Due to the rarity and the elusive nature of the Mediterranean monk seal, the demographics and conservation status of this species are logistically challenging to study (Karamanlidis et al., 2021; Valsecchi et al., 2022). Conventional techniques make detection and monitoring of rare, cryptic, and endangered species a difficult task that takes a substantial amount of time, effort and money (Beng & Corlett, 2020; Suarez-Bregua et al., 2022). A more effective conservation planning could be in action, by filling the current knowledge gaps of Mediterranean monk seal distribution (Valsecchi et al., 2022). Therefore, conservation actions would benefit greatly by an ecological survey that makes more accurate predictions about the distribution of this hard-to-study seal in the region of the eastern Mediterranean (Karamanlidis et al., 2021).

Current monitoring methods and their limitations

For the Mediterranean monk seals, current knowledge about distribution and abundance relies on data that was obtained by visual methods (Valsecchi et al., 2022). The available data comprises opportunistic, mostly coastal sightings, which are sporadic and in some cases unverified (Valsecchi et al., 2022). Apart from these sightings, camera trapping in marine caves is another visual method of monitoring the species (Székely et al., 2022; Valsecchi et al., 2022). The camera traps are placed in sites where adult females are known to return every year to reproduce (Beton et al., 2021; Valsecchi et al., 2022).

According to Hinds (1984), successful ecological monitoring programs must be ecologically relevant, statistically credible, and cost-effective (Peltier et al., 2012). The latter criterion entails justification of the costs, by the benefits of information that the monitoring program obtains (Caughlan, 2001). According to these criteria, the methods that are currently in use for monitoring marine mammal species do not suffice to be a successful conservation strategy. Limitations of visual methods include the boat-based or aerial surveys which demands a lot of time, money and expertise (Suarez-Bregua et al., 2022). In addition, outcomes of these surveys may be unreliable due to the wide dispersal and sub-surface activities of marine mammal species. Moreover, detection bias should be taken into account, restricting visual methods for monitoring to daylight hours and good weather conditions. In case of the Mediterranean monk seal specifically, additional limitations of visual methods for monitoring include the elusive nature of the species and their extended home range: they can travel dozens of kilometres per day (Valsecchi et al., 2022). Visual methods have limited the current knowledge of Mediterranean monk seal distribution observations along the shore, leaving knowledge gaps in their distribution in hard-to-study offshore waters (Valsecchi et al., 2022).

In addition to visual monitoring, passive acoustic monitoring (PAM) has become a feasible method for investigating temporal and spatial distributions of marine mammals (Frouin-Mouy et al., 2016). Passive acoustic monitoring can possibly overcome some of the hurdles involved in visual monitoring, like low population numbers, secretive behavior and inaccessible habitat (Charrier et al., 2023). For this technology, autonomous underwater recorders document vocalizations that are useful in management applications over all spatial scales (Van Parijs et al., 2009). Passive acoustic technologies can track, localize and track vocalizing fish and marine mammals by making use of their acoustic communication (Van Parijs et al., 2009). For Mediterranean monk seals, the first study of the underwater vocal repertoire was only performed recently. This research, performed at northern Evia Island in Greece, aimed to find the underwater vocal repertoire of Mediterranean monk seals, in order to develop new monitoring methods for effective conservation (Charrier et al., 2023). The results from this study could be used to set up acoustic monitoring schemes for the Mediterranean monk seal. Although acoustic methods look promising for monitoring

Mediterranean monk seals, PAM is still under development and major hardware and software hurdles need to be overcome. Moreover, PAM is only reliable when the data is taken into the right context of a species' acoustic behavioural ecology and regional and seasonal variation is taken into account (Frouin-Mouy et al., 2016; Van Parijs et al., 2009). Therefore, long-term, acoustic monitoring schemes for Mediterranean monk seals are needed for effective conservation planning. Due to the infancy of this technique these schemes are not available yet (Van Parijs et al., 2009).

