http://dx.doi.org/10.32360/78188
ISSN 0374-5686
e-ISSN 2526-7639

REVIEWS

Arquivos de Ciências do Mar

ENVIRONMENTAL DNA IN AN OCEAN OF CHANGE: STATUS, CHALLENGES AND PROSPECTS

DNA ambiental em um oceano de mudanças: status, desafios e perspectivas

Charlotte Havermans[1], Annkathrin Dischereit[1], Dmitrii Pantiukhin[1], Madlen Friedrich[1], Ayla Murray[1]

[1] Helmholtz Young Investigator Group ARJEL, Functional Ecology, Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research, Bremerhaven, Germany

ABSTRACT

Environmental DNA (eDNA) studies have burgeoned over the last two decades and the application of eDNA has increased exponentially since 2010, albeit at a slower pace in the marine system. We provide a literature overview on marine metazoan eDNA studies and assess recent achievements in answering questions related to species distributions, biodiversity and biomass. We investigate which are the better studied taxonomic groups, geographic regions and the genetic markers used. We evaluate the use of eDNA for addressing ecological and environmental issues through food web, ecotoxicological, surveillance and management studies. Based on this state of the art, we highlight exciting prospects of eDNA for marine time series, population genetic studies, the use of natural sampler DNA, and eDNA data for building trophic networks and ecosystem models. We discuss the current limitations, in terms of marker choice and incompleteness of reference databases. We also present recent advances using experiments and modeling to better understand persistence, decay and dispersal of eDNA in coastal and oceanic systems. Finally, we explore promising avenues for marine eDNA research, including autonomous or passive eDNA sampling, as well as the combined applications of eDNA with different surveillance methods and further molecular advances.

Keywords: environmental DNA, DNA metabarcoding, marine metazoa, biodiversity, population genetics, natural sampler DNA, diet analysis.

RESUMO

Os estudos com DNA ambiental (eDNA) cresceram nas últimas duas décadas e a aplicação de eDNA aumentou exponencialmente desde 2010, embora em um ritmo mais lento no sistema marinho. Apresentamos uma visão geral da literatura sobre estudos com eDNA de metazoários marinhos

e avaliamos as conquistas recentes em responder a perguntas relacionadas à distribuição de espécies, biodiversidade e biomassa. Investigamos quais são os grupos taxonômicos e as regiões geográficas melhor estudados assim como os marcadores genéticos utilizados. Avaliamos o uso de eDNA para abordar questões ecológicas e ambientais por meio de estudos de teia alimentar, ecotoxicologia, monitoramento e gestão. Com base nesse estado da arte, destacamos as perspectivas estimulantes do uso de eDNA para séries temporais marinhas, os estudos de genética populacional, o uso de DNA amostrador natural e dados de eDNA para a construção de redes tróficas e modelos de ecossistemas. Discutimos as limitações atuais, em termos de escolha do marcador e deficiências das bases de dados de referência. Também apresentamos avanços recentes usando experimentos e modelagem para melhor compreender a persistência, decaimento e dispersão de eDNA em sistemas costeiros e oceânicos. Finalmente, exploramos caminhos promissores para pesquisa com eDNA marinho, incluindo amostragem de eDNA autônoma ou passiva, bem como as aplicações combinadas de eDNA com diferentes métodos de monitoramento e outros avanços moleculares.

Palavras-chave: *DNA ambiental, metabarcoding DNA, metazoo marinho, biodiversidade, genética populacional, amostrador natural DNA, análise da dieta.*

INTRODUCTION

The world's oceans cover 71% of the Earth's surface and are drivers of global physical (circulation patterns) and biological processes (oxygen production, biological carbon pump). The oceans are responsible for around half of all primary production and provide crucial food, recreation, social and economic services (Falkowski & Raven, 2013). However, due to climate change and human activities, the physics and chemistry of the world's oceans are changing at an unprecedented pace, inducing an equally unprecedented biodiversity loss. These ever-mounting pressures on our Blue Planet will increasingly lead to changes in geographic distribution and population performances, fundamentally altering species communities and their functions. Hence, the use of an efficient toolkit for assessing and monitoring marine biodiversity is critical and urgent to improve existing management and conservation efforts.

The oceans contain the extra-organismal genetic material of all of its inhabitants, consisting of shed skin cells, mucus, damaged tissue, gametes, faeces and metabolic waste; all of which literally represents a "genetic soup" (Kelly *et al.*, 2014). Sampling this DNA, referred to as environmental DNA (eDNA), is a powerful way to assess entire communities and biodiversity. It is an increasingly common tool for conducting community and biodiversity surveys, rare and invasive species detection and impact assessment. Target organisms are detected through the extraction of their DNA from soil, water, and air samples as well as bulk (mixture of whole organisms) samples. eDNA represents a non-invasive, rapid method, based on the ever-advancing high-throughput sequencing technologies and bioinformatic processing, with a wide taxonomic applicability. It has already been shown to be a promising instrument for biodiversity studies, allowing major progress compared to, or in combination with, traditional sampling methods; which often are invasive, destructive (e.g., trawling) or costly. Furthermore, the subsequent taxonomic

inventory of these samples is dependent on sparse, localized expertise and can be extremely time-consuming, hence resource intensive.

Depending on the method used, eDNA studies focus on single species, groups of species or entire communities. The most accessible and cost-effective method used to target single species, is the quantitative real-time polymerase chain reaction (qPCR) analysis (reviewed in Langlois *et al.*, 2021). Communities can be studied at different levels of resolution, which can range from targeting different species of the same group (e.g., a particular family of fish) to the broadest scope, assessing all eukaryotic taxa in an environment. When multiple species are targeted and sequenced with general primer sets, we refer to the term DNA metabarcoding (Pompanon; Coissac & Taberlet, 2011). DNA metabarcoding is also increasingly being used to assess diversity in bulk samples, which contain a mixture of different organisms or parts of them (Ruppert *et al.*, 2019). DNA can be extracted from a sample of organisms isolated from sediment, or collected with nets or trawls (e.g., Creer *et al.*, 2016). Although DNA metabarcoding often refers to both bulk and environmental samples, the former is per definition not the same as eDNA. Samples collected for dietary analyses (e.g., gut contents or faeces) are in a grey area between bulk and environmental samples, as they do contain biological source material, but their DNA is often degraded (van der Loos & Nijland, 2021), so we will discuss their use here as well. Both single and multiple species approaches rely on the assumption that the detection of DNA indicates the presence of the species to which it belongs, and therefore need optimization of both sampling design and laboratory protocols to reduce false negatives and positives (Buxton *et al.*, 2021).

The aim of this paper is to provide a simplified overview of the current state of the art of eDNA research in the world's oceans, with a focus on metazoan-based studies. We will explore current trends and research gaps, before outlining the exciting prospects of eDNA as a growing field for ecological and evolutionary studies. Finally, we present future avenues of eDNA research in the marine environment.

Marine metazoan eDNA literature overview

To get a simplified overview of the state of the art of marine eDNA research, we conducted two topic searches in the Web of Science Core Collection in May 2021 (Table I). The aim of these searches was to find: (1) eDNA studies focusing on metazoans and (2) eDNA studies focusing on the analysis of stomach or faeces through metabarcoding in marine environments since 1970. The searches resulted in initial lists of 993 and 86 articles, respectively. For the first search, this was then reduced to 258 through the removal of reviews, papers focusing on non-metazoans (with the exception of macroalgae), terrestrial or freshwater studies, ancient DNA and diet analysis studies. For the diet analyses search, the initial list was reduced to 60, by removing papers with a terrestrial or freshwater focus, and microbiome papers. Diet papers recovered in our Topic 1 search were added to this list, resulting in a final list of 82 studies. It is inevitable that some relevant studies were left out of this search for reasons such as keywords not matching.

Based on the lists for topics 1 and 2, we assessed the studies based on their goals by classifying them into different categories: "biodiversity assessment", "impact assessment", "nonindigenous species (NIS) detection", "single/rare species detection" (studies aiming to detect a particular species), "methods" (i.e. studies with a major methodological focus)

and "primer testing" (i.e. studies in which primers were developed, compared or optimized) were counted for different habitats and different species. Target organisms and communities were identified to the lowest taxonomic level possible. These categories were assessed per habitat and target organism group. For the second dataset (Table I) on metabarcoding diet studies, the number of studies were evaluated per ocean and organism group.

Table I – Strategies used in Web of Science Core Collection search

Topic	Target literature	Keywords used in web of science with capitals for each word	Total (Initial/final)	Removed from search
1	eDNA studies in marine environments focusing on metazoans	TOPIC: (edna or "environmental DNA" or metabarcoding) *AND* TOPIC: (marine or ocean or sea or estuarine or coastal) *NOT* TOPIC: (freshwater)	993/258	Reviews, non-metazoans, terrestrial and freshwater environments, and diet analysis
2	eDNA studies in marine environments focusing on diet analyses	TOPIC: (diet or "stomach content" or faeces) AND TOPIC: ("DNA metabarcoding" or barcoding) AND TOPIC: (marine or ocean or sea or estuarine or coastal) NOT TOPIC: (freshwater or terrestrial)	86/82	Reviews, terrestrial and freshwater environments, gut microbiome studies

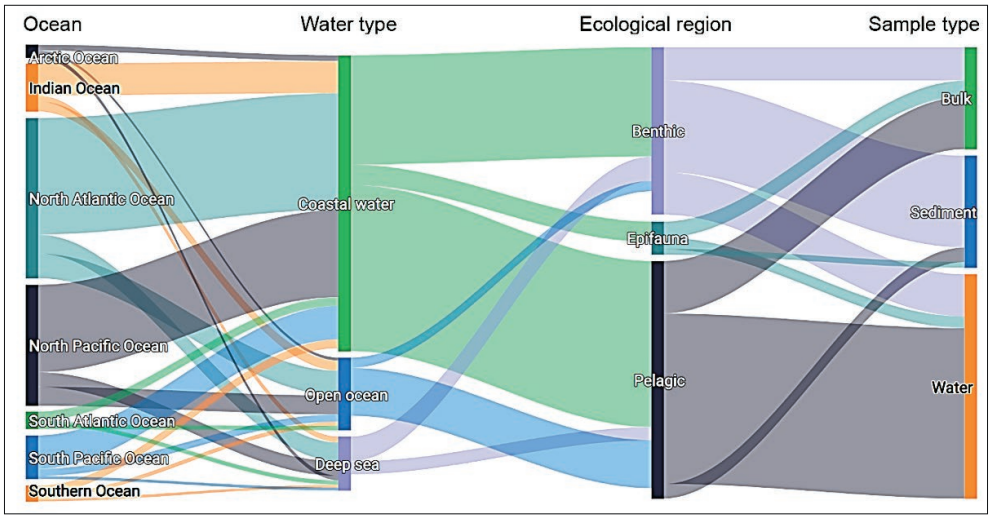
Current status and applications

1 Are eDNA studies omnipresent across oceans and organisms?

eDNA studies have been widely used in the marine system and have targeted a vast number of ecosystems, habitats and organisms. The majority of studies have focused on coastal waters in the North Atlantic and North Pacific oceans (together around 70% of all studies), whereas the South Atlantic and South Pacific oceans are comparably much less studied (Figure 1). However, the application of eDNA to the more inaccessible regions is far from uncommon. eDNA studies focusing on the deep sea have been carried out for more than a decade. The majority of these studies targeted foraminiferal diversity (not included in our metazoan literature search), from abyssal to hadal depths, and from the poles to temperate waters (Cordier *et al.*, 2019; Pawlowski *et al.*, 2011). Recently, a significant number of metazoan eDNA studies have greatly contributed to documenting deep-sea taxa, several of which revealed unprecedented levels of diversity (e.g., Kersten *et al.*, 2019; Le *et al.*, 2021; Lejzerowicz *et al.*, 2021). In contrast, eDNA studies in other remote areas such as the polar regions remain comparatively scarce (only 8% of eDNA studies in our search). Most of the studies investigated eDNA in coastal water, followed by the open ocean, and the least studied is the deep sea. Of the eDNA metazoan studies, the majority focused on the pelagic environment, followed by the benthic environment (Figure 1). Papers studying epifaunal communities or organisms were least common, and were carried out in coastal waters. Finally, a bit more than half of the studies used water samples to retrieve eDNA, whereas circa 26% used sediment samples and 23% investigated bulk

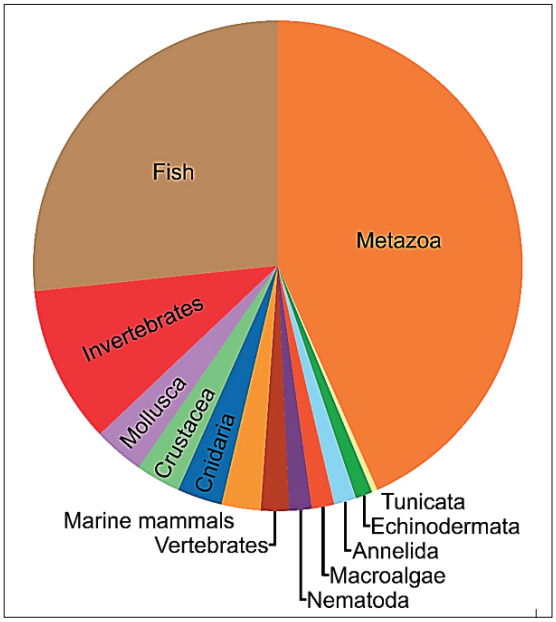
samples. Studies targeting benthic organisms or communities did not only work with sediment samples, but also used, albeit to a lesser extent, water or bulk samples. Epifaunal studies investigated bulk and water samples in almost equal proportions, whereas the majority of pelagic studies looked at eDNA in water, followed by bulk samples, and a small fraction worked with sediment samples.

Figure 1 – Sankey diagram of marine metazoan eDNA studies carried out per ocean, per "water type" (coastal, open ocean or deep sea), per ecological region (benthic, epifauna or pelagic) and per sample type (bulk, sediment or water). This figure was created with consideration that a single value of the node could have multiple connections (e.g., if the study was conducted within several oceans or its zones). The dataset was built on our literature search described in Table I. Out of the 258 research articles, 233 were considered on this graph, excluding articles with missing values for the studied features



Our literature search showed that metazoans (43%), fish (27%), and invertebrates (10%) are well represented among marine eDNA studies. However, the other taxa were each represented in three percent or less of studies, e.g., cnidarians (3%), marine mammals (3%), molluscs (3%), crustaceans (3%) and echinoderms (1%) (Figure 2).

Figure 2 – Comparison of the proportions of marine metazoan eDNA studies per targeted organism type, based on our literature search specified in Table I

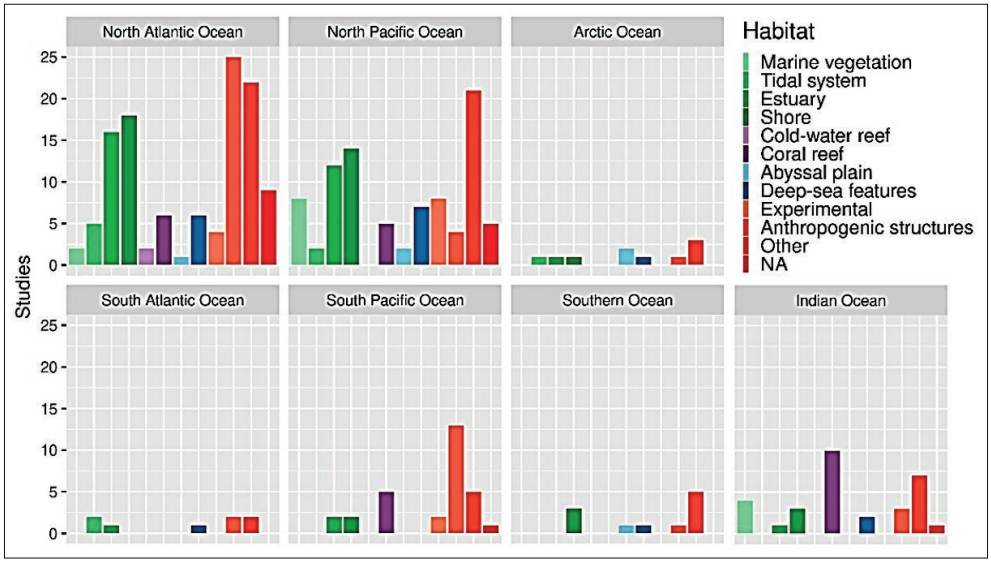


2 eDNA across a wide range of habitats

eDNA studies have focused on a wide range of habitats, from deep sea to estuarine and from coral reefs to ballast water. As reflected in Figure 1, showing that most studies were conducted in coastal areas, about 45% of all studies were carried out in estuaries, anthropogenic structures and shore habitats (Figure 3). An uneven distribution of surveys is also observed for reef habitats, with fractions of 1% and 10% for cold reef habitats and warm coral reefs, respectively (Figure 3). The North Atlantic Ocean has had the most eDNA studies across

the widest range of habitats, including the highest number of studies focusing on anthropogenic structures. The southern hemisphere has the least number of habitats studied, with the Southern Ocean and South Atlantic Ocean as least diverse in terms of habitats studied. The deep sea (including abyssal plain or deep-sea features such as seamounts, canyons, cold seeps and hydrothermal vents) has been studied with eDNA in all oceans except the South Pacific. The relatively large coverage of eDNA studies in deep-sea environments of the worlds' oceans is quite surprising considering its remoteness and associated sampling challenges.

