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Applications of environmental DNA (eDNA) to detect subterranean and aquatic invasive species: A critical review on the challenges and limitations of eDNA metabarcoding

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ABSTRACT

The world is struggling to solve a devastating biodiversity loss that not only affects the extinction of treasured species and irreplaceable genetic variation, but also jeopardizes the food production, health, and safety of people. All initiatives aimed to conserve biodiversity rely heavily on the monitoring of both species and populations to get accurate spatial patterns and overall population assessments. Conventional monitoring techniques, such as visual surveys and counting individuals, are problematic due to challenges in identifying cryptic species or immature life stages. Environmental DNA (eDNA) is a relatively new technology that has the potential to be a faster, non-invasive, and cost-effective tool for monitoring biodiversity, conservation, and management practices. eDNA has been extracted from materials that are both ancient and present, and its applications range from the identification of individual species to the study of entire ecosystems. In the past few years, there has been a substantial increase in the usage of eDNA in research pertaining to ecological preservation and conservation. However, several technological problems still need to be solved. To reduce the number of false positives and/or false negatives produced by current eDNA technologies, it is necessary to improve and optimize calibration and validation at every stage of the procedure. There is a significant need for greater information about the physical and ecological constraints on eDNA use, as well as its synthesis, current state, expected lifespan, and potential modes of movement. Due to the widespread use of eDNA research, it is also essential to assess the extent and breadth of these studies. In this article, we critically reviewed the primary applications of eDNA in subterranean and aquatic invasive species. Through this review, readers can better understand the challenges and limitations of eDNA metabarcoding.

1. Introduction

Ecosystems around the world have been changing at exponential levels and entering into a new geological epoch in which human effects drive significant changes across the globe mostly due to climate change, habitat destruction, and environmental pollution (Prakash and Verma, 2022; Steffen et al., 2011; Travis, 2003). Species respond to climate change in a variety of ways, including phenological changes, adjustments in distributional boundaries, acclimatization, and phenotypic adaptation (Chen et al., 2011; Pecl et al., 2017). However, in cases where these adjustments are inadequate, species may experience population declines and even loss to extinction (Barnosky et al., 2011). The molecular technique employing environmental DNA (eDNA) is considered a promising alternative to traditional surveys to overcome their limitations (Othman et al., 2023). eDNA is the short DNA fragments that organisms have left behind in nonliving elements such as soil (Frøslev et al., 2022), air (Redondo et al., 2020), water (Yang et al. 2023), silt (Nelson-Chorney et al. 2019), ice (Khalsa et al., 2020), or snow (Kinoshita et al., 2019) in the environment. eDNA can be accumulated in the environment and comes from the skin, blood, saliva, sperm,

Abbreviations: AIS, aquatic invasive species; CN, cellulose nitrate; COI, cytochrome oxidase subunit I; Cytb, cytochrome b; eDNA, environmental eDNA; GF, glass fiber; MCE, mixed cellulose acetate and nitrate; MCN, mixed cellulose nitrate; mtDNA, mitochondrial DNA; PC, polycarbonate; PCTE, polycarbonate track-etched; PCR, polymerase chain reaction; PES, polyethersulfone; qPCR, quantitative polymerase chain reaction.

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secretions, mucus, eggs, feces, urine, roots, fruit, pollen, and leaves in the ecosystems (Ruppert et al., 2019; Sahu et al., 2022). In comparison to conventional methods, the eDNA method can be carried out in practically any habitat with little skill or effort, and molecular identification appears to be more precise than visual detection (Biggs et al., 2015; Pilliod et al., 2013). eDNA-based biodiversity surveys have shown greater detection sensitivity, lower taxonomic selectivity, and superior cost-efficiency and these advantages make them an excellent choice for efficient and precise biomonitoring (Roger et al., 2022; Ruppert et al., 2019; Yao et al., 2022). eDNA has enormous promise for enhancing biodiversity conservation and management by replacing traditional monitoring systems. In the past ten years, eDNA has been widely employed by academics to analyze populations in marine (Foote et al., 2012), freshwater habitats (Yang et al. 2023), and terrestrial habitats (Allen et al., 2021; Van Der Heyde et al., 2020). The amplification of eDNA provides a non-invasive, reliable method for monitoring aquatic systems for the existence of invasive species (Coster et al., 2021). eDNA history was started in the mid-1980s when it was once referred to as DNA particle (Ruppert et al., 2022) and has been employed to identify and characterize microbes in marine sediments including phytoplankton populations in saline water starting in the early-1990s (Díaz-Ferguson and Moyer, 2014). The assessment of the variety of macro-organisms in prehistoric sediments led to the employment of techniques for monitoring and conserving aquatic species using eDNA (Willerslev et al., 2003). The first study in freshwater samples based on eDNA was recorded in 2008. Researchers utilized eDNA to follow the movements of American bullfrog (*Lithobates catesbeianus*) in both lab conditions and real wetlands, opening a new era for aquatic species detection in ecological and environmental research (Ficetola et al., 2008). A similar eDNA method was quickly adopted to survey different vertebrate and macroinvertebrate species. The eDNA technique has been tested effectively in freshwater, marine, estuarine, terrestrial, and subterranean environments. eDNA techniques have been employed to identify various aquatic organisms, including fish (Adrian-Kalchhauser and Burkhardt-Holm, 2016), molluscans (Sugawara et al., 2022), amphibians (Ficetola et al., 2008), reptiles (Ratsch et al., 2020), and mammals (Allen et al., 2023) in both laboratory and natural habitats. Notably, eDNA-based techniques significantly improve our ability to identify species in a wide range of habitats by collecting and retrieving shed cellular components from the environment (Coble et al., 2019; O'Malley et al., 2022). A species-specific quantitative polymerase chain reaction (qPCR) experiment employing eDNA is a promising technique for evaluating target aquatic and/or terrestrial species (Fu'adil Amin et al., 2021).

Notably, documenting aquatic species is typically more difficult than terrestrial species due to the difficulties involved in reaching aquatic habitats and creating techniques that can properly identify all species there (DiBattista et al., 2022). Capture-based conventional fish monitoring technologies, such as netting, trapping, or electrofishing, as well as underwater video surveys, have the potential to provide valuable insight into fish populations and species (Yao et al., 2022). However, to gather accurate data, conventional monitoring systems have relied on periodic fish surveys, which need a significant amount of specialized personnel, lengthy observation durations, and a considerable budget (Levi et al., 2019). In addition, traditional methods of species identification have their limitations because they are heavily reliant on skilled taxonomists who are also knowledgeable about the pertinent phenotaxonomical approaches (Hopkins and Freckleton, 2002). In addition to this, it is frequently invasive and damaging to aquatic ecosystems as well as their inhabitants (Brys et al., 2021). Due to the invasive character of capture-based approaches, traditional methods cannot be used to survey endangered species and small bodies of water (Yao et al., 2022).

In aquatic environments, typically the amount of water that is collected as a sample in the field has a significant impact on the sensitivity of eDNA surveys (Brys et al., 2021). The quantity of DNA that is finally analyzed is also affected by the elution volume (Capo et al., 2020) and extracted DNA amount (Piggot et al. 2016). During PCR, the presence of inhibitors greatly reduces the process of amplifying DNA (Goldberg et al., 2016; McKee et al., 2015). When next-generation sequencing techniques are used together, it is possible to identify whole faunas (Rees et al., 2014). eDNA is a great way to detect cryptic species that are difficult with the usual sampling methods and procedures (Adrian-Kalchhauser and Burkhardt-Holm, 2016). Nevertheless, despite the ecological and conservation importance of the issues that might possibly be solved with eDNA, there are a lot of obstacles and constraints to cope with. eDNA does not constantly function, and even when it "works," the outcomes are not always what is expected. Therefore, to emphasize the potential challenges and limitations of using eDNA techniques to detect subterranean and aquatic invasive species, we summarize existing eDNA studies.

2. Methodology

A literature review was carried out utilizing a variety of online indexes (PubMed, Springer Nature, Science Direct, Taylor & Francis, John Wiley, JSTOR, and Google Scholar) in order to highlight the uses of environmental DNA along with the challenges and limitations it encounters. The keywords used were mainly eDNA technology, conservation, global ecology, metabarcoding, challenges, and limitations. As eDNA just became usable as a survey instrument in 2008, we could only look for articles published between January 1, 2008, and January 31, 2023. In addition, the contents and citations in scholarly journals attest to the excellence of the chosen literature.

3. Standard methodology of eDNA to detect animals

3.1. Sample collection

There are no standard rules that can be followed regarding the volume of a sample, its depth, or the total amount of water. The purpose of the research, the extent and condition of the sample region, the number of species, and the technologies used to evaluate the eDNA samples are only a few of the elements that need to be evaluated. The samples (e.g., air, water, soil, silt, ice, snow, etc.) are collected from several sources (Figure 1). In the case of water, there is a wide range of possible sample volumes used ranging from 1.5 mL (Doi, Akamatsu, et al., 2017) to 45 L (Kumar et al., 2020). The sampling process needs to make use of the information concerning the environment of the target organisms (e.g., feeding areas or breeding grounds). It is required to collect many field sample replicates to improve the effectiveness of DNA capture and the possibility of intended eDNA detection (Ruppert et al., 2022). Notably, most of the studies used at least three samples from the same location for the collection of water samples (Hinlo et al., 2018; Uchii et al., 2017).

