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MINI REVIEW

A review on the applications and recent advances in environmental DNA (eDNA) metagenomics

Deviram Garlapati · B. Charankumar · K. Ramu · P. Madeswaran · M. V. Ramana Murthy

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Abstract Progressions accomplished in the field of molecular biology amid the most recent decade has changed the way in which we contemplate biodiversity on earth. Together with the accessibility of new molecular techniques like high-throughput sequencing (HTS), a prompt metamorphosis was attained in understanding the behaviour and biodiversity patterns of biological systems at a level never before possible. The DNA gathered from different environmental samples (named as environmental DNA or eDNA) when coupled with HTS offers a powerful tool by empowering the census of individual species on a global scale in real time. The applications of eDNA are transpiring in different domains, for example, trophic and community ecology (functional diversity, ecosystem dynamics and prey–predator interactions), biomonitoring, conservation biology (single and multi species detection, abundance estimates), invasion biology (early species detection, passive surveillance) and environmental assessment (detection of anthropogenic contamination, microbial source tracking). However, more empirical data is required to standardize the specific sampling procedures to achieve in the best possible way. Although the application of eDNA is intensifying swiftly at a global scale, there are still some knowledge gaps, especially with methods and applications. These procedures require some refinements and validations to diminish the burden of false positives/negatives. Considering these impediments, we mainly concentrated in pooling together the most recent outcome of research articles (2008–2019) available in eDNA analysis that converse about diverse ecosystems (freshwater, marine and terrestrial habitats). We likewise discussed developments and limitations that are generally concerned with eDNA exercise in the present review.

Keywords Biodiversity · Ecosystem · Environmental DNA · eDNA · Freshwater habitats · Invasive species · Marine habitats · Next-generation sequencing · High throughput sequencing · Species monitoring

D. Garlapati (✉) · B. Charankumar · K. Ramu · P. Madeswaran · M. V. Ramana Murthy
National Centre for Coastal Research (NCCR), Ministry of Earth Sciences (MoES), Chennai 600100, India
e-mail: deviram@nccr.gov.in; deviramgarlapati@gmail.com

1 Introduction

One of the prime concern of the twenty-first century is the mounting decline in Earths biodiversity and thereby ensuing change in productivity and sustainability of ecosystems (Butchart et al. 2010; Hooper et al. 2012). Augmentation of various quantitative scenario tools to signify these impacts on socioeconomic development pathways of biodiversity and ecosystem is documented on a broader note. As a

prime objective, these tools predicted that the biodiversity would continue to decline with a loss in habitat, distribution and species abundance. However, the projected rate of decline is broader than estimated, mainly because of significant uncertainties in projections (Pereira et al. 2010). Another cause is the majority of species present are undescribed by science (Thomsen and Willerslev 2015) besides the increased anthropogenic disturbances are responsible for the decline in the populations of flora and fauna (Dirzo et al. 2014; Gall and Thompson 2015). Under these circumstances, unswerving molecular tools offer an answer through quick, sensitive, cost-effective and noninvasive monitoring that promises a much better understanding in perceiving the universal biodiversity and therefore enabling effective and suitable management strategies (Valentini et al. 2016). There are many technological advancements and scientific breakthroughs observed within the last few decades, especially in the field of nucleic acid research. The generations of sequencing have drastically moved on from the first generation to the recent third generation of sequencing (Linnarsson 2010). These changes could be attributed to the inputs from many researchers around the globe who have invested a lot of time and resources to develop technologies that can underpin the DNA sequencing strategies (Heather and Chain 2016; Tatusova et al. 2016). Though there are traditional methods that decipher the results related to distribution pattern and population estimate, these methods mostly rely on physical identification and are believed to be less accurate due to factors like nonstandardized sampling, lack of expertise in modern taxonomy and invasive nature of some survey techniques (Pikitch 2018; Thomsen and Willerslev 2015). This necessity has led the scientific communities to find alternatives that are more precise and pertinent on a larger scale. It has long been proverbial that environment is a wealthy reservoir of DNA originated form resident organisms (Parro et al. 2007; Lasken and McLean 2014; Der Sarkissian et al. 2017). The emerging science of environmental DNA (eDNA) is a new molecular approach which mostly addresses the above-mentioned limitations where the technique mostly depends on the DNA material that is collected from specific and respective natural habitats (Mächler et al. 2014). The method is employed by many researchers due to its ease in handling, reliable data on phylogenetic assessment and cost-effective nature for analyzing the communities that drive the ecosystem (Yang et al. 2018). eDNA comprises of two distinct methods namely eDNA barcoding (species-specific approach) and eDNA metabarcoding (multispecific approach) which helps in identifying species present in the selected environmental samples from different ecosystems without prior species-specific knowledge (Valentini et al. 2016). The applications of eDNA are escalating and are mostly employed in monitoring biodiversity (Lacoursière-Roussel et al. 2018), and improving the probabilities of environmental management (Kelly et al. 2014b), to monitor benthic organic enrichment (Keeley et al. 2018), community assessment in microcosm study (Yang et al. 2018), to detect the anthropogenic contamination (Li et al. 2018), coastal sediments structure (Xie et al. 2018) and to study most complex ecosystems (Bovo et al. 2018). Even though there are research articles highlighting the significance of eDNA applications, the major problem arises with the rate of false positives or false negatives observed in the outcomes. These impediments can be attributed to the absence of standard conventional methods for eDNA testing both on field and laboratory (Roussel et al. 2015). In this review article, we address the most recent approaches for the eDNA analysis and gaps that delimit its intrinsic worth. Also, we endeavored to consolidate the literature accessible for eDNA based strategies.

2 Research methodology

With the advances in molecular biology, and thereby the expansion of sequencing analysis equipped humankind to analyze the silhouette of a particular ecosystem or habitat with no intricacy. To underline the importance of eDNA and its applications a thorough literature review was done using different scientific databases (Pubmed, Web of Science, Scopus, Science Direct, John Wiley, Taylor & Francis, ACS, Springer, Kluwer, Nature, and JSTOR). Many divergent research articles alluded for substance investigation in confining the center subject of the present review article by adopting a keyword-based search. The assistance of web indexes like PubMed, Google Scholar, Springer Link, and Science Direct were employed (Table 1). The keywords used are mainly eDNA, conservation, metabarcoding, monitoring, meta-genetics, high throughput sequencing

Table 1 Literature-based search (PubMed and Scopus)

Search word	Search fields	Number of hits in major databases		Last updated
		PubMed	Scopus	
"eDNA*"	Article title, Abstract, Keywords	1066	1444	16/01/2019
"eDNA AND aquatic*"	Article title, Abstract, Keywords	241	141	16/01/2019
"eDNA AND marine*"	Article title, Abstract, Keywords	94	93	16/01/2019
"eDNA AND freshwater*"	Article title, Abstract, Keywords	111	122	16/01/2019
"eDNA AND sediments*"	Article title, Abstract, Keywords	29	57	16/01/2019
"eDNA AND diversity*"	Article title, Abstract, Keywords	97	140	16/01/2019
"eDNA AND soil*"	Article title, Abstract, Keywords	59	92	16/01/2019

(HTS), next-generation sequencing (NGS), and community. The article search was limited to past ten years (2009–2019) to pitch light on the recent advances and qualitative studies in the review theme encompassing main themes of eDNA and their latest advances (Fig. 1a). To achieve the objective of the review, the searches were filtered using a thorough study of their subjective abstracts with the primacy of explanation. Subsequently, a secondary search was carried out for the recent scientific advances in the context of eDNA applications. Moreover, the quality of the selected literature is affirmed by the contents and their citations in peer-reviewed journals. Hence, journal articles which are by the review theme were specially selected, and their conclusions are discussed in detail.

3 History and advances in eDNA

During the year 1953, the three-dimensional structure of DNA was represented by Watson and Crick using the crystallographic data generated by Roslin Franklin and Maurice Wilkins (Watson and Crick 1953; Zallen 2003). Basing these studies, the conceptual framing of DNA replication and proteins encoding nucleic acids were postulated. Determining the order of nucleic acid residues in biological samples is an integral part of a broad type of research applications. However, the ability to read or sequence DNA took time to get into reality. Over the last 50 years, the advancements in the fields of molecular biology and instrument engineering brought considerable researchers making progress successfully to the assembly of techniques that facilitate sequencing DNA and RNA molecules (Heather and Chain 2016). The technology and methods applied are changing at a faster pace since the inception of the first generation sequencing by Robert Holley and colleagues in the year 1965 (Madison and Holley 1965) to the recent third generation of sequencing named single molecule real-time (SMRT) sequencing developed by pacific biosciences (Van Dijk et al. 2014). The idea to clone the DNA directly from environmental samples was proposed by Pace (1985), and this kind of work was first reported by cloning a phage vector by Schmidt et al. (1991). Experimental advances changed the way we visualize the microbial world, further from the works of Carl Woese, it became evident that rRNA is responsible for evolutionary chronometers (Woese 1987). Taking these leads Pace and colleagues used 5S and 16S rRNA for their analysis directly from uncultured environmental samples. Later with the introduction of PCR (Polymerase chain reaction), a technical breakthrough was attained wherein amplifying the entire gene is achieved with the help of predesigned primers (Handelsman 2004). With the expansion of sequencing methods, more technologies were derived that includes metagenomics, functional metagenomics, and the current next-generation sequencing (NGS). Out of different generations of sequencing methods (Fig. 2) the most advanced method was Nanopore sequencing method which is developed on the basis of SMRT technology and is capable of offering direct DNA/RNA sequencing at realtime. The benefits provided are ultra long reads up to 2 Mb, availability of scalable to portable or desktop models, less capital cost requirement, simple and automated library preparation with high yields or larger genomes.