Figure 3 – Comparison of habitat types sampled for metazoan eDNA studies in different oceans. The habitats were organized into eleven distinct categories. These include marine vegetation (incl. kelp forests, seaweeds, seagrass meadows), tidal systems (incl. intertidal, subtidal), estuary, shore (incl. caves, mangroves, lagoons, springs, bays, fjords, coves), cold-water reefs, coral reefs, abyssal plains (incl. polymetallic nodules), deep-sea features (incl. seamounts, canyons, hydrothermal vents, cold seeps, blue holes), experimental studies (incl. mesocosm, tank, lab-based), anthropogenic structures (incl. all man-made structures and settings) and other (incl. shelf, upwelling systems, water column, hard-bottom, pockmarks). The dataset was built on our literature search described in Table I

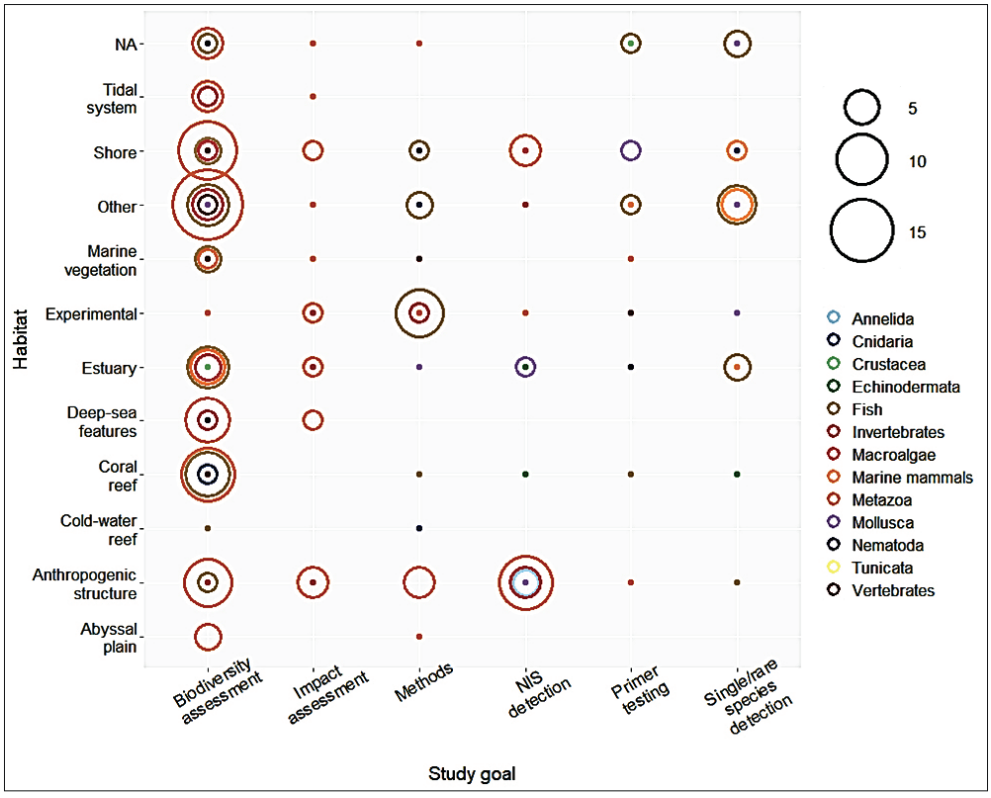


We compared marine eDNA studies and their major aims (biodiversity assessment, impact assessment, NIS detection, single/rare species detection, methods and primer testing) as well as their division over several habitat types and target organisms (Figure 4). Compared to other study goals, the most common are studies focusing on biodiversity assessment, often using DNA metabarcoding to assess communities in time and space. These studies focused on metazoan communities close to the shore, but also in coral reefs and both on and in the vicinity of deep-sea features.

Despite their remoteness, deep-sea habitats have been relatively well-characterized with eDNA. Several deep-sea eDNA studies explored diversity in abyssal plains, while others targeted seamounts (e.g., Kitahashi *et al.*, 2020; Laroche *et al.*, 2020), canyons (Andruszkiewicz *et al.*, 2017; Guardiola *et al.*, 2015), hydrothermal vents (e.g., Brandt *et al.*, 2021; Klunder *et al.*, 2020), and cold seeps (Macheriotou *et al.*, 2021). Artificial habitats, such as floating marine litter and shipping ballast water, have also been the focus of DNA metabarcoding research (Ibabe *et al.*, 2020). Colonization of the so-called "plastisphere" by fungi (Lacerda *et al.*, 2020) and eukaryotic communities (Davidov *et al.*, 2020) has been investigated, and is likely to be increasingly studied for marine metazoans. Ballast water

as an artificial habitat is becoming a focus when using eDNA as a tool in biosecurity monitoring, with both Rey *et al.* (2019) and Darling *et al.* (2018) demonstrating its potential as a cost-effective tool for monitoring the impacts of ballast water on local biodiversity and ecosystems.

Figure 4 – The number of marine metazoan eDNA studies based on our literature search specified in Table I organized for their target i) goals (biodiversity assessment, impact assessment, methods, primer testing, NIS (nonindigenous species) detection and single or rare species detection), ii) habitats and iii) organisms. Circle radius shows the number of conducted studies and colors indicate different organisms. Habitats were organized into different categories. These include deep-sea features (incl. seamounts, canyons, hydrothermal vents, blue holes and cold seeps), anthropogenic structures (incl. aquaculture settings, cages, ARMS – autonomous reef monitoring structures, harbors, offshore platforms, ballast or bilge water, and all other man-made structures), marine vegetation (incl. kelp forests, seagrass meadows and seaweeds), shore (incl. lagoons, bays, caves, fjords, springs, coves, mangroves), tidal systems (incl. intertidal, subtidal) and other (incl. upwelling systems, pockmarks, shelf, hard bottom, water column)



eDNA studies have not only been used to characterize diversity of particular habitats, but also targeted processes such as vertical carbon export, by identifying the major contributors to vertical flux. To do so, researchers have screened deep-sea sediments for organisms from the pelagic zone transported to the seafloor (Gutierrez-Rodriguez *et al.*, 2019; Laroche *et al.*, 2020). Another study compared eDNA of sediment taxa with eDNA found in the water column, revealing the presence of a wide array of pelagic taxa (from diatoms to scyphozoans and cephalopods) in both, supporting their contribution to pelagic-benthic coupling (Brandt *et al.*, 2021). The sinking particles themselves, too, have been investigated with eDNA, representing a microhabitat for organisms that re-work and process sinking particles on their way to the seafloor. In the study of Preston, Durkin and Yamahara (2020), sinking particulate organic matter (POM) was sampled from sediment traps, as aggregates on the seafloor and in seawater, and their communities assessed with

DNA metabarcoding over several seasons. The results highlighted seasonal community changes, including increases in diatoms or zooplankton grazing (Preston; Durkin & Yamahara, 2020).

3 Concurrent use and comparisons of eDNA with other methods

In order to validate the use of eDNA for diversity studies, many studies have used a combined approach, comparing community composition obtained with eDNA with other, more traditional methods in the field. Generally, for both terrestrial and aquatic systems, a meta-analysis has demonstrated that when there has been a direct comparison, eDNA outperforms traditional surveys (Fediajevaite *et al.*, 2021). A large number of marine studies have applied morphology-based identification in combination with eDNA. Often, community composition differed significantly between metabarcoding and morphology, as well as the alpha and beta diversity. In many cases, eDNA metabarcoding actually recovered more taxonomic groups than the morphological approach (e.g., Steyaert *et al.*, 2020). Hence, in a large number of case studies, eDNA metabarcoding has been suggested to provide a more holistic analysis than the traditional morphotaxonomy, despite existing limitations due to incomplete reference databases (see also section below). In Leduc *et al.* (2019), eDNA metabarcoding of water sampling was compared with standard invertebrate species sampling for assessing Arctic coastal community composition across different spatial scales. The authors found significant differences in species richness and community composition, with lesser taxa identified through species collection, as eDNA likely detects the species in their pelagic phases. However, eDNA revealed more homogeneous communities over larger spatial scales, concluding that the dispersal of eDNA may interfere with the evaluation of local community structure, emphasizing the need for the combination of both methods (Leduc *et al.*, 2019; Stoeckle *et al.*, 2021). A study comparing the seasonal diversity of two Antarctic coves demonstrated the effectiveness in combining morphotaxonomy and DNA metabarcoding to characterize different size classes in soft-sediment communities (Vause *et al.*, 2019).

Mock communities are another way to study the reliability of DNA metabarcoding, and are widely used for freshwater (e.g., Elbrecht & Leese, 2017) and terrestrial systems (e.g., Braukmann *et al.*, 2019) but to a much lesser extent in the marine community. Known species assemblages are used to assess the potential of DNA metabarcoding for amplifying all taxa present in a sample, and in some cases, to infer species abundance from read counts. Then polymerase and primer biases causing a differential amplification and other factors impeding the recovery of the DNA of all taxa present, can be considered when interpreting the results of metabarcoding studies in the field. An example in the marine environment is the study of Duke and Burton (2020), who used a mock community with a known relative proportion of eggs and larvae belonging to different fish species. DNA metabarcoding detected these even at very low input proportions, however, multiple markers were required to detect all species present (Duke & Burton, 2020).

eDNA has also been compared with other field methods, such as optics and acoustics. Baited remote underwater video systems (BRUVs) are commonly used to assess bony fish and elasmobranch diversity. Stat *et al.* (2019) compared video results with eDNA from water samples and concluded that the combination of both would yield more than 30% generic richness than each method used alone. eDNA also appeared to perform well in terms of spatial distribution revealed with the BRUVs. In the majority of comparative

studies, eDNA has proven to augment significantly traditional sampling. In the case of vertebrate fauna (fish and marine mammals), taxa identified with eDNA partially overlapped with those monitored through trawl and marine mammal surveys, but more taxa were identified with eDNA (Closek *et al*., 2019). On the contrary, when eDNA was compared with static acoustic monitoring devices to detect harbor porpoises, eDNA detection was less successful, although it did reveal species rarely sighted in the study area (Foote *et al*., 2012). Easson *et al.* (2020) combined the use of eDNA and acoustics for the analysis of diel vertical migration patterns of pelagic communities in the Gulf of Mexico, showing that eDNA can provide a fine-scale taxonomy where echosounders cannot.

In order to assess zooplankton diversity, eDNA sampling was compared with continuous plankton recorder (CPR) surveys (a regularly deployed monitoring tool for zooplankton in the Southern Ocean, which filters and collects zooplankton over large transects) (Suter *et al*., 2020). In this study, metabarcoding of eDNA water samples and bulk plankton samples collected with the CPR, as well as conventional morphological identification of CPR samples were all compared. Of all three methods, metabarcoding of the bulk plankton revealed the most species, while eDNA from filtered water, detected 1.6 times more species than morphological analyses. Plankton studies in the mesopelagic, which compared eDNA from water samples with that recovered from net sampling (MOCNESS), showed that the number of taxa detected per liter was significantly higher for eDNA, although DNA metabarcoding of zooplankton from the net hauls recovered more taxa (Govindarajan *et al*., 2021). This discrepancy can be explained by the delicate gelatinous zooplankton fraction, which remains undersampled with nets.

For fish surveys, eDNA sampling has been compared with trawling efforts. eDNA metabarcoding identified over 30% more fish species than bottom trawling (Zou *et al*., 2020). Similar results were obtained for finfish, comparing the sensitivity of traditional surveys and eDNA metabarcoding, where the latter detected species rarely observed otherwise (Liu *et al*., 2019). Stoeckle *et al.* (2021) compared eDNA and fish trawls over the period of a year and found that the two methods largely agreed, with eDNA showing more sensitive in detecting species richness, relative abundance and seasonality.

4 The elusive, rare and endangered: eDNA as a non-invasive method

Traditional sampling methods are often invasive, destructive, and time-consuming. The development of eDNA methods represents major progress in monitoring organisms that are not easily sampled or detected. One of the most famous examples of such a species is the elusive giant squid (*Architeuthis dux*), gigantic in size but hidden to humans despite intense surveying efforts. For a long time, it was only known as washed up carcasses or from fishermen's nets, while its ecology and distribution have remained a mystery. A newly developed species-specific PCR probe was able to detect *A. dux* DNA in water samples in the Sea of Japan (Wada *et al*., 2020). Similarly, for other elusive animals such as the octopus *Octopus vulgaris* in the Cantabrian Sea, qPCRs protocols were developed with an experimental approach and validated with water samples collected in the field (Mauvisseau *et al*., 2017). eDNA has also been applied within highly species-rich coral reef environments to reveal otherwise undetected species, i.e. those inhabiting hidden spaces within the reef matrix. This "reef cryptobiome" was successfully studied with DNA metabarcoding, revealing many rare and localized lineages of sessile and mobile organisms (Carvalho *et al*., 2019).

Traditional net sampling has appeared particularly poor for recovering gelatinous zooplankton diversity and abundances. Species richness and biomass of gelatinous zooplankton such as ctenophores and cnidarians are consistently underestimated due to their fragile and watery bodies that are easily damaged and destroyed in nets (Hosia *et al.*, 2017). Recently, a study compared the zooplankton taxa recovered with nets and with eDNA of filtered water samples, and demonstrated eDNA to be particularly suitable for revealing gelatinous diversity (Govindarajan *et al.*, 2021). However, eDNA studies focusing on gelatinous zooplankton still remain relatively scarce (Ames *et al.*, 2021; Gaynor *et al.*, 2017; Minamoto *et al.*, 2017; Takasu *et al.*, 2019).

eDNA yields many promises for many other vertebrate taxa too. One example is sharks, of which several species are endangered and difficult to monitor due to their elusiveness and high mobility. An elasmobranch-specific metabarcoding assay was proven effective in characterizing shark diversity around Reunion Island (Mariani *et al.*, 2021) and detecting temporal fluctuations during the study period. Great white shark (*Carcharodon carcharias*) eDNA was also identified in the open ocean (Truelove; Andruszkiewicz & Block, 2019), showing the potential for eDNA to be used in concert with visual surveys to eventually replace invasive capture-based techniques. Other rare but emblematic animals such as whales have been detected with eDNA. Droplet digital PCR technology with species-specific probes was applied for identifying cetaceans from seawater samples, which were collected during encounters with orcas (*Orcinus orca*) in inshore waters of the Salish Sea. eDNA remained detectable up to 2 hours after the passage of the orcas, which were monitored with sightings and hydrophone recordings (Baker *et al.*, 2018). Another study targeting the rarely sighted pygmy (*Kogia breviceps*) and dwarf (*K. sima*) sperm whales with eDNA metabarcoding was successful in detecting their presence in Colombian waters (Juhel *et al.*, 2021). However, compared to other study goals, the detection of single or rare species is still rather scarcely applied in the marine environment, and most of these studies were focused on fish species (Figure 4).

5 eDNA for large-scale monitoring of nonindigenous and invasive species

Nonindigenous species (NIS) detection is, after biodiversity assessment, the second most important goal of all marine metazoan eDNA studies carried out so far (Figure 4). Of all habitats, NIS studies have focused mostly on communities or organisms on anthropogenic structures, such as harbors, aquaculture settings and ballast waters (Figure 4), followed by shore habitats and estuaries. A lot of the studies looking at anthropogenic structures were carried out in the North Atlantic Ocean (Figure 3), in particular the European seas. Most of the NIS studies carried out so far targeted metazoan and invertebrate communities, but several also detected single species (e.g., echinoderms, annelids or mollusks) (Figure 4).

Invasions of NIS are heavily facilitated in marine environments by the global shipping industry, and are becoming an increasingly pervasive global issue (reviewed by Bowers *et al.*, 2021; Duarte *et al.*, 2020). Timely detection, and management responses are of paramount importance (Lehtiniemi *et al.*, 2015), and traditional surveillance may overlook NIS when the population is still localized and in low densities (Freire *et al.*, 2014). Hence, eDNA is becoming a crucial tool in biosecurity monitoring in marine environments all over the world (Bowers *et al.*, 2021). Even in the most intensively studied regions, such as the Baltic Sea (Ojaveer *et al.*, 2010; Zaiko *et al.*, 2015), eDNA could exclusively detect four of five NIS, all considered invasive. When applied for plankton surveillance, eDNA has the

advantage over traditional sampling that it detects meroplankton more effectively (Zaiko *et al.*, 2015) and also circumvents the problem that morphological identification of larvae and juveniles is often impossible (Pochon *et al.*, 2013). eDNA retrieved from only some liters of water allowed the early detection of NIS and harmful algae in a Mediterranean lagoon (Suarez-Menendez *et al.*, 2020). The use of eDNA as biosurveillance tool has the potential to implement eradication efforts in a timely manner for mitigating the devastating effects of NIS on marine communities worldwide (Westfall; Therriault & Abbott, 2020).

