

NEEDLES IN AN OCEAN HAYSTACK: USING ENVIRONMENTAL DNA TO STUDY MARINE MAMMALS IN THE NORTH ATLANTIC

Dóra Székely¹, Kristina M. Cammen², Morten Tange Olsen¹

¹ *Globe Institute, University of Copenhagen, Copenhagen, Denmark. Corresponding author: dora.szekely@sund.ku.dk*

² *School of Marine Sciences, University of Maine, Orono, United States*

ABSTRACT

Marine mammals in the North Atlantic have experienced severe depletions due to overexploitation. While some species and populations have now recovered, there are numerous other anthropogenic activities impacting their North Atlantic ecosystem. Studying marine mammals in their natural habitat is often associated with logistical challenges, and many species have an elusive nature, resulting in substantial knowledge gaps on the distribution, abundance and diversity of marine mammals in the North Atlantic. Environmental DNA (eDNA) is an emerging tool in biodiversity monitoring that benefit from advances in molecular methods to extract, detect and/or sequence the genetic material of marine organisms from a seawater sample. The ease of sampling and ability to detect otherwise cryptic species demonstrates the power of eDNA to complement traditional monitoring methods for a wide range of marine taxonomic groups. We present a literature review of eDNA studies of marine mammals and discuss the potential applications and practical challenges of using eDNA in marine mammal research, management and conservation in the North Atlantic. Environmental DNA has already been introduced to a wide range of applications within marine mammal science, from detection of endangered species to population genetic assessments. Furthermore, eDNA has the power to capture other biologically important species in the marine ecosystem and food web, which could facilitate insight into the spatiotemporal variation of different marine communities in a changing environment. With methodological and technological standardization, eDNA based approaches have a promising potential to be integrated into regular monitoring practices and management strategies.

Keywords: eDNA, cetacean, pinniped, biodiversity monitoring, ecosystem, population genetics, conservation, exploitation

INTRODUCTION

Following centuries of overexploitation of marine mammals (Olsen & Galatius, 2018; Tønnessen & Johnsen, 1982), the gradual implementation of monitoring, management programmes and hunting quotas has resulted in the recovery of many (but not all) species and populations (Roman et al., 2013). However, the ecosystems inhabited by North Atlantic marine mammals are facing new threats. On a global scale, some of the largest cumulative environmental impacts due to global warming are predicted in the North Atlantic (Albouy et al., 2020; Ramírez et al., 2017) and several marine mammal species are consequently undergoing range shifts (Chambault et al., 2018, 2020; Insley et al., 2021). In addition, human activities are heavily impacting the North Atlantic (Halpern et al., 2017) with many species affected by ocean noise (Haver et al., 2017), increased shipping activities (Hauser et al., 2018), incidental bycatch (Reeves et al., 2013), ship strike (van der Hoop et al., 2012), and high contaminant loads (Desforges et al., 2018; Dietz et al., 2019). Some species (e.g., narwhal) are still hunted at unsustainable levels (NAMMCO-North Atlantic Marine Mammal Commission, 2021).

The North Atlantic Marine Mammal Commission (NAMMCO) considers 23 marine mammal species (i.e., 10 toothed whales, six baleen whales, six phocids and the walrus) as permanent

residents in the NAMMCO management area (NMA) (Table 1). In addition, a few marine mammal species are classified as visitors or rare residents, and there are multiple species occurring in the temperate waters of the North Atlantic that are currently considered to have a range outside of the NMA. These marine mammal species could become more regular visitors or permanent residents as climate change drives species ranges northward. Together, the North Atlantic marine mammal species represent a large diversity in terms of abundance (hundreds to millions), distribution (Arctic, subarctic, temperate), habitats (coastal, shelf, oceanic), behaviours (migratory, stationary), prey (microfauna, cephalopods, fish, mammals) and sociality (solitary, pods). This diversity also includes levels of past and present exploitation, vulnerability to current and future human impacts and environmental change, and hence conservation needs (Albouy et al., 2020; Hauser et al., 2018).

Assessing the diversity, distribution and abundance of marine mammals is crucial for understanding species- and ecosystem-level dynamics, and for informing management and conservation strategies. The most common scientific methods for such assessments include sighting surveys (Hammond et al., 2013), acoustic monitoring (Kyhn et al., 2012) and mark-

Table 1. North Atlantic marine mammals, their presence in the NAMMCO Management Area (NMA), conservation status and genetic resources. The status of the species on the IUCN Red list refers to the global population, unless assessments are available for populations in the North Atlantic (asterisk)