Environmental DNA technology

In recent years, a new and rapidly developing tool for long-term monitoring programs has emerged: eDNA technology (Beng & Corlett, 2020; Suarez-Bregua et al., 2022). Environmental DNA or eDNA is genetic material present in environmental samples. eDNA can come from skin, hair, mucous, saliva, sperm, secretions, eggs, feces, urine, blood, roots, leaves, fruit, pollen, rotting bodies of larger organisms and micro-organisms (Ruppert et al., 2019). Depending on the environment, eDNA can persist from hours, up to hundreds or thousands of years (Beng & Corlett, 2020). eDNA samples can be taken from water, soil or sediment. In case of aquatic samples, eDNA can be extracted by means of filtration, precipitation or centrifugation (Suarez-Bregua et al., 2022). After sample collection, specific targeted DNA sequences are amplified by Polymerase Chain Reaction (PCR) (Suarez-Bregua et al., 2022). PCR exponentially amplifies targeted fragments of DNA by enzymatic activity (Wages, 2005). PCR uses specific synthetic oligonucleotides (primers) for DNA amplification that detect the presence of a single species of multiple species at the same time (Valsecchi et al., 2022). Usually, primers are designed to amplify segments of mitochondrial DNA (mtDNA), because of the high copy number of mtDNA in eukaryotic cells and the availability of reference databases for this type of DNA (Liu & Zhang, 2021; Suarez-Bregua et al., 2022). The primers often amplify short mtDNA fragments (<300 basepairs) from the control region (D-loop), 12s rRNA, 16s rRNA, or cytochrome b (cyt b) (Suarez-Bregua et al., 2022).

The first use of eDNA as a survey tool in macro-ecology was as recent as 2008, but several different applications of this technique are already in use: indicating presence of invasive species, assessing changes in taxa assemblages over time, and tracing ecologically important species (Beng & Corlett, 2020; Closek et al., 2019). Two major approaches of eDNA are barcoding and metabarcoding (Fig. 2) (Beng & Corlett, 2020). The main difference between these two techniques is the type of primer that is used for detection: eDNA barcoding uses species-specific primers to detect DNA fragments of a single species, while metabarcoding uses universal primers to detect millions of DNA fragments from a range of species at the same time (Beng & Corlett, 2020; Suarez-Bregua et al., 2022). In addition, the type of assay used for the eDNA after extraction differs between the two approaches: eDNA barcoding uses quantitative PCR (qPRC) and, more recently, digital PCR (dPCR) for detecting a species' presence or absence and for quantifying the relative abundance of DNA sequences (as a measure of relative species abundance or biomass) (Beng & Corlett, 2020; Eble et al., 2020). In case of eDNA metabarcoding, PCR is followed by next-generation sequencing (NGS) (Beng & Corlett, 2020). NGS eDNA analysis has the ability to simultaneously determine the presence or absence of large numbers of taxa (Eble et al., 2020). DNA barcoding and metabarcoding both have their own applications in addressing current ecological and conservation questions. eDNA barcoding has been proven useful for detecting invasive, rare and cryptic species and possibly estimating their abundance (Beng & Corlett, 2020; Eble et al., 2020). By making use of this approach of eDNA, the presence of a species could even be detected in areas that are hard to access and this information has been used to map species distributions and design a management strategy (Beng & Corlett, 2020; Reinhardt et al., 2019). In contrast to barcoding, eDNA metabarcoding focuses on a range of species at the same time. This approach has successfully unravelled past and present biodiversity patterns, trophic interactions and dietary preferences, spawning ecology of elusive species and ecosystem health and dynamics (Beng & Corlett, 2020; Closek et al., 2019).