6 Application of eDNA for impact assessment, biodiversity monitoring and conservation

The impact of anthropogenic activities can be monitored with environmental impact studies, and in this context, eDNA has appeared to be a very useful and rapid tool. eDNA has been applied for impact assessments across a wide range of habitats (Figure 3). A large fraction of them assessed species present on anthropogenic structures (e.g., offshore platforms and coastal aquaculture settings) and targeted diversity at low resolution (metazoans, invertebrate communities). When validated and following standardized sampling protocols, eDNA holds many promises for being used as a proxy for ecological changes and impact monitoring over regular intervals. Examples include monitoring of the impact of oil production platforms (Laroche *et al.*, 2017) and offshore gas platforms (Cordier *et al.*, 2019) on surrounding benthic assemblages, the impact of tourist visits on meiofauna inhabiting sandy beaches (Martínez *et al.*, 2020), and the impact of metal pollution caused by a mine tailing disaster on benthic estuarine fauna (Bernardino *et al.*, 2019). Finally, the impacts of coastal salmon farming can be monitored with eDNA metabarcoding, to detect benthic community changes across different spatial scales (He *et al.*, 2020) to develop biotic indices to quantify such changes (Keeley; Wood & Pochon, 2018).

Despite its apparent inaccessibility, the deep sea is threatened by mining and other anthropogenic activities. As mentioned above, eDNA has already contributed to major advances in the understanding of deep-sea communities and the processes controlling them (Macheriotou *et al.*, 2020). In an area claimed for deep-sea mining activities, the Clarion Clipperton Fracture Zone (CCZ, Pacific Ocean), DNA metabarcoding of the meroplankton assemblages was carried out to assess biodiversity and spatial variability in larval communities. It provided baseline findings that can serve future monitoring studies, and to better understand larval dispersal and recolonization, essential for assessing the patchiness and vulnerability of these communities (Kersten *et al.*, 2019). Just like polymetallic nodules on the abyssal plains, seafloor massive sulfide (SMS) deposits at hydrothermal vents are of interest for deep-sea mining for exploiting their valuable metals. The species inhabiting this particular habitat may be exposed to mining-related sediment plumes. By looking at benthic communities with eDNA from sediment and water in the vicinity of natural plumes, Klunder *et al.* (2020) reported the fauna to be heavily influenced by the plume's fall out and communities varying over small spatial scales, which may hinder their restoration after mining activities. Cowart, Murphy and Cheng (2018) used eDNA to study recolonization dynamics of benthic communities associated with hard-substrata after an experimentally induced faunal clearance. Such baseline and impact studies will facilitate future monitoring efforts and allow us to better understand the resilience and recovery potential of these unique ecosystems in view of conservation and management efforts.

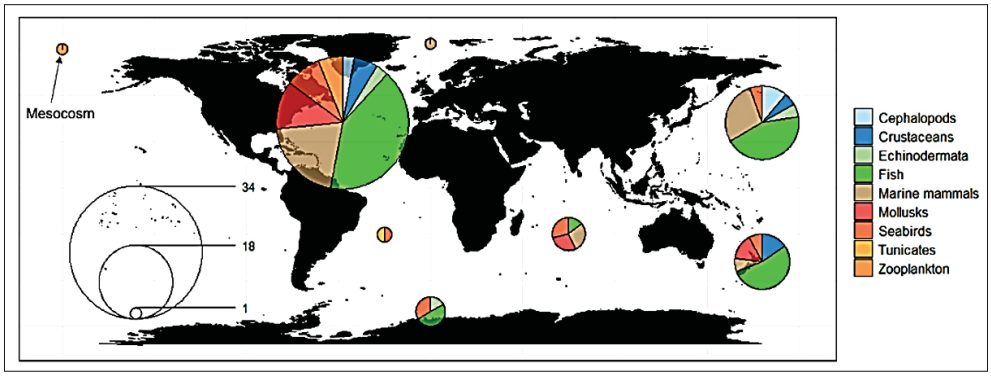
Finally, eDNA appeared to be a successful strategy for revealing macrophyte contribution to coastal sediment as sources of blue carbon (i.e. carbon stored in coastal

and marine ecosystems) (Geraldi *et al.*, 2019; Ortega; Geraldi & Duarte, 2020). It was proven to provide a high-resolution picture of the diversity of macroalgae, seagrasses and mangroves, but eDNA abundances could also be correlated with the organic carbon content. The role of eDNA in identifying the drivers of natural carbon sinks may become crucial to ensure restoration and protection of such blue carbon habitats, in the light of climate mitigation measures.

7 DNA metabarcoding for assessing diets from gut contents, faeces and scats

Most diet studies using DNA metabarcoding have targeted different fish species (e.g., Bachiller *et al.*, 2020; Kume *et al.*, 2021; Shink *et al.*, 2019), followed in number by studies targeting marine mammals (e.g., Granquist *et al.*, 2018; Nielsen *et al.*, 2007; Tverin *et al.*, 2019) (Figure 5). Regarding regions, most studies were performed in the North Atlantic Ocean, followed by the North Pacific Ocean. The polar regions seem to be understudied, with only one study, focusing on marine mammals, carried out in the Arctic Ocean (Sonsthagen *et al.*, 2020) and five Southern Ocean studies, targeting seabirds, fish and echinoderms (Clarke *et al.*, 2020; Heindler *et al.*, 2018; McInnes *et al.*, 2017a; McInnes *et al.*, 2017b; Yoon *et al.*, 2017). The South Atlantic Ocean is underrepresented, with only two studies carried out on tunicates and seabirds (see Figure 5) and one mesocosm study characterizing the diet of copepods (Ray *et al.*, 2016).

Figure 5 – Diet studies for different target organisms using DNA metabarcoding conducted in the world's oceans based on our literature search specified in Table I



Diet studies using DNA metabarcoding have, compared to biomarker analyses, the unique advantage of providing a high-resolution prey spectrum, which, depending on the reference database, allows an identification of all the different prey items to the species level. Hence, DNA metabarcoding revealed previously unknown trophic links in many instances. Its application has also contributed to studying predation on endangered species or populations. This was the case for the endangered European eel, of which the larvae were found to be preyed upon by several mesopelagic fish species (Jensen *et al.*, 2018). The use of DNA metabarcoding has also revealed a hitherto unknown level of complexity, in terms of intraspecific variation in diet and foraging habits of apex predators. Indeed, metabarcoding of scat samples of harbor seals (*Phoca vitulina*) resulted in a very comprehensive examination of intraspecific variation in feeding specialization and foraging styles, which was revealed to be much more important than initially assumed. This unexpected feeding diversity varied in time and space, as well as between sexes (Voelker *et al.*, 2020). Similar unexpected levels of complexity of feeding habits were uncovered by

using DNA metabarcode assays on ray (*Mobula*) species, revealing unprecedented diversity and occurrences of bony fish (Bessey *et al.*, 2019). Such studies show that we can expect feeding habits to be much more complex in other organisms as well, and we recommend in-depth diet studies comparing feeding strategies within and between life stages, sexes, habitats and populations.

DNA metabarcoding in diet analysis has also revealed predation of otherwise undetectable prey. An example of this is gelatinous zooplankton, which are easily digested in predators' stomachs and therefore not revealed by traditional microscopic stomach content analyses. Major insights on this came from the Southern Ocean, where mesopelagic myctophid fish were shown to feed on gelatinous zooplankton from metabarcoding of stomach contents (Clarke *et al.*, 2020; Nirazuka *et al.*, 2021), corroborating that their role as prey has been significantly underestimated. The same was true for seabirds, such as the little penguin (*Eudyptula minor*), which extensively preys on gelatinous zooplankton including salps, appendicularians and cnidarians (Cavallo *et al.*, 2018), as well as albatrosses regularly consuming scyphozoan jellyfish (McInnes *et al.*, 2017a). Similarly, larvae of mesopelagic fish in the coastal Pacific were demonstrated to feed on appendicularians (Kume *et al.*, 2021).

Despite the major advances this method brings to trophic ecology, there are still some uncertainties and caveats. One such remaining issue is the challenge of pinpointing secondary predation. Some studies hypothesize ingestion as secondary prey based on their size (e.g., appendicularians and copepods for penguins). Another way to identify secondary predation is the use of probabilistic co-occurrence analysis, as suggested in Tercel, Symondson and Cuff (2021). With such analyses, the co-occurrence patterns of different prey items are visualized to identify items that have a high likelihood to result from secondary predation (Tercel; Symondson & Cuff, 2021). However, since this approach is only an explorative analysis, it should be interpreted with care (Tercel; Symondson & Cuff, 2021). Another difficulty for interpreting the DNA metabarcoding results for diet analysis, is the occurrence of cannibalistic behavior, as for most commonly used markers, it may not be possible to easily distinguish between the DNA from ingested conspecific individuals or that originating from the predator itself. Additionally, predator DNA is often of good quality, while prey DNA can be highly degraded, resulting in a higher probability of predator DNA being amplified and dominating the recovered sequences (Piñol *et al.*, 2015). Two approaches are commonly applied to overcome this problem. The first approach is the use of predator-specific blocking oligonucleotides, preventing the predator's DNA from being amplified during the PCR (Deagle *et al.*, 2009; Deagle *et al.*, 2010; Deagle *et al.*, 2013; Shehzad *et al.*, 2012; Vestheim & Jarman, 2008). However, Vestheim and Jarman (2008) pointed out that such blocking primers may inhibit the amplification of DNA from (often closely-related) prey items. The second approach deals with predator read predominance by increasing the sequencing depth through newly emerging sequencing technologies, which are able to reveal a higher diversity and therefore provide a more comprehensive picture of the dietary spectrum of a species (e.g., NovaSeq, van der Loos & Nijland, 2021; Singer *et al.*, 2019).

DNA metabarcoding also holds many more promising avenues for diet analyses (see also section below). An exciting example is the use of metabarcoding of scats to determine whether microplastic exposure is linked to the type of prey consumed, which appeared to be the case for grey seals (*Halichoerus grypus*) (Nelms *et al.*, 2019). Furthermore, DNA

metabarcoding of gut contents not only reveals the presence of the prey but also allows the discovery of endoparasites and the characterization of the microbiome of an organism. For the latter, the application of metagenomics can have a twofold outcome: characterizing both the diet spectrum as well as the microbiome of a certain species (e.g., for fish; Pan *et al.*, 2021).

Prospects for eDNA in a changing ocean

1 Target areas for future eDNA studies: the lesser explored oceans, habitats and organisms

So far, the northern hemisphere is much better studied for metazoan eDNA than the southern hemisphere marine systems (Figure 1). The least well studied oceans are the South Atlantic and Southern oceans, in terms of the number of studies and range of habitats covered (Figure 3). A similar pattern can be observed for diet studies, where the South Atlantic Ocean clearly stands out as being underrepresented. Cold-water reefs and marine vegetation (kelp forests, seagrass or seaweed) have only scarcely been studied (Figure 3). We also determined a gap in studies of epifauna in the open ocean and deep sea, as well as the open ocean benthic realm.

We could also highlight the comparatively low coverage of eDNA studies in both the Arctic and the Southern oceans. Polar regions are changing faster than others, with range contractions of native species and poleward range expansions of temperate species already underway (e.g., Frainer *et al.*, 2017; Freer; Daase & Tarling, 2021; Schröter *et al.*, 2019). The need for baseline studies and comprehensive biodiversity assessments to be used for mitigating these impacts is more pressing than ever. Nevertheless, these regions also represent major research limitations in terms of year-round and spatial accessibility. Logically, these regions are comparatively less studied (Figures 1 and 3), but where applied, eDNA has shown to be a particularly useful tool for detecting incoming species and assessing diversity. In Antarctica, eDNA was used to document benthic diversity from shelf water samples collected around the West Antarctic Peninsula, and allowed the detection of king crabs, known to expand their ranges poleward and colonizing the Antarctic shelf (Cowart; Murphy & Cheng, 2018). eDNA also holds many promises for studying marine mammals in a non-invasive way. As a case study, Howell, LaRue and Flanagan (2021) proposed to use snow-based eDNA for monitoring Weddell seals hauling out on fast ice. Recently, eDNA has been increasingly applied for monitoring coastal communities in the Canadian Arctic (Lacoursière-Roussel *et al.*, 2018; Sevellec *et al.*, 2021) highlighting the effectiveness of eDNA in documenting diversity over different spatial and temporal scales but also linking the timing of different life stages (occurrence of larvae, spawning) with the most abundant sequence data (Sevellec *et al.*, 2021). Mesozooplankton communities from the Pacific Arctic have also recently been characterized with DNA metabarcoding (Hirai; Tachibana & Tsuda, 2020; Questel *et al.*, 2021). However, pelagic eDNA studies from elsewhere in the Arctic region are still missing. Benthic communities have only been characterized with sediment eDNA in Kongsfjorden (Svalbard) for NIS detection (van den Heuvel-Greve *et al.*, 2021), and in the Beaufort Sea (Alaska, Barrow). In the latter study, DNA metabarcoding was used to investigate sea ice and sediment samples for metazoan diversity throughout winter, spring and summer (Leasi *et al.*, 2021). The majority of the aforementioned studies were carried out in coastal or shore areas of the Arctic and Southern oceans. Hence, we appeal for a wider application of eDNA methods

in the polar regions, across broader spatial and temporal scales than those of the studies that have been carried out so far.

Another unstudied habitat so far for metazoans are oxygen minimum zones (OMZ). OMZs have been explored with eDNA for picoeukaryote diversity assessment, comparing different environmental gradients (De la Iglesia *et al.*, 2020). eDNA holds much potential for evaluating metazoan species distributions in dysoxic and suboxic conditions, bringing new insights into tolerance limits of pelagic organisms. Candidate species for future eDNA studies could be identified for their occurrence in such zones, just like a limpet species endemic to hydrothermal vents was suggested as candidate to detect presence of active vents (Collins *et al.*, 2020).

eDNA has so far been only scarcely applied in Marine Protected Areas (MPAs), for their biodiversity assessment (baseline studies) and subsequent monitoring. In an MPA in California, eDNA surveys were combined with visual surveys to compare fish communities inside and outside the area, and eDNA was shown to pick up many species that divers did not (Gold *et al.*, 2021). eDNA was also used to compare fish communities inside a marine no-take reserve and in its surrounding fishing grounds in the Mediterranean (Boulanger *et al.*, 2021). While most other traditional methods are lethal or stressful for the targeted species, eDNA can greatly enhance monitoring of marine reserves and MPAs and reduce collateral damage.

In terms of target organisms, the majority of eDNA studies in our literature search focused on metazoan or invertebrate species and communities but research focusing on a lower taxonomic rank of invertebrates is still missing. Finally, eDNA has been largely applied to fish species and communities, but other marine vertebrates such as seabirds and mammals remain understudied.

2 The use of eDNA in marine time series

Despite its rapidly increasing use as a tool for marine biodiversity studies and ecological monitoring, eDNA is still greatly underutilized for marine time series analyses, particularly those including the study of metazoans. However, temporal eDNA datasets, especially those collected over longer time spans, will become increasingly valuable to identify biotic shifts related to environmental changes (Balint *et al.*, 2018). For example, water sampling, using CTD (conductivity-temperature-depth) rosette samplers or pumps, demands much less station time than deploying various nets and trawls to capture planktonic and nektonic communities across their different size ranges. Furthermore, the eDNA datasets obtained from these filtered water samples provide information simultaneously across a wide array of taxa, whereas traditional sampling and processing may be limited to certain size ranges or taxonomic groups. The integration of eDNA in long-term monitoring programs, conveniently allows for less ship time for sampling particular communities, facilitates the use of standardized protocols, as well as the reliance on generally sparse and dispersed taxonomic expertise. Marine eDNA time series could infer seasonality in community patterns as well as long-term dynamics of species and communities. Such temporal eDNA datasets have the potential for detecting changes in abundances and range expansions, when focusing on the species level. When targeting the community level, decreases in species richness, biotic tipping points and regime shifts can be identified. Before they are used to test hypotheses concerning the long-term drivers of community changes, eDNA data can be validated with the traditional data sources for

assessing their efficiency in capturing biodiversity changes and providing estimates of biomass. eDNA time series can also serve to study the impact of extreme events on communities and species' abundances. The five-year study of Berry and colleagues (2019), assessed eDNA by metabarcoding of monthly bulk zooplankton samples at a locality in Western Australia, which was subjected to a marine heatwave during the time span of the time series. The authors were not only able to track biotic shifts in response to seasonal and annual changes but also identify changes that occurred in the zooplankton community due to the witnessed temperature anomaly. Such time-stamped eDNA samples could be collected in view of their long-term storage in curated "environmental biobanks" to allow for comparability of studies as molecular technologies evolve (Jarman; Berry & Bunce, 2018).

3 Spatial up- and downscaling

The use of eDNA for surveying whole animal communities in large, dynamic marine systems has been slowed down due to significant unknowns regarding error rates (false negatives and false positives) in species' detection and an in-depth understanding of the relevant spatial resolution for eDNA surveys (Port *et al.*, 2016). Like a lack of temporal sampling, a low spatial resolution of monitoring programs can severely influence biodiversity monitoring and hamper NIS detection (e.g., Zaiko *et al.*, 2015). Recent studies have shown that eDNA metabarcoding showed a high sensitivity to elucidate fine-scale patterns in oceanographically very dynamic and complex regions (West *et al.*, 2021). In a study targeting eDNA from fish and mammals, communities could be distinguished from habitats separated by as little as 60m (Port *et al.*, 2016). However, spillover of eDNA between habitats has also been detected (Lafferty *et al.*, 2021). Wide-scale oceanographic surveys using eDNA have shown to represent well the catches obtained by trawling and also match known species distribution patterns of fish species, corroborating that eDNA can be used across wide spatial scales (Fraija-Fernández *et al.*, 2020).