	Species	NMA	IUCN	Mitogenome
MYSTICETES	Common minke whale (<i>Balaenoptera acutorostrata</i>)	yes	LC	NC_005271.1
	Humpback whale (<i>Megaptera novaeangliae</i>)	yes	LC	NC_006927.1
	Fin whale (<i>Balaenoptera physalus</i>)	yes	VU	NC_001321.1
	Sei whale (<i>Balaenoptera borealis</i>)	yes	EN	NC_006929.1
	Blue whale (<i>Balaenoptera musculus</i>)	yes	EN	NC_001601.1
	North Atlantic right whale (<i>Eubalaena glacialis</i>)	no	CR	NC_037444.1
	Bowhead whale (<i>Balaena mysticetus</i>)	yes	EN*	NC_005268.1
	Bryde's whale (<i>Balaenoptera brydei</i>)	no	DD	NC_006928.1
ODONTOCETES	Narwhal (<i>Monodon monoceros</i>)	yes	LC	NC_005279.1
	Beluga (<i>Delphinapterus leucas</i>)	yes	LC	NC_034236.1
	Harbour porpoise (<i>Phocoena phocoena</i>)	yes	VU*	NC_005280.1
	Sperm whale (<i>Physeter macrocephalus</i>)	yes	VU*	NC_002503.2
	False killer whale (<i>Pseudorca crassidens</i>)	no	NT	NC_019577.1
	Pygmy killer whale (<i>Feresa attenuata</i>)	no	LC	NC_019588.1
	Killer whale (<i>Orcinus orca</i>)	yes	DD	NC_064558.1
	Short-finned pilot whale (<i>Globicephala macrorhynchus</i>)	no	LC	NC_019578.2
	Long finned pilot whale (<i>Globicephala melas</i>)	yes	LC	NC_019441.1
	Northern bottlenose whale (<i>Hyperoodon ampullatus</i>)	yes	NT	NC_005273.1
	Common bottlenose dolphin (<i>Tursiops truncatus</i>)	yes	LC	NC_012059.1
	White-beaked dolphin (<i>Lagenorhynchus albirostris</i>)	yes	LC	NC_005278.1
	Atlantic white-sided dolphin (<i>Lagenorhynchus acutus</i>)	yes	LC	NC_050265.1
	Atlantic spotted dolphin (<i>Stenella frontalis</i>)	no	LC	NC_060612.1
	Rough-toothed dolphin (<i>Steno bredanensis</i>)	no	LC	NC_042761.1
	Short-beaked common dolphin (<i>Delphinus delphis</i>)	no	LC	NC_036415.1
	Striped dolphin (<i>Stenella coeruleoalba</i>)	rare/visitor	LC	NC_012053.1
	Risso's dolphin (<i>Grampus griseus</i>)	rare/visitor	LC	NC_012062.1
	Cuvier's beaked whale (<i>Ziphius cavirostris</i>)	rare/visitor	DD	NC_021435.1
	Sowerby's beaked whale (<i>Mesoplodon bidens</i>)	rare/visitor	LC	NC_042218.1
True's beaked whale (<i>Mesoplodon mirus</i>)	rare/visitor	LC	NC_042217.1	
Blainville's beaked whale (<i>Mesoplodon densirostris</i>)	rare/visitor	LC	NC_021974.2	
Gervais's beaked whale (<i>Mesoplodon europaeus</i>)	no	LC	NC_021434.2	
PINNIPEDS	Mediterranean monk seal (<i>Monachus monachus</i>)	no	EN	NC_044972.1
	Harbour seal (<i>Phoca vitulina</i>)	yes	LC	NC_001325.1
	Grey seal (<i>Halichoerus grypus</i>)	yes	LC	NC_001602.1
	Harp seal (<i>Pagophilus groenlandicus</i>)	yes	LC	NC_008429.1
	Bearded seal (<i>Erignathus barbatus</i>)	yes	LC	NC_008426.1
	Hooded seal (<i>Cystophora cristata</i>)	yes	VU	NC_008427.1
	Ringed seal (<i>Pusa hispida</i>)	yes	LC	NC_008433.1
	Atlantic walrus (<i>Odobenus rosmarus ssp. rosmarus</i>)	yes	NT	NC_004029.2
	Polar bear (<i>Ursus maritimus</i>)	no	VU	NC_003428.1

Box 1. Seawater eDNA in a nutshell

Emerging approaches of marine biodiversity studies include the collection and processing of environmental DNA (eDNA) from seawater samples. Seawater samples are complex, containing genetic material from a wide variety of marine life in the form of both intact micro- and planktonic organisms (organismal eDNA), as well as shed cellular material from larger organisms such as marine mammals (extra-organismal eDNA) (Rodríguez-Ezpeleta et al., 2021). The origin of extra-organismal DNA in an environment is most typically sloughed epithelial cells from the skin, digestive tract, excretory system, or respiratory system. The persistence of eDNA in the marine environment is presumed to be the function of several biotic and abiotic factors that vary across habitat types and taxonomic groups, and affect DNA dispersal, dilution and degradation rates. Though it is generally acknowledged that eDNA has a relatively short persistence in the marine environment (hours to days), thus captures a snapshot of species presence, diversity and interactions. Despite its low quantity and quality in comparison to tissue based genetic material, molecular methods have been designed to sample, concentrate, and extract this eDNA from seawater. It has been demonstrated that these methods were proven successful for studying marine mammals, despite the fact that marine mammal eDNA is found in very low concentrations in the sea compared to other more abundant organisms (e.g., fish, invertebrates, zooplankton, phytoplankton). While there is no golden standard for the ideal sampling volume for robust detections, most studies have found that 1 litre of seawater is practically feasible and performs well in capturing the marine community at the time of sampling.