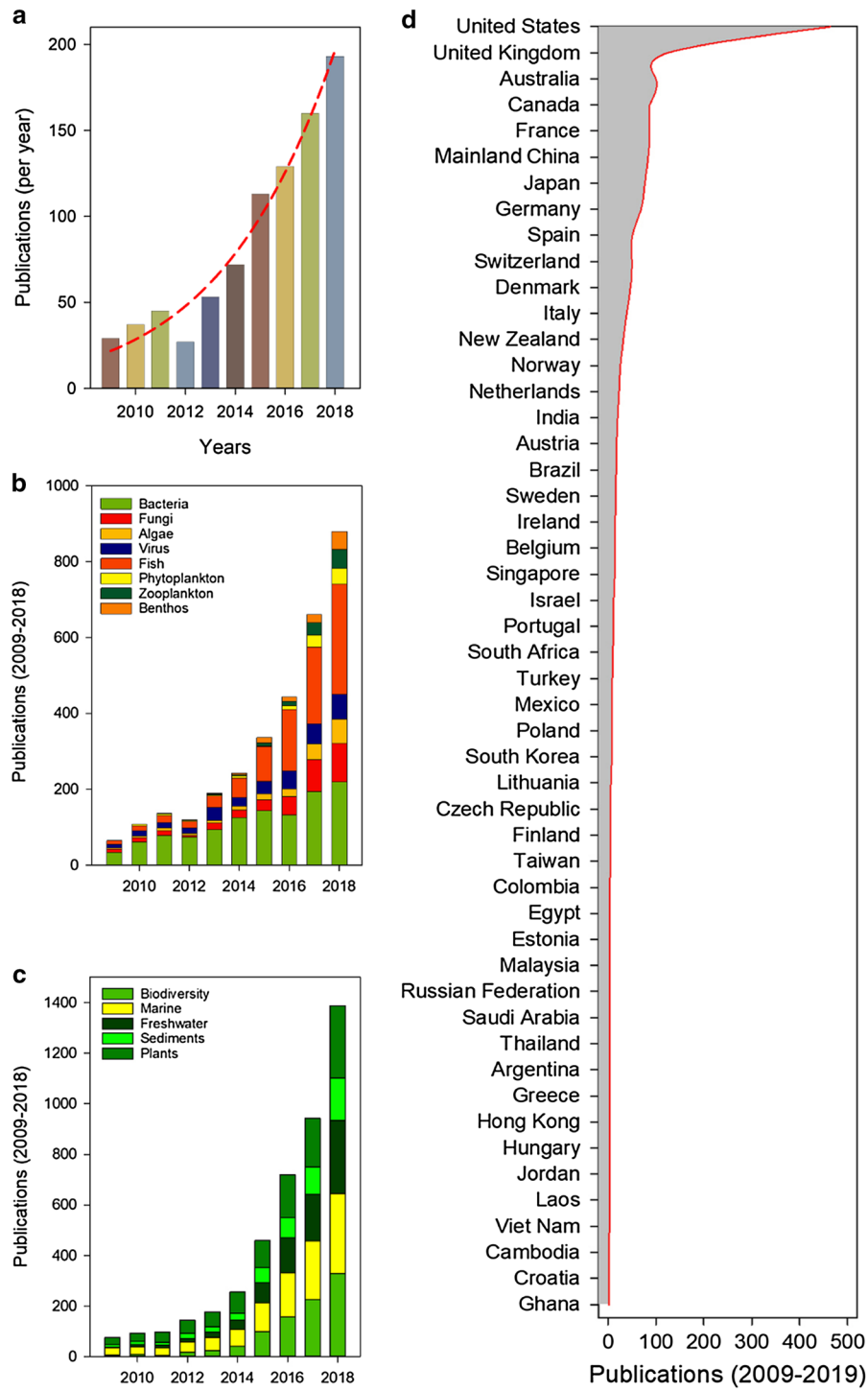

Fig. 1 **a** Publication trends of eDNA from 2009 to 2019 Jan retrieved from the publication database using the search term 'eDNA'. **b–d** eDNA analysis based on area of utilization and publications from different countries (top 50 countries in terms of publications from 2009 to 2019) **a** *Source*: https://www.ncbi.nlm.nih.gov/pubmed, **b–d** *Source*: https://www.scopus.com

The functional aspects of the Nanopore technology was designed with a sequence of flowcell comprising more number of microwells that are surrounded by synthetic bilayer comprising biological nanopores. These pores are based by the molecular motor proteins which detect the characteristic current flow changes

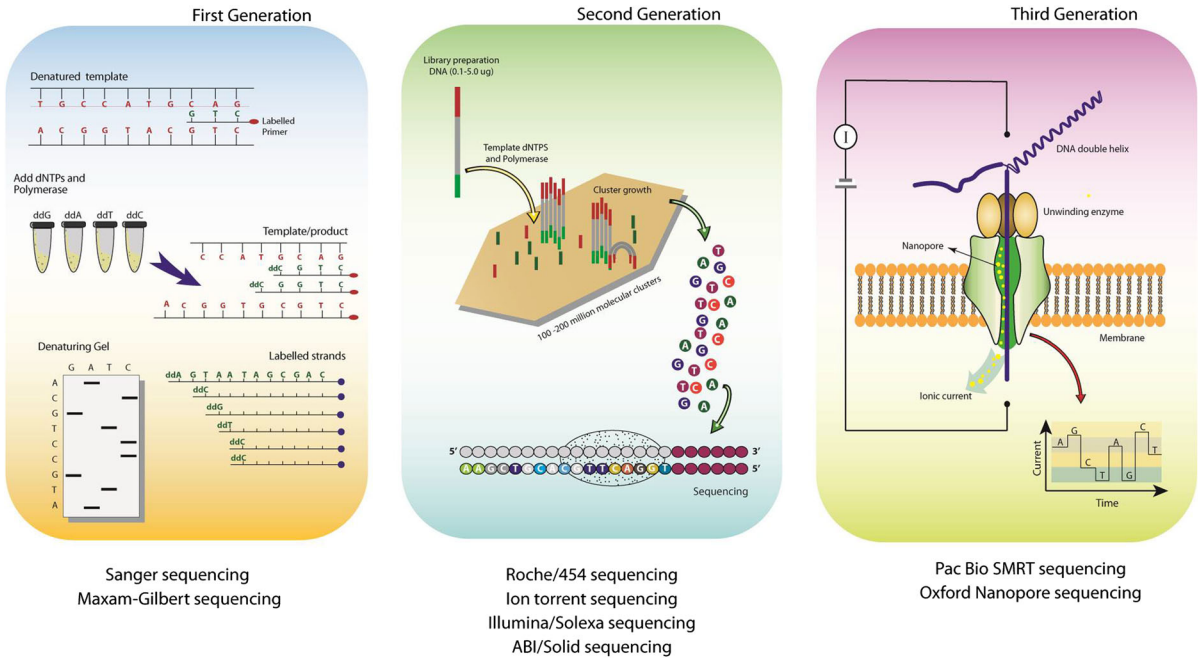

Fig. 2 Generations of sequencing technologies

during the sequencing process. The library preparation is also at ease involving only fragmentation of DNA and ligation of adapters. This method was commercialized by the Oxford Nanopore technologies with the development of MinION (a small 100 g instrument that can be connected to a laptop or computer using high speed USB 3.0) which can generate 10–30 gb of sequence data and not constrained to laboratory environment. GridION (series of five MinION flow cells connected together) a compact benchtop system and PromethION suitable for large scale sequencing like plant genomics. The same company has developed a smaller device SmidgION (can be used with smartphones) which caters the broad range of field analysis (Table 2). The persistence of DNA from higher animals in the environment, brought ease in sampling, extracting, and analyzing, which is one of the breakthroughs in the last decade. This kind of methodology which relies mostly on the environmental DNA holds the potential to answer many of the challenges related to biodiversity assessment (Foote et al. 2012). Although perceived as modern day technique, eDNA is in use from the mid 1980s for the detection of bacterial communities in marine sediments (Ogram et al. 1987) at later stages it's being used in monitoring phytoplankton blooms and in comprehending their biomass regulation (Bailiff and Karl 1991; Paul et al. 1996). The DNA extracted was more discriminated based on size. Hence eDNA in aggregates higher than 0.2 µm was treated as particulate DNA (P-DNA), whereas below 0.2 µm is considered as dissolved DNA (D-DNA). The primary literature on eDNA was by Henne et al. (2000) wherein they prepared eDNA libraries of *Escherichia coli* (lipolytic activity genes) from three different soil samples and demonstrated the feasibility of using eDNA in identifying the natural biodiversity (Fig. 3). Over the last decade, it is dynamically distinct that assessment of eDNA from different environments, when pooled with non-destructive sampling, can reveal information on the events of targeted organisms (e.g., endangered, rare and invasive species) with high precision and plasticity (Biggs et al. 2015). The momentum was further escalated when eDNA, coupled with high-throughput sequencing, could uncover the biotic composition of the entire ecosystem (Clare 2014). The application of eDNA is now applicable at diversified fields right from the new diet analysis to the level of understanding the food web interactions, niches and biodiversity assessments (Taberlet et al. 2012), population genetics and genomics (Barnes and Turner 2016) (Fig. 1b–d).