4 Natural samplers in the ocean and invertebrate-derived DNA

Recently, the principle of natural sampler DNA (nsDNA), also frequently called biological samplers, has been put forward. It refers to DNA naturally captured by organisms occurring in the field. As an example, sponges have been considered for nsDNA due to their efficiency in filtering water. In a recent study from a coral reef environment, sponges appeared to be very successful at collecting nsDNA in their tissues: one third of the total local ichthyofauna has been recovered in sponges. Not only local coral reef fish species but also pelagic, migratory and deep-sea fish species were revealed from sponge tissues (Turon *et al.*, 2020). A number of studies have targeted predators for assessing the occurrence of their prey, such as the study by Queiros and colleagues (2021), which used Antarctic toothfish as natural samplers of cephalopods. In order to assess diversity at the community level across a wide array of taxa, generalist predators/scavengers have been targeted as natural benthic samplers in several studies. Gut content analyses of scampi were shown efficient in sampling deep-sea benthic ecosystems (van der Reis; Jeffs & Lavery, 2020) and the diet of the European brown shrimp *Crangon crangon* for monitoring biodiversity in estuarine systems (Siegenthaler *et al.*, 2019).

An offshoot of this approach is invertebrate-derived DNA or iDNA, which is the sampling of flesh-eating or hematophagous invertebrate parasites for retrieving the DNA

of their hosts. The use of iDNA is widely used in terrestrial systems, using flies, ticks or leeches (e.g., Schnell *et al.*, 2018; Schubert *et al.*, 2015); but until recently, there were no similar studies for the marine environment, despite its potential as a minimally invasive method to assess endangered or elusive species. A pioneering example from the marine realm is the sampling of parasitic or commensal copepods for assessing their host, the whale shark *Rhincodon typus*, and infer its population genetic structure based on mitochondrial DNA (mtDNA) (Meekan *et al.*, 2017).

5 A huge step for evolutionary ecology: from eDNA to population genetics

eDNA studies have shown large potential as a source of population genetic information. eDNA metabarcoding studies generate millions of reads for studying different taxa across multiple sampling sites, areas and seasons. When hypervariable metabarcoding markers are used, which were recently suggested to be applied for understanding phylogeographic patterns under the term "metaphylogeography" (Turon *et al.*, 2020), the generated large datasets also contain information at the intraspecific level. Similar results were obtained when comparing typical phylogeographic analyses (haplotype networks, AMOVA) based on Cytochrome c oxidase subunit I (COI) metabarcoding reads and traditional Sanger sequence data (Turon *et al.*, 2020). This and similar findings (Sigsgaard *et al.*, 2021) open up new possibilities to test new hypotheses in evolutionary ecology and connectivity, especially since one of the major current limitations for population genetic analyses is the need to collect a sufficient number of tissue samples. Using eDNA for assessing population dynamics is particularly useful when it acts as an indirect sampling tactic for targeting elusive, endangered, or inaccessible marine taxa. It also allows for a larger sampling window in time and space, as it increases the likelihood to detect individuals, including those that are not present at the exact time and place of sampling (Baker *et al.*, 2018). For studying harbor porpoises (*Phocoena phocoena*), eDNA from surface water was validated as novel approach for detecting genetic differentiation throughout the population's range (Parsons *et al.*, 2018). The same was true for orcas (*Orcinus orca*) (Baker *et al.*, 2018), whale sharks (Sigsgaard *et al.*, 2016) and bowhead whales (*Balaena mysticetus)* (Székely *et al.*, 2021). Since this application is still in its infancy, it will require more fine-tuning of bioinformatical and statistical analyses to correct for errors, biases and random variation in sequences and their relative abundances (Sigsgaard *et al.*, 2020). Such population genetic studies will also largely benefit from the expansion of public sequence repositories, in order to more exhaustively cover intra- and interspecific variation. Since several individuals can share the same mtDNA haplotype, targeting longer fragments or higher polymorphic regions will enhance the potential to distinguish individuals from eDNA reads (Dugal *et al.*, 2021). Genome-wide approaches based on eDNA are also becoming reality with whole mitochondrial genomes being recovered from eDNA water samples (Deiner *et al.*, 2017). Newest methods, such as Haplotype Count from eDNA or HaCeD-Seq, applicable to organisms with individual specific mitochondrial D-loop sequences, allow the determination of population sizes (Yoshitake *et al.*, 2019). This method has been successfully applied for the Pacific bluefin tuna (*Thunnus orientalis*), and was shown to detect 94% of the haplotypes present in an experimental setting (Yoshitake *et al.*, 2021).

6 eDNA for food web reconstructions, network analyses and ecosystem models

Recently, eDNA metabarcoding studies have not only been used for community composition assessments, but also for providing information about biotic interactions (e.g., predator-prey relationships, trophic linkages) that can be linked to ecosystem changes. This can be achieved using community network analyses, as in the study of (Djurhuus *et al.*, 2020). Here, metabarcoding of metazoan communities based on time-series samples provided evidence for seasonal shifts and putative interactions among taxa, through the detection of correlations between the communities and environmental conditions over time. When applied to an ecosystem over time, eDNA analyses may also allow for the identification of potential indicator species for different ecosystem states. D'Alessandro and Mariani (2021) proposed a novel method for reconstructing trophic networks based on DNA metabarcoding datasets of marine communities, combined with a literature review to identify all possible consumer-resource interactions. With this approach of simplified and rapid food web reconstructions, the authors were able to identify noteworthy features of food webs, which can be used to make predictions about impacts of environmental changes, NIS, and loss of keystone species. Finally, by using metrics for quantifying predator-prey interactions, DNA metabarcoding studies of gut contents can also be used to reconstruct food webs and evaluate the trophic niche of species with niche-base modelling (Casey *et al.*, 2019).

7 eDNA for monitoring marine resources and their exploitation

Marine resource monitoring and management is another field where eDNA has the potential to bring major advances. In fishery science, there is an increased use of data coming from fishing vessels to monitor commercially exploited fish stocks, as they may act as sentinels of changes in stock sizes and distributions. eDNA performs well for providing spatial and abundance information (evaluated in terms of relative read abundances) for several commercially exploited fish species (Fraija-Fernández *et al.*, 2020; Salter *et al.*, 2019). Hence, it could be incorporated into stock assessment programs to provide a less costly method that can cover a broad geographic space, is non-lethal for the fish and not damaging for its environment in comparison with standardized trawling efforts. Erroneous detections, or false positives, may complexify management decisions, because "there is no fish in hand" and contribute to current skepticism on the use of eDNA for such purposes (Jerde; Wilson & Dressler, 2019). However, further research will continue to further minimize this uncertainty linked to the nature of eDNA technologies. The combination of eDNA and eRNA detection could also tackle the current drawback of eDNA in that there is no way to conclude that it is sourced from a living organism or not. "Legacy" eDNA can lead to false positives whereas eRNA distinguishes the living portion of a community (Pochon *et al.*, 2017). Finally, a better understanding of the behavior of eDNA molecules in the marine environment (see section below) will increase our confidence in eDNA for management purposes.

eDNA also represents a useful tool for controlling fishery activities and fisheries enforcement. Such catch assessments rely on visual identification and quantification at sea or when landed. This can be difficult, particularly when fish is processed on board as silage (dissolved in acid) or block frozen. Here too, DNA metabarcoding and qPCR analyses were able to identify and quantify DNA from fish species in both processed catches, from run-off water, and exterior swabs of the frozen blocks (Hansen *et al.*, 2020a). Similarly,

another quantitative eDNA study to characterize catch composition based on DNA metabarcoding of the drain water from fish nets after hauling operations was shown to reliably represent the fish assemblages found and could demonstrate a relationship between species abundances in the catch and read abundances (Russo *et al.*, 2021).

eDNA has also proven its efficiency for providing crucial insights into the ecology and migration patterns of fish species. Species-specific qPCR arrays can be used to characterize spawning grounds where traditional surveys fail to identify the precise areas. For example, the Japanese eel (*Anguilla japonica*) spawns at sea and subsequently migrates to freshwater sites where it is extensively raised for aquaculture. For this species, a near real-time identification of spawning aggregations was achieved with highly sensitive eDNA probes (Takeuchi *et al.*, 2019). DNA metabarcoding of gentoo penguin scats within an MPA was used to track their diet and showed that there is an increase in potential of spatial overlap between gentoo penguin foraging and krill fisheries (Ratcliffe *et al.*, 2021). This is an example of eDNA providing a basis for informed management decisions in commercially important krill fisheries.

Drawbacks, challenges and uncertainties in marine eDNA studies

1 To count or not to count, that is the question

The question whether eDNA can be used beyond mere presence-absence detections, to reliably assess biomass and abundances of species, has been discussed since the early years of eDNA research (Bohmann *et al.*, 2014). For several fish species, it has been demonstrated that eDNA successfully monitors the abundances of particular species, over temporal or spatial scales. A large number of DNA metabarcoding studies show a strong overall correlation between frequency of species detection and read abundance. A range of evidence exists from fish studies, comparing eDNA with other methods such as visual surveys or traditional trawling assessments (Mariani *et al.*, 2021; Stoeckle *et al.*, 2021; Thomsen *et al.*, 2016). However, in some cases eDNA failed to reliably assess fish biomass, when compared with trawling efforts (Knudsen *et al.*, 2019). Detection discrepancies can occur when abundances are very low (Salter *et al.*, 2019).

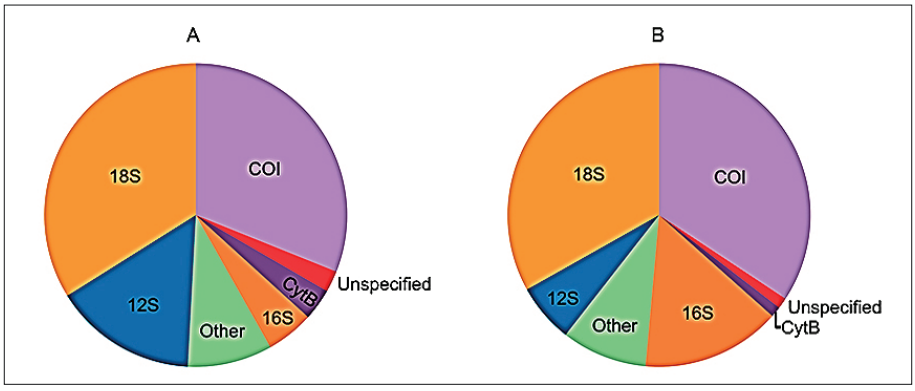
qPCR provides a quantitative measurement of eDNA molecules, which have been extensively validated through both experimental and field studies, again, mostly with fish (Horiuchi *et al.*, 2019; Kuwae *et al.*, 2020; Tsuji *et al.*, 2020). A similar study, quantifying eDNA with qPCR in experimental settings and subsequently validating the arrays in the field, was also achieved for jellyfish (Minamoto *et al.*, 2017). Attempts to assess abundances or biomass with eDNA also included other taxa. Examples include a cold-water coral (Kutti *et al.*, 2020) and a sea star species (Uthicke; Lamare & Doyle, 2018), both developing qPCR assays based on aquarium experiments prior to field assessments. However, as is the case with qPCR, too low concentrations of the target taxa DNA may prevent them from being accurately quantified (e.g., Postaire *et al.*, 2020).

2 The dominance of single over multiple markers in marine eDNA studies

In order to be able to assess entire communities, one has to use universal or generic primers for metabarcoding. A major drawback when doing so, is the reputed primer bias, leading to certain sequences (from certain species) amplifying less efficiently than others (Deagle *et al.*, 2014). Hence, species with the highest primer affinity will be overrepresented

in the community, or even inhibit the amplification of rarer taxa or those with a lower primer affinity. Therefore, studies focusing on communities at the eukaryote or metazoan level will often use multiple markers (e.g., Zhang *et al.*, 2020). The variable regions within the conserved nuclear small-subunit ribosomal RNA genes 18S and 28S rRNA are often used for studies aiming to identify taxa from the phylum to the family level but are generally not able to identify the sequences to species level. On the contrary, mitochondrial markers (e.g., COI, 16S and 12S rRNA) provide a better taxonomic resolution, allowing for species differentiation, but owing to less conserved primer regions (e.g., in COI), certain taxa may not be amplified (false negatives) (Laakmann *et al.*, 2020). In marine eDNA studies, almost 70% of all studies evaluated here used only a single marker, the majority of them using COI and 18S rRNA (Figure 6). Those studies that applied multiple markers often used a combination of those two markers. Few studies have focused so far on developing primers applicable for the study of specific marine organisms (Figure 4). 12S rRNA was more frequently used in single marker studies, for those targeting fish communities. 16S rRNA was more widely used in combination with other markers than it was in single-gene studies. The choice of a certain marker is also largely influenced by the availability of reference sequences for comparison. A certain marker may more reliably allow species differentiation of a particular taxon, whereas it may be limited for primer design and in terms of completeness of the reference database. Moreover, some markers may reveal diverse and abundant communities of a certain taxonomic group whereas these are barely detected with another conventional marker (Günther *et al.*, 2018; Santoferrara, 2019). COI of many ctenophores are practically impossible to amplify with universal primers (Christianson *et al.*, 2021), and therefore these taxa remain undetected, whereas 18S rRNA analyses do detect them (Günther *et al.*, 2018). As an example, abundant octocoral species were not detected in a coral reef eDNA metabarcoding community study, which could be due to primer biases, amongst other factors influencing eDNA detection (DiBattista *et al.*, 2019). Such issues warrant a concurrent use of several markers, which clearly deserves further attention in marine eDNA studies.

Figure 6 – Proportions of studies using either A. single, or B. multiple markers, specifying the markers used in those studies. "Other" category includes markers that appeared in 4 or less studies: ITS, D-loop, rbcL, 28S, ND4, Mitochondrial control region, Microsatellites, 23S, mutS, tRNA and species-specific assays



3 The need for comprehensive and taxonomically validated reference databases

The accuracy of DNA read assignment to a certain species in DNA metabarcoding largely depends on the completeness of the reference database. Typically, public sequence

repositories (GenBank, BOLD) are used, however, despite the great advantages they offer, these massive public databases are naturally error-rich and incomplete (Radulovici *et al.*, 2021). The design of appropriate species-specific qPCR markers and probes also largely depends on the availability of sequences of the target taxa, and as many different haplotypes as possible, to ensure that the markers are specific to one target species without cross-amplification with closely related species (Ardura, 2019). The database coverage of both GenBank and BOLD were tested for all reported macrofaunal species from the North Sea, for the most commonly used metabarcoding genes COI and 18S rRNA. A coverage of 50% or less was found for COI, and for 18S, only ca. 36% (Hestetun *et al.*, 2020), showing that even for a well-surveyed area, a high number of missing reference sequences can severely hamper biodiversity studies.

Recent progress in the further development of interactive tools linked to barcodes and reads from public databases will significantly enhance future eDNA research, as well as the use of eDNA studies for in-depth biogeographic and population genetic studies. The Ocean Barcode Atlas web service (Vernette *et al.*, 2021) directly links sequences to environmental parameters at the locality and time of sampling and mapping. Curated databases also provide a solution, such as the MARine Eukaryote Species (MARES) reference database, including all COI sequences available in GenBank and BOLD for marine taxa, unified into a single taxonomy (Arranz *et al.*, 2020). The COI barcode library for plankton studies has also seen significant improvement during last years (Bucklin *et al.*, 2021). Nonetheless, no consensus has yet been achieved on a general marker to be used for zooplankton, as the completeness of reference databases varies strongly between taxa (Bucklin *et al.*, 2021).

A number of studies have concentrated efforts on improving reference databases for certain localities or organisms. Oliveira and colleagues (2016) have done so for marine fish, and assigned the publicly available barcodes to the different species. Discordances were assessed and evaluated, species with a high intraspecific variability were detected, and species in need of taxonomic re-evaluation were highlighted. A similar approach was applied by Radulovici *et al.* (2021), who reviewed and annotated publicly available sequences of crustaceans, echinoderms, molluscs and polychaetes. Moreover, several studies have enriched the databases with sequences of species present in their target area, accompanied with a comprehensive set of metadata. An example is the Biocode approach, used to construct a regional reference database characterizing the marine fauna of a marine park in Singapore, along with a rich documentation, including photographs of all specimens sequenced (Ip *et al.*, 2019). Major initiatives such as the Tara Oceans expedition made genomics datasets publicly available, from viruses to metazoan marine plankton sequences together with their metadata (Alberti *et al.*, 2017). Finally, other techniques are being developed, which do aim to feed the databases but rather overcome biases linked to eDNA sequences remaining unassigned, for example, using supervised machine learning (Cordier *et al.*, 2017).