The majority of eDNA studies use either quantitative PCR (qPCR, or digital droplet PCR [ddPCR]) or metabarcoding for detection of target species. The quantitative PCR approach relies on the design of species-specific primers and probes that can be used to detect eDNA from a target species and quantify the amount of target-species DNA in a sample of known volume. Metabarcoding leverages primers that target highly conserved regions of the mitochondrial genome for taxon specificity but sufficient variation for species distinction (e.g., COI, 16S, 12S). These regions are amplified from multiple organisms for high-throughput sequencing and simultaneous identification of multiple taxa in a single environmental sample. Parallel identification of multiple species from different taxonomic groups and trophic levels could allow for characterizing biodiversity and community interactions through space and time (Djurhuus et al., 2020). In addition to detection-based studies using qPCR and metabarcoding, eDNA studies have explored the use of sequencing genetically more informative genetic markers for population genetic analyses of marine mammals (see Supplementary Table 1). While to date mitochondrial markers have traditionally been favoured in eDNA studies due to their higher copy number in the cell, some recent studies have also shown promise in sequencing nuclear eDNA (Andres et al., 2021; Reinholdt Jensen et al., 2020).

recapture (Palsbøll et al., 1997). While providing essential information for management and conservation, these methods are typically costly and laborious, weather dependent, require specialist expertise, may involve risks to human observers, can be invasive, and may result in large uncertainties in population estimates (Desportes et al., 2020). Moreover, inter- and intraspecific differences in distribution, abundance and biology of North Atlantic marine mammals imply that specific monitoring methods often are limited to certain species, geographical regions and seasons. As a result, some species are only surveyed at infrequent intervals e.g., every 5-10 years; and some not at all (Pike et al., 2009, 2019). Thus, a top priority for implementing national, regional and international strategies and goals for the conservation of North Atlantic marine mammals is to develop and integrate innovative, yet relatively simple, non-invasive and cost-effective methods for monitoring their diversity, distribution and abundance.

Environmental DNA, or eDNA, is an emerging tool in marine biodiversity assessments (Box 1). In a broad sense, eDNA-based approaches seek to characterize organisms in an environment through the analysis of the genetic material they leave behind. In this way, organisms can be detected and studied without ever directly sampling, or even seeing, the target study species. Since it was first deployed less than two decades ago for invasive species detection in freshwater systems (Ficetola et al., 2008) and for detecting marine mammals and fish in the marine environment (Foote et al., 2012; Thomsen et al., 2016), the use of eDNA for biodiversity assessments has grown rapidly (Ruppert et al., 2019).

AIM AND METHODS

Here we outline the potentials of seawater eDNA for monitoring North Atlantic marine mammals. First, we review the current

literature on marine mammal eDNA and describe its demonstrated use and future potential for species detections, population genetic analyses, inferring ecological interactions, and abundance estimation. Although our focus is on marine mammals of the North Atlantic, we include studies of marine mammal eDNA in other regions, as well as general eDNA literature when marine mammal studies are lacking. The literature was compiled through a search of Web of Science and Google Scholar databases with the search term “environmental DNA” or “eDNA” and marine mammal keywords (e.g., “cetacean”, “pinniped”, “dolphin”, “whale”, “manatee”, etc.). Drawing from this literature, and our personal experience, we then discuss practical challenges for using eDNA to study marine mammal populations. Our aim with this review is not to provide a best practice guide for field or laboratory work, but to offer insights on the current and future potential of eDNA for monitoring the distribution, diversity, interactions and abundance of North Atlantic marine mammals.

APPLICATIONS OF eDNA IN MARINE MAMMAL SCIENCE

Species detections

In the North Atlantic and elsewhere, the promise of eDNA approaches for detection of species, including rare and invasive species, has been validated in studies that range in focus from dinoflagellates (Drouet et al., 2021) and sessile marine invertebrates (Matejusova et al., 2021; Schill & Galbraith, 2019) to highly mobile pelagic species, including marine mammals (Gargan et al., 2017; Székely et al., 2021; Valsecchi et al., 2022). Across such studies, eDNA approaches have proven successful in a range of habitats, from the Arctic (Székely et al., 2021) to the tropics (Bakker et al., 2017; Hunter et al., 2018) and from the sea surface (Székely et al. 2021) to pelagic waters (Thomsen et al., 2016) and the deep sea (Brandt et al., 2021).