Table 2 Current available sequencing platforms

Company platform	Platforms	Amplification of libraries	Sequencing carrier	Principle	Nucleotide modifications	Detection methods	Most frequent sequencing error
Roche	GS 454 FLX Titanium GS 454 FLX+ GS Junior Titanium system	emPCR on microbeads	Picotiter plate	Pyrosequencing	None	Optical detection of light	Indels in homopolymeric regions
Illumina	Illumina GAIIx Illumina Hiseq 1000 Illumina Hiseq 1500 Illumina Hiseq 2000 Illumina Hiseq 2500 Illumina Hiseq 3000/4000 Novaseq 6000 system Illumina iseq 100 Illumina NextSeq 500 Illumina HiSeq X ten	Bridge PCR on flow cell surface	Flow Cell	Reversible terminator sequencing by synthesis	End blocked fluorescent nucleotide	Optical detection of fluorescent emission	Substitution in particular at the end of the read
Life Technologies	Solid 4 Solid 5500 Solid 5500 xL Solid 5500 W Solid 5500 xL W	emPCR on microbeads, PCR on flowchip surface for the 5500 W models	Flow Chip	Sequencing by ligation	2-base encoded fluorescent oligonucleotides	Optical detection of fluorescent emission	Substitution in particular at the end of the read
Life Technologies	Ion PGM Ion Proton Abi/Solid	emPCR on microbeads	Ion chip, a semiconductor chip	Semiconductor-based sequencing by synthesis	None	Transistor-based H+ shift upon nucleotide incorporation	Indels
Pacific Biosciences	PacBio RS RSII	Not applied	SMRT Cell	Single-molecule real-time DNA sequencing by synthesis	Phosphor linked fluorescent nucleotides	Real-time optical detection of fluorescent dye in polymerase active site	Indels
Helicos	Heliscope	Helicos flow cell plate	SMRT Cell	Single-molecule real-time DNA sequencing by synthesis		Fluorescence, Virtual terminator	

Table 2 continued

Company platform	Platforms	Amplification of libraries	Sequencing carrier	Principle	Nucleotide modifications	Detection methods	Most frequent sequencing error
Oxford Nanopore Technologies	Flongle MinION GridIONx5 PromethION SmidgION (designed for use with a smart phone at any location)	VolTRAX Cartridge—used with a small USB powered device	SMRT Cell	Sequencing by measuring the changes induced in bases passed through a pore by a molecular motor protein	Minimal modifications are required like fragmentation of DNA and ligation of adapters	Single channel recording method	Indels

3.1 Freshwater

Freshwater habitats are of high conservation value and present a wide range of environmental services. Successful management of these systems needs regular monitoring. However, typical methods depending on direct surveillance or specimen collection are invasive, high-priced, and labor-intensive (Lim et al. 2016). The eDNA approach demonstrates its potential in freshwater habitats by surveying the biodiversity with lesser inputs and field observations. With continuous advancements, eDNA approach successfully established the ability to differentiate organisms at a noteworthy rate pivoting on the molecular barcoding or eDNA copy number for the biomass of different water systems (Darling and Mahon 2011; Rees et al. 2014b). Currently, we lack the knowledge that understands ecological factors that control eDNA construction in realistic, complex, and heterogeneous streaming waters. To attain this, utilizing an exact methodology and a fundamental applied model is of extreme importance. Three important points in connection with the above theme were proposed by Shogren et al. (2017) highlighting transport, retention, and resuspension of eDNA from the water column, and considered to be crucial factors for sampling eDNA from natural systems. As the eDNA is known to be ploydisperse (made up of different sized particles from diverse sources, i.e., tissues, cells, mucous and feces, etc.) and has the ability to degarde at a faster rate in flowing waters, it is important to note the factors that regulate the eDNA complexity (Turner et al. 2014; Wilcox et al. 2015). Lim et al. (2016) using metazoan primers demonstrated the eDNA signatures of more than 500 metazoan types where 105 were recognized to genus/species level by applying DNA bar codes. Davy et al. (2015) successfully demonstrated the use of eDNA in monitoring and conserving the threatened freshwater turtle species. The validation of eDNA study was a big concern earlier, to overcome this issue, many researchers have analyzed both indoor and outdoor conditions. The presence of non-native freshwater fish and Benthic hard-shelled crustacean have been successfully amplified from the aquarium samples and also in the brackish water environments (Forsström and Vasemägi 2016; Davison et al. 2016). By making use of specific primers pairs a wide range of taxa was identified from the Cuyahoga River (Ohia, USA), showing the presence of 15 species of fishes, 17

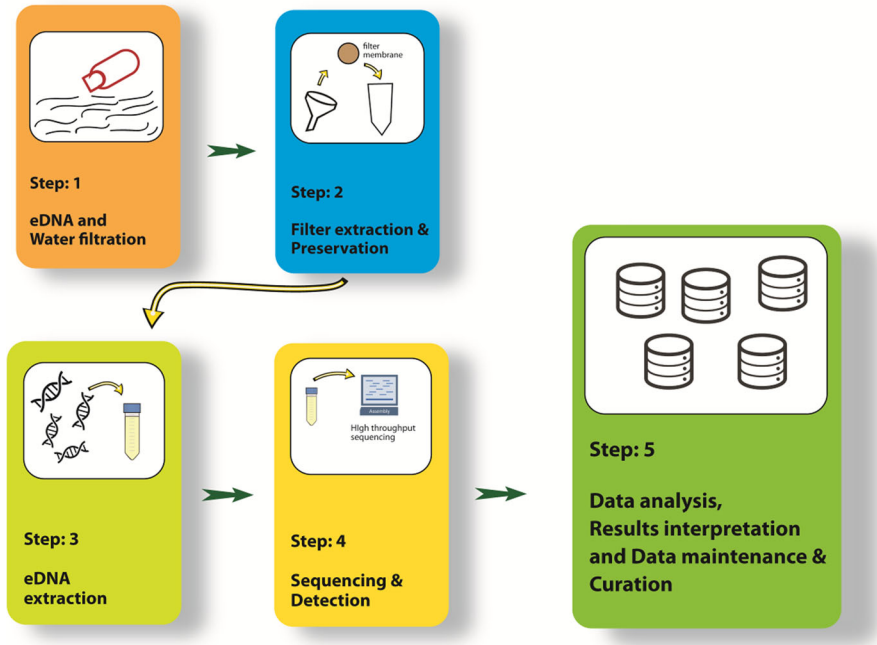

Fig. 3 Schematic representation of steps involved in eDNA analysis

species of mammals, 8 species of birds, 15 species of arthropods, interestingly the samples also showed the presence of DNA of the terrestrial species that inhabit near the river (Cannon et al. 2016). Detection of virus like particles (VLP), especially bacteriophages was achieved through eDNA metagenomic studies (Mohiuddin and Schellhorn 2015). Several studies have suggested that eDNA analysis coupled with deep sequencing technologies can be a good molecular approach for ecological and environmental research (Table 3).

3.2 Marine

Marine ecosystems are under constant stress from the mounting anthropogenic contamination, fishing activities and habitat degradation driving into a condition where there is an immediate requirement for the efficient marine management system (Cordier et al. 2017). This management system ought to be fit for rendering a wide scope of information on the status of marine biodiversity. Biomonitoring tool takes on a key function in rendering the data that supplements the sustainability in the ocean resources (Stat et al. 2017). Harnessing information encoded in eDNA from marine waters has the perspective to reforming marine biomonitoring. Using information encoded in eDNA from marine waters can reform ocean biomonitoring. By using organism-specific quantitative PCR assays or perhaps metabarcoding in concurrence with amplicon sequencing, scientists have established that pragmatic organism censuses can be deduced from eDNA (Andruszkiewicz et al. 2017). eDNA coupled with high throughput sequencing of West Antarctic Peninsula (WAP) waters collected from shallow shelf regions ($\leq$ 300 m) showed abundant signatures of benthic invertebrate fauna, endemic notothenioid fishes and litholid king crabs. The research team successfully demonstrated that eDNA derived mitochondrial *ND2* gene copies of WAP icefish persisted in WAP ice water for more than 20 days (Cowart et al. 2018). It is mostly believed that rare marine species are often very challenging to study, due to their scarcity and patchy distribution. Studying endangered or rare marine species using eDNA can be a promising tool in terms of rapidness, sensitivity, safety, and cost-effectiveness. The metagenomic study performed against the zooplankton communities from marine pelagic environments showed a clear difference between the filtered samples (PF-eDNA) and direct surface samples (eDNA) highlighting the importance of sample collection process (Pikitch 2018; Djurhuus et al. 2018). eDNA analysis is also helpful in detecting the presence of algal taxa like