4 eDNA in the physical environment: decay vs. persistence and dispersal

Compared to other aquatic systems, the marine system is a much larger water body and a much more complex three-dimensional environment, with various processes at play influencing eDNA persistence in the water. As mentioned before, the detection of eDNA is no evidence that the species in question is present, since the location, time and nature of the

eDNA source remain unknown. Environmental and physical processes in the marine environment (e.g., processes of advection, mixing, decay, and gravitational settling) may rapidly dilute and disperse eDNA fragments, hence influencing the concentrations in the water column (Foote *et al.*, 2012). The transport of eDNA with currents over great distances may induce false-positive species detection, generating inaccurate community assessments (Jeunen *et al.*, 2020).

Compared to freshwater systems and organisms, less marine eDNA studies have been carried out with an experimental focus to determine the shedding and detection of eDNA for a known biomass of organisms. From freshwater-focused studies, we know that temperature, ultraviolet radiation and pH influence eDNA degradation (Strickler; Fremier & Goldberg, 2015). Hence, eDNA is hypothesized to remain detectable for longer periods in colder, less irradiated and more alkaline waters (Strickler; Fremier & Goldberg, 2015). In the same line, in the coastal Arctic eDNA study of Sevellec *et al.* (2021), the rather homogeneous eDNA signal over a wider spatial scale could be explained by the colder temperatures preserving eDNA over a longer time. Such differences in eDNA persistence need a better understanding for the marine environment and future studies need to consider the potential differences between different oceanic regions. Sediment is also known to harbor much higher concentrations of eDNA than found in the water column, likely due to particle settling or a delayed degradation of eDNA molecules that adsorb to sediment particles (Turner; Uy & Everhart, 2015). This was also confirmed for the marine environment, in the case of jellyfish (Minamoto *et al.*, 2017). In the marine system, microbial nutrient limitation has been demonstrated to play an additional role in the persistence of dissolved eDNA (Salter, 2018). Murakami and colleagues (2019) performed experiments with caged fish in a dynamic coastal system, showing that eDNA can be successfully detected 1h after removal of the fish and up to 1000m away.

In-situ studies have also aimed to assess the influence of different oceanographic features on eDNA signal. Tides are likely to influence eDNA detection and concentrations. However, a study targeting a sand-flat showed that there was no significant variation in species composition recovered with eDNA on the incoming tide compared with the outgoing tide (Lafferty *et al.*, 2021). On the contrary, water stratification, i.e. in the form of a strong halocline, was demonstrated to restrict the vertical dispersal of eDNA in the water column (Jeunen *et al.*, 2020).

Although abiotic factors are quite often considered to be the main factors influencing eDNA decay, consideration of biotic factors can be just as significant. As an example, Friebertshauser *et al.* (2019) showed that bivalve filtration activity may lead to obstructed detection of targeted species in near-bottom eDNA samples. An example of biotic factors influencing the detection of eDNA is the presence of the colonial waterbirds, acting as a potential source of contamination by contributing DNA of their prey species to the water body sampled (Guilfoyle & Schultz, 2017). The aforementioned studies, investigating eDNA detection and decay *in situ*, were focused on freshwater organisms; similar studies are currently missing for the marine realm.

The next steps for eDNA studies in the marine realm

The drive to develop more cost effective, less invasive and less labour-intensive methods has led to some interesting developments in eDNA technology. Williams et al. (2021) developed a CRISPR-Cas-based eDNA assay for rapid detection of a single species.

In a field-based validation experiment, it performed similarly to qPCR for detecting absence and presence of Atlantic salmon (*Salmo salar*), showing potential as an accurate and cheap on-site detection tool. More emerging technologies, such as the MinION sequencing device (Oxford Nanopore Technologies), will render eDNA in-field analyses more accessible and adaptable (e.g., Gowers *et al.*, 2019; Truelove; Andruszkiewicz & Block, 2019). The passive collection of eDNA with submerged filter membranes, as opposed to active filtration of water samples, which is often time-consuming and varies in methodology across studies, has shown potential in both tropical and marine systems (Bessey *et al.*, 2021). Yamahara and colleagues (2019) recently developed an autonomous sampling device installed on an autonomous underwater vehicle (AUV), which was able to effectively collect eDNA from a broad range of taxa (microbes to fish), in areas without human presence. This Environmental Sample Processor (ESP) can perform water sample filtration, storage and *in-situ* genetic identification (Hansen *et al.*, 2020b) via 'ecogenomic sensors' (Scholin, 2010), which should drastically enhance spatial and temporal coverage of the sampling regions (Yamahara *et al.*, 2019). It has been shown that such devices can take dozens of samples per time while moving freely across the ocean for several days, and the quality of the samples taken is comparable to classical sampling methods (Yamahara *et al.*, 2019). Moreover, a recent comparison of *in-situ* ESP and laboratory qPCR analyses showed that both methods can well detect the most abundant organisms (Hansen *et al.*, 2020b).

As discussed earlier, location, time, and nature of the eDNA source are unknown. An eDNA fate and transport modelling framework can help to address these unknowns. Such modelling frameworks should include high resolution regional oceanographic models combined together with particle tracking models (Andruszkiewicz *et al.*, 2019, Dagestad *et al.*, 2017). However, setting up the combination of such models requires the clarification of multiple parameters and steps: 1) quantifying eDNA concentration in the environment; 2) quantifying the eDNA source; 3) quantifying the decay rate of eDNA molecules; and 4) model application and validation. When solving these parameters, we will be able to more precisely trace the eDNA particles and find their origin. To do so, autonomous vehicles such as AUVs, equipped with ESPs, could be programmed to detect and follow the potential 'hot spots' of the targeted organisms.

eDNA research provides a promising opportunity for both the utilization of citizen science as well as further involvement of indigenous peoples and members of communities who are often excluded or disconnected from scientific research in their surroundings (Handsley-Davis *et al.*, 2021). Citizen science holds the potential to allow for rapid and widescale, simultaneous sampling with minimal training and reduced costs, across a wide range of habitats and ecosystems. A successful citizen science campaign was undertaken with minimal training by Biggs *et al.* (2015) on the Great Crested Newt in freshwater environments. Its use is so far still limited for the marine environment. Howell, LaRue and Flanagan (2021) suggest the Antarctic research communities and tourists to join efforts for standardized eDNA surveys, of which Cusick *et al.* (2020), using tourist activities for research, is an example. Lacoursière-Roussel *et al.* (2018) successfully combined both by involving indigenous community members and local research staff in a coastal biodiversity study across two remote ports in the Canadian Arctic.

One thing that is clear: eDNA has the prospect to significantly enhance, improve, and facilitate a better understanding of the global biogeography, macroecology and biodiversity changes. This all-encompassing system of observations has been referred to as "the

macroscope" (Dornelas *et al*., 2019). Together with an array of other advanced technologies and recent methods, such as satellites, drones, passive samplers and biologgers, we will be able to achieve an unprecedented, comprehensive monitoring effort, turning these efficient methods into more than the sum of their parts, as cited by Dornelas *et al.* (2019). As an example, in a recent biologger-based investigation into predator niche segregation of the Risso's dolphin and Cuvier's beaked whales, eDNA was used to simultaneously assess the community composition of their preferred prey (cephalopods) (Visser *et al*., 2021). Further developments in molecular technologies and sequencer performances will further boost eDNA research, which will be increasingly combined with metagenomics, meta-transcriptomics, proteomics, metabolomics and epigenetics. As mentioned above, simultaneous eDNA and eRNA surveys can help to distinguish "legacy" DNA from living organisms present in the environment. Such comparative studies can significantly improve our understanding of eDNA transport and decay. Whereas eDNA mostly serves to characterize species and communities in terms of taxonomy and diversity, these different methods will shed light on the functional processes characterizing them.

Strengthening interactions between eDNA and physical platforms around the world ocean can bring unprecedented amounts of high-quality biodiversity data tied to its environmental data. These data can subsequently be used in community distribution modelling (Foster *et al.*, 2013; Ovaskainen & Abrego, 2020), which will help us more clearly define species niches, interactions between species, identify ecoregions, and determine which of these regions are most vulnerable to ongoing and predicted environmental changes, in terms of NIS arrivals or range shifts leading to the disruption of local food webs. Such places may have a special priority for further eDNA monitoring, which can be in the shape of surveys with autonomous technologies such as AUVs. Subsequently, in these areas, the most susceptible species to climate change can be identified as being most threatened. Based on this information, vulnerable species niches, as well as parts of ecoregions, can be considered for further protection actions. To conclude, eDNA certainly takes biodiversity studies to the next level and has the potential to tremendously facilitate conservation efforts over the next decade, which is also the United Nations Decade of Ocean Science for Sustainable Development.

Acknowledgements – This study has been conducted in the framework of the Helmholtz Young Investigator Group "ARJEL – Arctic Jellies" (project number VH-NG-1400), funded by the Helmholtz Society and the Alfred Wegener Institute Helmholtz Centre for Polar and Marine Research.

REFERENCES

Ames, C.L.; Ohdera, A.H.; Colston, S.M.; Collins, A.G.; Fitt, W.K.; Morandini, A.C.; Erickson, J.S. & Vora, G.J. Fieldable environmental DNA sequencing to assess Jellyfish Biodiversity in Nearshore Waters of the Florida Keys, United States. *Frontiers in Marine Science,* v. 8, n. 369, 2021.

Alberti, A.; Poulain, J.; Engelen, S.; Labadie, K.; Romac, S.; Ferrera, I.; Albini, G.; Aury, J.-M.; Belser, C.; Bertrand, A.; Cruaud, C.; Da Silva, C.; Dossat, C.; Gavory, F.; Gas, S.; Guy, J.; Haquelle, M.; Jacoby, E.; Jaillon, O.; Lemainque, A.; Samson, G.; Wessner, M., Genoscope

Technical Team, Acinas, S.G.; Royo-Llonch, M.; Cornejo-Castillo, F.; Logares, R.; Fernandez-Gomez, B.; Bowler, C.; Cochrane, G.; Amid, C.; Ten Hoopen, P.; De Vargas, C.; Grimsley, N.; Desgranges, E.; Kandels-Lewis, S.; Ogata, H.; Poulton, N.; Sieracki, M.E.; Stepanauskas, R.; Sullivan, M.B.; Brum, J.R.; Duhaime, M.B.; Poulos, B.T.; Hurwitz, B.L.; Tara Oceans Consortium Coordinators; Pesant, S.; Karsenti, E. & Wincker, P. Viral to metazoan marine plankton nucleotide sequences from the Tara Oceans expeditions. *Scientific Data*, v. 4, p. 170093, 2017.

Andruszkiewicz, E.A.; Koseff, J.R.; Fringer, O.B.; Ouellette, N.T.; Lowe, A.B.; Edwards, C.A. & Boehm, A.B. Modeling environmental DNA transport in the coastal ocean using Lagrangian particle tracking. *Frontiers in Marine Science,* v. 6, n. 477, 2019.

Andruszkiewicz, E.A.; Starks, H.A.; Chavez, F.P.; Sassoubre, L.M.; Block, B.A. & Boehm, A.B. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PloS One,* v. 12, n. 4, e0176343, 2017.

Ardura, A. Species-specific markers for early detection of marine invertebrate invaders through eDNA methods: gaps and priorities in GenBank as database example. *Journal for Nature Conservation,* v. 47, p. 51-57, 2019.

Arranz, V.; Pearman, W.S.; Aguirre, J.D. & Liggins, L. MARES, a replicable pipeline and curated reference database for marine eukaryote metabarcoding. *Scientific Data,* v. 7, n. 1, p. 1-8, 2020.

Bachiller, E.; Albo-Puigserver, M.; Giménez, J.; Pennino, M.G.; Marí-Mena, N.; Esteban, A.; Lloret-Lloret, E.; Jadaud, A.; Carro, B. & Bellido, J.M. A trophic latitudinal gradient revealed in anchovy and sardine from the Western Mediterranean Sea using a multi-proxy approach. *Scientific Reports,* v. 10, n. 1, p. 1-18, 2020.

Baker, C.S.; Steel, D.; Nieukirk, S. & Klinck, H. Environmental DNA (eDNA) from the wake of the whales: droplet digital PCR for detection and species identification. *Frontiers in Marine Science,* v. 5, 133, 2018.

Balint, M.; Pfenninger, M.; Grossart, H.-P.; Taberlet, P.; Vellend, M.; Leibold, M.A.; Englund, G. & Bowler, D. Environmental DNA time series in ecology. *Trends in Ecology & Evolution,* v. 33, n. 12, p. 945-957, 2018.

Bernardino, A.F.; Pais, F.S.; Oliveira, L.S.; Gabriel, F.A.; Ferreira, T.O. Queiroz, H.M. & Mazzuco, A.C.A. Chronic trace metals effects of mine tailings on estuarine assemblages revealed by environmental DNA. *PeerJ,* v. 7, p. e8042, 2019.

Berry, T.E.; Saunders, B.J.; Coghlan, M.L.; Stat, M.; Jarman, S.; Richardson, A.J.; Davies, C.H.; Berry, O.; Harvey, E.S. & Bunce, M. Marine environmental DNA biomonitoring reveals seasonal patterns in biodiversity and identifies ecosystem responses to anomalous climatic events. *PLoS Genetics, v. 15,* n. 2, p. e1007943, 2019.

Bessey, C.; Jarman, S.N.;, Simpson, T.; Miller, H.; Stewart, T.; Keesing, J.K. & Berry, O. Passive eDNA collection enhances aquatic biodiversity analysis. *Communications biology,* v. 4, n. 1 , p. 1-12, 2021.

Bessey, C.; Jarman, S.N.; Stat, M.; Rohner, C.A.; Bunce, M.; Koziol, A.; Power, M.; Rambahiniarison, J.M.; Ponzo, A. & Richardson, A.J. DNA metabarcoding assays reveal a

diverse prey assemblage for *Mobula* rays in the Bohol Sea, Philippines. *Ecology and Evolution,* v. 9, n. 5, p. 2459-2474, 2019.

Biggs, J.; Ewald, N.; Valentini, A.; Gaboriaud, C.; Dejean, T.; Griffiths, R.A.; Foster, J.; Wilkinson, J.W.; Arnell, A. & Brotherton, P. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation,* v. 183, p. 19-28, 2015.

Bohmann, K.; Evans, A.; Gilbert, M.T.P.; Carvalho, G.R.; Creer, S.; Knapp, M.; Douglas, W.Y. & De Bruyn, M. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution,* v. 29, n. 6, p. 358-367, 2014.

Boulanger, E.; Loiseau, N.; Valentini, A.; Arnal, V.; Boissery, P.; Dejean, T.; Deter, J.; Guellati, N.; Holon, F. & Juhel, J.-B. Environmental DNA metabarcoding reveals and unpacks a biodiversity conservation paradox in Mediterranean marine reserves. *Proceedings of the Royal Society B,* v. 288, n. 1949, p. 20210112, 2021.

Bowers, H.A.; Pochon, X.; von Ammon, U.; Gemmell, N.; Stanton, J.-A.L.; Jeunen, G.-J.; Sherman, C.D. & Zaiko, A. Towards the optimization of eDNA/eRNA sampling technologies for Marine Biosecurity Surveillance. *Water,* v. 13, n. 8, p. 1113, 2021.

Brandt, M.I.; Pradillon, F.; Trouche, B.; Henry, N.; Liautard-Haag, C.; Cambon-Bonavita, M.-A.; Cueff-Gauchard, V.; Wincker, P.; Belser, C. & Poulain, J. Evaluating sediment and water sampling methods for the estimation of deep-sea biodiversity using environmental DNA. *Scientific Reports,* v. 11, n. 1, p. 1-14, 2021.

Braukmann, T.W.; Ivanova, N.V.; Prosser, S.W.; Elbrecht, V.; Steinke, D.; Ratnasingham, S.; De Waard, J.R.; Sones, J.E.; Zakharov, E.V. & Hebert, P.D. Metabarcoding a diverse arthropod mock community. *Molecular Ecology Resources,* v. 19, n. 3, p. 711-727, 2019.

Bucklin, A.; Peijnenburg, K.T.; Kosobokova, K. & Machida, R.J. New insights into biodiversity, biogeography, ecology, and evolution of marine zooplankton based on molecular approaches. *ICES Journal of Marine Science,* 2021.

Buxton, A.; Matechou, E.; Griffin, J.; Diana, A. & Griffiths, R.A. Optimising sampling and analysis protocols in environmental DNA studies. *Scientific Reports,* v. 11, n. 1, p. 1-10, 2021.

Carvalho, S.; Aylagas, E.; Villalobos, R.; Kattan, Y.; Berumen, M. & Pearman, J.K. Beyond the visual: using metabarcoding to characterize the hidden reef cryptobiome. *Proceedings of the Royal Society B, v. 286,* n. 1896, p. 20182697, 2019.

Casey, J. M.; Meyer, C.P.; Morat, F.; Brandl, S.J.; Planes, S. & Parravicini, V. Reconstructing hyperdiverse food webs: gut content metabarcoding as a tool to disentangle trophic interactions on coral reefs. *Methods in Ecology and Evolution,* v. 10, n. 8, p. 1157-1170, 2019.

Cavallo, C.; Chiaradia, A.; Deagle, B.E.; McInnes, J.C.; Sanchez, S.; Hays, G.C. & Reina, R.D. Molecular analysis of predator scats reveals role of salps in temperate inshore food webs. *Frontiers in Marine Science,* v. 5, n. 381, 2018.