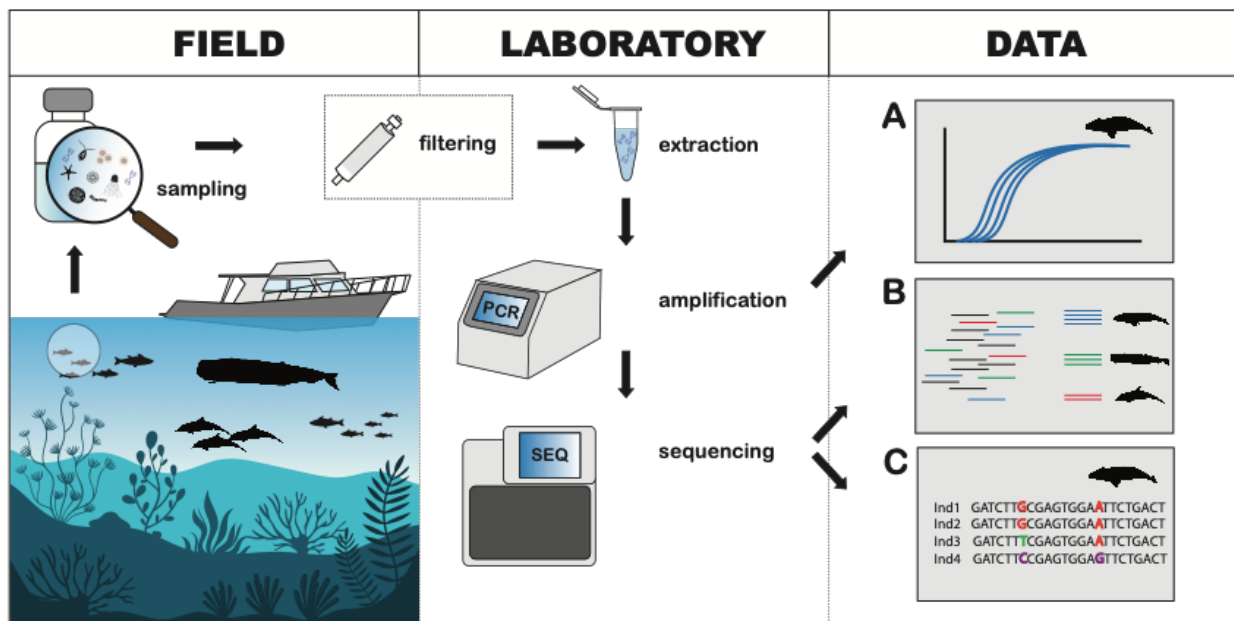


Figure 1. Schematic overview of the workflow associated with eDNA sampling, sample processing and data generation options. Samples either in the field or in a laboratory. The “DATA” panel explains the commonly used methods to study marine mammals with eDNA, including A) absolute quantification of the presence of DNA from target species with e.g. qPCR, B) metabarcoding for species detection and community inference, and C) the identification of SNPs and haplotype sequencing for population genetics.

Through spatio-temporal eDNA sampling, this approach can further be used to characterize species distributions and seasonal phenology (Djurhuus et al., 2020; Drouet et al., 2021; Liu et al., 2019; Sevellec et al., 2021; Stoeckle et al., 2021), with recognized challenges and limitations as discussed further below.

Marine mammal eDNA can be detected in seawater samples by species-specific assays (e.g., qPCR and ddPCR) (Figure 1A) and community metabarcoding (Figure 1B). To date, targeted species-specific assays have been designed and validated for several North Atlantic species, including the humpback whale (*Megaptera novaeangliae*), killer whale (*Orcinus orca*), harbour porpoise (*Phocoena phocoena*), Mediterranean monk seal (*Monachus monachus*) and bowhead whale (*Balaena mysticetus*) (Andruszkiewicz et al., 2020; Baker et al., 2018; Foote et al., 2012; Székely et al., 2021; Valsecchi et al., 2022) (Supplementary Table 1). In addition to these targeted approaches, marine mammal species have been detected in seawater eDNA samples analysed using primers that target a broader taxonomic group (e.g., vertebrates) as part of community metabarcoding studies. Metabarcoding allows for the amplification and identification of DNA fragments from multiple species at the same time and has been used to assess the biodiversity and community composition of different habitats (Bohmann et al., 2014; Closek et al., 2019). In such studies, species from almost all major marine mammal groups present in the North Atlantic have been detected, including rorqual whales, dolphins, porpoises, seals, and polar bears (Supplementary Table 1).

The power of eDNA for marine mammal detections has been assessed through direct comparison with conventional survey

methods, including visual and acoustic surveying approaches. Though such validation has not yet been published for the North Atlantic, other studies conducted across diverse global habitats report a strong concordance between eDNA and conventional methods (Closek et al., 2019; Tang et al., 2019; Valsecchi et al., 2021). Though species detections can be missed in all approaches, eDNA can in some cases facilitate finer taxonomic resolution of species identification than possible by visual survey, particularly for species that are visually hard to distinguish in the field, e.g., dwarf and pygmy sperm whale (Juhel et al., 2021), oceanic dolphin species, rorqual species, and pinnipeds (Closek et al., 2019). In understudied areas, eDNA surveys have also resulted in new species records, demonstrating the benefit of eDNA for monitoring species, habitats or regions where traditional visual and acoustic surveys are limited or unavailable (Madduppa et al., 2021). This benefit of eDNA may be particularly advantageous in regions of the North Atlantic that are challenging for conventional survey methods such as the Arctic or the mid-Atlantic.