3.2. Selection of filters for capture eDNA

eDNA has been efficiently extracted from water samples using cellulose nitrate (CN) (Schabacker et al., 2020), cellulose acetate (CA) (Spens et al., 2017), mixed cellulose acetate and nitrate (MCE) (Liang and Keeley, 2013), glass fiber (GF) (Lacoursière-Roussel et al., 2016), polyethersulfone (PES) (Thomas et al., 2019), polycarbonate (PC) (Eichmiller et al., 2016), mixed cellulose nitrate (MCN) (Hinlo et al., 2018), polycarbonate track-etched (PCTE) (Spens et al., 2017), and nylon filters (Jeunen et al., 2022). The most frequent pore size of the filter is 0.45 μm, whereas researchers have discovered that fish DNA molecules also extracted from water tend to be between 1 and 10 μm size of filters (Capo et al., 2020; Cooper et al., 2022; Schabacker et al., 2020; Turner et al., 2014). There is some debate as to whether cellulose-based filters or glass fiber filters are better for fish DNA capture, even though cellulose-based filters regularly outperformed other filters in eDNA capture for aquatic animals (Kumar et al., 2020). Researchers who want to employ multiple varieties of different filters at the same time may

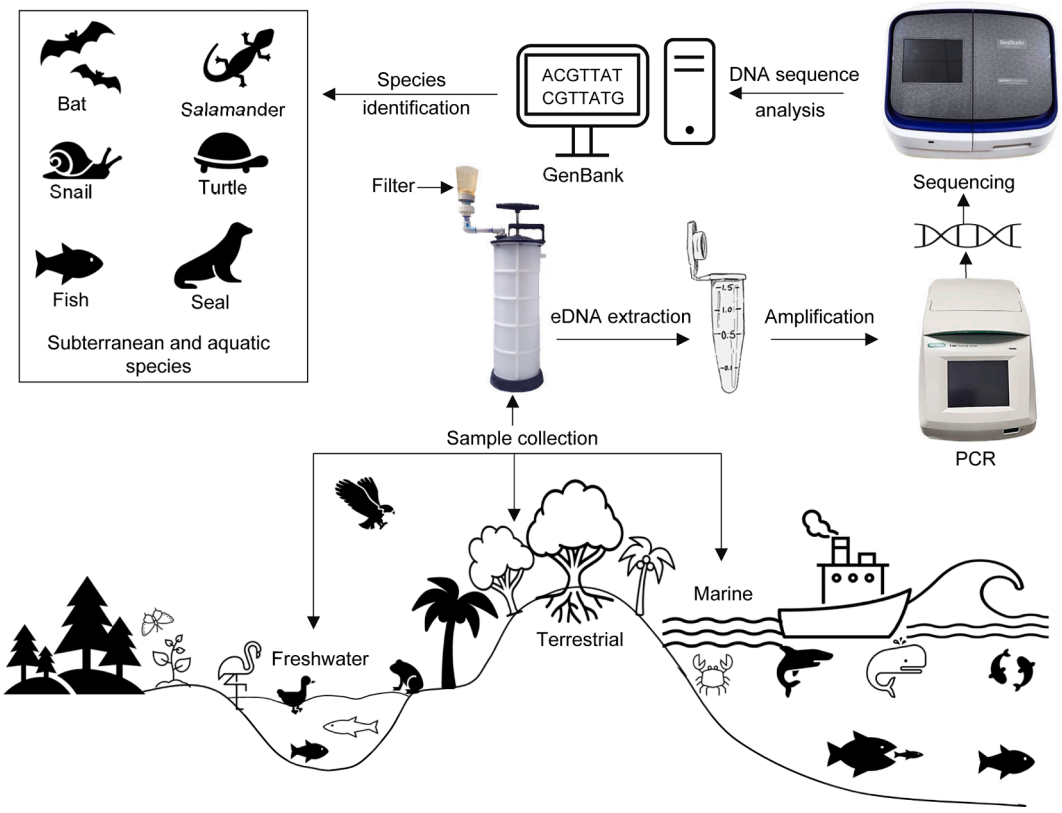

Fig. 1. Schematic diagram of global ecosystem and biodiversity monitoring with eDNA metabarcoding to detect subterranean and aquatic species.

need to use capsule filters, which can also comprise two membranes with distinct pore diameters and materials (Spens et al., 2017).

3.3. eDNA extraction procedure

The two most commonly used commercially available eDNA extraction processes are the DNeasy Blood & Tissue Kit (Qiagen, Hilden, Germany) (Chen et al., 2020; Coster et al., 2021) and Power Water DNA Isolation Kit (MoBio, Hilden, Germany) (Deiner et al., 2017; Li et al., 2021). The DNeasy Blood & Tissue Kit was found the best option for eDNA extraction in most situations since it is cheap, easy to use, does not contain any hazardous chemicals, tasks as efficient PCR amplification, and has a quality of information (Goldberg et al., 2016; Kelly, Shelton and Gallego, 2019). Stoeckle et al. (2017) conducted a comprehensive study to investigate the influence of a variety of environmental factors and inhibitors and found that sediment causes decreased eDNA detection in the water samples, and this was the case regardless of whether the water was moving or stationary. If such information is determined in advance, it can assist decide whether a method that involves the removal of inhibitors is necessary.

The methods of centrifugation (Eichmiller et al., 2016), precipitation with isopropanol or ethanol (Doi, Uchii, et al., 2017), and filtration (Spens et al., 2017) are utilized in the process of eDNA concentration. On-site filtering and preservation is preferable for the preservation of eDNA since it stops eDNA from degrading during transport (Yamanaka et al., 2016). On the other hand, laboratory filtering can reduce the amount of time spent in the field and avoid contamination risk (Di Muri et al., 2020). However, when water turbidity is low, the number of samples is small, and the portable pump has enough power to handle the sampling, on-site filtering is recommended (Strickland and Roberts, 2019; Sutter and Kinziger, 2019). If the water to be sampled is turbid or the number of samples collected is large in volume, some authors suggest that the water should be brought back to the lab in a clean bottle so that it can be filtered (Fukaya et al., 2021; Ghosal et al., 2018).

3.4. Selection of genetic biomarker

eDNA detection assays have previously used both mitochondrial and nuclear genes as genetic markers; however, nuclear genes are regarded as the gold standard due to their fast evolution and ability to provide a more accurate description of biodiversity (Andres et al., 2021; Garagnani et al., 2014). In addition, it has been demonstrated that mitochondrial DNA (mtDNA) is accurate for assessing whether DNA has been degraded or not; it is informative for determining the species of vertebrates, and well-suited to surveys of fish diversity (Rees et al., 2014; Freeland, 2017). Markers for species-specific detection in aquatic organisms eDNA includes the cytochrome b gene (Keskin et al., 2016; Sakai et al., 2019; Zhang et al., 2020), the cytochrome oxidase subunit I (COI) gene (Holman et al., 2022; Uchida et al., 2020; Xia et al., 2018), and the D-loop region (Yoshitake et al., 2019). However, the cytochrome b gene (Cytb) is the most used genetic marker for characterizing eDNA from fish (Hunter et al., 2018; Santas et al., 2013; Yusishen et al., 2020).

3.5. eDNA detection systems using PCR

The vast majority of eDNA detection systems that are used in the monitoring of biodiversity typically make use of eDNA metabarcoding in addition to species-specific detection (Ardura et al., 2015; Gold et al., 2021; Rees et al., 2014). Most eDNA investigations have placed a focus on species-specific detection, which includes the detection of invasive and vulnerable species (Hernandez et al., 2020). This is because the early detection of invading species is critical for the creation of conservation policies that will maintain the diversity of native species (Larson et al., 2020). In recent years, there has been a growth in the use of eDNA technology as a management technique for tracking animal populations prior to and after eradication operations. For example, Asian carp in the reservoirs of the United States and Canada are being monitored in this manner (LeBlanc et al., 2020; Mahon et al., 2013).

Initial eDNA detection of combined PCR (cPCR) (Farrington et al.,

2015) utilizing cloning or Sanger sequencing (Hernandez et al., 2020) technologies in obtaining target eDNA for the purpose of species-specific identification. In single-species detection, qPCR with specific probes is suggested over cPCR due to its superior sensitivity and reproducibility (Amberg et al., 2015; Langlois et al., 2021; Wilcox et al., 2013). Furthermore, qPCR approaches have been utilized to determine fish population and biomass by quantifying the sample's target eDNA (Baldigo et al., 2017; Mizumoto et al., 2018). Additionally, droplet digital PCR (ddPCR)-based techniques are employed to detect or quantify target species because of enhanced sensitivity even in the presence of small amounts of eDNA (Doi et al., 2015; Hänfling et al., 2016). However, the ddPCR test costs are typically more expensive than qPCR assays (Doi et al., 2015; Hamaguchi et al., 2018).