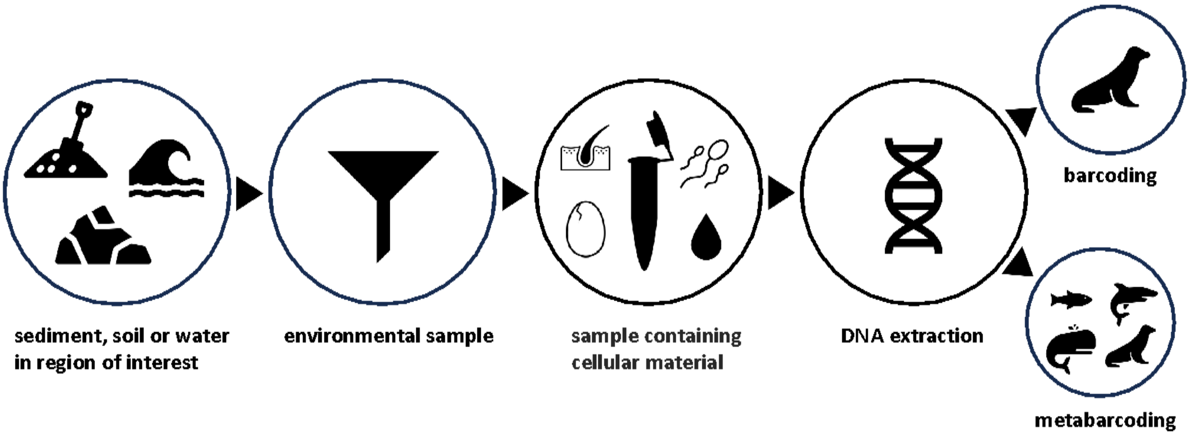

Fig. 2. A schematic overview of the overall workflow for two main approaches of environmental DNA: barcoding and metabarcoding.

Applications of eDNA in marine vertebrates

Some of the major hurdles of traditional survey methods can be overcome by using eDNA as a technique of detecting rare, cryptic, and endangered species (Beng & Corlett, 2020). Being relatively simple, fast and cost-effective, eDNA seems a promising tool for population genetic monitoring of marine mammals (Székely et al., 2021). As opposed to visual monitoring, eDNA analysis does not call for experienced observers and is not restricted by time of day or weather conditions (Székely et al., 2021). eDNA biomonitoring circumvents the need to directly sample or even sight living organisms completely and therefore offers a tool that is both non-invasive and suitable to detect rare or elusive species (Székely et al., 2021; Valsecchi et al., 2022).

For the Mediterranean monk seal, the first example of detection based on seawater eDNA analysis was published in a study by Valsecchi et al. (2022). In this study, three species-specific qPCR-assays targeting the 12S/16S rRNA were developed. The efficiency of this approach was tested on eDNA samples from the Tyrrhenian Sea and the Strait of Sicily: areas where presence of Mediterranean monk seals cannot be ruled out (Valsecchi et al., 2022). The results show that the qPCR-assays successfully detect small amounts of Mediterranean monk seal eDNA (e.g., 5.7×10^{-8} mg/L), while negative controls for tissue and eDNA samples both produced no signals in all qPCR runs. In addition, the presence of Mediterranean monk seal DNA was verified in 50% of opportunistic eDNA samples of Mediterranean monk seals (from previous and ongoing studies) after screening. This confirms that this assay can detect the presence/absence of Mediterranean monk seals without confirmation by visual sightings. The approach that was developed by Valsecchi et al. is the first to offer the potential to unravel previously unknown aspects of habitat use. It was found that eDNA monitoring could possibly detect the presence of Mediterranean monk seals in contexts that go beyond the scope of traditional monitoring techniques, like offshore waters and during night-time. By obtaining this new information on spatial distribution of Mediterranean monk seals, potential areas for conservation targets can be defined.

In addition, previous publications have shown that eDNA barcoding can provide important information about the presence/absence of specific marine vertebrate species other than the Mediterranean monk seal. For example, a genus-specific primer was designed to detect the presence of three species of the cryptic, low density and logistically difficult-to-study manatee (Hunter et al., 2018). Results of this study found that detection estimates were higher than those based on aerial survey data from the west coast of Florida, an area containing a large manatee population. The DNA assay that was described in this study made it possible to detect the presence of manatees from as few as 3 copies μl^{-1} of genetic material. In addition, this assay appeared to obtain results that are specific to the target species and did not cross-react with other species, offering possibilities for further use in population monitoring. Another study has investigated the utility of eDNA to determine the presence of the rare and endangered Maugean skate (*Zearaja maugeana*) (Weltz et al., 2017). This study made use of a species-specific primer to detect eDNA of the species in the wild. By using eDNA amplification, the presence of the Maugean skate in Macquarie Harbour, Tasmania, was confirmed. Results from these previous studies look promising for determining presence of marine mammals by using eDNA barcoding, especially in comparison to traditional surveys, like fishing and visual monitoring (Hunter et al., 2018; Weltz et al., 2017).