In general, because different survey methods often produce distinct lists of species assemblages with a significant but not complete overlap, eDNA-based approaches may be most valuable when used to complement, rather than replace, conventional survey methods. For rare or elusive marine mammal species (e.g., beaked whale species), eDNA surveys may also be particularly useful for identifying new areas to target for research and conservation (Lozano Mojica & Caballero, 2021), or for detecting species that serve as sentinels or early indicators of climate and ecosystem shifts (Closek et al., 2019; Djurhuus et al., 2020)

Genetic diversity and differentiation

An interesting, more recent advance in eDNA science is the assessment of intraspecific genetic diversity for population genetic analyses, known as eDNA haplotyping or genetic profiling (Adams et al., 2019; Sigsgaard et al., 2016, Sigsgaard et al., 2020; Székely et al., 2021) (Figure 1C). Given the higher copy number of mitochondrial DNA compared to nuclear DNA, eDNA studies of intraspecific diversity typically target regions of the mitochondrial genome with high levels of variation (e.g., the mitochondrial D loop). The reliability of eDNA for obtaining population genetic data has thus far been validated for fish and shark species by the comparison of DNA sequences derived from eDNA samples versus concurrently collected tissue samples (Sigsgaard et al., 2016; Tsuji et al., 2020). In marine mammals, eDNA haplotyping approaches have been successfully employed in the North Atlantic, where Székely et al. (2021) captured the genetic diversity of bowhead whale individuals by sampling eDNA in their “footprint” (i.e., the turbulent surface water that marine mammals leave behind following their breathing and diving sequence). Similarly in the Northeast Pacific, seawater eDNA samples collected in the vicinity of killer whales and harbour porpoise have been sequenced to identify the individuals’ ecotypes (Baker et al., 2018) and to evaluate genetic differentiation within a management stock (Parsons et al., 2018).

An important strength of the eDNA approach for population genetic inference is the relatively non-invasive nature of seawater collection from the vicinity of an animal, as compared to the collection of a tissue biopsy. Population genetic analyses based on eDNA samples may be particularly valuable for marine

mammal species that are elusive and hard to sample using traditional biopsy approaches. However, as outlined below, the applications of seawater eDNA for population genetic assessments still face several challenges, including low DNA quantity and quality, as well as potential difficulties in disentangling individual genotypes in a mixed sample, e.g. collected from a pod of cetaceans.

Trophic interactions

Scaling further up from the focus on detection and genetic profiling on an individual, seawater eDNA also holds the potential to draw inferences about the role of marine mammals in their broader ecosystem. While understanding the role of all species in a food web is challenging, Djuurhus et al. (2020) successfully demonstrated that eDNA can capture seasonal changes in ecosystem composition and trophic networks, thereby potentially providing important information on e.g., marine mammal foraging behaviour, dietary preferences, energetics, inter- and intraspecific competition, presence of pathogens, and their sensitivity to environmental change and human activities. Assessments of trophic interactions by eDNA may also include concurrent collection of oceanographic data (e.g., salinity, temperature, chlorophyll A) for a more detailed understanding of the abiotic and biotic processes that drive spatiotemporal variation in marine ecosystem and community structure. Though few eDNA studies of marine mammal trophic ecology have been published so far, this is an area of eDNA science experiencing high interest and rapid development, with several ongoing studies in the North Atlantic and elsewhere.