4. Applications of eDNA in global ecology and conservation

4.1. Species distribution and estimation

Although there is rising global awareness of decreases in wildlife populations, it is still challenging to monitor the growth or decline of specific species' populations. This is due to methodological limitations, the limited knowledge of their lives, the intricacy of their life cycles, and the vastness of their geographic distributions contributing to this lack of certainty (Beng and Corlett, 2020). In the past, monitoring of organisms has typically consisted of collecting or examining individuals and identifying them based on their outward appearance using visual morphology (Rauf et al., 2019; Rosel et al., 2021). The traditional method comprises the employment of multiple techniques for the capture of aquatic animals such as fishing with gill nets, cast nets, and sometimes trawls (Jo et al., 2019; Senapati et al., 2019). Underwater drones and scuba diving have recently become popular methods for conducting visual research on aquatic habitats (Minamoto, 2022). Estimating the distribution of species has been made easier in the past twenty years because of developments in eDNA and metabarcoding. By combining eDNA sampling with hydro-geomorphological features of the network, we can get a better idea of where sessile and migratory species are distributed in aquatic systems (Carraro et al., 2018). Researchers have found a correlation between the concentration of environmental DNA and the distribution of species and biomass in waterbodies (Díaz-Ferguson and Moyer, 2014). In 2007, Matter et al. combined intrinsic prospective habitat simulation with occupancy assessment relied on eDNA and identified an effective, quicker method to anticipate and then evaluate the abundance of juvenile Chinook salmon in previous unsampled tributary areas in the Alaska river system (Matter et al., 2018). Thalinger et al., 2019, looked at the spawning movement patterns of *Alburnus mento* and *Vimba vimba*, and they found a strong relationship between the regular traditional fish monitoring data and the downstream eDNA signals that were derived from filtered water samples (Thalinger et al., 2019). Using sensitive and standardized methods, eDNA analysis can more easily track the location and abundance of species and habitats over large areas and over long periods of time (Hobbs et al., 2019; Matter et al., 2018).

4.2. Applications of eDNA to detect animals in subterranean environments

Subterranean habitats not only play a critical role in providing mankind with vital resources such as food, agriculture, and industrial water reserves, but they also support complex ecosystems whose diversity and function are only now being explored (Danielopol et al., 2003; Mammola et al., 2019). Establishing the distribution and ecology of species is a crucial step in protecting subterranean ecosystems (Boyd et al., 2020). When compared to their counterparts in surface freshwater, subterranean habitats are more difficult to access, and the inhabitants of these habitats tend to be more cryptic. Therefore, underground environments present unique and challenging natural environments to study ecological dynamics and evolutionary tendencies (Saccò et al., 2022). Understanding the distribution and ecology of subterranean organisms (e.g., crickets, fish, salamanders, etc.) is crucial for their conservation, but this is difficult to do using conventional methods (Hashemzadeh et al., 2022). Due to the inaccessibility of underneath voids and interconnections, subterranean ecosystems have received less exploration and are difficult to assess using more traditional methods (West et al., 2020). A number of studies in the last few years have demonstrated that eDNA is a useful methodology for identifying and tracking biodiversity, including scarce, threatened, and endangered organisms (Boyd et al., 2020). Multiple researchers have examined the effectiveness of eDNA and traditional survey methods for identifying uncommon, cryptic, and threatened species, and they observed that eDNA has a higher or equivalent chance of detecting a target species than conventional surveys (Deiner et al., 2017). Analysis of eDNA has the potential to enlighten the biodiversity of the subterranean world both locally and globally (Hashemzadeh et al., 2022). Using traditional methods to detect and monitor uncommon, cryptic, and unusual species is a challenging endeavor that can take a significant amount of time and resources (Qu and Stewart, 2019).

Two methods for DNA metabarcoding involve sequencing either the "haystack" (i.e., wide biological variety) or even the "needle" (i.e., single-species detection assay/specific taxon) of environment DNA sequences (Saccò et al., 2022). Numerous surveys focusing on the detection of a particular species using eDNA were carried out (Table 1). Examples of them are the Alabama cave crayfish, *Cambarus speleocoopi* (Boyd et al., 2020), Caney mountain cave crayfish, *Orconectes stygocaney* (DiStefano et al., 2020), the cave amphibian, *Proteus anguinus* (Vörös et al., 2017), the spring amphipod, *Stygobromus hayi* (Niemiller et al., 2018), the Pilbara blind cave eel, *Ophisternon candidum* (White et al., 2020), five species of cave crayfishes (*Cambarus aculabrum, C. setosus, C. subterraneus, C. tartarus, Orconectes stygocaneyi*) (Mouser et al., 2021). For the "haystack" or "wide biological diversity" method, eDNA metabarcoding research has been conducted for alluvial reservoirs in Australia (Korbel et al., 2017), a calcrete aquifer in Western Australia (Saccò et al., 2022), karst ecosystems on Christmas Island (West et al., 2020), and twenty springs in Iran (Hashemzadeh Segherloo et al., 2022). The results of studies conducted on the fauna of subterranean ecosystems have shown that the "haystack technique" is an effective method for conducting eDNA analysis and is particularly helpful for identifying meiofauna, which is extremely challenging to recognize by employing microscopy. Even though, it is fairly apparent that no single technique can positively identify every taxonomic category (West et al., 2020). The "needle" technique demonstrated the necessity of sample replication when dealing with subterranean organisms with relatively poor identification chances (Mouser et al., 2021).

4.3. Applications of eDNA to detect aquatic invasive species

There are significant threats to aquatic ecosystems from the introduction and spread of aquatic invasive species (AIS), hence it is crucial to detect these invaders as early as possible (LeBlanc et al., 2020). Aquatic, introduced species have devastating effects on native biodiversity because they may eat native species, compete with them, degrade habitats, or spread diseases (Dubreuil et al., 2022). Their appearance can lead to inconsistencies in the biodiversity of a region and have profound impacts on the firms that operate nearby (Lovell et al., 2006). Numerous attempts have been made to identify invasive species as soon as possible so that they can be dealt with quickly and effectively (Thomas et al., 2020). In the past, scientists tracked aquatic invasive species by techniques such as mark-recapture experiments, net surveys, oocyte collecting, electroshocking, and netting juveniles (Erickson et al., 2016). These techniques are expensive to implement because of require intensive personnel efforts. Additionally, there are constraints on these approaches. Many of these drawbacks can be avoided when using more recent methods of detection, such as eDNA, which enables samples to

Table 1
eDNA studies in freshwater ecosystems.

Study site/ Sample habitat/ Substrate	Taxon Studied	Uses	Geographical Location	Barcoding	References
Reservoir, Pond	Parasites	Detection of freshwater myxozoan communities	Czech Republic	Metabarcoding	(Lisnerová et al., 2023)
Lake	Fish	Invasive species detection	USA	Species–specific	(Przybyla–Kelly et al., 2023)
River	Unionid mussels	Population declines monitoring	Japan	Species–specific	(Hata et al., 2022)
River, Lake	Fish	Assess the fish diversity and spatial characteristics of river system	China	Metabarcoding	(He et al., 2022)
River	Fish	Monitoring of diverse fish communities	Austria	Metabarcoding	(Pont et al., 2022)
Lake	Freshwater Mussel	Detection of freshwater mussel	Canada	Metabarcoding	(Coghlan et al., 2021)
River	Rusty crayfish	Detection of the invasive crayfish	USA	Species–specific	(Coster et al., 2021)
River	Freshwater mussels	Quantification of eDNA shedding rates	USA	Metabarcoding	(Klymus et al., 2021)
River	Freshwater mussels	Monitoring of freshwater mussels	USA	Metabarcoding	(Preece et al., 2021)
River	Sharptooth catfish	Tracking of catfish	Egypt	Species–specific	(Elberri et al.,2020)
River	Spectaclecase Mussel	Detection of the endangered mussel	USA	Species–specific	(Lor et al., 2020)
Lake	European eel	eDNA concentrations comparison	Ireland	Species–specific	(Weldon et al., 2020)
River	Fish	Detect the spawning distribution	UK	Species–specific	(Antognazza et al., 2019)
Pond	Fish	Surveillance of the threatened crucian carp	UK	Species–specific	(Harper et al., 2019)
River	Fish	Invasive populations detection	USA	Species–specific	(Guan et al., 2019)
River	Fish	Detection of rare and invasive fish species	Canada	Metabarcoding	(Balasingham et al., 2018)
River	Fish	Detect and quantify elusive benthic fish	Australia	Species–specific	(Hinlo et al., 2018)
Pond	Crayfishes	Detecting invasive crayfishes	France	Species–specific	(Mauvisseau et al., 2018)
Lakes	Fish	Quantifying the elusive round goby	USA	Species–specific	(Nevers et al., 2018)
Pond	Parasites	Prediction of protozoan parasites outbreaks in fish farms	Australia	Species–specific	(Gomes et al., 2017)
Laboratory	Freshwater mussel	eDNA Shedding and decay rates determination	USA	Species–specific	(Sansom & Sassoubre, 2017)
River	Fish	Invasive Fish Species detection	Switzerland	Species–specific	(Adrian– Kalchhauser & Burkhardt– Holm, 2016)
Lake	Fish	Detection of rare and invasive freshwater fish	Turkey	Metabarcoding	(Keskin et al., 2016)
Ponds	Amphibians (great crested newt)	Monitoring of the great crested newt	England, Wales, Scotland	Species–specific	(Biggs et al., 2015)
Plain wetlands	Amphibia	Detecting presence of amphibian species	USA	Metabarcoding	(McKee et al., 2015)
Ponds	Fish (common carp)	Sample processing effect on the detection rate of eDNA	Japan	Species–specific	(Takahara et al., 2015)
Artificial containers	Fish	Detection of freshwater mussels	USA	Species–specifc	(Klymus et al., 2015)
Ponds	Crustacean (red swamp cray fish)	Surveillance of invertebrate species	France	Species–specific	(Tréguier et al. 2014)
Lakes	Fish (common carp)	Distribution of microbial eDNA	USA	Species–specific	(Turner et al., 2014)
Wetlands	Reptiles (Burmese python)	Detecting an invasive species	USA	Species–specific	(Piaggio et al., 2014)
River	Mollusk (Mud snail)	Early detection of mudsnails	USA	Species–specific	(Goldberg et al.,2013)
River	Fish	eDNA surveillance sensitivity for detection of fish	USA	Metabarcoding	(Mahon et al., 2013)
Streams	Frog	Estimating abundance of amphibians	USA	Species–specific	(Pilliod et al., 2013)
Rivers, streams	Amphibians (eastern hellbender)	Survey of eastern hellbenders	USA	Species–specific	(Santas et al., 2013)
Ponds	American bullfrog	Detection of an alien invasive species	France	Species–specific	(Dejean et al., 2012)
Rivers, dammed pool	Fish	Surveillance of fish species composition	Japan	Metabarcoding	(Minamoto et al., 2012)
Lagoon, artificial	Fish	Estimation of fish	Japan	Species–specific	(Takahara et al. 2012)
Ponds, lakes, Streams	Amphibians, fish, mammals, insects, crustaceans	Monitoring endangered freshwater biodiversity	Northern Europe	Metabarcoding	(Thomsen et al., 2012)
Streams	Frogs	Detection of vertebrates	USA	Species–specific	(Goldberg et al., 2011)
Rivers	Fish	Detection of rare aquatic species	USA	Species–specific	(Jerde et al., 2011)
Ponds	Amphibians	Species detection	France	Species–specific	(Ficetola et al., 2008)