Table 3 Environmental DNA (eDNA) applications in freshwater ecosystems

References	Ecosystem	Organism/sample	Use	Remarks/conclusion
Sansom and Sassoubre (2017)	Freshwater	Mussel *Lampsilis siliquoidea*	Quantification	eDNA has the potential to be a complementary tool to survey mussels and protect biodiversity
Bista et al. (2017)	Freshwater	Macroinvertebrates *Chironomidae*	Temporal shifts in ecosystem biodiversity	eDNA metabarcoding is useful for tracking seasonal diversity at the ecosystem scale
Apothéloz-Perret-Gentil et al. (2017)	Freshwater and Streams	Epilithic samples	Molecular index	A complementary tool allowing fast and cost-effective assessment of the biological quality of watercourses
Song et al. (2017)	Chicago Area Waterway System (CAWS)	Silver carp *Hypophthalmichthys molitrix*	The spread of aquatic invasive species	The influence of hydrology on eDNA sampling results, which will help to make eDNA sampling a robust and useful tool for monitoring aquatic invasive species
Agersnap et al. (2017)	Freshwater	Crayfish *Astacus astacus* *Pacifastacus leniusculus* *Astacus leptodactylus*	Species-specific detection	The study shows promising potential for future monitoring and management of freshwater crayfish using eDNA
Klymus et al. (2017)	Freshwater	Invasive bivalve and gastropod species	Surveying biodiversity	Environmental DNA metabarcoding surveys can significantly augment efforts for identifying and eradicating invasive species and conserving native species
Rees et al. (2017)	Freshwater	The great crested newt *Triturus cristatus*	Species-specific detection	The efficacy and reproducibility of eDNA detection for determining the presence of great crested newts
Erickson et al. (2016)	Freshwater	Bigheaded carp movements	Monitoring species abundance	Current applications of eDNA are widespread, but the relatively new technology requires further refinement
Cannon et al. (2016)	Freshwater	Mammals, fish, amphibians, birds, bryophytes, arthropods, copepods, plants and microorganism taxa	Surveying biodiversity	The application of eDNA coupled with deep sequencing can constitute an excellent foundation for ecological and environmental research
Carraro et al. (2017)	Freshwater	*Salmonoid* species	Proliferative kidney disease (PKD)	eDNA along with hydrological and geomorphological surveys and metacommunity barcoding reproduce the main reason for Proliferative kidney disease
Davison et al. (2016)	Freshwater	Freshwater fish *Lepomis gibbosus* *Leucaspius delineatus* *Pimephales promelas* *Pseudorasbora parva*	Species-specific detection	eDNA coupled with conventional PCR was capable of detecting species with random of patchy distribution
Deiner et al. (2016)	Freshwater	Metazoan eukaryotes	Biodiversity patterns	eDNA the novel and spatially integrated way to assess the total biodiversity for whole landscapes and will transform biodiversity data acquisition in ecology

Table 3 continued

References	Ecosystem	Organism/sample	Use	Remarks/conclusion
Davy et al. (2015)	Freshwater	Sympatric freshwater turtles	Monitoring and conservation of threatened species	Traditional methods are 2–10× higher than eDNA method and this could further help in the conservation of threatened species
Clusa et al. (2017)	Freshwater	Molluscs *Corbicula fluminea* *Melanoides tuberculata* *Mytilopsis leucophaeata* *Corbicula fluminea* *Sinanodonta woodiana* *Melanoides tuberculata*	Species-specific detection	The designed primers for eDNA analysis will be helpful for the detection of invasive molluscs
Matsuhashi et al. (2016)	Freshwater	Submerged aquatic plants	Management and conservation	eDNA analysis was helpful in providing useful insights into analyzing the aquatic plant population along with considering the factors like water condition, environmental factors and growth process
Lim et al. (2016)	Freshwater	Metazoan biodiversity	Surveying biodiversity	eDNA can be used to find the origin of the sample and single sample richness comparisons. It also outperforms the conventional methods used for biodiversity assessment
Adrian-Kalchhauser and Burkhardt-Holm (2016)	Freshwater	Ponto-Caspian gobies	Species-specific detection	eDNA analysis requires less time, equipment, manpower skills, financial resources when compared with conventional methods
Gustavson et al. (2015)	Freshwater	Sea lamprey *Petromyzon marinus* Brown trout *Salmo trutta*	Species-specific detection	Presence of low abundance taxa and relative abundance of rare or invasive species
Clusa et al. (2016)	Freshwater	New Zealand mud snail *Potamopyrgus antipodarum*	Species-specific detection	The eDNA tools developed can detect New Zealand mud snail populations away from its native range
Mohiuddin and Schellhorn (2015)	Freshwater	Sequence associated with pathogens	Source tracking or estimation of public health risk	eDNA in combination with virus-like particles (VLP) fractions are useful in detecting the viral load in the environment
Dunker et al. (2016)	Freshwater	Northern Pike *Esox lucius*	Species-specific detection	eDNA can help in informing about the eradicated species with multiple lines of inquiry
Deiner et al. (2016)	Freshwater	Metazoan Eukaryotes Annelida, Arthropoda, Ascomycota, Basidiomycota, Bryozoa, Chordata, Cnidaria, Gastrotricha	Biodiversity patterns	eDNA analysis asses the biodiversity in for whole landscapes and ecological data

Table 3 continued

References	Ecosystem	Organism/sample	Use	Remarks/conclusion
Li et al. (2018)	Freshwater	Bateria Protista, Metazoan communities and Anthropogenic contamination	Ecological status	eDNA analysis helped to understand the diversity monitoring the impact of anthropogenic contamination on the river ecosystem
Ushio et al. (2018)	Freshwater	Microbial diversity and Anthropogenic contamination	Biodiversity patterns	eDNA helps in predicting structured microbial communities, abiotic conditions, animal ecology, and anthropogenic disturbances

Chattonella, Heterosigma, Karlodinium, and *Noctiluca* representing the advantage of molecular mapping tools in the detection of toxin producing algal strains (Shaw et al. 2019). The main advantage in eDNA analysis is the small amount of sample that is used for the analysing and predicting the large quantities of microbial diversities from diverse environments. The detection of 834 prokaryotic cyanobacteria, 346 eukaryotic Alveolata, and 254 unique virus phylotypes of *Phycodnaviridae,* and *mimiviridae* were made possible with the extraction of pelagic double-stranded DNA (dsDNA) from 250 ml of seawater (Flaviani et al. 2017). Population genetics aids in deciphering the local biological communities and also provides information that highlights the human intervened impacts, combining this kind of studies with eDNA analysis will prove their ability in ecological assessments. Cytochrome oxidase I (COI) and 18S ribosomal RNA (rRNA) are used to study the eukaryotic diversity along the coral reef tract of Florida Keys National Marine Sanctuary (FKNMS), and the results showed distinct domination by copepods along with some phytoplankton communities. The 18S rRNA gene results were dominated by dinoflagellates and COI gene by coccolithophores. Even though the samples collected were from surface waters, the presence of sponges, crustaceans, and corals were also detected (Sawaya et al. 2019). In another study named Rapid universal metabarcoding surveys (RUMS) 18S rRNA gene marker was used to study the eukaryotic subtropical biodiversity along depth gradients ranging from 10 to 40 m (Okinawa, Japan). The results showed a significant difference among the different depths and the dominant operational taxonomic unit (OTU) was Demospongiae with phylum Porifera constituting more than 81% signifying the usefulness of eDNA analysis in providing baseline information (DiBattista et al. 2019) (Table 4).

3.3 Terrestrial

The applications of eDNA were also employed in terrestrial habitats to augment the information of diversity. Wakelin et al. (2016) demonstrated the soil environmental genomics to highlight the functional state of the soil ecosystem. They employed the use of high-density functional gene microarray analysis (GeoChip5) of eDNA for more than 50 pastoral soils collected from 11 major soil groups of more than ten

Table 4 Environmental DNA (eDNA) applications in marine ecosystems

References	Ecosystem	Organism/sample	Use	Remarks/conclusion
Bitok et al. (2017)	Sediment	Organic enrichment of sediment samples	Anthropogenic disturbances (to evaluate the effect of finfish farming activities)	eDNA technology provides more cost-effective and practical biotic measures than traditional morpho-taxonomy
Levi et al. (2018)	Marine	Pacific salmon Sockeye and coho spawners	Standard detection and monitoring and enumeration	eDNA when used in combination with robust data, daily sampling and knowledge of species life cycles prooves to be accurate and efficient enumeration method
Tessler et al. (2017)	Pelagic water samples	Bacterioplankton	Global comparison	The study establishes the efficacy of eDNA studies for broad, rapid comparisons of freshwater bacterioplankton
Jeunen et al. (2018)	Marine	Fish Crustacean and eukaryotic organisms	Standard detection and monitoring	eDNA helps in detecting a broad range of taxa, but a refinement of assay choice is required to unleash the full potentials of this current method
Forsström and Vasemägi (2016)	Brackish water	Mud crab *Rhithropanopeus harrisii*	Standard detection and monitoring	Studies focusing on the optimization of different sampling and DNA extraction procedures combined with fine-scale vertical and horizontal characterization of the concentration of eDNA in the water column is needed
Guo et al. (2018)	Estuary Biofilm, sediment, and water	–	Antibiotic resistance genes (ARG)	eDNA may be more stable and persistent in the environment when associated with organic matter
Ramírez et al. (2018)	Sedimentary habitats	Extracellular 16S rRNA genes	Standard detection and monitoring	Extracellular 16S rRNA genes have little influence on community composition, community richness and relative abundance particularly in subseafloor sediments
Lacoursière-Roussel et al. (2018)	Marine	Arctic biodiversity	Biodiversity assessment	COI-based eDNA metabarcoding may improve large scale Arctic biomonitoring both in the open water and under the ice. eDNA samples should be standardized
Parsons et al. (2018)	Marine	Surface seawater samples	Population genetic analysis	Population-specific eDNA sampling coupled with NGS (Next-generation sequencing) helpful in characterizing small and endangered marine mammals
Forster et al. (2018)	Marine	Benthic Ciliate communities	Environmental monitoring	eDNA marker V9 used for monitoring was better than traditional method making this the best choice for implementing routine monitoring programs
Djurhuus et al. (2018)	Marine	Zooplankton communities Surface seawater samples zooplankton tissue samples	Biodiversity assessment	Dominant Copepoda taxa were detected, and the tissue and eDNA metabarcoding is a promising technique for assessing pelagic zooplankton communities