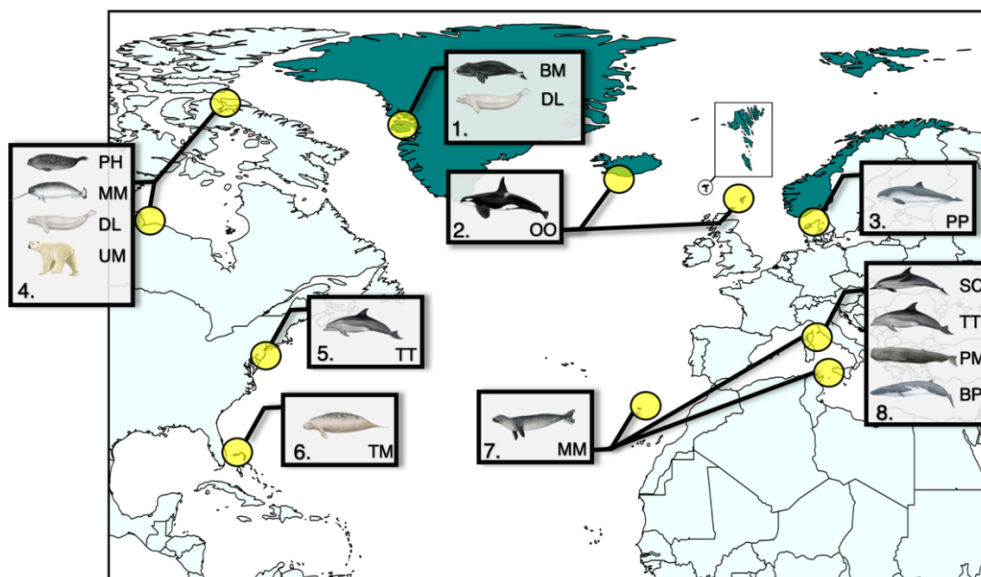


Figure 2. North Atlantic marine mammal species that have been studied/detected with eDNA. Countries shaded in dark green are members of NAMMCO. Yellow circles represent areas studied by: 1) Székely et al. 2021, 2) Pinfield et al. 2019, 3) Foote et al. 2012, 4) Sevellec et al. 2020, 5) Stoeckle et al. 2018, 6) Hunter et al. 2018, 7) Valsecchi et al. 2022, 8) Valsecchi et al. 2021. Detected species include the bowhead whale (BM), beluga (DL), killer whale (OO), harbour porpoise (PP), ringed seal (PH), narwhal (MM), polar bear (UM), bottlenose dolphin (TT), West Indian manatee (TM), Mediterranean monk seal (MM), striped dolphin (SC), sperm whale (PM), and fin whale (BP). All cetacean and pinniped illustrations are by courtesy of NOAA Fisheries, the polar bear by courtesy of Encyclopaedia Britannica, Inc., copyright 2007; used with permission and West Indian manatee by courtesy of Encyclopaedia Britannica, Inc., copyright 2003; used with permission.

Abundance estimation

Conventional marine mammal monitoring approaches typically aim to obtain abundance estimates that are used in stock assessment and management models. While yet to be tested thoroughly for marine mammals, eDNA studies have yielded mixed results when evaluating the reliability of eDNA for estimating species abundance on other marine vertebrate groups (primarily fish), (Knudsen et al., 2019; Rourke et al., 2021; Thomsen et al., 2016). For example, Knudsen et al. (2019) report that the measured concentration of eDNA from several fish species correlates with their known distribution and abundance in the Baltic Sea, but not with their biomass estimates from concurrent trawl surveys (i.e., catch per unit effort). In contrast, other studies have reported positive correlations between fish biomass inferred from trawl surveys and eDNA sampling (Salter et al., 2019; Thomsen et al., 2016). Metabarcoding studies also typically report a general concordance between the number of DNA sequence reads and biomass, though both field and laboratory factors can affect species detection and quantification (Afzali et al., 2021; Fraija-Fernández et al., 2020; Stoeckle et al., 2021).

The main obstacles associated with estimating abundance by eDNA is understanding the factors that affect organismal and environmental variation in eDNA shedding, dispersion and decay, and how to integrate such variation into statistical models in order to translate eDNA yield to real animal abundance or biomass (Tillotson et al., 2018). Thus, while eDNA without doubt will find uses in estimating marine mammal relative densities and mapping species hotspots, the knowledge generated so far from other taxonomic groups is insufficient to evaluate the potential of eDNA to quantify the absolute abundance of marine mammals. We anticipate that studies testing the power of eDNA to monitor marine mammal abundance will be carried out in the near future as this field progresses forward.

PRACTICAL CONSIDERATIONS OF STUDYING MARINE MAMMALS WITH eDNA

With its wide-ranging application, there is a lot of potential in employing eDNA-based approaches to the study of marine mammals in the North Atlantic and elsewhere (Figure 2). Yet, marine mammal eDNA studies must be carefully designed to consider challenges in eDNA collection, detection, analysis, and interpretation. Here, we highlight some of the main challenges for applications of eDNA in marine mammal science.

Acknowledging the uncertainty of eDNA persistence in seawater

The ability to obtain marine mammal DNA from a seawater sample depends on eDNA shedding, dispersion and degradation rates, which can vary between habitats, seasons and taxonomic groups (Andruszkiewicz Allan et al., 2021; Andruszkiewicz et al., 2017; Harrison et al., 2019). For instance, animal body size, metabolic rate, population density and behaviour appear to be important determinants (Rourke et al., 2021), but ocean currents, water temperature, depth and DNA-degrading microbiota can also affect eDNA persistence. Several studies have examined the persistence of seawater eDNA in various experimental setups, documenting its stochastic and highly context specific nature. In marine settings, DNA from different invertebrate and vertebrate taxonomic groups can be reliably

detected for the first 24-48 hours, but thereafter tend to decay at an exponential rate with very little if any DNA left after seven days (Collins et al., 2018; Holman et al., 2021; Moushomi et al., 2019; Skinner et al., 2020). In contrast, results from field studies indicate a shorter period of eDNA persistence. For instance, Székely et al. (2021) reported that the detectability of bowhead whale eDNA collected in a footprint of a diving whale was significantly reduced after 10 minutes, while Baker et al. (2018) reported successful detections of killer whale eDNA up to two hours after a pod travelled through an area. To fully understand the spatiotemporal snapshot of the marine ecosystem that is captured by a seawater eDNA sample more studies are needed that evaluate the persistence of marine mammal eDNA in the environment. While such studies are likely to reveal some generalities (e.g., lower persistence in warmer, faster moving waters), the field needs empirical data and guidance on how to efficiently design and implement studies that characterize eDNA persistence times for individual environmental contexts. Until such knowledge is available, we encourage researchers to carefully consider the factors affecting eDNA persistence and hence marine mammal species detectability in their specific study system.