identify invasive species (Lodge et al. 2012). eDNA sample coupled with qPCR analysis has become an effective technique, notably for AIS identification, among the instruments available for underwater species tracking (Goldberg et al., 2013; Klymus et al., 2015). Therefore, managers can take rapid action to detect the spread and settlement of invasive species in aquatic environments by confirming their presence using eDNA in a period of hours or days rather than weeks or months (Darling and Mahon, 2011). eDNA research on AIS has included the American bullfrog in France (Ficetola et al., 2008); the Bluegill sunfish in Japan (Takahara et al., 2013), Asian carps in the United States reservoirs (Jerde et al., 2013); African jewelfish (*Hemichromis letourneuxi*) and Rusty crayfish (*Orconectes rusticus* (Dougherty et al., 2016) in the United States (Díaz-Ferguson and Moyer, 2014); New Zealand mud snail in the Portneuf river; Burmese python in the southern Florida, USA (Hunter et al., 2019; Hunter et al., 2015; Piaggio et al., 2014); the spread of Ruffe (*Gymnocephalus cernua*) in the Great Lakes (Tucker et al., 2016); Round goby (*Neogobius melanostomus*) in the North America (Balasingham et al., 2018); the suckermouth/armored catfish (*Hypostomus robinii*) in the tropical island of Martinique (Dubreuil et al., 2022); wedge clam (*Rangia cuneata*) in Europe (Ardura et al., 2015); invasive golden mussel (*Limnoperna fortune*) in China (Xia et al., 2018); sessile marine fouling species (*Bugula neritina*) in South Korea (Kim et al., 2018); cray fish (*Pacifastacus leniusculus, Procambarus clarkia*) in Europe and China (Cai et al., 2017; Mauvisseau et al., 2019; Porco et al., 2022); black carp (*Mylopharyngodon piceus*) in the Unites States (Guan et al., 2019); European green crab (*Carcinus maenas*) (Danziger and Frederich, 2022) (Table 1 and 2).

In addition to fish and shellfish species, eDNA detection surveys for

Table 2
eDNA studies in marine ecosystems.

Study site/ Sample habitat/ Substrate	Taxon Studied	Uses	Geographical Location	Barcoding	References
Peninsula	Fish	Biomass evaluation	China	Species–specific	(Sun et al., 2023)
Archipelago	Green turtles	Diet analyses of sea turtles	West Africa	Metabarcoding	(Díaz–Abad et al., 2022)
Ocean	Pelagic fish	Assessment of fish	Kiribati, Tuvalu	Metabarcoding	(Li et al., 2022)
Archipelago	Monk seal	Range expansion determination	Portugal	Species–specific	(Valsecchi et al., 2022)
Coast	Sessile benthic	Sessile benthic survey	Australia	Metabarcoding	(West et al., 2022)
Sea	Fish	Assessment of fish biodiversity	China	Metabarcoding	(Zhou et al., 2022)
Gulf	Sawfishes	Detection of endangered species	Mexico	Species–specific	(Bonfil et al., 2021)
Island	Fish	Used as a biomonitoring tool for marine protected areas	USA	Metabarcoding	(Gold et al., 2021)
Island	Marine vertebrates	Biodiversity monitoring	New Zealand	Metabarcoding	(Jeunen et al., 2023)
Island	Shark and ray	Diversity, abundance and temporal variation of shark and ray	France	Metabarcoding	(Mariani et al., 2021)
Reefs	Narrow sawfish	Endangered sawfish detection	Indonesia	Species–specific	(Sani et al., 2021)
Indian Ocean	Corals	Monitoring coral diversity	Australia	Metabarcoding	(Alexander et al., 2020)
Estuaries	European eel	Detection and monitoring of the endangered eel	Spain	Species–specific	(Cardás et al., 2020)
Coastal region	Fish	Detection of cryptic seahorse taxa	Australia	Species–specific	(Nester et al., 2020)
Marine Sanctuaries	Marine vertebrates	Marine vertebrate biodiversity and distribution	USA	Metabarcoding	(Closek et al., 2019)
Sea	Fish	Detection of fish species	Denmark	Species–specific	(Knudsen et al., 2019)
Sea	Sea snail	Detection of snail	Spain	Species–specific	(Miralles et al., 2019)
Bay	Fish	Estimates of a threatened salmon species	USA	Species–specific	(Shelton et al., 2019)
Ocean	Corals	Exploring deep–water coral communities	USA	Metabarcoding	(Everett & Park,2018)
Coastal water	Shark	Detection of shark	USA	Species–specific	(Lafferty et al., 2018)
Sea Water	Tubeworm	Detection of Tubeworm species	New Zealand, France, Spain	Species–specific	(Muñoz– Colmenero et al., 2018)
Ocean	Vertebrates	Biomonitoring of marine vertebrates	USA	Metabarcoding	(Andruszkiewicz et al., 2017)
Sea	Invertebrates	Assessment of non–indigenous species	France	Metabarcoding	(Ardura & Planes, et al. 2017)
Ocean	Invertebrates	Survey for early alerts of invasive species	Spain	Metabarcoding	(Borrell et al., 2017)
Ocean	Ray	Survey of pelagic biodiversity	Azores	Species–specific	(Gargan et al., 2017)
Sea	Fish	Used as a conservation tool for fish species	Israel	Metabarcoding	(Karahan et al., 2017)
Bay	Jellyfish	Spatial and temporal jellyfish distribution	Japan	Metabarcoding	(Minamoto et al., 2017)
Harbour	Skate	Detection of an endangered marine skate	Australia	Species–specific	(Weltz et al., 2017)
Bay	Vertebrates	Assessing vertebrate biodiversity in kelp forest ecosystem	USA	Metabarcoding	(Port et al. 2016)
Lagoon	Fish	Monitoring of an endangered aquatic species	USA	Species–specific	(Schmelzle & Kinziger, 2016)
Gulf	Shark	Population characteristics of a large whale	Qatar	Species–specific	(Sigsgaard et al. 2016)
Bay	Fish	Fish distribution estimation	Japan	Metabarcoding	(Yamamoto et al., 2016)
Lagoon	Mollusk	Early detection of invasive species	Russia	Metabarcoding	(Ardura et al., 2015)
Sea	Fish	Detection of more than 230 subtropical marine species	Japan	Metabarcoding	(Miya et al.,2015)
Bay	Fish	Census marine fishes in a large mesocosm	USA	Metabarcoding	(Kelly et al., 2014)
Sea	Marine mammal	Monitoring of marine mammals	Denmark	Species–specific	(Foote et al., 2012)

invasive aquatic vegetation were done for the first time in 2015 (Scriver et al., 2015) due to the difficulty in identifying adequate barcoding markers in flora (Ford et al., 2009). They looked into the potential utility of three regions of the chloroplast genome in aquatic vegetation and showed that all these markers could recognize species from eDNA retrieved from water samples, suggesting that eDNA could be used to monitor aquatic invasive plants (Scriver et al., 2015). The findings of this study might prove useful in the further development of eDNA as a mechanism for the early diagnosis and ongoing monitoring of aquatic invasive species.