Discussion

Monitoring programmes are crucial for assessing population status and anthropogenic threats to populations of marine mammals, like the Mediterranean monk seal (Suarez-Bregua et al., 2022). By looking at the population structure (i.e., demographic structure and genetic structure) and diversity of the Mediterranean monk seal over time, an assessment can be made about the possible scenarios of local extinction together with genetic drift and inbreeding in extant populations of Mediterranean monk seals (Salmona et al., 2022; Slatkin, 1994). Therefore, monitoring the species is important to unravel the population structure in order to make recommendations for evidence-based conservation strategies (Karamanlidis et al., 2021; Salmona et al., 2022). Distribution, but also abundance, are essential parameters for population monitoring, providing information about the population structure (Suarez-Bregua et al., 2022). Despite the significance of long-term biomonitoring programs for marine mammal species that are in need of conservation measures, several factors often limit the collection of reliable data over long time periods (Suarez-Bregua et al., 2022). That is to say, visual and acoustic detections that are traditionally used for monitoring, are costly, laborious and can be ineffective for detecting rare, cryptic and elusive marine mammals (Suarez-Bregua et al., 2022; Székely et al., 2022). On the other hand, the power of using eDNA for biomonitoring has been demonstrated by the ease of sampling and the power to detect otherwise cryptic species (Székely et al., 2022).

Marine ecosystems are great reservoirs of eDNA: the high density of marine biota allows for a high availability of naturally shed cellular material (Díaz-Ferguson & Moyer, 2014; Suarez-Bregua et al., 2022). However, current limitations of using eDNA for testing presence involve the relatively very low concentrations of eDNA from marine mammals, in comparison to the dominating taxonomic groups in marine ecosystems (Székely et al., 2022). Despite the high sensitivity of eDNA assays, low eDNA concentrations decrease the probability for accurate detection of the targeted species, possibly leaving room for false-positive and false-negative results (Mauvisseau et al., 2017; Suarez-Bregua et al., 2022). False positive results occur when eDNA of the species is detected in a sample, while the species itself is not present. False negative results occur when eDNA analysis fails to detect the presence of the target species, while the species itself is present on the site where the sample was taken (Mauvisseau et al., 2017). Because concentrations of marine mammals in eDNA samples can be low, stochasticity in sampling, laboratory processing and data analysis decrease the reliability of correct detection from a single seawater sample (Székely et al., 2022). Analysis of data that represented all DNA from a bowhead whale (*Balaena mysticetus*) footprint illustrates the low probability of detecting marine mammals in an eDNA sample: only 1-2% of the DNA sequences matched the DNA of a bowhead whale, while 98-99% matched DNA sequences from bacteria and phytoplankton (Székely et al., 2021). To increase the probability of correctly detecting the presence of the target species and decrease stochasticity, the volume of seawater filtered per sample and the number of replicates can be increased (Székely et al., 2022). This solution was also proposed in the previously mentioned studies on manatee and skate species: for future research, it was recommended that sample sizes are increased and a larger volume is sampled to prevent these errors (Hunter et al., 2018; Weltz et al., 2017). Apart from low amounts of eDNA being shed into the environment by individual animals, eDNA concentrations of the manatee species and the Maugean skate in seawater are also expected to be low due their compromised conservation status (Hunter et al., 2018; Weltz et al., 2017). This adds another argument in favour of sampling larger volumes and increasing sample size, to decrease the chance of false positives and negatives.

Analysis of eDNA samples to collect information about a species' presence has to take into account the rate by which DNA is released and degraded by biotic and abiotic factors, to prevent errors in detection (Díaz-Ferguson & Moyer, 2014; Székely et al., 2022). Sea water occupies a great volume in comparison to biomass and marine environments are dynamic in nature (e.g., tides, current systems and oceanographic events). Consequently, eDNA from marine samples may be diluted, dispersed and hard to process due to the high salinity environment. Salinity, along with factors like temperature, pH, UV and microbial activity, increase degradation of eDNA, at a rate that is currently not completely understood (Rees et al., 2014; Székely et al., 2021). Although eDNA in samples from temperate regions degrade less quickly than in samples from tropical areas, it has been found that eDNA from all marine environments degrades rapidly (less than seven days) (Díaz-Ferguson & Moyer, 2014). In comparison, eDNA persistence in freshwater lentic systems can be as long as 30 days (Díaz-Ferguson & Moyer, 2014). Apart from variation in eDNA shedding, dispersion and decay due to environmental factors, these processes also differ between habitat, seasons and taxonomic groups, increasing the chance of unjust interpretation of presence/absence information of a target species (Díaz-Ferguson & Moyer, 2014; Székely et al., 2022). Therefore, presence/absence information on its own can still be misleading: eDNA can be present in the environment in absence of the target species, or the target species can be present, while eDNA is not found (Beng & Corlett, 2020). Therefore, abundance data allows for more robust assessments of the factors that affect populations, since it provides more information on the status of the population (Beng & Corlett, 2020).