Table 4 continued

References	Ecosystem	Organism/sample	Use	Remarks/conclusion
Grey et al. (2018)	Marine	Metazoan phyla	Global biodiversity similarity patterns	eDNA sampling reveals the biodiversity patterns among a broad array of taxa and can help in detecting nonindigenous species (NIS). Species richness is extremely sensitive to primer choice indicating proper care while selecting for analysis
Weltz et al. (2017)	Marine	Endangered Maugean skate *Zearaja maugeana*	Species-specific detection	eDNA analysis was employed for detecting the rare species Maugean skate. Proper care should be taken, and the samples should be filtered within 4–16 h as dissolved oxygen plays a role in quantification of eDNA
Laroche et al. (2017)	Oil production platform	Macro-infauna (benthic invertebrates)	Anthropogenic disturbances (offshore oil production impacts)	The data obtained from both eDNA and eRNA is robust and can be employed in biomonitoring surveys
Flaviani et al. (2017)	Marine	Virus and unicellular phytoplankton	Diversity and Biological complexity	eDNA analysis using a small volume of a sample (250 ml) assessing the microbial diversity is possible (834 prokaryotic, 346 eukaryotic and 254 unique viruses detected)
Won et al. (2017)	Water and sediment (sand mining and control areas)	Microbial communities	Anthropogenic disturbances (microbial communities)	Revealed the presence of microbial taxa (Marinobacter, Alcanivorax and Novosphingobium and some pathogens) capable of degrading toxic chemicals
Guardiola et al. (2016)	Marine	Deep-sea communities	Biodiversity assessment	eDNA and eRNA studies should be done parallel, and these will become a cornerstone in deep-sea biomonitoring
Sassoubre et al. (2016)	Marine	Fish *Engraulis mordax* *Sardinops sagax* *Scomber japonicas*	Shedding and decay rates and biodiversity assessment	eDNA monitoring helps in monitoring ecologically and economically important marine biodiversity on local and global scales
Simpson et al. (2017)	Marine	Ascidians *Didemnum perlucidum* *Didemnum vexillum*	Nonindegenous species (NIS)	The successful detection of NIS using developed eDNA assay from water samples at sites where it could not be visually identified opens a path for biosecurity surveys for *Didemnid* sp.
Kim et al. (2018)	Marine	Bryozoan *Bugula neritina*	Invasive species detection	The results obtained showed a simple eDNA based methods could help understand the early distribution and monitoring of specific species
Kelly et al. (2016)	Marine	Metazoan communities Eelgrass communities	Anthropogenic disturbances (effects of urbanization along nearshore)	eDNA clearly shows an advantage over traditional ecological sampling and the genetic data generated is a powerful tool for uncovering human-ecosystem interactions
Pochon et al. (2017)	Marine Bilgewater samples	Operational taxonomic units	Diversity difference between OTU of eDNA and eRNA	The data collected for eDNA and eRNA can be used by taking similar OTU of both analysis for biosecurity application

geographic areas in New Zealand. The results mentioned the key elements linked with alterations in soil functional ecology and nitrogen cycling metalloenzymes. Thomsen and Sigsgaard (2019) used the eDNA metagenomics analysis to study the terrestrial arthropod communities by collecting arthropod eDNA from different wildflowers. They used the genomic markers like 16S rRNA and COI and obtained data of more than 135 arthropod species (67 families and 14 orders) from different ecological groups like gall inducers, pollinators, predators and phytophagous species. Galan et al. (2018) used rigorous metabarcoding approach coupled with COI and two-step PCR protocol and detected the diet variability in bats to understand the biology of bats and conservation strategies. Using the COI gene variant from arthropods they detected the presence of 18 orders, 117 families, 282 genus and 290 species from fecal matter and mock insect communities of France. The introduction of eDNA analysis has facilitated many researchers and scientific communities to carry the works in multidimensional aspects. Hunter et al. (2015) developed a species-specific eDNA assays using quantitative PCR to detect the python communities and were successful in detecting the Burmese python eDNA from 37 of 63 sampled locations in Southern Florida (Table 5).

3.4 Other advantages of eDNA

Along with freshwater, marine and terrestrial habitats, eDNA applications are also spearheading in environmental monitoring, conservation genetics and forest ecology, etc. eDNA was chosen by many scientific communities because of high detection sensitivity and precision. Bovo et al. (2018) used the honey eDNA and demonstrated an overall picture of the colony ecosystem and the landscapes from which they take their nutrients. This information is helpful for predicting the honey bee pathosphere and the factors that contribute to colony collapse disorder. Diet analysis is one of the earliest applications of eDNA analysis which is labour intensive and requires expert morphological identification of faecal matter or stomach contents especially with small, rare and soft-bodied organisms (Berry et al. 2017). In boreal ecosystems, small herbivores play a crucial role between vegetation and predators. Stomach content analysis of these herbivores coupled with eDNA and microhistological studies opens up new possibilities to understand the plant–herbivore interactions and also provides a comprehensive picture on the food utilization by these herbivores (Soininen et al. 2009). Pollinator interactions with the plants is another field that is being analyzed as pollinators are very much essential in maintaining the ecosystem health. A comparative study between the eDNA and traditional microscopic analysis of pollen collected from pollinators revealed the presence of additional 13 taxa with eDNA analysis over the conventional method (Keller et al. 2015). This kind of approach can also help in understanding the pollen from migratory pollinators and the distances and geographic range of migratory species (Chang et al. 2018). Using the same method Suchan et al. (2019) used a model butterfly species along the European Mediterranean coast to detecte the pollen native to Africa, suggesting the insect-mediated transcontinental pollination. eDNA analysis is also now applied in monitoring air quality for human and ecosystem health. Airborne fungal diversity analysis conducted by Banchi et al. (2018) showed a 10×, more comprehensive identification of taxa to the regular microscopic identification. Similarly, Oh et al. (2014) identified more than 1000 unique OTUs, of which 40 are potentially allergy-inducing genera.

4 Drawbacks and challenges

Attempts in eDNA investigations are seen at a dynamic scale by different research networks to drive the thought of managers, stakeholders, and end users. These endeavors are centered around expounding the uncertainties in data elucidation while advancing standardization and similarity (Darling and Mahon 2011). Nevertheless, these strategies demonstrated a variety of contrasts in results as compared with the traditional methods and found concern particularly with biodiversity findings. This can be surprising since current biomonitoring applications are costly, frequently include negative results, and are hard to pertain due to the lack of taxonomic expertise (Kelly et al. 2014a). In spite of these elements, eDNA-based strategies are seldom used for ecological management. One encouraging case is the validation by the United Kingdom for eDNA qPCR outcomes as facts for the existence of the secure great crested newt, *Triturus cristatus* (Rees et al. 2014a). Similarly, New Zealand and Australia were early proponents for DNA

Table 5 Environmental DNA (eDNA) applications in soil habitats

References	Ecosystem	Organism/sample	Use	Remarks/conclusion
Katz et al. (2016)	Soil	Microbial biosynthetic diversity	Screening secondary metabolite biosynthesis process	eDNA bases metagenomics offer alternative methods for natural product discovery
Gellie et al. (2017)	Soil	Bacterial communities	Monitoring changes in bacterial communities	eDNA based high throughput amplicon sequencing provides a cost-effective, scalable and uniform ecological monitoring
Parducci et al. (2017)	Soil	Reconstruction of palaeofloras	Profiling lake sediments	The majority of sequences obtained cannot be aligned with reference currently available in databases. eDNA can be a future tool for determining the local vegetation
Bitok et al. (2017)	Soil	Biosynthetic gene clusters	Identification clones containing biosynthetic gene clusters (BGC)	The screening of soil eDNA led to the identification of the gene clones containing BGCs
Khaliq et al. (2018)	Soil	*Phytophthora* communities	To determine the *Phytophthora* communities in natural ecosystems	eDNA soil samples contained 13 *Phytophthora* species making it as the best choice for detecting at the species level
Liu et al. (2018)	Soil	Archeal community	Biodiversity assessment	The community composition varied from the control and the rotational plots based on the *mcrA* gene studies
Lemetre et al. (2017)	Soil	Bacterial community	Biodiversity assessment	The bacterial biosynthetic communities hidden in the environment varies in latitude and lesser extent changes in pH
Prosser and Hedgpeth (2018)	Soil	Benthic fauna *Eisenia fetida*	To study the natural movement of genetic material through the soil	The study revealed the capability of benthic fauna in distributing the genetic material through the soil matrix

barcoding to support biosurveillance (Darling and Blum 2007). However, the effective use of eDNA tools would depend on addressing a few uncertainties that exist in data elucidation.