4.4. Monitoring ecosystem health using eDNA

Human activities, such as changes in land use, algae blooms, industrial pollution, pesticides (e.g., insecticides, herbicides, fungicides, etc.), or even global warming, pose a direct risk to ecosystems all over the world (Johnstone et al., 2019; Lacy et al., 2022; Lacy and Rahman, 2022; Nash et al., 2019; Vörösmarty et al., 2010). Existing native populations might have significant negative effects both demographically and genetically, as a result of the presence of aquatic invasives as well as introduced viral or fungal pathogens (Billah and Rahman, 2021; Blanc, 2001). One of the greatest dangers to biodiversity is posed by biological invaders, pests, and diseases, which have negative effects on ecosystems, economies, and human health around the world (Beng and Corlett, 2020). Using eDNA, managers can monitor the prevalence of viruses and the spread of invasive species, two indicators of ecosystem health (Minamoto et al., 2009).

The health and yield of plants are intimately connected to the biological and functional variability of the soil microbe composition (Delgado-Baquerizo et al., 2017). To detect soil microbe composition, DNA tests have served as the standard method for over twenty years, in contrast to most other ecosystem monitoring initiatives (Rolf, 2005). As a result of predators, competitors, and parasites, biologically rich soils are more effective at controlling soil-borne pathogens and illnesses, which are advantageous to the development of crops (Barrios, 2007). eDNA has allowed the classification of important archaea, eukaryotes, bacteria, and fungi that constitute the soil microbial populations in agro-ecosystems (Frøslev et al., 2022; Wang et al., 2020). For instance, Frøslev et al. (2022) gathered mass samples of soil and extracted and amplified eDNA from microorganisms such as bacteria, fungi, and eukaryotes to assess whether or not the tillage regimes linked to various agricultural methods altered the diversity and abundance of soil biota. The authors came to the conclusion that even though lower tillage can increase soil diversification; this technique might not be the ideal option

in all farming scenarios because they observed that few intensive tillage regimens only led to slight compositional alterations in soil bacteria. eDNA-based observation of soil microbial diversity has the potential to become an important tool for identifying soil diversification linked with various farming techniques, which might ultimately assist in increasing agricultural yields and ecosystem health (de Graaff et al., 2019).

Furthermore, eDNA can be used as a substitute for monitoring ecosystem health by specifically focusing on shifts in community structure and declines in species diversity. To be more specific, alteration of species diversity can have direct or indirect effects on the dynamics of a whole ecosystem by lowering the quality of water, changing how nutrients move through the system, or influencing submerged macrophytes distribution (Didham et al., 2005; Strayer, 2010). Early identification, examination of distribution patterns, and assessment of population dynamics have all indicated that eDNA is an effective sampling method for tracking the expansion and establishment of threatening biological organisms (Amberg et al., 2019; Nardi et al., 2019; Rudko et al., 2019). Consequently, further environmental impact studies may benefit from the use of eDNA as a decision-making tool based on risk factors (Veldhoen et al., 2012).

4.5. Monitoring biodiversity using eDNA

The ongoing loss of the planet's animal and plant diversity is still one of the most significant problems facing humanity in the 21st century. Populations of natural flora and fauna are decreasing all over the world due to anthropogenic activities, and the pace of species extinctions currently exceeds that of pre-human periods, which has a significant influence on both the health of humans and the long-term viability of our planet (Thomsen and Willerslev, 2015). Over the last decade, eDNA has emerged as a promising tool for biodiversity assessment from the genetic marker of inferring species' existence. The management of biodiversity involves detecting species in danger, analyzing biosecurity threats, and avoiding the entrance of invasive species, etc. (Cristescu and Hebert, 2018).

DNA metabarcoding is a term that refers to the process of identifying multiple species through the usage of eDNA samples (Taberlet et al., 2012). The methodology relies on next-generation sequencing, which enables the sequencing of millions of 100-base-pair reads and is employed to construct taxonomic reference libraries like the Barcode of Life (Díaz-Ferguson and Moyer, 2014). Because of this, an eDNA metabarcoding approach could potentially recognize the eDNA of every taxon found in a sample taken, as long as the nucleotide sequences have already been documented in a database. eDNA has made it easier for us to monitor past and current biodiversity by alleviating some of the challenges posed by time-consuming and labor-intensive traditional survey methods (Cai et al., 2022). Therefore, metabarcoding may be utilized as a technique to develop estimates of biodiversity that are easier to construct, and less dependent on taxonomic skill than earlier methods. (Ji et al., 2013). It is now possible at a reasonable cost to evaluate the diversity of whole communities, as well as draw conclusions about the diversity and assemblage patterns of many different taxonomic groupings (Stat et al., 2019; Zinger et al., 2018).

4.6. Trophic interactions and dietary studies

Understanding and measuring biotic relationships, such as the relationships between predators and prey and hosts and parasites, and ecological traits such as dietary patterns, trophic niches, and food webs are important parts of ecological research. However, despite their significance, these crucial biological operations have received insufficient research attention, mostly due to difficulties associated with various methodological obstacles. For example, using stomach contents or excreta pellets, traditional methods of research make it possible to quantify and estimate the link between herbivores and plants as well as the association between herbivores and their food (Zarzoso-Lacoste et al., 2013). However, it can be challenging to observe or identify prey in the stomach or fecal waste, which can make the taxonomic resolution less clear or lead to biases.

DNA metabarcoding is a noninvasive method for analyzing animal diets that have been validated as both precise and cost-effective and have gained widespread acceptance (Ando et al., 2020). Symondson (2002) reviewed the first investigations that used DNA barcoding to analyze wildlife feeding patterns, with a focus on invertebrates. In 1992, the earliest DNA-based research on diet identified particular foods by amplifying them with a taxon-specific PCR (Höss et al., 1992). This experiment was designed to evaluate a mammal's diet and undertaken to investigate if vegetative DNA might survive the stomach in the European brown bear (*Ursus arctos*) (Höss et al., 1992). These scientists amplified a fragment of the chloroplast rbcL gene from excrement using PCR, which suggests that barcoding methodologies might be utilized to analyze the nutrition of endangered animals utilizing noninvasive sampling methods. Four years later, a different team was able to identify many plants to order and family level by sequencing the rbcL extracted from the coprolites (prehistoric excrement) of the extinct ground sloth, *Nothrotheriops shastensis* (Poinar et al., 1998). Simultaneously, another group came up with a plan to use microsatellites to identify and differentiate between different kinds of waterfowl based on the contents of the digestive systems of glaucous gull, *Larus hyperboreus* (Scribner and Bowman, 1998). After that, dietary DNA sequences were extracted from a mix of feces DNA from many different species by cloning and Sanger sequencing (Deagle et al., 2007). Using a DNA barcoding procedure, researchers were able to determine the species from which the retrieved sequences originated (Hebert and Gregory, 2005). In 2009, some of the earliest investigations on fecal metabarcoding were publicly released; these studies demonstrated that fecal metabarcoding could be used to estimate an animal's diet by comparing the findings of visual assessment of feces and earlier diet documentation of each particular species (Deagle et al., 2009; Valentini et al., 2009).

The initial research to use molecular methods to investigate the diets of aquatic animals focused on determining classes of prey, such as krill and fish species, using samples taken from the digestive tracts or excrement of sand shrimp, *Crangon affinis* (Asahida et al., 1997); whales, *Balaenoptera musculus* (Jarman et al., 2002); giant squid, *Architeuthis sp.* (Jarman et al., 2004); penguins, *Pygoscelis adeliae* and *Eudyptes chrysolophus* (Deagle et al., 2007; Jarman et al., 2004); sea lions, *Eumetopias jubartus* (Deagle et al., 2005); seals, *Halichoerus grypus* and *Phoca vitulina* (Kvitrud et al., 2005; Parsons et al., 2005).

Over the past decade, the frequency of dietary research on aquatic biota employing molecular techniques has expanded substantially, and they are now nearly as common as investigations on terrestrial species. For nutritional and trophic investigations, using eDNA fragments or a metabarcoding approach with gastrointestinal material as target DNA. Rather than relying on visual inspection or feces identification, this method can now be employed (Zarzoso-Lacoste et al., 2013). Investigating how plants and animals interact and the significance of these relationships in maintaining ecosystem functions and services may also be conducted using the DNA left by pollinators on flowers or by seed-spreaders on seeds (Thomsen and Sigsgaard, 2019).

4.7. Monitoring spawning ecology using eDNA

Reproduction is a crucial component of an aquatic organism's life cycle and is especially true for rare species, and species that are important targets for fisheries as well as aquaculture. It is important to know when and where spawning happens for effective conservation and/or population management (Danylchuk et al., 2011; Spear et al., 2015). Researchers have relied on the collection of eggs, larvae, and adults in the spawning phase to advance their knowledge of the natural reproductive ecology of aquatic organisms (Beng and Corlett, 2020). Traditional techniques of surveying by capture can pose a risk to the survival of species or populations, particularly for uncommon and

endangered species, due to the increased mortality they cause among the spawning population (Wei et al., 2009). In many cases, the methodologies used to identify life stages are flawed because they are biased, damaging, or reliant on a dwindling group of experienced taxonomists (Maruyama et al., 2018). Traditional spawning surveys, in which individuals or eggs are directly observed are difficult, time-consuming, and prone to investigator biased, territorial constraints, and erroneous spawning count estimates (Caswell et al., 2004; Ko et al., 2013).