Apart from detecting the presence of rare and elusive species, quantification of eDNA in the sea has been used to provide an indication of biomass in marine ecosystems (Foote et al., 2012). Estimating species abundance by using eDNA is yet to be tested thoroughly for marine mammals (Székely et al., 2022). However, some studies have found that the concentration of eDNA can be used to estimate the biomass of a target species. A study in Maizuru bay in Japan monitored Japanese Jack Mackerel (*Trachurus japonicus*) biomass using echo sounder technology and compared these findings with eDNA concentrations from surface water samples (Yamamoto et al., 2016). A significant partial association between spatial variation in estimated eDNA concentration and echo intensity were found for the Japanese Jack Mackerel, suggesting that the concentration of eDNA reflects the fish distribution and biomass across west Maizuru Bay. Likewise, a study on the common Octopus revealed a significant positive correlation between the species' total biomass and the total amount of eDNA that was detected by using species-specific primers (Mauvisseau et al., 2017). This study aimed at detecting the presence and abundance of the common octopus (*Octopus vulgaris*) by using eDNA samples taken from aquaria and a study area in the Cantabrian Sea. For the aquarium experiment, 2 tanks were filled with different numbers of octopus and different water volumes. The results of this experiment showed a significant positive correlation between the total biomass (g of octopus inside the tank) and the amount of the species' eDNA detected. In this study, better quantifications of common octopus eDNA were obtained in samples from the tank experiments than in sea samples, possibly attributable to the differences between a controlled and a non-controlled environment (Mauvisseau et al., 2017). The water samples taken from the Cantabrian Sea showed significant variations in the amounts of detected eDNA, possibly correlating with differences in common octopus biomass. However, variation in the detected amounts of the species' eDNA from sea samples may also be attributed to migration to greater depths, dilution and several other unknown factors. The studies on the Japanese Jack Mackerel and the common octopus both conclude that eDNA analysis could potentially be a quick and cheap tool for assessing abundance of a targeted species (Mauvisseau et al., 2017; Yamamoto et al., 2016).

Although some studies show promising results for using eDNA for estimating biomass, other studies question the extent to which eDNA concentration can represent species abundance (Beng & Corlett, 2020; Knudsen et al., 2019; Weltz et al., 2017). One example is the previously mentioned study on the Maugean skate (Weltz et al., 2017). In this study, an assay was designed to quantify the amount of eDNA concentration detected in the water samples, in order to estimate the absolute abundance of the species in Macquarie Harbour. The unlikeliness of eDNA concentration being a good proxy for species abundance was attributed to the lack of information about the age of the DNA in the sample. In addition, possibly incorrect assumptions could have been made about the number of individuals from which the DNA originates and the amount of eDNA that is shed by one individual (Weltz et al., 2017). Another study by Knudsen et al. (2019) found that concentration of eDNA from several fish species in the Baltic Sea correlated with their known distribution and abundance, but not with their biomass estimates from concurrent trawl surveys. These results are not surprising, since trawling represents a real-time picture of the fish distribution, while eDNA can only be used to make implications about the amount of shedding during the past days/weeks (Knudsen et al., 2019). As was the case for the Maugean skate, the skew in fish density and corresponding species-specific eDNA concentrations could be due to eDNA shedding and degradation rates or could possibly be attributed to the process of collecting and analysing eDNA samples (e.g., stochasticity in sampling method, DNA extraction method, PCR reactions and primer chemistry) (Knudsen et al., 2019; Weltz et al., 2017).

The four aforementioned studies that used eDNA as an indicator for biomass show mixed results: two of them found a significant positive correlation between the targeted species' biomass and eDNA concentration (Mauvisseau et al., 2017; Yamamoto et al., 2016). However, for the common octopus, the correlation was found in tank experiments. The studies that did not find a significant positive correlation between biomass estimates and eDNA concentrations, were performed in uncontrolled environments (Knudsen et al., 2019; Weltz et al., 2017). Hence, this supports the reasoning that dynamics of eDNA due to environmental conditions in uncontrolled environments could confound biomass estimates. Therefore, the four studies that have either successfully or unsuccessfully estimated biomass by using eDNA all suggest that more research on the dynamics of eDNA under environmental conditions (e.g., patterns of release, degradation, and diffusion of eDNA) is needed, in order to use eDNA quantification to estimate biomass (Knudsen et al., 2019; Mauvisseau et al., 2017; Weltz et al., 2017; Yamamoto et al., 2016).