Christianson, L.; Johnson, S.; Schultz, D. & Haddock, S. Hidden diversity of Ctenophora revealed by new mitochondrial COI primers and sequences. *Authorea Preprints*, 2021.

Clarke, L.J.; Trebilco, R.; Walters, A.; Polanowski, A.M. & Deagle, B.E. DNA-based diet analysis of mesopelagic fish from the southern Kerguelen Axis. *Deep Sea Research Part II: Topical Studies in Oceanography,* v. 174, 2020.

Closek, C.J.; Santora, J.A.; Starks, H.A.; Schroeder, I.D.; Andruszkiewicz, E.A.; Sakuma, K.M.; Bograd, S.J.; Hazen, E.L.; Field, J.C. & Boehm, A.B. Marine vertebrate biodiversity and distribution within the central California Current using environmental DNA (eDNA) metabarcoding and ecosystem surveys. *Frontiers in Marine Science,* v. 6, n. 732, 2019.

Collins, P.C.; Hunter, W.R.; Carlsson, J. & Carlsson, J. Fortuitous insights into the ecology of a recently charted deep-sea hydrothermal vent, using snails' feet. *Deep Sea Research Part I: Oceanographic Research Papers, v. 163*, n. 103358, 2020.

Cordier, T.; Esling, P.; Lejzerowicz, F.; Visco, J.; Ouadahi, A.; Martins, C.; Cedhagen, T.; & Pawlowski, J. Predicting the ecological quality status of marine environments from eDNA metabarcoding data using supervised machine learning. *Environmental Science & Technology, v. 51, n.* 16, p. 9118-9126, 2017.

Cordier, T.; Frontalini, F.; Cermakova, K.; Apothéloz-Perret-Gentil, L.; Treglia, M.; Scantamburlo, E.; Bonamin, V. & Pawlowski, J. Multi-marker eDNA metabarcoding survey to assess the environmental impact of three offshore gas platforms in the North Adriatic Sea (Italy). *Marine Environmental Research,* v. 146, p. 24-34, 2019.

Cowart, D.A.; Murphy, K.R. & Cheng, C.-H.C. Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula. *Marine Genomics,* v. 37, p. 148-160, 2018.

Creer, S.; Deiner, K.; Frey, S.; Porazinska, D.; Taberlet, P.; Thomas, W.K.; Potter, C. & Bik, H.M. The ecologist's field guide to sequence-based identification of biodiversity. *Methods in Ecology and Evolution,* v. 7, n. 9, p. 1008-1018, 2016.

Cusick, A.M.; Gilmore, R.; Bombosch, A.; Mascioni, M.; Almandoz, G.O. & Vernet, M. Polar tourism as an effective research tool. *Oceanography,* v. 33, n. 1, p. 50-61, 2020.

D'Alessandro, S. & Mariani, S. Sifting environmental DNA metabarcoding data sets for rapid reconstruction of marine food webs. *Fish and Fisheries,* 2021.

Dagestad, K.F.; Röhrs, J.; Breivik, Ø. & Adlandsvik, B. OpenDrift v1.0: a generic framework for trajectory modeling. *Geoscientific Model Development*, v. 11, p. 1-28, 2017.

Darling, J. A.; Martinson, J.; Gong, Y.; Okum, S.; Pilgrim, E.; Lohan, K.M.P.; Carney, K.J. & Ruiz, G.M. Ballast water exchange and invasion risk posed by intracoastal vessel traffic: an evaluation using high throughput sequencing. *Environmental Science & Technology,* v. 52, n. 17, p. 9926-9936, 2018.

Davidov, K.; Iankelevich-Kounio, E.; Yakovenko, I.; Koucherov, Y.; Rubin-Blum, M. & Oren, M. Identification of plastic-associated species in the Mediterranean Sea using DNA metabarcoding with Nanopore MinION. *Scientific Reports,* v. 10, n. 1, p. 1-11, 2020.

De la Iglesia, R.; Echenique-Subiabre, I.; Rodríguez-Marconi, S.; Espinoza, J.P.; von Dassow, P.; Ulloa, O. & Trefault, N. Distinct oxygen environments shape picoeukaryote assemblages

thriving oxygen minimum zone waters off central Chile. *Journal of Plankton Research,* v. 42, n. 5, p. 514-529, 2020.

Deagle, B.E.; Chiaradia, A.; McInnes, J. & Jarman, S.N. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? *Conservation Genetics,* v. 11, n. 5, p. 2039-2048, 2010.

Deagle, B.E.; Jarman, S.N.; Coissac, E.; Pompanon, F. & Taberlet, P. DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biology letters,* v. 10, n. 9, p. 20140562, 2014.

Deagle, B.E.; Kirkwood, R. & Jarman, S.N. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology,* v. 18, n. 9, p. 2022-2038, 2009.

Deagle, B.E.; Thomas, A.C.; Shaffer, A.K.; Trites, A.W. & Jarman, S.N. Quantifying sequence proportions in a DNA-based diet study using Ion Torrent amplicon sequencing: which counts count? *Molecular Ecology Resources,* v. 13, n. 4, p. 620-633, 2013.

Deiner, K.; Renshaw, M.A.; Li, Y.; Olds, B.P.; Lodge, D.M. & Pfrender, M.E. Long-range PCR allows sequencing of mitochondrial genomes from environmental DNA. *Methods in Ecology and Evolution,* v. 8, n. 12, p. 1888-1898, 2017.

DiBattista, J.D.; Reimer, J.D.; Stat, M.; Masucci, G.D.; Biondi, P.; De Brauwer, M. & Bunce, M. Digging for DNA at depth: rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. *PeerJ,* v. 7, n. e6379, 2019.

Djurhuus, A.; Closek, C.J.; Kelly, R.P.; Pitz, K.J.; Michisaki, R.P.; Starks, H.A.; Walz, K.R.; Andruszkiewicz, E.A.; Olesin, E. & Hubbard, K. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nature Communications,* v. 11, n. 1, p. 1-9, 2020.

Dornelas, M.; Madin, E.M.; Bunce, M.; DiBattista, J.D.; Johnson, M.; Madin, J.S.; Magurran, A.E.; McGill, B.J.; Pettorelli, N. & Pizarro, O. Towards a macroscope: leveraging technology to transform the breadth, scale and resolution of macroecological data. *Global Ecology and Biogeography*, 2019.

Duarte, S.; Vieira, P.E.; Lavrador, A.S. & Costa, F.O. Status and prospects of marine NIS detection and monitoring through (e) DNA metabarcoding. *Science of The Total Environment*, 141729, 2020.

Dugal, L.; Thomas, L.; Reinholdt Jensen, M.; Sigsgaard, E.E.; Simpson, T.; Jarman, S.; Thomsen, P.F. & Meekan, M. Individual haplotyping of whale sharks from seawater environmental DNA. *Molecular Ecology Resources*, 2021.

Duke, E.M. & Burton, R.S. Efficacy of metabarcoding for identification of fish eggs evaluated with mock communities. *Ecology and Evolution,* v. 10, n. 7, p. 3463-3476, 2020.

Easson, C.G.; Boswell, K.M.; Tucker, N.; Warren, J.D. & Lopez, J.V. Combined eDNA and acoustic analysis reflects diel vertical migration of mixed consortia in the Gulf of Mexico. *Frontiers in Marine Science*, v. 7, n. 552, 2020.

Elbrecht, V. & Leese, F. Validation and development of COI metabarcoding primers for freshwater macroinvertebrate bioassessment. *Frontiers in Environmental Science*, v. 5, n. 11, 2017.

Falkowski, P.G. & Raven, J.A. *Aquatic photosynthesis*. Princeton University Press, 2013.

Fediajevaite, J.; Priestley, V.; Arnold, R. & Savolainen, V. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecology and Evolution*, v. 11, n. 9, p. 4803-4815, 2021.

Foote, A.D.; Thomsen, P.F.; Sveegaard, S.; Wahlberg, M.; Kielgast, J.; Kyhn, L.A.; Salling, A.B.; Galatius, A.; Orlando, L. & Gilbert, M.T.P. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PloS One*, v. 7, n. 8, 2012.

Foster, S.; Givens, G.; Dornan, G.; Dunstan, P. & Darnell, R. Modelling biological regions from multi-species and environmental data. *Environmetrics*, v. 24, n. 7, p. 489-499, 2013.

Fraija-Fernández, N.; Bouquieaux, M.C.; Rey, A.; Mendibil, I.; Cotano, U.; Irigoien, X.; Santos, M. & Rodríguez-Ezpeleta, N. Marine water environmental DNA metabarcoding provides a comprehensive fish diversity assessment and reveals spatial patterns in a large oceanic area. *Ecology and Evolution*, v. 10, n. 14, p. 7560-7584, 2020.

Frainer, A.; Primicerio, R.; Kortsch, S.; Aune, M.; Dolgov, A.V.; Fossheim, M. & Aschan, M.M. Climate-driven changes in functional biogeography of Arctic marine fish communities. *Proceedings of the National Academy of Sciences*, v. 114, n. 46, p. 12202-12207, 2017.

Freer, J.J.; Daase, M. & Tarling, G.A. Modelling the biogeographic boundary shift of Calanus finmarchicus reveals drivers of Arctic Atlantification by subarctic zooplankton. *Global Change Biology*, 2021.

Freire, M.; Genzano, G.N.; Neumann-Leitão, S. & Pérez, C.D. The non-indigenous medusa *Blackfordia virginica* (Hydrozoa, Leptothecata) in tropical Brazil: 50 years of unnoticed presence. *Biological Invasions*, v. 16, n. 1, p. 1-5, 2014.

Friebertshauser, R.; Shollenberger, K.;, Janosik, A.; Garner, J.T. & Johnston, C. The effect of bivalve filtration on eDNA-based detection of aquatic organisms. *PloS One*, v. 14, n. 11, e0222830, 2019.

Gaynor, J.J.; Bologna, P.A.; Restaino, D.J. & Barry, C.L. qPCR detection of early life history stage *Chrysaora quinquecirrha* (sea nettles) in Barnegat Bay, New Jersey. *Journal of Coastal Research,* n. 78 (10078), p. 184-192, 2017.

Geraldi, N.R.; Ortega, A.; Serrano, O.; Macreadie, P.I.; Lovelock, C.E.; Krause-Jensen, D.; Kennedy, H.; Lavery, P.S.; Pace, M.L. & Kaal, J. Fingerprinting blue carbon: rationale and tools to determine the source of organic carbon in marine depositional environments. *Frontiers in Marine Science*, v. 6, n. 263, 2019.

Gold, Z.; Sprague, J.; Kushner, D.J.; Zerecero Marin, E. & Barber, P.H. eDNA metabarcoding as a biomonitoring tool for marine protected areas. *PloS One*, v. 16, n. 2, e0238557, 2021.

Govindarajan, A.; Francolini, R.; Jech, J.M.; Lavery, A.C.; Llopiz, J.; Wiebe, P.H. & Zhang, W.G. Exploring the use of environmental DNA (eDNA) to detect animal taxa in the mesopelagic zone. *Frontiers in Ecology and Evolution*, v. 9, n. 146, 2021.

Gowers, G.-O.F.; Vince, O.; Charles, J.-H.; Klarenberg, I.; Ellis, T. & Edwards, A. Entirely off-grid and solar-powered DNA sequencing of microbial communities during an ice cap traverse expedition. *Genes,* v. 10, n. 11, 902, 2019.

Granquist, S.M.; Esparza-Salas, R.; Hauksson, E.; Karlsson, O. & Angerbjörn, A. Fish consumption of harbour seals (*Phoca vitulina*) in north western Iceland assessed by DNA metabarcoding and morphological analysis. *Polar Biology*, v. 41, n. 11, p. 2199-2210, 2018.

Guardiola, M.; Uriz, M.J.; Taberlet, P.; Coissac, E.; Wangensteen, O.S. & Turon, X. Deep-sea, deep-sequencing: metabarcoding extracellular DNA from sediments of marine canyons. *PloS One*, v. 10, n. 10, e0139633, 2015.

Guilfoyle, M.P. & Schultz, M.T. The contribution of double-crested cormorants (*Phalacrocorax auritus*) to silver carp (*Hypophthalmichthys molitrix*) DNA loads in the Chicago Area Waterway System. *Journal of Great Lakes Research*, v. 43, n. 6, p. 1181-1185, 2017.

Günther, B.; Knebelsberger, T.; Neumann, H.; Laakmann, S. & Arbizu, P.M. Metabarcoding of marine environmental DNA based on mitochondrial and nuclear genes. *Scientific Reports*, v. 8, n. 1, p. 1-13, 2018.

Gutierrez-Rodriguez, A.; Stukel, M.R.; dos Santos, A.L.; Biard, T.; Scharek, R.; Vaulot, D.; Landry, M.R. & Not, F. High contribution of Rhizaria (Radiolaria) to vertical export in the California Current Ecosystem revealed by DNA metabarcoding. *The ISME Journal*, v. 13, n. 4, p. 964-976, 2019.

Handsley-Davis, M.; Kowal, E.; Russell, L. & Weyrich, L.S. Researchers using environmental DNA must engage ethically with Indigenous communities. *Nature Ecology & Evolution*, v. 5, n. 2, p. 146-148, 2021.

Hansen, B.K.; Farrant, G.K.; Ogden, R.; Humble, E.; Ólafsdóttir, G.; Bekkevold, D.; Knudsen, S.W.; Møller, P.R. & Nielsen, E.E. From DNA to biomass: opportunities and challenges in species quantification of bulk fisheries products. *ICES Journal of Marine Science,* v. 77, n. 7-8, p. 2557-2566, 2020.

Hansen, B.K.; Jacobsen, M.W.; Middelboe, A.L.; Preston, C.M.; Marin, R.; Bekkevold, D.; Knudsen, S.W.; Møller, P.R. & Nielsen, E.E. Remote, autonomous real-time monitoring of environmental DNA from commercial fish. *Scientific Reports*, v. 10, n. 1, p. 1-8, 2020.

He, X.; Gilmore, S.R.; Sutherland, T.F.; Hajibabaei, M.; Miller, K.M.; Westfall, K.M.; Pawlowski, J. & Abbott, C.L. Biotic signals associated with benthic impacts of salmon farms from eDNA metabarcoding of sediments. *Molecular Ecology*, 2020.

Heindler, F.M.; Christiansen, H.; Frédérich, B.; Dettaï, A.; Lepoint, G.; Maes, G.E.; Van de Putte, A.P. & Volckaert, F.A. Historical DNA metabarcoding of the prey and microbiome of trematomid fishes using museum samples. *Frontiers in Ecology and Evolution*, v. 6, n. 151, 2018.

Hestetun, J.T.; Bye-Ingebrigtsen, E.; Nilsson, R.H.; Glover, A.G.; Johansen, P.-O. & Dahlgren, T.G. Significant taxon sampling gaps in DNA databases limit the operational use of marine macrofauna metabarcoding. *Marine Biodiversity*, v. 50, n. 5, p. 1-9, 2020.

Hirai, J.; Tachibana, A. & Tsuda, A. Large-scale metabarcoding analysis of epipelagic and mesopelagic copepods in the Pacific. *PloS One*, v. 15, n. 5, e0233189, 2020.

Horiuchi, T.; Masuda, R.; Murakami, H.; Yamamoto, S. & Minamoto, T. Biomass-dependent emission of environmental DNA in jack mackerel *Trachurus japonicus* juveniles. *Journal of Fish Biology*, v. 95, n. 3, p. 979-981, 2019.

Hosia, A.; Falkenhaug, T.; Baxter, E.J. & Pagès, F. Abundance, distribution and diversity of gelatinous predators along the northern Mid-Atlantic Ridge: a comparison of different sampling methodologies. *PloS One*, v. 12, n. 11, e0187491, 2017.

Howell, L.; LaRue, M. & Flanagan, S.P. Environmental DNA as a tool for monitoring Antarctic vertebrates. *New Zealand Journal of Zoology*, 1-18, 2021.

Ibabe, A.; Rayon, F.; Martinez, J.L. & Garcia-Vazquez, E. Environmental DNA from plastic and textile marine litter detects exotic and nuisance species nearby ports. *PloS One*, v. 15, n. 6, e0228811, 2020.

Ip, Y.C.A.; Tay, Y.C.; Gan, S.X.; Ang, H.P.; Tun, K.; Chou, L.M.; Huang, D. & Meier, R. From marine park to future genomic observatory? Enhancing marine biodiversity assessments using a biocode approach. *Biodiversity Data Journal*, v. 7, 2019.

Jarman, S.N.; Berry, O. & Bunce, M. The value of environmental DNA biobanking for long-term biomonitoring. *Nature Ecology & Evolution*, v. 2, n. 8, p. 1192-1193, 2018.

Jensen, M.R.; Knudsen, S.W.; Munk, P.; Thomsen, P.F. & Møller, P.R. Tracing European eel in the diet of mesopelagic fishes from the Sargasso Sea using DNA from fish stomachs. *Marine Biology*, v. 165, n. 8, p. 1-11, 2018.

Jerde, C.L.; Wilson, E.A. & Dressler, T.L. *Measuring global fish species richness with eDNA metabarcoding*. Wiley Online Library, 2019.

Jeunen, G.J.; Lamare, M.D.; Knapp, M.; Spencer, H.G.; Taylor, H.R.; Stat, M.; Bunce, M. & Gemmell, N.J. Water stratification in the marine biome restricts vertical environmental DNA (eDNA) signal dispersal. *Environmental DNA*, v. 2, n. 1, p. 99-111, 2020.