The foremost problem of eDNA examination is the rate of false positives (the target microorganisms are not present but its DNA is recovered) and false negatives (organism will be present, but DNA cannot be recovered) that turn up during the results. False negatives are one of the major concern with regards to studies aiming at identifying intrusive, rare, or perhaps endangered species (Guillera-Arroita et al. 2017). The probability of detection depends on many factors. Even though the concentration of eDNA resulting from each species in the environment is key to its identification, other factors get involved. The isolation of eDNA depends on sampling effort, extraction efficiency, barriers to analysis (e.g., PCR inhibitors), and also the sensitivity of analytical methods (Goldberg et al. 2016). As a result, non-target species can reveal the failure to recuperate eDNA from the prospective species due to its low population intensity, inadequate sample, low effectiveness in DNA extraction, or perhaps inhibitors that prevent amplification. However, false positives are of concern due to their mirror contamination on the design at some stage in the scheduled process, besides, poor primer selection or design, probes that lack satisfactory specificity, or perhaps ambiguities presented during sequencing processing results in the issue (Ficetola et al. 2016). These false positives that arise due to the method errors can be redressed with the use of negative controls at all steps in the analytical chain. This kind of approach will administer the power to validate the reference sequence by identifying the source of contamination and thereby allowing to adjust the protocol errors and sampling methods (sample collection and filtration) (Darling and Mahon 2011).

Notwithstanding, method errors are extremely responsible for creating false positives that are difficult to identify and are elusive. These errors are mainly due to the general assumptions that are made during the analysis. For instance, when eDNA is identified, most investigations predict that the source organisms are available in the examined environment. However, their recognition could reflect eDNA transported from somewhere else or from the activation of eDNA discharged earlier. Observing that eDNA in surface waters normally degrades within a few hours to days, the species detected by these methods usually indicate their recent presence (< 1 month) and at the same time providing the information about their communities (Thomsen et al. 2012). Though this comparatively short determination is ideal for analyses of current biodiversity (Lindahl 1993; Friedberg 2003), eDNA in sediments can endure far much longer and is generally present at a much superior concentration than eDNA inside the water column, the comparative species richness based on the analysis of eDNA extracted from normal water versus sediments found that eDNA from water demonstrates predominant species composition (Shaw et al. 2016).

Since eDNA is accessible throughout the environment (Thomsen and Willerslev 2015), its geographic source is often challenging to establish. For example, the average velocity of Amazon river is 2 km per hour, eDNA can be transported nearly for 1500 km in less than one month which is equal to one-quarter of the total river length. Deiner et al. (2015) noticed the presence of eDNA of two invertebrate species, *Daphnia longispina* and *Unio tumidus* at 20 km down the alpine lake. Adding to it terrestrial eDNA runoffs from the catchment area can move to the aquatic systems and can reveal the presence of non-native species. The studies conducted by (Pansu et al. 2015) on ancient DNA (aDNA) lake sediments showed the presence of both terrestrial plant communities and livestock, indicating that water bodies are one of the major sinks of eDNA. Evidently, the organic, physical, and chemical elements are responsible for the origin, movement, degradation, or tenacity of eDNA (Wilcox et al. 2013). Some recent eDNA studies have reported broad biotic range that has been uncovered through classic survey strategies and has figured eDNA as a sensitive procedure (Valentini et al. 2016). Nevertheless, this summary fails to consider or control for the differences in timescale and location reflected by methods. Calculating the comparative abundance of species continues to be a great problem for eDNA-based methods (Deiner et al. 2017). Even though eDNA concentrations are often favourably correlated with population densities, biomass evaluation is usually problematic (Biggs et al. 2015). Examination in a well-controlled aquatic natural environment suggests that eDNA can be used to determine abundance if one engages species-specific primer models and qPCR (Deiner et al. 2017; Lacoursière-Roussel et al. 2016). However, regaining abundance via whole

neighbourhoods using sequencing is less promising because of amplification tendency among species during PCR and to species- and size-specific variation in the release of eDNA (Clare 2014). Relating eDNA reads to different abundance takes a shift out of standard PCR (Valentini et al. 2016).

Optimization of bioinformatics starts prior to data collection, as it commences with primer design (Cristescu and Hebert 2018). In silico explanations can help to recognize markers that offer an adequate resolution and can help primer design, functionality and style (Freeland 2016; Lacoursière-Roussel et al. 2018). A perfect metabarcoding gene region couples simplicity of recuperation with a high degree of species in the target assemblage. With this, marker attributes should keep primer binding sites or perhaps consolidate degenerate bases to empower their recuperation coming from assorted taxa (Rees et al. 2014b). One more complication in data examination arises from the simple fact that eDNA is often degraded (Deiner et al. 2017), so primers often strive to amplify division of approximately 150 bp (Cristescu 2014). In contrast, amplicons utilized in conventional DNA barcoding or metabarcoding are typical $>$ 500 bp, aiding the discrimination of carefully related species and often divulging intraspecies genetic variations that are unnoticed by short amplicons. There is certainly a requirement of basic and vigorous screening of the significant presumptions involved with the usage of eDNA, specifically around the sampling, steadiness and transportation (Ruppert et al. 2019; Adams et al. 2019). Prevalent skepticism, particularly in the administration the eDNA, will be brought down by refined strategies and reinforced promotions for information and understanding. Correlation is also required among results from eDNA investigation acquired through conventional techniques created by various labs under unique conditions (Mauvisseau et al. 2019). The key prerequisite should be to accomplish harmony among utilizing conventions that are geared to address the assessment, especially conditions under exploration and advancing a predictable aspect of validation and standardization.

5 Future prospects

The science of molecular biology is evolving at a faster rate and is believed to progress furthermore. Reliable detection tools for evaluating biodiversity are vital aspects of developing environmental monitoring, management, and policy making. These developments in DNA sequencing technology has altogether extended the potential outcomes of employing eDNA and is expected to comprehend the traditional methods. However, the initial experimental research using eDNA relied upon cloning and subsequent Sanger sequencing of PCR items (many nonetheless pursue an identical strategy), undoubtedly the developments in sequencing techniques will have an undeniable impact on eDNA research. eDNA analysis is gaining momentum swiftly over the traditional identification methods and transforming into a necessary device for the examination of biotic communities due to its high detection rates and cost-effectiveness. While the eDNA method seems to be a promising source, the success rate mostly depends on the articulated hypothesis and proper experimental design. The success rate and execution of eDNA banks on few elements like the accessibility to trained manpower, access to expensive instruments (e.g., PCR devices or Highend sequencers) and computational assets capable of handling contemporary bioinformatics strategies.

The subsequent generations will have access to additional versatile and reasonable eDNA studies provided:

- First, *standardization* of the available protocols to implement across different localities in a given type of habitat at a global scale.
- Second, *miniaturization*, development of portable instruments (handheld qPCR like Biomeme, nanopore DNA sequencers like MinION) that carry out on field analysis rapidly and can perform to the scale of the fully functional sequencer to avoid the misconceptions that occur during the sample preservation and carrying.
- Third, *data accumulation/mining,* which is believed to be a key element in every experiment that contemplates for future use. The data generated from eDNA analysis usually is huge, and henceforth proper care should be taken to preserve the data and to stay away from the reiteration. Furthermore, to give access to different analysts to counter check their outcomes.
- Fourth, *Cost-effectiveness* a fundamental factor for every analysis. It would be wrong to say that eDNA is more cost-efficient than traditional methods, but

many of the research communities argue the reducing costs in sequencing per base-pair will make eDNA a superior power especially in metagenomics approach.