Field sampling design - transect or footprint

There are two primary sampling strategies that currently are appropriate for field collection of marine mammal eDNA samples: transect surveys or close approaches. Transect surveys, where samples are collected at multiple points along a predefined route, may provide a record of multiple species present in an area and thereby capture their distribution and trophic interactions (Djurhuus et al., 2020; Valsecchi et al., 2021). Upon close approach, water samples can be collected from the vicinity of sighted animals, including the footprint that an animal leaves on the surface when it dives. Footprint samples are best suited for obtaining individual non-invasive genetic samples for population genetic analyses (Baker et al., 2018; Parsons et al., 2018; Székely et al., 2021).

The use of large research vessels for eDNA transect surveys may allow for sampling of offshore species and populations, and during bad weather or polar conditions. However, such sampling comes with trade-offs in terms of reduced manoeuvrability, which may prevent close and safe approaches to individual animals, as well as the substantial economic costs associated with chartering a large research vessel. Alternatively, for coastal species or geographically limited studies, eDNA footprint samples and/or short surface transects can easily be collected from smaller vessels such as dinghies by researchers or local communities (e.g., citizen science).

Tackling contamination every step of the way

Marine mammal eDNA occurs at very low concentrations in the marine environment and samples are prone to contamination from other DNA sources. Tracking contamination is crucial for the reliable use of eDNA as a tool in marine mammal research. Contamination in the context of eDNA means DNA from other species besides the target species, which is potentially introduced during field collection, sample storage and laboratory work. Contamination may also be from the target species but deriving from other sources than the eDNA seawater sample. Recently, multiple field guides have become available for the best practices of sample processing and contamination tracking specific to the marine environment

(Bruce et al., 2021; Goldberg et al., 2016). Thus, our aim is mainly to mention some critical considerations for marine mammal eDNA collection, filtration and DNA extraction.

Firstly, sampling (and filtering, if this occurs shipboard) seawater for eDNA in the field should be conducted in isolation from other types of sampling efforts. Specifically, if the research includes e.g., biopsy collection, scat sampling or fish trawl surveys, it is highly recommended that such samples are handled on another boat (e.g., if several dinghies are involved in field work) or by staff and equipment not involved in eDNA sampling. Moreover, in order to avoid cross contamination in the field, eDNA sampling instruments should be cleaned thoroughly between sampling events.

Secondly, it is recommended that DNA extraction of low concentration eDNA samples, such as seawater samples, are conducted in a designated eDNA laboratory (clean laboratory), isolated from other tissue-based sample processing. Establishing a clean laboratory might not be an option for every institution, thus as a minimum, important mitigation steps should include separating sample processing into pre- and post-PCR areas, processing samples in a laminar flowhoods, wearing protective gear, and decontaminating laboratory equipment regularly with UV light, bleach and ethanol.

To complement these precautions to avoid contamination, it is important to track potential contamination sources by using negative controls (blanks) at each step along the way, from sample collection through laboratory processing. Negative seawater controls, for example collected in locations with known absence of the target species, can be used to test for contamination in sampling gear. Tap water controls can be filtered alongside environmental samples to test for contamination in filtering equipment. In the laboratory, negative controls without added DNA should be included at each step from eDNA extraction to amplification (PCR blanks) and reported when interpreting sequencing data (Sepulveda et al., 2020). For further detailed information on best practices related to the use of negative controls in eDNA research and additional techniques to avoid contamination, we refer our readers to recently published practical guides on these topics (Bruce et al., 2021; Goldberg et al., 2016)

Interpreting marine mammal eDNA detections (or lack thereof)

Despite the relatively large body size of marine mammals, the abundance of their DNA in the sea is typically very low compared to the dominating taxonomic groups in marine ecosystems (Stat et al., 2017; Székely et al., 2021). For instance, an analysis of shotgun sequencing data representing all DNA in a sample from a bowhead whale footprint found that as little as 1-2% of the DNA sequences matched bowhead whale DNA in a reference database, while the remaining 98%-99% matched DNA sequences from bacteria and phytoplankton (Székely et al. 2021). Therefore, the probability of detecting marine mammals from a single seawater sample is low and influenced by stochasticity in sampling, laboratory processing and data analysis. To address such stochasticity and increase the probability of detection, researchers can increase the volume of seawater filtered per sample, increase the number of geographical locations sampled, and use biological replicates (replication of seawater samples from the same sampling location) and technical replicates (replication of PCR reactions).

Ultimately, the number, type, and location of samples must be tailored to the biology and assumed occurrence of the target species or taxonomic group (Bruce et al., 2021; Goldberg et al., 2016).