Juhel, J.B.; Marques, V.; Fernández, A.P.; Borrero-Pérez, G.H.; Martinezguerra, M.M.; Valentini, A.; Dejean, T.; Manel, S.; Loiseau, N. & Velez, L. Detection of the elusive Dwarf sperm whale (*Kogia sima*) using environmental DNA at Malpelo island (Eastern Pacific, Colombia). *Ecology and Evolution*, v. 11, n. 7, p. 2956, 2021.

Keeley, N.; Wood, S.A. & Pochon, X. Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. *Ecological Indicators*, v. 85, p. 1044-1057, 2018.

Kelly, R.P.; Port, J.A.; Yamahara, K.M. & Crowder, L.B. Using environmental DNA to census marine fishes in a large mesocosm. *PloS One*, v. 9, n. 1, e86175, 2014.

Kersten, O.; Vetter, E.W.; Jungbluth, M.J.; Smith, C.R. & Goetze, E. Larval assemblages over the abyssal plain in the Pacific are highly diverse and spatially patchy. *PeerJ, v. 7*, e7691, 2019.

Kitahashi, T.; Sugime, S.; Inomata, K.; Nishijima, M.; Kato, S. & Yamamoto, H. Meiofaunal diversity at a seamount in the pacific ocean: a comprehensive study using environmental DNA and RNA. *Deep Sea Research Part I: Oceanographic Research Papers*, v. 160, n. 103253, 2020.

Klunder, L.; de Stigter, H.; Lavaleye, M.S.; van Bleijswijk, J.D.; van der Veer, H.W.; Reichart, G.-J. & Duineveld, G.C. A molecular approach to explore the background benthic fauna around a hydrothermal vent and their larvae: implications for future mining of deep-sea SMS deposits. *Frontiers in Marine Science*, v. 7, n. 134, 2020.

Knudsen, S.W.; Ebert, R.B.; Hesselsøe, M.; Kuntke, F.; Hassingboe, J.; Mortensen, P.B.; Thomsen, P. F.; Sigsgaard, E.E.; Hansen, B.K. & Nielsen, E.E. Species-specific detection and quantification of environmental DNA from marine fishes in the Baltic Sea. *Journal of Experimental Marine Biology and Ecology*, v. 510, p. 31-45, 2019.

Kume, G.; Kobari, T.; Hirai, J.; Kuroda, H.; Takeda, T.; Ichinomiya, M.; Komorita, T.; Aita-Noguchi, M. & Hyodo, F. Diet niche segregation of co-occurring larval stages of mesopelagic and commercially important fishes in the Osumi Strait assessed through morphological, DNA metabarcoding, and stable isotope analyses. *Marine Biology*, v. 168, n. 1, p. 1-14, 2021.

Kutti, T.; Johnsen, I.A.; Skaar, K.S.; Ray, J.L.; Husa, V. & Dahlgren, T.G. Quantification of eDNA to map the distribution of cold-water coral reefs. *Frontiers in Marine Science*, v. 7, n. 446, 2020.

Kuwae, M.; Tamai, H.; Doi, H.; Sakata, M.K.; Minamoto, T. & Suzuki, Y. Sedimentary DNA tracks decadal-centennial changes in fish abundance. *Communications Biology*, v. 3, n. 1, 1-12, 2020.

Laakmann, S.; Blanco-Bercial, L. & Cornils, A. The crossover from microscopy to genes in marine diversity: from species to assemblages in marine pelagic copepods. *Philosophical Transactions of the Royal Society B*, v. 375, n. 1814, 20190446, 2020.

Lacerda, A.L. d. F.; Proietti, M.C.; Secchi, E.R. & Taylor, J.D. Diverse groups of fungi are associated with plastics in the surface waters of the Western South Atlantic and the Antarctic Peninsula. *Molecular Ecology*, v. 29, n. 10, p. 1903-1918, 2020.

Lacoursière-Roussel, A.; Howland, K.; Normandeau, E.; Grey, E.K.; Archambault, P.; Deiner, K.; Lodge, D.M.; Hernandez, C.; Leduc, N. & Bernatchez, L. eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. *Ecology and Evolution*, v. 8, n. 16, p. 7763-7777, 2018.

Lafferty, K.D.; Garcia-Vedrenne, A.E.; McLaughlin, J.P.; Childress, J.N.; Morse, M.F. & Jerde, C.L. At Palmyra Atoll, the fish-community environmental DNA signal changes across habitats but not with tides. *Journal of Fish Biology*, v. 98, n. 2, p. 415-425, 2021.

Langlois, V.S.; Allison, M.J.; Bergman, L.C.; To, T.A. & Helbing, C.C. The need for robust qPCR-based eDNA detection assays in environmental monitoring and species inventories. *Environmental DNA*, v. 3, n. 3, p. 519-527, 2021.

Laroche, O.; Kersten, O.; Smith, C.R. & Goetze, E. From sea surface to seafloor: a benthic allochthonous eDNA survey for the abyssal ocean. *bioRxiv*, 2020.

Laroche, O.; Wood, S.A.; Tremblay, L.A.; Lear, G.; Ellis, J.I. & Pochon, X. Metabarcoding monitoring analysis: the pros and cons of using co-extracted environmental DNA and RNA data to assess offshore oil production impacts on benthic communities. *PeerJ*, v. 5, e3347, 2017.

Le, J.T.; Levin, L.A.; Lejzerowicz, F.; Cordier, T.; Gooday, A.J. & Pawlowski, J. Scientific and budgetary tradeoffs between morphological and molecular methods for deep-sea biodiversity assessment. *Integrated Environmental Assessment and Management*, 2021.

Leasi, F.; Sevigny, J.L. & Hassett, B.T. Meiofauna as a valuable bioindicator of climate change in the polar regions. *Ecological Indicators*, v. 121, n. 107133, 2021.

Leduc, N.; Lacoursière-Roussel, A.; Howland, K.L.; Archambault, P.; Sevellec, M.; Normandeau, E.; Dispas, A.; Winkler, G.; McKindsey, C.W. & Simard, N. Comparing eDNA metabarcoding and species collection for documenting Arctic metazoan biodiversity. *Environmental DNA*, v. 1, n. 4, p. 342-358, 2019.

Lehtiniemi, M.; Ojaveer, H.; David, M.; Galil, B.; Gollasch, S.; McKenzie, C.; Minchin, D.; Occhipinti-Ambrogi, A.; Olenin, S. & Pederson, J. Dose of truth-monitoring marine non-indigenous species to serve legislative requirements. *Marine Policy*, v. 54, p. 26-35, 2015.

Lejzerowicz, F.; Gooday, A.J.; Barrenechea Angeles, I.; Cordier, T.; Morard, R.; Apothéloz-Perret-Gentil, L.; Lins, L.; Menot, L.; Brandt, A. & Levin, L.A. Eukaryotic biodiversity and spatial patterns in the Clarion-Clipperton Zone and other abyssal regions: insights from sediment DNA and RNA metabarcoding. *Frontiers in Marine Science*, v. 8, n. 536, 2021.

Liu, Y.; Wikfors, G.H.; Rose, J.M.; McBride, R.S.; Milke, L.M. & Mercaldo-Allen, R. Application of environmental DNA metabarcoding to spatiotemporal finfish community assessment in a temperate embayment. *Frontiers in Marine Science*, v. 6, n. 674, 2019.

Macheriotou, L.; Rigaux, A.; Derycke, S. & Vanreusel, A. Phylogenetic clustering and rarity imply risk of local species extinction in prospective deep-sea mining areas of the Clarion-Clipperton Fracture Zone. *Proceedings of the Royal Society B*, v. 287, n. 1924, 20192666, 2020.

Macheriotou, L.; Rigaux, A.; Olu, K.; Zeppilli, D.; Derycke, S. & Vanreusel, A. Deep-sea nematodes of the Mozambique Channel: evidence of random community assembly dynamics in seep sediments. *Frontiers in Marine Science*, v. 8, 2021.

Mariani, S.; Fernandez, C.; Baillie, C.; Magalon, H. & Jaquemet, S. Shark and ray diversity, abundance and temporal variation around an Indian Ocean Island, inferred by eDNA metabarcoding. *Conservation Science and Practice*, v. 3, n. 6, e407, 2021.

Martínez, A.; Eckert, E.M.; Artois, T.; Careddu, G.; Casu, M.; Curini-Galletti, M.; Gazale, V.; Gobert, S.; Ivanenko, V.N. & Jondelius, U. Human access impacts biodiversity of microscopic animals in sandy beaches. *Communications Biology*, v. 3, n. 1, p. 1-9, 2020.

Mauvisseau, Q.; Parrondo, M.; Fernández, M.; García, L.; Martínez, J.; García-Vázquez, E. & Borrell, Y. On the way for detecting and quantifying elusive species in the sea: The Octopus vulgaris case study. *Fisheries Research*, v. 191, p. 41-48, 2017.

McInnes, J.C.; Alderman, R.; Lea, M.A.; Raymond, B.; Deagle, B.E.; Phillips, R.A.; Stanworth, A.; Thompson, D.R.; Catry, P. & Weimerskirch, H. High occurrence of jellyfish predation by black-browed and Campbell albatross identified by DNA metabarcoding. *Molecular Ecology*, v. 26, n. 18, p. 4831-4845, 2017a.

McInnes, J.C.; Jarman, S.N.; Lea, M.-A.; Raymond, B.; Deagle, B.E.; Phillips, R.A.; Catry, P.; Stanworth, A.; Weimerskirch, H. & Kusch, A. DNA metabarcoding as a marine conservation and management tool: A circumpolar examination of fishery discards in the diet of threatened albatrosses. *Frontiers in Marine Science*, v. 4, n. 277, 2017b.

Meekan, M.; Austin, C.M.; Tan, M.H.; Wei, N.-W.V.; Miller, A.; Pierce, S.J.; Rowat, D.; Stevens, G.; Davies, T.K. & Ponzo, A. iDNA at sea: recovery of whale shark (Rhincodon typus) mitochondrial DNA sequences from the whale shark copepod (*Pandarus rhincodonicus*) confirms global population structure. *Frontiers in Marine Science*, v. 4, n. 420, 2017.

Minamoto, T.; Fukuda, M.; Katsuhara, K.R.; Fujiwara, A.; Hidaka, S.; Yamamoto, S.; Takahashi, K. & Masuda, R. Environmental DNA reflects spatial and temporal jellyfish distribution. *PloS One*, v. 12, n. 2, e0173073, 2017.

Murakami, H.; Yoon, S.; Kasai, A.; Minamoto, T.; Yamamoto, S.; Sakata, M.K.; Horiuchi, T.; Sawada, H.; Kondoh, M. & Yamashita, Y. Dispersion and degradation of environmental DNA from caged fish in a marine environment. *Fisheries Science*, v. 85, n. 2, p. 327-337, 2019.

Nelms, S.E.; Parry, H.E.; Bennett, K.A.; Galloway, T.S.; Godley, B.J.; Santillo, D. & Lindeque, P.K. What goes in, must come out: Combining scat-based molecular diet analysis and quantification of ingested microplastics in a marine top predator. *Methods in Ecology and Evolution*, v. 10, n. 10, p. 1712-1722, 2019.

Nielsen, K.M.; Johnsen, P.J.; Bensasson, D. & Daffonchio, D. Release and persistence of extracellular DNA in the environment. *Environmental Biosafety Research*, v. 6, n. 1-2, p. 37-53, 2007.

Nirazuka, S.; Makabe, R.; Swadling, K.M. & Moteki, M. Phyto-detritus feeding by early-stage larvae of *Electrona antarctica* (Myctophidae) off Wilkes Land in the Southern Ocean, austral summer 2017. *Polar Biology*, 1-11, 2021.

Ojaveer, H.; Jaanus, A.; MacKenzie, B.R.; Martin, G.; Olenin, S.; Radziejewska, T.; Telesh, I.; Zettler, M.L. & Zaiko, A. Status of biodiversity in the Baltic Sea. *PloS One*, v. 5, n. 9, e12467, 2010.

Oliveira, L.; Knebelsberger, T.; Landi, M.; Soares, P.; Raupach, M. & Costa, F.O. Assembling and auditing a comprehensive DNA barcode reference library for European marine fishes. *Journal of Fish Biology*, v. 89, n. 6, p. 2741-2754, 2016.

Ortega, A.; Geraldi, N.R. & Duarte, C.M. Environmental DNA identifies marine macrophyte contributions to Blue Carbon sediments. *Limnology and Oceanography*, v. 65, n. 12, p. 3139-3149, 2020.

Ovaskainen, O. & Abrego, N. *Joint species distribution modelling: with applications in R.* Cambridge: Cambridge University Press, 2020.

Pan, W.; Qin, C.; Zuo, T.; Yu, G.; Zhu, W.; Ma, H. & Xi, S. Is Metagenomic Analysis an Effective Way to Analyse Fish Feeding Habits? A Case of the Yellowfin Sea Bream *Acanthopagrus latus* (Houttuyn) in Daya Bay. *Frontiers in Marine Science*, v. 8, n. 37, 2021.

Parsons, K.M.; Everett, M.; Dahlheim, M. & Park, L. Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. *Royal Society Open Science*, v. 5, n. 8, p. 180537, 2018.

Pawlowski, J.; Fontaine, D.; da Silva, A.A. & Guiard, J. Novel lineages of Southern Ocean deep-sea foraminifera revealed by environmental DNA sequencing. *Deep Sea Research Part II: Topical Studies in Oceanography*, v. 58, n. 19-20, p. 1996-2003, 2011.

Piñol, J.; Mir, G.; Gomez-Polo, P. & Agustí, N. Universal and blocking primer mismatches limit the use of high-throughput DNA sequencing for the quantitative metabarcoding of arthropods. *Molecular Ecology Resources*, v. 15, n. 4, p. 819-830, 2015.

Pochon, X.; Bott, N.J.; Smith, K.F. & Wood, S.A. Evaluating detection limits of next-generation sequencing for the surveillance and monitoring of international marine pests. *PloS One*, v. 8, n. 9, e73935, 2013.

Pochon, X.; Zaiko, A.; Fletcher, L.M.; Laroche, O. & Wood, S.A. Wanted dead or alive? Using metabarcoding of environmental DNA and RNA to distinguish living assemblages for biosecurity applications. *PloS One*, v. 12, n. 11, e0187636, 2017.

Pompanon, F.; Coissac, E. & Taberlet, P. Metabarcoding, a new way of analysing biodiversity. *Biofutur*, v. 319, p. 30-32, 2011.

Port, J.A.; O'Donnell, J.L.; Romero-Maraccini, O.C.; Leary, P.R.; Litvin, S.Y.; Nickols, K. J.; Yamahara, K.M. & Kelly, R.P. Assessing vertebrate biodiversity in a kelp forest ecosystem using environmental DNA. *Molecular Ecology*, v. 25, n. 2, p. 527-541, 2016.

Postaire, B.D.; Bakker, J.; Gardiner, J.; Wiley, T.R. & Chapman, D.D. Environmental DNA detection tracks established seasonal occurrence of blacktip sharks (*Carcharhinus limbatus*) in a semi-enclosed subtropical bay. *Scientific Reports*, v. 10, n. 1, p. 1-8, 2020.

Preston, C.M.; Durkin, C.A. & Yamahara, K.M. DNA metabarcoding reveals organisms contributing to particulate matter flux to abyssal depths in the North East Pacific ocean. *Deep Sea Research Part II: Topical Studies in Oceanography*, v. 173, 104708, 2020.

Queirós, J.P.; Ramos, J.A.; Cherel, Y.; Franzitta, M.; Duarte, B.; Rosa, R.; Monteiro, F.; Figueiredo, A.; Strugnell, J.M. & Fukuda, Y. Cephalopod fauna of the Pacific Southern Ocean using Antarctic toothfish (*Dissostichus mawsoni*) as biological samplers and fisheries bycatch specimens. *Deep Sea Research Part I: Oceanographic Research Papers*, 103571, 2021.

Questel, J.M.; Hopcroft, R.R.; DeHart, H.M.; Smoot, C.A.; Kosobokova, K.N. & Bucklin, A. Metabarcoding of zooplankton diversity within the Chukchi Borderland, Arctic Ocean: improved resolution from multi-gene markers and region-specific DNA databases. *Marine Biodiversity*, v. 51, n. 1, p. 1-19, 2021.

Radulovici, A.E.; Vieira, P.E.; Duarte, S.; Teixeira, M.A.; Borges, L.M.; Deagle, B.; Majaneva, S.; Redmond, N.; Schultz, J.A. & Costa, F.O. Revision and annotation of DNA barcode records for marine invertebrates: report of the 8th iBOL conference hackathon. *bioRxiv*, 2021.

Ratcliffe, N.; Deagle, B.; Love, K.; Polanowski, A.; Fielding, S.; Wood, A.G.; Hill, S.; Grant, S.; Belchier, M. & Fleming, A. Changes in prey fields increase the potential for spatial overlap between gentoo penguins and a krill fishery within a marine protected area. *Diversity and Distributions*, v. 27, n. 3, p. 552-563, 2021.