Inevitably, connecting these kinds of new progress with classic field methods that are typically intended to estimate population structure, abundance, biomass, or condition of individuals will perform much to improve the functionality of eDNA.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Adams CI, Knapp M, Gemmell NJ, Jeunen G-J, Bunce M, Lamare MD, Taylor HR (2019) Beyond biodiversity: Can environmental DNA (eDNA) cut it as a population genetics tool? Genes 10(3):192

Adrian-Kalchhauser I, Burkhardt-Holm P (2016) An eDNA assay to monitor a globally invasive fish species from flowing freshwater. PLoS ONE 11(1):e0147558. https://doi.org/10.1371/journal.pone.0147558

Agersnap S, Larsen WB, Knudsen SW, Strand D, Thomsen PF, Hesselsøe M, Mortensen PB, Vrålstad T, Møller PR (2017) Monitoring of noble, signal and narrow-clawed crayfish using environmental DNA from freshwater samples. PLoS ONE 12(6):e0179261. https://doi.org/10.1371/journal.pone.0179261

Andruszkiewicz EA, Sassoubre LM, Boehm AB (2017) Persistence of marine fish environmental DNA and the influence of sunlight. PLoS ONE 12(9):e0185043. https://doi.org/10.1371/journal.pone.0185043

Apothéloz-Perret-Gentil L, Cordonier A, Straub F, Iseli J, Esling P, Pawlowski J (2017) Taxonomy-free molecular diatom index for high-throughput eDNA biomonitoring. Mol Ecol Resour 17(6):1231–1242. https://doi.org/10.1111/1755-0998.12668

Bailiff MD, Karl DM (1991) Dissolved and particulate DNA dynamics during a spring bloom in the Antarctic Peninsula region, 1986–1987. Deep Sea Res Part A Oceanogr Res Pap 38(8–9):1077–1095

Banchi E, Ametrano CG, Stanković D, Verardo P, Moretti O, Gabrielli F, Lazzarin S, Borney MF, Tassan F, Tretiach M (2018) DNA metabarcoding uncovers fungal diversity of mixed airborne samples in Italy. PLoS ONE 13(3):e0194489

Barnes MA, Turner CR (2016) The ecology of environmental DNA and implications for conservation genetics. Conserv Genet 17(1):1–17. https://doi.org/10.1007/s10592-015-0775-4

Berry TE, Osterrieder SK, Murray DC, Coghlan ML, Richardson AJ, Grealy AK, Stat M, Bejder L, Bunce M (2017) DNA metabarcoding for diet analysis and biodiversity: a case study using the endangered Australian sea lion (*Neophoca cinerea*). Ecol Evol 7(14):5435–5453

Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson JW, Arnell A, Brotherton P, Williams P, Dunn F (2015) Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). Biol Conserv 183:19–28. https://doi.org/10.1016/j.biocon.2014.11.029

Bista I, Carvalho GR, Walsh K, Seymour M, Hajibabaei M, Lallias D, Christmas M, Creer S (2017) Annual time-series analysis of aqueous eDNA reveals ecologically relevant dynamics of lake ecosystem biodiversity. Nat Commun 8:14087. https://doi.org/10.1038/ncomms14087

Bitok JK, Lemetre C, Ternei MA, Brady SF (2017) Identification of biosynthetic gene clusters from metagenomic libraries using PPTase complementation in a Streptomyces host. FEMS Microbiol Lett 364(16):fnx155. https://doi.org/10.1093/femsle/fnx155

Bovo S, Ribani A, Utzeri VJ, Schiavo G, Bertolini F, Fontanesi L (2018) Shotgun metagenomics of honey DNA: evaluation of a methodological approach to describe a multikingdom honey bee derived environmental DNA signature. PLoS ONE 13(10):e0205575. https://doi.org/10.1371/journal.pone.0205575

Butchart SH, Walpole M, Collen B, Van Strien A, Scharlemann JP, Almond RE, Baillie JE, Bomhard B, Brown C, Bruno J (2010) Global biodiversity: indicators of recent declines. Science 28:1164–1168

Cannon MV, Hester J, Shalkhauser A, Chan ER, Logue K, Small ST, Serre D (2016) In silico assessment of primers for eDNA studies using PrimerTree and application to characterize the biodiversity surrounding the Cuyahoga River. Sci Rep 6:22908. https://doi.org/10.1038/srep22908

Carraro L, Bertuzzo E, Mari L, Fontes I, Hartikainen H, Strepparava N, Schmidt-Posthaus H, Wahli T, Jokela J, Gatto M, Rinaldo A (2017) Integrated field, laboratory, and theoretical study of PKD spread in a Swiss prealpine river. Proc Natl Acad Sci 114(45):11992–11997. https://doi.org/10.1073/pnas.1713691114

Chang H, Guo J, Fu X, Liu Y, Wyckhuys K, Hou Y, Wu K (2018) Molecular-assisted pollen grain analysis reveals spatiotemporal origin of long-distance migrants of a *Noctuid moth*. Int J Mol Sci 19(2):567

Clare EL (2014) Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. Evol Appl 7(9):1144–1157. https://doi.org/10.1111/eva.12225

Clusa L, Ardura A, Gower F, Miralles L, Tsartsianidou V, Zaiko A, Garcia-Vazquez E (2016) An easy phylogenetically informative method to trace the globally invasive potamopyrgus mud snail from river's eDNA. PLoS ONE 11(10):e0162899. https://doi.org/10.1371/journal.pone.0162899

Clusa L, Miralles L, Basanta A, Escot C, García-Vázquez E (2017) eDNA for detection of five highly invasive molluscs. A case study in urban rivers from the Iberian Peninsula. PLOS ONE 12(11):e0188126. https://doi.org/10.1371/journal.pone.0188126

Cordier T, Esling P, Lejzerowicz F, Visco J, Ouadahi A, Martins C, Cedhagen T, Pawlowski J (2017) Predicting the ecological quality status of marine environments from eDNA

metabarcoding data using supervised machine learning. Environ Sci Technol 51(16):9118–9126. https://doi.org/10.1021/acs.est.7b01518

Cowart DA, Murphy KR, Cheng CHC (2018) Metagenomic sequencing of environmental DNA reveals marine faunal assemblages from the West Antarctic Peninsula. Mar Genom 37:148–160. https://doi.org/10.1016/j.margen.2017.11.003

Cristescu ME (2014) From barcoding single individuals to metabarcoding biological communities: towards an integrative approach to the study of global biodiversity. Trends Ecol Evol 29(10):566–571

Cristescu ME, Hebert PDN (2018) Uses and misuses of environmental DNA in biodiversity science and conservation. Annu Rev Ecol Evol Syst 49(1):209–230. https://doi.org/10.1146/annurev-ecolsys-110617-062306

Darling JA, Blum MJ (2007) DNA-based methods for monitoring invasive species: a review and prospectus. Biol Invasions 9(7):751–765. https://doi.org/10.1007/s10530-006-9079-4

Darling JA, Mahon AR (2011) From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. Environ Res 111(7):978–988. https://doi.org/10.1016/j.envres.2011.02.001

Davison PI, Créach V, Liang W-J, Andreou D, Britton JR, Copp GH (2016) Laboratory and field validation of a simple method for detecting four species of non-native freshwater fish using eDNA. J Fish Biol 89(3):1782–1793. https://doi.org/10.1111/jfb.13086

Davy CM, Kidd AG, Wilson CC (2015) Development and validation of environmental DNA (eDNA) markers for detection of freshwater turtles. PLoS ONE 10(7):e0130965. https://doi.org/10.1371/journal.pone.0130965

Deiner K, Walser J-C, Mächler E, Altermatt F (2015) Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. Biol Conserv 183:53–63

Deiner K, Fronhofer EA, Mächler E, Walser J-C, Altermatt F (2016) Environmental DNA reveals that rivers are conveyer belts of biodiversity information. Nat Commun 7:12544. https://doi.org/10.1038/ncomms12544

Deiner K, Bik HM, Mächler E, Seymour M, Lacoursière-Roussel A, Altermatt F, Creer S, Bista I, Lodge DM, de Vere N, Pfrender ME, Bernatchez L (2017) Environmental DNA metabarcoding: transforming how we survey animal and plant communities. Mol Ecol 26(21):5872–5895. https://doi.org/10.1111/mec.14350

Der Sarkissian C, Pichereau V, Dupont C, Ilsøe PC, Perrigault M, Butler P, Chauvaud L, Eiriksson J, Scourse J, Paillard C (2017) Ancient DNA analysis identifies marine mollusc shells as new metagenomic archives of the past. Mol Ecol Resour 17(5):835–853

DiBattista JD, Reimer JD, Stat M, Masucci GD, Biondi P, De Brauwer M, Bunce M (2019) Digging for DNA at depth: rapid universal metabarcoding surveys (RUMS) as a tool to detect coral reef biodiversity across a depth gradient. PeerJ 7:e6379. https://doi.org/10.7717/peerj.6379

Dirzo R, Young HS, Galetti M, Ceballos G, Isaac NJB, Collen B (2014) Defaunation in the anthropocene. Science 345(6195):401–406. https://doi.org/10.1126/science.1251817

Djurhuus A, Pitz K, Sawaya NA, Rojas-Márquez J, Michaud B, Montes E, Muller-Karger F, Breitbart M (2018) Evaluation of marine zooplankton community structure through environmental DNA metabarcoding. Limnol Oceanogr Methods 16(4):209–221. https://doi.org/10.1002/lom3.10237

Dunker KJ, Sepulveda AJ, Massengill RL, Olsen JB, Russ OL, Wenburg JK, Antonovich A (2016) Potential of environmental DNA to evaluate northern pike (*Esox lucius*) eradication efforts: an experimental test and case study. PLoS ONE 11(9):e0162277. https://doi.org/10.1371/journal.pone.0162277

Erickson RA, Rees CB, Coulter AA, Merkes CM, McCalla SG, Touzinsky KF, Walleser L, Goforth RR, Amberg JJ (2016) Detecting the movement and spawning activity of bighead carps with environmental DNA. Mol Ecol Resour 16(4):957–965. https://doi.org/10.1111/1755-0998.12533

Ficetola GF, Taberlet P, Coissac E (2016) How to limit false positives in environmental DNA and metabarcoding? Mol Ecol Resour 16(3):604–607. https://doi.org/10.1111/1755-0998.12508

Flaviani F, Schroeder DC, Balestreri C, Schroeder JL, Moore K, Paszkiewicz K, Pfaff MC, Rybicki EP (2017) A pelagic microbiome (viruses to protists) from a small cup of seawater. Viruses 9(3):47