DNA barcoding was utilized by Chen et al. (2021) to evaluate the species composition of the eggs as well as to make predictions on the spawning cycles of the species that were detected (Chen et al., 2021). They were able to accurately classify 392 eggs as well as 13 larvae among 14 different species and discovered that spawning cycles are likely to be species-specific. The accurate determination of eggs can provide important information on the spawning habitats of several species. Lima et al. (2020) employed a DNA barcode for the purpose of identifying fish eggs found in river streams. They used the database of the systems to conduct an analysis of 928 sequences and found that 99.8% of those sequences could be recognized at a specific level, suggesting a high rate of success for egg detection (Lima et al., 2020). Meulenbroek et al. (2018) employed DNA barcoding to validate the first-ever species-level identification of fish larvae in the Danube river in

Table 3
eDNA studies in terrestrial ecosystems.

Study site/ Sample habitat/ Substrate	Taxon Studied	Uses	Geographical Location	Barcoding	References
Soil	Earthworms	Comparing earthworms' biodiversity	Denmark	Metabarcoding	(Lilja et al., 2023)
Soil	Fungi	Fungal diversity and community composition variation determination	Spain	Metabarcoding	(Krah & March- Salas, 2022)
Soil	Plant	Plant biodiversity assessment	Norway	Metabarcoding	(Ariza et al., 2022)
Soil	Terrestrial reptile	Terrestrial reptile survey	USA	Species–specific	(Kyle et al., 2022)
Soil	Fungi	Comparison of soil fungal communities	Mexico	Metabarcoding	(Navarro–Noya et al. 2021)
Soil and Vane	Fungi and Arthropods	Characterize ecosystem diversity	Germany	Metabarcoding	(Agerbo Rasmussen., 2021)
Soil	Fungi	Comparison the patterns of functional diversity among different fungal	Costa Rica	Metabarcoding	(Sternhagen et al., 2020)
Soil	Earthworms	Earthworm communities tracking	France	Metabarcoding	(Bienert et al.,2012)
Grassland (soil)	Plants	Soil extracellular DNA analyses	France	Metabarcoding	(Taberlet et al., 2012)
Soil and permafrost, enchytraeids, beetle, birds	Fungi, bryophytes	Analyzing soil DNA	Norway; Siberia	Metabarcoding	(Epp et al., 2012)
Cropland	Metazoa	Determine land–use impacts on soil invertebrate communities.	New Zealand	Metabarcoding	(Dopheide et al., 2020)
Agricultural field	Leptospira sp. and bacteria	Understanding leptospirosis co–epidemiology	Sri Lanka	Species–specific, Metabarcoding	(Gamage et al., 2020)
Coffee farms (Soil)	Archaea and bacteria	Prokaryotic diversity analysis	Brazil	Metabarcoding	(Caldwell et al., 2015)
Crop lands (Soil)	Archaea and bacteria	Achaea and bacteria detection	China	Metabarcoding	(Jiang et al., 2014)
Cropland	Bacteria and Eukaryotes	Soil microbial succession	China	Metabarcoding	(Wang et al., 2020)
Cropland (Soil, root and leaf)	Bacteria, fungi, and oomycetes	Plant pathogen detection	New Zealand	Metabarcoding	(Makiola et al., 2019)
Rice field	Bacteria	Transmission and biogeography of bacteria	China	Metabarcoding	(Zhou et al., 2020)
Farmland	Plants and moths	Construction, validation, and application of nocturnal pollen transport networks	England	Metabarcoding	(Macgregor et al.,2019)
Forests	Fungi	Determines spore deposition	Sweden	Metabarcoding	(Redondo et al., 2020)
Forests	Snake	Detection of Kirtland's snake microbiota	USA	Species–specific	(Ratsch et al., 2020)
Agricultural field and forest	Insects	Assessing insect biodiversity	Brazil	Metabarcoding	(Zenker et al.,2020)
Agricultural fields	Bugs	Detection of invasive exotic insect	USA	Species–specific	(Valentin et al.,2018)
Agricultural landscapes	Pollen	Diversity of collected pollen	Germany	Metabarcoding	(Danner et al., 2017)
Agricultural fields	Insects	Prey detection	Europe	Metabarcoding	(Aizpurua et al., 2017)
Barley fields	Fungi	Characterizes fungal endophyte diversity	Western Australia	Metabarcoding	(Milazzo et al., 2021)
Nunatak sediments	Plants	Vegetational stability determination	Greenland	Metabarcoding	(Jørgensen et al.,2012)
Sand sediment	Mammals	Ancient 'dirt' DNA analyses	South–west Greenland	Metabarcoding	(Hebsgaard et al., 2009)
Island habitat	Insects	Pollen grain analysis	China	Species–specific	(Chang et al., 2018)
Orchards	Insects	Information gathers from plant–sucking insects	Europe	Metabarcoding	(Utzeri et al., 2018)
Macadamia orchards	Arthropod	Diet and explore pest–reduction services of sympatric bird	Australia	Metabarcoding	(Crisol–Martínez et al., 2016)
Several Vegetation	Fungi	Assess air borne fungal	Italy	Metabarcoding	(Tordoni et al., 2021)
Grass land	Plants	Comparison of grassland plant–pollinator networks	France	Metabarcoding	(Michelot–Antalik et al., 2021)
Vineyards	Bacteria and yeast	Grape microbiome detection	Italy	Metabarcoding	(Mezzasalma et al., 2017)
Palm plantations	Insects	Quantify the biological impacts of plantations	Malaysia	Metabarcoding	(Edwards et al.,2014)
Tree bark, Soil	Mammals (Bats)	Detection of cryptic arboreal mammals	USA	Metabarcoding	(Allen et al., 2023)
Fruit and leaf surfaces	Insects	State, transport, and fate of eDNA	USA	Species–specific	(Valentin et al.,2021)
Leaf and stem surfaces	Insects	Detecting an invasive pest insect	USA	Species–specific	(Allen et al., 2021)
Apple and pear orchards	Bacteria	Temporal and spatial variation in bacterial communities.	Belgium	Metabarcoding	(Smessaert et al., 2019)
Permafrost	Plants	Molecular reconstruction of arctic vegetation	Siberia	Metabarcoding	(Sønstebø et al.,2010)
Compost	Bacteria	Analysis of the bacterial succession	India	Metabarcoding	(Srivastava et al.,2021)
Air	Bat, Mammals	Tropical bat, and other mammal	Belize	Metabarcoding	(Garrett et al., 2023)

Austria. The researchers discovered that the seasonality and length of larval drifting were relatively unique to each species (Meulenbroek et al., 2018). Using DNA barcoding, Hou et al. (2021) were able to locate spawning areas and determine the viability of eggs from the hairtail fish, *Trichiurus*, in the northern part of the South China Sea (Hou et al., 2021). In most cases, synchronous multi-specific coral spawning takes place once a year and is an essential component of the entire lifespan of corals (Ip et al., 2022). Ip et al. (2022) studied eDNA as an additional monitoring method by examining its potential in recognizing spawning species and observing the relative abundances of coral as well as fish eDNA. Their findings showed that eDNA has the potential to be an effective monitoring tool in the future. In the study conducted by Di Muri et al. (2022), the spatial pattern of Arctic charr, *Salvelinus alpinus* L., in the lake was determined using a DNA (eDNA) metabarcoding method at various times throughout the year. The goal of this research was to determine whether this method could help us identify spawning regions and associated fish behavior.

4.8. Monitoring agricultural ecosystems using eDNA

Environmental and anthropogenic stressors reduce worldwide food production and make it more difficult for 8.9% of the world's population to obtain adequate nutrition (Cole et al., 2018; UNICEF, 2020; Yue et al., 2020). It will be more difficult to improve global food security when agricultural systems are threatened by factors such as climate change, the disappearance of arable land, a shortage of water, pests, pathogens, and species that are important for pollination (Hossain et al., 2020; Lesk and Anderson, 2021; Lippert et al., 2021; Savary et al., 2019). In order to fulfill the demand for food in the world population agricultural and horticultural methods need to be sped up, with a focus on improving soil health and plant nutrition, eradicating disease, and encouraging the growth of beneficial organisms (i.e., modulating bacteria) (Amari et al., 2021; Potts et al., 2010). Detection of mesofauna and macrofauna within the soil, crop, animal pests/pathogens, and pollinating species is mainly reliant on laboring processes (Ashfaq and Hebert, 2016; Gerlach et al., 2013; Pardo and Borges, 2020; Tsoi et al., 2020). The new plant pathogens introduction as well as alterations in the pathogenicity and distribution of existing plant pests and pathogens may be jeopardized conventional agricultural systems; especially, if modern inventions and tools are not used to track the spread of new pests and diseases (Jones, 2009; Wintermantel and Hladky, 2010).