Conclusions and future directions

Monitoring Mediterranean monk seals is challenging due to their rarity and elusive nature, leaving many gaps in our understanding of the distribution of this threatened species (Valsecchi et al., 2022). Molecular detection of Mediterranean monk seals through eDNA analysis could be an advancement in the traditional monitoring methods: eDNA monitoring is cheaper than visual monitoring and offers a non-invasive approach (Beng & Corlett, 2020; Suarez-Bregua et al., 2022). Studies that have evaluated the methodological efficiency of eDNA surveys in detecting rare, cryptic, and endangered species, have demonstrated that eDNA has a higher or comparable ability to detect a target species (Beng & Corlett, 2020). The first eDNA-based assay that aimed to detect the presence of Mediterranean monk seals was successful, in both coastal and offshore samples (Valsecchi et al., 2022). These results look promising for further use in defining the Mediterranean monk seals' actual distribution and home range. Unlike visual monitoring techniques, eDNA monitoring is not limited by time-of-day, weather conditions, experience of observers, and accessibility of possible habitat. This even offers the possibility to unravel previously unstudied aspects of the Mediterranean monk seal and other marine mammals (Juhel et al., 2021; Valsecchi et al., 2022).

Despite limitations of eDNA methods that are yet to resolve, results from previous studies have shown that eDNA analysis offers the opportunity to detect marine mammals in areas where they were not yet or poorly reported in previous visual surveys (Suarez-Bregua et al., 2022). According to Díaz-Ferguson & Moyer (2014), the next steps for improving eDNA as a method for species detection will be revolving eDNA methodologic issues, improving eDNA technologies, and exploring new eDNA applications. Marine mammal eDNA studies must be carefully designed to consider challenges in eDNA collection, detection, analysis and interpretation (Székely et al., 2022). To optimize sampling methods and support interpretation of eDNA detections and non-detections, technical advances and development of guidelines and protocols are needed (Székely et al., 2022). The number of eDNA studies has substantially increased in the last decade and given the potential for marine mammal monitoring programs, this trend is likely to continue (Székely et al., 2022). To optimize eDNA techniques, future studies could focus on exploring eDNA collection methods and striving for validation and standardization of sample collection, data generation and interpretation protocols (Székely et al., 2022). In order to incorporate eDNA techniques into monitoring programs, these aspects are vital for the generation of reliable and comparable data (Jerde, 2021; Székely et al., 2022).

While eDNA techniques are under development, eDNA could be used as a complementary method, along with traditional monitoring methods, like visual or acoustic surveys (Beng & Corlett, 2020; Székely et al., 2021). Since eDNA and traditional survey methods can give such different information, they can be used in combination, instead regarding them as alternative methods. It is often the case that eDNA surveys require additional information from traditional surveys (Beng & Corlett, 2020). Since visual monitoring involves inherent difficulties for the Mediterranean monk seal (i.e., low population numbers, secretive behaviour, inaccessible habitat) and acoustic monitoring schemes for the species are still under development, these methods could be paired with eDNA monitoring (Charrier et al., 2023; Suarez-Bregua et al., 2022). Data obtained by eDNA analysis can guide these traditional surveys in the right direction, for example by using eDNA to validate sporadic sightings of Mediterranean monk seals (Valsecchi et al., 2022). More profound sampling in waters where encounters with the species were made, provides the opportunity to verify whether sightings are accidental or indicate an expansion of the distributional range. Another way of combining eDNA analysis and visual monitoring is by using eDNA to identify appropriate conditions to position camera traps (Valsecchi et al., 2022). Due to the elusive nature of Mediterranean monk seals, the species are known to occupy difficult-to-study sites, like marine caves (Karamanlidis et al., 2019). Camera traps are useful to observe Mediterranean monk seal presence and their behavior, but extremely expensive to place in locations where the seal has not already been documented. Complementing traditional monitoring tools by using eDNA analysis contributes to achieving improved monitoring programs for assessment and conservation of marine mammals, like the Mediterranean monk seals (Suarez-Bregua et al., 2022; Valsecchi et al., 2022).

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