The success of marine mammal eDNA detections relies not just on the amount of marine mammal eDNA in the seawater sample, but also the quality of the molecular probes or primers that are used to detect that marine mammal eDNA. For qPCR-based eDNA assays, there exist published validation scales to evaluate the readiness of eDNA assays for species monitoring (Thalinger et al., 2021). Before widespread use, primers need careful evaluation *in silico*, *in vitro* with tissue-based DNA, and eventually *in situ* using real eDNA samples (Goldberg et al. 2016); a process which is time- and resource-consuming. The potential difficulty with designing reliable primers is illustrated by Pinfield et al. (2019), where qPCR primers designed specifically for killer whale detections failed to detect killer whales in seawater samples collected in the immediate vicinity of multiple individuals, despite successful testing of the primers on killer whale DNA in the laboratory prior to field work.

In contrast to the thorough testing and validation of species-specific qPCR-based eDNA assays, the metabarcoding primers available for detection of marine mammals have been developed more broadly for marine vertebrates (Miya et al., 2015; Valsecchi et al., 2020). As a result, the primers may not match equally well to all marine mammal species and hence fail to detect them with equal likelihood. Moreover, there is little consensus about what constitutes a positive detection for metabarcoding studies in terms of the number of DNA sequences or the degree of sequence similarity to a reference database. Thus, eDNA metabarcoding results may be biased by false positive or false negative detections. Some of these obstacles can be addressed through the development of more specific marine mammal metabarcoding primers and testing of primers using mock communities composed of known mixtures of species derived from synthetic or tissue-derived DNA. Finally, all eDNA metabarcoding approaches are limited to the reliability and completeness of reference DNA sequence databases such as NCBI GenBank. Complete mitochondrial genomes have been generated all North Atlantic marine mammals (Table 1), but lack of reference data could limit the detection of other marine organisms, e.g., for eDNA-based reconstruction of marine biodiversity and trophic interactions (Mugnai et al., 2021).

FUTURE POTENTIALS IN MARINE MAMMAL RESEARCH

Despite the many challenging aspects of working with eDNA, technical advances and the development of recommended guidelines and protocols (Bruce et al., 2021; Goldberg et al., 2016) have resulted in a substantial increase in eDNA studies in the last decade. In this review, we have highlighted that eDNA is also an emerging and promising tool for marine mammal research, with several future applications.

Emerging approaches to how and where eDNA is collected have the potential to advance the spatial and temporal scale of current marine mammal monitoring strategies in the North Atlantic. The relatively simple and cheap nature of eDNA collection enables broad participation in sampling, making it amenable to volunteer-based citizen science projects (Agersnap et al., 2022; Chiovitti et al., 2019), and implementation aboard

commercial ferries and ecotourist platforms (Valsecchi et al., 2021). Paralleling trends in advances in the collection of oceanographic data, automated eDNA samplers have also been developed and tested in recent years. Automated eDNA samplers can be deployed in ocean settings as stationary or drifting buoys for spatiotemporal collections, or in the form of autonomous vehicles that sample over larger spatial scales e.g., along predefined transects (Aguzzi et al., 2019; Yamahara et al., 2019). While some platforms collect and archive samples, others include processing capacity that enables real-time reporting of the presence of certain target species (Hansen et al., 2020).

With broader capacity for eDNA collection, we anticipate broader uptake of eDNA based methods in diverse marine mammal monitoring programs, including those that are associated with monitoring the impacts of human activities. For example, eDNA biodiversity monitoring has recently been employed to track changes in benthic and pelagic marine communities due to offshore oil drilling (Cordier et al., 2019). Marine mammals are known to be affected by different marine industrial operations, including offshore wind farms, oil exploration, fishing and commercial shipping. Traditionally these industries would employ marine mammal observers on site to mitigate impacts on marine mammals, but with further development and validation, automated eDNA-based detection of species may be an effective alternative.

Given its applied potential, in addition to pushing the envelope on eDNA collection methods, we envision that the near-future of marine mammal eDNA science will continue to strive for validation and standardization of sample collection, data generation and interpretation protocols. These efforts are critical to the generation of reliable and comparable data that can be routinely incorporated into monitoring programs and management strategies. Overcoming, or accepting, the uncertainties associated with eDNA data interpretation will be necessary to integrate this new approach into management decision making (Jerde, 2021). Similar to the emergence of acoustic monitoring tools to supplement or replace visual marine mammal surveys, eDNA-based approaches are adding a new tool to the toolbox of marine mammal scientists and managers (Howe et al., 2019). In conclusion, the future of eDNA based marine mammal studies is rife with opportunities, from studies that advance our foundational understanding of the ecology of eDNA itself to the development of management frameworks that integrate eDNA data into the decision-making process.

ADHERENCE TO ANIMAL WELFARE PROTOCOLS

The research presented in this article has been done in accordance with the institutional and national laws and protocols for animal welfare that are applicable in the jurisdictions where the work was conducted.

AUTHOR CONTRIBUTION STATEMENT

All authors conceived the study, drafted the manuscript and approved the final version.

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