In agriculture, identifications based on eDNA are extremely useful since they allow for the rapid and reliable detection of pathogens in leaf litter, soil, and air (Table 3). Wheat blast fungus (*Magnaporthe oryzae*) and (*Ramularia* sp.) are pathogenic fungi that are difficult to identify/cultivate and have rapidly spread over country borders, with some farms reporting annual output losses of up to 100% and 70%, respectively (Ceresini et al., 2019; Havis et al., 2015). The geographical and temporal differences of airborne mold spores in the forest or agricultural habitats were determined by Redondo et al. (2020) using passive and active air samplers in combination with eDNA techniques. Tordoni et al. (2021) identified more fungal species with eDNA metabarcoding than with the conventional identification method. They collected fungal spores from the air and showed that this eDNA is a potential technology that was able to recognize and distinguish pathogenic microbes in cultivated environments (Tordoni et al., 2021). Farmers could use fungicides more effectively by spraying them only in regions where pathogen infestation has been detected. This would extend the amount of time they work and enhance the investment return. In addition, the danger of causing harm to the environment can be reduced by prioritizing the application of fungicides, in comparison to other spraying methods that are more ubiquitous (Sowunmi et al., 2019). When applied to plant material or bulk insect traps, barcoding and metabarcoding of the DNA of herbivorous insects can be an efficient method for immediately assessing the presence of both harmful and helpful insects on large-scale agricultural and horticultural plants (Thomsen and Sigsgaard, 2019; Young et al., 2021). An economical eDNA detection approach for the devastating pest species (*Halymorpha halys*) was more successful than conventional methods, and it utilized the rinse water extracted from washed apples (Valentin et al., 2016). However, these advanced detection technologies are not just necessary for treating crops after they have been harvested; they also hold promise for use in pre-harvest detections which would enable targeted pesticide treatments before crops suffer widespread harm (Leskey et al., 2012; Valentin et al., 2018).

Zoonotic infections in cattle provide a risk to the welfare of animals by elevating animal stress levels. They can cause miscarriages, and reduce the overall production of livestock (Mohamed, 2020; Saadi et al., 2020). Zoonotic illnesses are transmissible between people and create direct and indirect health risks to humans (Mohamed, 2020). It is still difficult to identify and eradicate these infections in many countries across the world, especially in developing countries (Gebreyes et al., 2014; Thomas et al., 2020). The detection of both common and unusual zoonotic infections with a small number of non-invasive samples is now possible through the use of eDNA analysis from animal waste (Brunner, 2020). The earliest use of technologies based on eDNA gave health metrics for animals by assessing the feces microbes (Fouts et al., 2012). Then, the eDNA approach was expanded to include universal and species-specific assays in agricultural water in order to detect which organisms served as potential hosts (Gamage et al., 2020). Researchers would be able to identify a wide variety of zoonotic infections if the widespread adoption of a method that involves collecting samples of feces, urine, or saliva and applying numerous metabarcoding assays was used. Rapid and accurate diagnoses of developing corona infections (e.g., COVID-19; Jafarinejad et al., 2021) and zoonotic infestations (Cabodevilla et al., 2022) may be possible with the use of eDNA detection techniques, leading to preventive interventions that are good for the health of animals and the efficiency of herds.

4.9. Population genetic analysis using eDNA

Conservation, management, and optimal use of natural diversity require an in-depth knowledge of the molecular basis of fundamental biological processes in a species. As increased attention is paid to climatic changes and ecological imbalances, it is essential to recognize the changes and problems that wildlife populations experience and to apply the conservation and governance techniques that are currently available (Hohenlohe et al., 2021). It is important to understand the forms of connectivity among communities and the degree of self-recruitment in order to investigate the population biology of animals and manage and conserve resources effectively (Bay et al., 2006). The use of genomics technologies enables the generation of accurate estimates of fundamental aspects of animal populations, such as effective population size, demographic history, inbreeding, and population structure, all of which are essential for the effectiveness of conservation initiatives (Hohenlohe et al., 2021).

Due to the similarity between fish eDNA extracted from water bodies and grouped DNA from a specific population, the sequence variations obtained from eDNA sequencing offer a cheap, effective, non-invasive, and feasible method to approximate genetic diversity in wild communities (Sigsgaard et al., 2020). The efficacy of the eDNA approach in aquatic population genetics is shown by its application to the assessment of genetic features such as allele frequencies, population genetic structure, and effective population size in a variety of fish species across a wider geographical area (Andres et al., 2021; Ruppert et al., 2019; Székely et al., 2021; Weitemier et al., 2021). Along with providing information about genetic diversity within a species, sequence variations detected among organisms can shed light on a population's origins. For example, to determine the subspecies-specific colonization trends of native trout (*Oncorhynchus clarkia bouvieri*) and invasive trout (*O. c. lewisi*), Nelson-Chorney et al. (2019) employed eDNA extracted from lake sediments and a determined mtDNA variability.

5. Challenges and limitations of eDNA metabarcoding

5.1. Contamination in eDNA studies

The most significant disadvantage of eDNA is most likely the chance of contamination, which can lead to the generation of false positive results (Sepulveda et al., 2020). It is possible for samples to get contaminated at any stage of the analysis process, beginning with the collection of the samples in the field and continuing through every stage of laboratory analysis (Furlan and Gleeson, 2016; Valdivia-Carrillo et al., 2021). This is a significant problem in eDNA surveys because of the high sensitivity of the method (Table 4), and it has the potential to lead to false positive findings as well as incorrect conclusions being drawn from the data (Darling et al., 2021; Hutchins et al., 2022; Sepulveda et al., 2020).

There are many chances for contamination throughout the entire eDNA analysis process, from filtering in the field, transportation, sample storage, eDNA extraction, PCR preparation, and sequencing (Goldberg et al., 2016; Huerlimann et al., 2020; Valdivia-Carrillo et al., 2021). Unintentional transfer of DNA from one or more samples to another sample, either from a different location within the same research or from an unknown locality, constitutes contamination in the field (Thomsen and Willerslev, 2015). This usually takes place when the same sample equipment such as filters, gloves, and corers are used in many places without being properly cleaned (e.g., sterilization) (Goldberg et al., 2016; Thomas et al., 2018). If samples are taken from several different places in the field one after the other, there is a possibility of cross-contamination because the target DNA was accidentally moved from one place to another (Bylemans et al., 2019). When the same laboratory equipment is used for multiple studies without proper cleaning, contamination can arise in the form of residual DNA from previous genetic studies extending into new samples (Rodgers, 2017; Shaw et al., 2016). The potential contamination concerns can be greatly reduced by strictly adhering to a clean-lab strategy that includes decontamination processes and the physical separation of laboratories for pre- and post-PCR processing (Carraro et al., 2020; McClenaghan et al., 2020).

5.2. Short lifespan of eDNA

The persistence of DNA fragments is a phenomenon that occurs when DNA remains present in a system after the DNA source has been taken out of the system (Dejean et al., 2011). DNA released into the surrounding environment by organisms is not usually confined in one place; rather, it is dispersed over space and degrades over time (Pilliod et al., 2014; Sansom and Sassoubre, 2017). Density, life cycle features, species interactions, and size of the target species all have an influence on the persistence of eDNA; however, biotic factors such as the concentrations of bacteria and fungus also influence the persistence of eDNA (Dejean et al., 2011; Stewart, 2019). The rates at which eDNA degrades are also affected by factors such as nuclease activity, pH, oxygen content, conductivity, temperature, salinity, and ultraviolet exposure (Barnes et al., 2021; Saito and Doi, 2021; Strickler et al., 2015). When analyzing eDNA persistence, another crucial factor to consider is the fragment size of the target eDNA. Reports indicate that fragments with a base pair count of 300–400 can sustain in an aqueous environment for at least a week under controlled conditions (Alvarez et al., 1996; Zhu, 2006). However, research has shown that shorter DNA fragments (~100 base pairs or fewer) can survive in their original form for months or even years, depending on the conditions in which they are placed (Díaz-Ferguson and Moyer, 2014).

5.3. PCR primer biases for eDNA studies

For accurate species identification, it is crucial to carefully identify the molecular markers to be used in the study of eDNA, whether the

Table 4
Problems and solutions in eDNA studies

Problems	Solutions	References
Challenges arising from selecting an acceptable sample method	Utilize eDNA sampling procedures that are completely integrated into the environment.	(Thomas et al., 2018)
The short–term persistence of eDNA in the environment	Several PCR and field tests should be conducted and determine the rates of detection by utilizing the other models.	(Alberdi et al., 2018; Jo & Minamoto, 2021; Joseph et al., 2022; Ratsch et al., 2020; Valentini et al., 2016)
False positives are caused by sample contamination.	Establishing clean and consistent field collecting techniques. Negative controls will be utilized during the field, water filtering, DNA extraction, and amplification stages.	(Antognazza et al., 2021; Bista et al., 2017; Carim et al., 2016)
Due to faulty sampling, ancient DNA (aDNA) has been retrieved.	Validate the presence of the species using the conventional survey techniques. Increase the number of replications.	(Ficetola et al., 2015; Wu et al., 2018)
Primer biases	Use of several marker and primer combinations, even when aiming for the same taxa, is one option for resolving this issue. This approach has the potential to decrease primer bias and expand taxonomic coverage, however, it is more time–consuming and expensive.	(Alberdi et al., 2018; Collins et al., 2019; Cristescu, 2014; Drummond et al., 2015)
False positive because of a dead organism or animal waste containing target eDNA	Amplification should be performed on both longer and shorter eDNA fragments. Check the findings against the results of traditional community composition surveys.	(Laroche et al., 2017; Mathieu et al., 2020; Nathan et al., 2015; Rasmussen et al., 2021)
Difficulties in estimating abundance and biomass arise from the uncertainty in eDNA deposition and preservation.	Determine the quantitative nature of the relationship that exists between the release of eDNA and both biotic and abiotic variables. Some preservation method was restricted to a specific species.	(Sales et al., 2019; Sassoubre et al., 2016)
A limited knowledge of the ecology of eDNA, including its origin, condition, density, movement, and fate.	Perform experimental verification in controlled and uncontrolled environments. Recover longer barcodes, which might assist in targeting less damaged eDNA.	(Barnes & Turner, 2016; Bohmann et al., 2014; Murakami et al., 2019)
There is little data available regarding the individuals' ages, genders, and sizes.	Markers such as age–specific and gender–specific can be used as a more practical approach to overcoming this challenging limitation. Concurrently use both eDNA and conventional survey methods.	(Biggs et al., 2015; Valentin et al., 2016)
Results from different studies are difficult to compare because of the absence of established protocols.	Make a side–by–side comparison of the new procedures with the old ones, preferably in separate labs and under a variety of conditions. Analyze the effectiveness of different eDNA metabarcoding methods using established benchmarks.	(Beja-Pereira et al., 2009; Hunter et al., 2017; Rees et al., 2014)