Ray, J.L.; Althammer, J.; Skaar, K.S.; Simonelli, P.; Larsen, A.; Stoecker, D.; Sazhin, A.; Ijaz, U.Z.; Quince, C. & Nejstgaard, J.C. Metabarcoding and metabolome analyses of copepod grazing reveal feeding preference and linkage to metabolite classes in dynamic microbial plankton communities. *Molecular Ecology*, v. 25, n. 21, p. 5585-5602, 2016.

Rey, A.; Carney, K.J.; Quinones, L.E.; Pagenkopp Lohan, K.M.; Ruiz, G.M.; Basurko, O.C. & Rodríguez-Ezpeleta, N. Environmental DNA metabarcoding: a promising tool for ballast water monitoring. *Environmental Science & Technology*, v. 53, n. 20, p. 11849-11859, 2019.

Ruppert, K.M.; Kline, R.J. & Rahman, M.S. Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: A systematic review in methods, monitoring, and applications of global eDNA. *Global Ecology and Conservation*, v. 17, e00547, 2019.

Russo, T.; Maiello, G.; Talarico, L.; Baillie, C.; Colosimo, G.; D'Andrea, L.; Di Maio, F.; Fiorentino, F.; Franceschini, S. & Garofalo, G. All is fish that comes to the net: metabarcoding for rapid fisheries catch assessment. *Ecological Applications*, v. 31, n. 2, e02273, 2021.

Salter, I. Seasonal variability in the persistence of dissolved environmental DNA (eDNA) in a marine system: The role of microbial nutrient limitation. *PloS One*, v. 13, n. 2, e0192409, 2018.

Salter, I.; Joensen, M.; Kristiansen, R.; Steingrund, P. & Vestergaard, P. Environmental DNA concentrations are correlated with regional biomass of Atlantic cod in oceanic waters. *Communications Biology*, v. 2, n. 1, p. 1-9, 2019.

Santoferrara, L.F. Current practice in plankton metabarcoding: optimization and error management. *Journal of Plankton Research*, v. 41, n. 5, p. 571-582, 2019.

Schnell, I.B.; Bohmann, K.; Schultze, S.E.; Richter, S.R.; Murray, D.C.; Sinding, M.H.S.; Bass, D.; Cadle, J.E.; Campbell, M.J. & Dolch, R. Debugging diversity – a pan-continental exploration of the potential of terrestrial blood-feeding leeches as a vertebrate monitoring tool. *Molecular Ecology Resources*, v. 18, n. 6, p. 1282-1298, 2018.

Scholin, C.A. What are "ecogenomic sensors?" A review and thoughts for the future. *Ocean Science*, v. 6, n. 1, p. 51-60, 2010.

Schröter, F.; Havermans, C.; Kraft, A.; Knüppel, N.; Beszczynska-Möller, A.; Bauerfeind, E. & Nöthig, E.-M. Pelagic amphipods in the Eastern Fram Strait with continuing presence of *Themisto compressa* based on sediment trap time series. *Frontiers in Marine Science*, v. 6, n. 311, 2019.

Schubert, G.; Stockhausen, M.; Hoffmann, C.; Merkel, K.; Vigilant, L.; Leendertz, F.H. & Calvignac-Spencer, S. Targeted detection of mammalian species using carrion fly-derived DNA. *Molecular Ecology Resources*, v. 15, n. 2, p. 285-294, 2015.

Sevellec, M.; Lacoursière-Roussel, A.; Bernatchez, L.; Normandeau, E.; Solomon, E.; Arreak, A.; Fishback, L. & Howland, K. Detecting community change in Arctic marine ecosystems using the temporal dynamics of environmental DNA. *Environmental DNA*, v. 3, n. 3, p. 573-590, 2021.

Shehzad, W.; Riaz, T.; Nawaz, M.A.; Miquel, C.; Poillot, C.; Shah, S.A.; Pompanon, F.; Coissac, E. & Taberlet, P. Carnivore diet analysis based on next-generation sequencing: Application to the leopard cat (*Prionailurus bengalensis*) in Pakistan. *Molecular Ecology*, v. 21, n. 8, p. 1951-1965, 2012.

Shink, K.G.; Sutton, T.M.; Murphy, J.M. & López, J.A. Utilizing DNA metabarcoding to characterize the diet of marine-phase Arctic lamprey (*Lethenteron camtschaticum*) in the

eastern Bering Sea. *Canadian Journal of Fisheries and Aquatic Sciences*, v. 76, n. 11, p. 1993-2002, 2019.

Siegenthaler, A.; Wangensteen, O.S.; Soto, A.Z.; Benvenuto, C.; Corrigan, L. & Mariani, S. Metabarcoding of shrimp stomach content: Harnessing a natural sampler for fish biodiversity monitoring. *Molecular Ecology Resources*, v. 19, n. 1, p. 206-220, 2019.

Sigsgaard, E.E.; Jensen, M.R.; Winkelmann, I.E.; Møller, P.R.; Hansen, M.M. & Thomsen, P.F. Population-level inferences from environmental DNA - Current status and future perspectives. *Evolutionary Applications*, v. 13, n. 2, p. 245-262, 2020.

Sigsgaard, E.E.; Nielsen, I.B.; Bach, S.S.; Lorenzen, E.D.; Robinson, D.P.; Knudsen, S.W.; Pedersen, M.W.; Al Jaidah, M.; Orlando, L. & Willerslev, E. Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology & Evolution*, v. 1, n. 1, p. 1-5, 2016.

Sigsgaard, E.E.; Olsen, K.; Hansen, M.D.; Hansen, O.L.P.; Høye, T.T.; Svenning, J.C. & Thomsen, P.F. Environmental DNA metabarcoding of cow dung reveals taxonomic and functional diversity of invertebrate assemblages. *Molecular Ecology*, v. 30, n. 13, p. 3374-3389, 2021.

Singer, G.; Fahner, N.A.; Barnes, J.; McCarthy, A. & Hajibabaei, M. Comprehensive biodiversity analysis via ultra-deep patterned flow cell technology: a case study of eDNA metabarcoding seawater. *Scientific Reports*, v. 9, n. 1, p. 1-12, 2019.

Sonsthagen, S.A.; Jay, C.V.; Cornman, R.S.; Fischbach, A.S.; Grebmeier, J.M. & Talbot, S.L. DNA metabarcoding of feces to infer summer diet of Pacific walruses. *Marine Mammal Science*, v. 36, n. 4, p. 1196-1211, 2020.

Stat, M.; John, J.; DiBattista, J.D.; Newman, S.J.; Bunce, M. & Harvey, E.S. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Conservation Biology*, v. 33, n. 1, p. 196-205, 2019.

Steyaert, M.; Priestley, V.; Osborne, O.; Herraiz, A.; Arnold, R. & Savolainen, V. Advances in metabarcoding techniques bring us closer to reliable monitoring of the marine benthos. *Journal of Applied Ecology*, v. 57, n. 11, p. 2234-2245, 2020.

Stoeckle, M.Y.; Adolf, J.; Charlop-Powers, Z.; Dunton, K.J.; Hinks, G. & VanMorter, S.M. Trawl and eDNA assessment of marine fish diversity, seasonality, and relative abundance in coastal New Jersey, USA. *ICES Journal of Marine Science*, v. 78, n. 1, p. 293-304, 2021.

Strickler, K.M.; Fremier, A.K. & Goldberg, C.S. Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms. *Biological Conservation*, v. 183, p. 85-92, 2015.

Suarez-Menendez, M.; Planes, S.; Garcia-Vazquez, E. & Ardura, A. Early alert of biological risk in a coastal lagoon through eDNA metabarcoding. *Frontiers in Ecology and Evolution*, v. 8, n. 9, 2020.

Suter, L.; Polanowski, A.M.; Clarke, L.J.; Kitchener, J.A. & Deagle, B.E. Capturing open ocean biodiversity: comparing environmental DNA metabarcoding to the continuous plankton recorder. *Molecular Ecology*, 2020.

Székely, D.; Corfixen, N.L.; Mørch, L.L.; Knudsen, S.W.; McCarthy, M.L.; Teilmann, J.; Heide-Jørgensen, M.P. & Olsen, M.T. Environmental DNA captures the genetic diversity of bowhead whales (*Balaena mysticetus*) in West Greenland. *Environmental DNA*, v. 3, n. 1, p. 248-260, 2021.

Takasu, H.; Inomata, H.; Uchino, K.; Tahara, S.; Mori, K.; Hirano, Y.; Harada, K.; Yamaguchi, M.; Nozoe, Y. & Akiyama, H. Spatio-temporal distribution of environmental DNA derived from Japanese sea nettle jellyfish *Chrysaora pacifica* in Omura Bay, Kyushu, Japan. *Plankton and Benthos Research*, v. 14, n. 4, p. 320-323, 2019.

Takeuchi, A.; Watanabe, S.; Yamamoto, S.; Miller, M.J.; Fukuba, T.; Miwa, T.; Okino, T.; Minamoto, T. & Tsukamoto, K. First use of oceanic environmental DNA to study the spawning ecology of the Japanese eel *Anguilla japonica*. *Marine Ecology Progress Series*, v. 609, p. 187-196, 2019.

Tercel, M.P.; Symondson, W.O. & Cuff, J.P. *The problem of omnivory: a synthesis on omnivory and DNA metabarcoding*. Wiley Online Library, 2021.

Thomsen, P.F.; Møller, P.R.; Sigsgaard, E.E.; Knudsen, S.W.; Jørgensen, O.A. & Willerslev, E. Environmental DNA from seawater samples correlate with trawl catches of subarctic, deepwater fishes. *PloS One*, v. 11, n. 11, e0165252, 2016.

Truelove, N.K.; Andruszkiewicz, E.A. & Block, B.A. A rapid environmental DNA method for detecting white sharks in the open ocean. *Methods in Ecology and Evolution*, v. 10, n. 8, p. 1128-1135, 2019.

Tsuji, S.; Shibata, N.; Sawada, H. & Ushio, M. Quantitative evaluation of intraspecific genetic diversity in a natural fish population using environmental DNA analysis. *Molecular Ecology Resources*, v. 20, n. 5, p. 1323-1332, 2020.

Turner, C.R.; Uy, K.L. & Everhart, R.C. Fish environmental DNA is more concentrated in aquatic sediments than surface water. *Biological Conservation*, v. 183, p. 93-102, 2015.

Turon, X.; Antich, A.; Palacín, C.; Præbel, K. & Wangensteen, O.S. From metabarcoding to metaphylogeography: separating the wheat from the chaff. *Ecological Applications*, v. 30, n. 2, e02036, 2020.

Tverin, M.; Esparza-Salas, R.; Strömberg, A.; Tang, P.; Kokkonen, I.; Herrero, A.; Kauhala, K.; Karlsson, O.; Tiilikainen, R. & Vetemaa, M. Complementary methods assessing short and long-term prey of a marine top predator – Application to the grey seal-fishery conflict in the Baltic Sea. *PloS One*, v. 14, n. 1, e0208694, 2019.

Uthicke, S.; Lamare, M. & Doyle, J.R. eDNA detection of corallivorous seastar (Acanthaster cf. solaris) outbreaks on the Great Barrier Reef using digital droplet PCR. *Coral Reefs*, v. 37, n. 4, p. 1229-1239, 2018.

Van den Heuvel-Greve, M.J.; van den Brink, A.M.; Glorius, S.T.; de Groot, G.A.; Laros, I.; Renaud, P.E.; Pettersen, R.; Węsławski, J.M.; Kuklinski, P. & Murk, A.J. Early detection of marine non-indigenous species on Svalbard by DNA metabarcoding of sediment. *Polar Biology*, v. 44, n. 4, p. 653-665, 2021.

Van der Loos, L.M. & Nijland, R. Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. *Molecular Ecology*, v. 30, n. 13, p. 3270-3288, 2021.

Van der Reis, A.; Jeffs, A. & Lavery, S. From feeding habits to food webs: exploring the diet of an opportunistic benthic generalist. *Marine Ecology Progress Series*, v. 655, p. 107-121, 2020.

Vause, B.J.; Morley, S.A.; Fonseca, V.G.; Jażdżewska, A.; Ashton, G.V.; Barnes, D.K.; Giebner, H.; Clark, M.S. & Peck, L.S. Spatial and temporal dynamics of Antarctic shallow soft-bottom benthic communities: ecological drivers under climate change. *BMC Ecology*, v. 19, n. 1, p. 1-14, 2019.

Vernette, C.; Henry, N.; Lecubin, J.; de Vargas, C.; Hingamp, P. & Lescot, M. The Ocean barcode atlas: A web service to explore the biodiversity and biogeography of marine organisms. *Molecular Ecology Resources*, v. 21, n. 4, p. 1347-1358, 2021.

Vestheim, H. & Jarman, S.N. Blocking primers to enhance PCR amplification of rare sequences in mixed samples–a case study on prey DNA in Antarctic krill stomachs. *Frontiers in Zoology*, v. 5, n. 1, p. 1-11, 2008.

Visser, F.; Merten, V.; Bayer, T.; Oudejans, M.; de Jonge, D.; Puebla, O.; Reusch, T.B.; Fuss, J. & Hoving, H. Deep-sea predator niche segregation revealed by combined cetacean biologging and eDNA analysis of cephalopod prey. *Science Advances*, v. 7, n. 14, eabf5908, 2021.

Voelker, M.R.; Schwarz, D.; Thomas, A.; Nelson, B.W. & Acevedo-Gutiérrez, A. Large-scale molecular barcoding of prey DNA reveals predictors of intrapopulation feeding diversity in a marine predator. *Ecology and Evolution*, v. 10, n. 18, p. 9867-9885, 2020.

Wada, T.; Doi, H.; Togaki, D.; Kaida, R.; Nagano, M.; Katano, I.; Suzuki, M.; Ohtani, T. & Mitsuhashi, H. Exploring a legendary giant squid: An environmental DNA approach. *Marine Biology*, v. 167, n. 11, p. 1-6, 2020.

West, K.; Travers, M.J.; Stat, M.; Harvey, E.S.; Richards, Z.T.; DiBattista, J.D.; Newman, S.J.; Harry, A.; Skepper, C.L. & Heydenrych, M. Large-scale eDNA metabarcoding survey reveals marine biogeographic break and transitions over tropical north-western Australia. *Diversity and Distributions*, 2021.

Westfall, K.M.; Therriault, T.W. & Abbott, C.L. A new approach to molecular biosurveillance of invasive species using DNA metabarcoding. *Global Change Biology*, v. 26, n. 2, p. 1012-1022, 2020.

Williams, M.A.; Hernandez, C.; O'Sullivan, A.M.; April, J.; Regan, F.; Bernatchez, L. & Parle-McDermott, A. Comparing CRISPR-Cas and qPCR eDNA assays for the detection of Atlantic salmon (*Salmo salar* L.). *Environmental DNA*, v. 3, n. 1, p. 297-304, 2021.

Yamahara, K.M.; Preston, C.M.; Birch, J.; Walz, K.; Marin III, R.; Jensen, S.; Pargett, D.; Roman, B.; Ussler III, W. & Zhang, Y. In situ autonomous acquisition and preservation of marine environmental DNA using an autonomous underwater vehicle. *Frontiers in Marine Science*, v. 6, n. 373, 2019.

Yoon, T.-H.; Kang, H.-E.; Lee, S.R.; Lee, J.-B.; Baeck, G.W.; Park, H. & Kim, H.-W. Metabarcoding analysis of the stomach contents of the Antarctic Toothfish (*Dissostichus mawsoni*) collected in the Antarctic Ocean. *PeerJ*, v. 5, e3977, 2017.

Yoshitake, K.; Fujiwara, A.; Matsuura, A.; Sekino, M.; Yasuike, M.; Nakamura, Y.; Nakamichi, R.; Kodama, M.; Takahama, Y. & Takasuka, A. Estimation of tuna population by the improved analytical pipeline of unique molecular identifier-assisted HaCeD-Seq (haplotype count from eDNA). *Scientific Reports*, v. 11, n. 1, p. 1-12, 2021.

Yoshitake, K.; Yoshinaga, T.; Tanaka, C.; Mizusawa, N.; Reza, M.S.; Tsujimoto, A.; Kobayashi, T. & Watabe, S. HaCeD-Seq: a novel method for reliable and easy estimation about the fish population using haplotype count from eDNA. *Marine Biotechnology*, v. 21, n. 6, p. 813-820, 2019.

Zaiko, A.; Samuiloviene, A.; Ardura, A. & Garcia-Vazquez, E. Metabarcoding approach for nonindigenous species surveillance in marine coastal waters. *Marine Pollution Bulletin*, v. 100, n. 1, p. 53-59, 2015.

Zhang, Y.; Pavlovska, M.; Stoica, E.; Prekrasna, I.; Yang, J.; Slobodnik, J.; Zhang, X. & Dykyi, E. Holistic pelagic biodiversity monitoring of the Black Sea via eDNA metabarcoding approach: From bacteria to marine mammals. *Environment International*, v. 135, n. 105307, 2020.

Zou, K.; Chen, J.; Ruan, H.; Li, Z.; Guo, W.; Li, M. & Liu, L. eDNA metabarcoding as a promising conservation tool for monitoring fish diversity in a coastal wetland of the Pearl River Estuary compared to bottom trawling. *Science of The Total Environment*, v. 702, n. 134704, 2020.