sample under investigation contains a single species or multiple species (Freeland, 2017; Othman et al., 2021). However, the primer's specificity, sensitivity, and efficiency are extremely important factors in determining whether an eDNA amplification is effective (Schultz and Lance, 2015; Xia et al., 2018; Yang et al., 2023). Obtaining optimal primer-target sequence complementarity in the polymerase chain reaction is difficult when dealing with eDNA samples because of the large number of distinct taxa or haplotypes present in these samples (Nichols et al., 2018; Wei et al., 2018). Disparities between primers and templates can cause the primer-template pair to break down prematurely and can limit the effectiveness with which the polymerase amplifies the primer, both of which can result in erroneous results or the complete failure of the PCR process (Stadhouders et al. 2010). In contrast to metabarcoding, primer partiality is not a significant problem for barcoding. For this reason, eDNA barcoding should emphasize targeted sample amplification during PCR with species-specific primers rather than universal primers (Wilcox et al. 2013; Cannon et al. 2016). The qPCR method is thought to be more reliable than the traditional cPCR method which might result in cross-amplification, and consequently, false positive findings (Guan et al., 2019; Langlois et al., 2021; Wilcox et al., 2013).

5.4. False positive detections of eDNA

The current approach for eDNA metabarcoding entails using polymerase chain reaction (PCR) to amplify gene fragments derived from extremely small quantities of DNA in aquatic settings in order to facilitate high-throughput sequencing (Miya et al., 2022). False-positive detections are nearly impossible to avoid due to contamination with DNA introduced from outside of the organisms in the field or in a variety of steps before conducting PCR in the laboratory (Klepke et al., 2022; Thalinger et al., 2021). There are different types of prerequisites needed for eDNA metabarcoding. One of them is the establishment of an experimental setting that is less vulnerable to contamination by exogenous DNA (Hänfling et al., 2016). Minimum requirements for laboratory facilities (such as having a separate room for eDNA extraction, pre- and post-PCR steps, each with their own equipment), guidelines for individuals directing experiments (including instructions to eliminate contamination spreading from the post-PCR room to adjoining rooms), and disinfection process to remove exogenous DNA from lab equipment (e.g., UV sterilization) (Bylemans et al., 2016; Piggott, 2016; Spens et al., 2017).

Exogenous DNA can come from a variety of places outside the lab such as wastewater from fishing vessels or ports, the fish processing industry, fish farms, aquariums, raw materials during processing, induced breeding of fish, etc. (Yamamoto et al., 2016). There is also a significant quantity of eDNA that is obtained from dead-fish carcasses, in addition to the feces of piscivorous animals including fish, mammals, and migratory marine birds (Rees et al., 2014). So, water sampling locations should be selected with care for preventing the collection of exogenous DNA from these kinds of sources in the environment. In addition to these exogenous DNA sources, it was noted that habitat-specific studies utilizing eDNA need to take into consideration the possibility of eDNA overflow from one area to another because of tidal movements or ocean circulation (Lafferty et al., 2021).

5.5. False-negative detections of eDNA

In every ecological field survey methodology, there is the possibility of obtaining false negative results, which refer to the absence of species that are really found in the study region (Brys et al., 2021; Xia et al., 2018; Schultz and Lance, 2015). In eDNA metabarcoding, the rate of detection improves or the number of false negatives goes down in proportion to the amount of sampling effort and the volume of filtered water used (Bessey et al., 2020). The experimental methods, such as filtration (e.g., Filterselectionand pore size), and the DNA extraction process (e.g., the reagents amount and eDNA concentration), have a significant impact on the amount of eDNA that can be extracted and may lead to false negatives results when detecting low-abundance species (Kawato et al., 2021; Spens et al., 2017; Wong et al., 2020). There is no universal primer combination that can successfully work for amplifying different kinds of marine fishes because of their diversity (Miya et al., 2020). The second issue is that the amplicons of congeners that are has a close relation do not alter significantly which invariably results in an underestimate of the total number of species present in a particular sample, community, or geographical region (Miya, 2022). The species detected by eDNA method is dependent on factors other than the PCR primers, such as the sample number and the number of times the same sample was run through PCR (Bessey et al., 2021; Yamamoto et al., 2017). If there are minor species in eDNA extracts, it would be best to do PCR with numerous replicates to retain a relatively high detection probability (Ficetola et al., 2015).

6. Future directions

The eDNA technique has provided novel opportunities for the environmental research and management of ecosystems, along with a lot of potential for the future. The advance in DNA sequencing technologies has significantly expanded the possibilities of using eDNA and is expected to continue improving in the future. However, all monitoring methods have both advantages and disadvantages. In this article, we reviewed the existing situation of eDNA research, as well as its useful applications, and the constraints that still need to be resolved before they can be widely used. Now, we advocate that future research based on eDNA techniques might concentrate on the following:

1 The application of eDNA technologies in toxicology seems to have the possibility of serving as a successful technique for investigating the adverse effect of toxic chemicals on ecosystems. Chemical toxins, insecticides, and heavy metals can all have drastic effects of the organisms living in contact with them. By using eDNA to characterize populations in water, soils, and sediment samples, the effects on the populations can be compared. Ecosystem health and toxicological assessments can benefit from this non-invasive and very accurate approach.
2 Mangrove species, particularly those that are challenging to observe using conventional methods, can be assessed for their existence in a zone utilizing eDNA techniques. Conservation efforts depend heavily on understanding the species' genetic diversity, which may be revealed through this method. For example, by employing eDNA technology, investigators are able to figure out what species are existing in a specific location and assess trends in abundance and diversity over the period, enabling more specific recovery efforts. Moreover, eDNA technology may assist to identify favorable locations for mangrove restoration by trying to identify regions with appropriate environmental factors for mangrove expansion. With this knowledge, restoration experts will have the ability to prioritize their efforts where they will have the major influence.
3 Finally, eDNA technology hasn't been employed as the primary approach to determine COVID-19 presently, but it looks promising as a complementary technique for upcoming public health surveillance activities. The accuracy of eDNA procedures for COVID-19 diagnosis is not currently well-established. However, it is likely to recognize residues of the virus's RNA from environmental samples including sewage or atmospheric samples (Anand et al., 2021). Screening wastewater for indications of the virus's genetic information (a method called "wastewater-based epidemiology") has already been suggested as one possible approach for assessing the distribution of COVID-19 among populations (Fuschi et al., 2021; Innes et al., 2022). This technique was utilized in order to monitor outbreaks and monitor the transmission of the virus in numerous countries (Innes et al., 2022).

7. Conclusion

In conclusion, the use of technologies based on eDNA has the potential to significantly increase our capability for the scientific study and protection of biodiversity and conservation. Using eDNA for monitoring biodiversity may provide a simple, cheap, and standardized technique to collect crucial information on the subterranean and aquatic invasive species range and population size, allowing for more effective use of minimal conservation funds and taxonomic knowledge. The study of eDNA will provide valuable information for studies that aim to identify diversity fluctuations, species hot spots, and the presence of invasive species, as well as those aimed at focusing on conservation programs or exposing ecosystem-level processes. Now, the most practical implementation of eDNA-based biomonitoring is as a supplementary tool to conventional assessment paradigms, which have been gradually improved over a considerable period of time (Table 4). eDNA analysis is changing the way we develop and carry out projects for biodiversity monitoring and conservation. In addition to this, it has shown the possibility of opening new doors in the future. Although this method shows potential in aquatic and terrestrial systems for biodiversity monitoring, hypothesis testing, and understanding eDNA, it faces several difficulties related to these ecosystems in addition to the typical challenges experienced in all habitats. The use of eDNA for tracking cannot take the place of the field observation methods by skilled environmental scientists and taxonomic experts, who can collect and store data that goes beyond quantitative and qualitative observations. So, techniques still need to be standardized, and results still need to be shown consistently. In addition, further research is necessary to determine the ecological and physical constraints of employing eDNA. The assessment of biodiversity cannot be resolved globally using eDNA-based methods. Another significant obstacle is the requirement for coordinated action, not only for the purpose of benchmarking methodologies but as well as for the purpose of integrating conventional methods such as taxonomic and ecological data when simultaneously deploying and continually upgrading new technology.

Consent for publication

The authors have approved to submission of the final version of the manuscript.

CRediT authorship contribution statement

Sakib Tahmid Rishan: Methodology, Writing – original draft. **Richard J. Kline:** Methodology, Supervision, Writing – review & editing. **Md Saydur Rahman:** Conceptualization, Methodology, Supervision, Writing – review & editing.

Declaration of Competing Interest

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

Data availability

No data was used for the research described in the article.

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