Foote AD, Thomsen PF, Sveegaard S, Wahlberg M, Kielgast J, Kyhn LA, Salling AB, Galatius A, Orlando L, Gilbert MTP (2012) Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. PLoS ONE 7(8):e41781. https://doi.org/10.1371/journal.pone.0041781

Forsström T, Vasemägi A (2016) Can environmental DNA (eDNA) be used for detection and monitoring of introduced crab species in the Baltic Sea? Mar Pollut Bull 109(1):350–355. https://doi.org/10.1016/j.marpolbul.2016.05.054

Forster D, Filker S, Kochems R, Breiner HW, Cordier T, Pawlowski J, Stoeck T (2018) A comparison of different ciliate metabarcode genes as bioindicators for environmental impact assessments of salmon aquaculture. J Eukaryot Microbiol 66:294–308

Freeland JR (2016) The importance of molecular markers and primer design when characterizing biodiversity from environmental DNA. Genome 60(4):358–374

Friedberg EC (2003) DNA damage and repair. Nature 421(6921):436–440. https://doi.org/10.1038/nature01408

Galan M, Pons J-B, Tournayre O, Pierre É, Leuchtmann M, Pontier D, Charbonnel N (2018) Metabarcoding for the parallel identification of several hundred predators and their prey: application to bat species diet analysis. Mol Ecol Resour 18(3):474–489. https://doi.org/10.1111/1755-0998.12749

Gall SC, Thompson RC (2015) The impact of debris on marine life. Mar Pollut Bull 92(1):170–179. https://doi.org/10.1016/j.marpolbul.2014.12.041

Gellie NJC, Mills JG, Breed MF, Lowe AJ (2017) Revegetation rewilds the soil bacterial microbiome of an old field. Mol Ecol 26(11):2895–2904. https://doi.org/10.1111/mec.14081

Goldberg CS, Turner CR, Deiner K, Klymus KE, Thomsen PF, Murphy MA, Spear SF, McKee A, Oyler-McCance SJ, Cornman RS, Laramie MB, Mahon AR, Lance RF, Pilliod DS, Strickler KM, Waits LP, Fremier AK, Takahara T, Herder JE, Taberlet P (2016) Critical considerations for the application of environmental DNA methods to detect aquatic species. Methods Ecol Evol 7(11):1299–1307. https://doi.org/10.1111/2041-210x.12595

Grey EK, Bernatchez L, Cassey P, Deiner K, Deveney M, Howland KL, Lacoursière-Roussel A, Leong SCY, Li Y, Olds B, Pfrender ME, Prowse TAA, Renshaw MA, Lodge DM (2018) Effects of sampling effort on biodiversity patterns estimated from environmental DNA metabarcoding surveys. Sci Rep 8(1):8843. https://doi.org/10.1038/s41598-018-27048-2

Guardiola M, Wangensteen OS, Taberlet P, Coissac E, Uriz MJ, Turon X (2016) Spatio-temporal monitoring of deep-sea communities using metabarcoding of sediment DNA and RNA. PeerJ 4:e2807. https://doi.org/10.7717/peerj.2807

Guillera-Arroita G, Lahoz-Monfort JJ, van Rooyen AR, Weeks AR, Tingley R (2017) Dealing with false-positive and false-negative errors about species occurrence at multiple levels. Methods Ecol Evol 8(9):1081–1091. https://doi.org/10.1111/2041-210x.12743

Guo X, Yang Y, Lu D, Niu Z, Feng J, Chen Y, Tou F, Garner E, Xu J, Liu M, Hochella MF (2018) Biofilms as a sink for antibiotic resistance genes (ARGs) in the Yangtze Estuary. Water Res 129:277–286. https://doi.org/10.1016/j.watres.2017.11.029

Gustavson MS, Collins PC, Finarelli JA, Egan D, Conchuir RO, Wightman GD, King JJ, Gauthier DT, Whelan K, Carlsson JE, Carlsson J (2015) An eDNA assay for Irish *Petromyzon marinus* and *Salmo trutta* and field validation in running water. J Fish Biol 87(5):1254–1262. https://doi.org/10.1111/jfb.12781

Handelsman J (2004) Metagenomics: application of genomics to uncultured microorganisms. Microbiol Mol Biol Rev 68(4):669–685. https://doi.org/10.1128/mmbr.68.4.669-685.2004

Heather JM, Chain B (2016) The sequence of sequencers: the history of sequencing DNA. Genomics 107(1):1–8. https://doi.org/10.1016/j.ygeno.2015.11.003

Henne A, Schmitz RA, Bömeke M, Gottschalk G, Daniel R (2000) Screening of environmental DNA libraries for the presence of genes conferring lipolytic activity on *Escherichia coli*. Appl Environ Microbiol 66(7):3113–3116

Hooper DU, Adair EC, Cardinale BJ, Byrnes JEK, Hungate BA, Matulich KL, Gonzalez A, Duffy JE, Gamfeldt L, O'Connor MI (2012) A global synthesis reveals biodiversity loss as a major driver of ecosystem change. Nature 486:105. https://doi.org/10.1038/nature11118

Hunter ME, Oyler-McCance SJ, Dorazio RM, Fike JA, Smith BJ, Hunter CT, Reed RN, Hart KM (2015) Environmental DNA (eDNA) sampling improves occurrence and detection estimates of invasive burmese pythons. PLoS ONE 10(4):e0121655. https://doi.org/10.1371/journal.pone.0121655

Jeunen G-J, Knapp M, Spencer HG, Lamare MD, Taylor HR, Stat M, Bunce M, Gemmell NJ (2018) Environmental DNA (eDNA) metabarcoding reveals strong discrimination among diverse marine habitats connected by water movement. Mol Ecol Resour. https://doi.org/10.1111/1755-0998.12982

Katz M, Hover BM, Brady SF (2016) Culture-independent discovery of natural products from soil metagenomes. J Ind Microbiol Biotechnol 43(2):129–141. https://doi.org/10.1007/s10295-015-1706-6

Keeley N, Wood SA, Pochon X (2018) Development and preliminary validation of a multi-trophic metabarcoding biotic index for monitoring benthic organic enrichment. Ecol Ind 85:1044–1057

Keller A, Danner N, Grimmer G, Ankenbrand M, Von Der Ohe K, Von Der Ohe W, Rost S, Härtel S, Steffan-Dewenter I (2015) Evaluating multiplexed next-generation sequencing as a method in palynology for mixed pollen samples. Plant Biol 17(2):558–566

Kelly RP, Port JA, Yamahara KM, Crowder LB (2014a) Using environmental DNA to census marine fishes in a large mesocosm. PLoS ONE 9(1):e86175. https://doi.org/10.1371/journal.pone.0086175

Kelly RP, Port JA, Yamahara KM, Martone RG, Lowell N, Thomsen PF, Mach ME, Bennett M, Prahler E, Caldwell MR (2014b) Harnessing DNA to improve environmental management. Science 344(6191):1455–1456

Kelly RP, O'Donnell JL, Lowell NC, Shelton AO, Samhouri JF, Hennessey SM, Feist BE, Williams GD (2016) Genetic signatures of ecological diversity along an urbanization gradient. PeerJ 4:e2444. https://doi.org/10.7717/peerj.2444

Khaliq I, Hardy GESJ, White D, Burgess TI (2018) eDNA from roots: a robust tool for determining Phytophthora communities in natural ecosystems. FEMS Microbiol Ecol 94(5):fiy048. https://doi.org/10.1093/femsec/fiy048

Kim P, Kim D, Yoon TJ, Shin S (2018) Early detection of marine invasive species, *Bugula neritina* (Bryozoa: Cheilostomatida), using species-specific primers and environmental DNA analysis in Korea. Mar Environ Res 139:1–10. https://doi.org/10.1016/j.marenvres.2018.04.015

Klymus KE, Marshall NT, Stepien CA (2017) Environmental DNA (eDNA) metabarcoding assays to detect invasive invertebrate species in the Great Lakes. PLoS ONE 12(5):e0177643. https://doi.org/10.1371/journal.pone.0177643

Lacoursière-Roussel A, Côté G, Leclerc V, Bernatchez L (2016) Quantifying relative fish abundance with eDNA: a promising tool for fisheries management. J Appl Ecol 53(4):1148–1157

Lacoursière-Roussel A, Howland K, Normandeau E, Grey EK, Archambault P, Deiner K, Lodge DM, Hernandez C, Leduc N, Bernatchez L (2018) eDNA metabarcoding as a new surveillance approach for coastal Arctic biodiversity. Ecol Evol 8(16):7763–7777. https://doi.org/10.1002/ece3.4213

Laroche O, Wood SA, Tremblay LA, Lear G, Ellis JI, Pochon X (2017) 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 5:e3347. https://doi.org/10.7717/peerj.3347

Lasken RS, McLean JS (2014) Recent advances in genomic DNA sequencing of microbial species from single cells. Nat Rev Genet 15(9):577

Lemetre C, Maniko J, Charlop-Powers Z, Sparrow B, Lowe AJ, Brady SF (2017) Bacterial natural product biosynthetic domain composition in soil correlates with changes in latitude on a continent-wide scale. Proc Natl Acad Sci 114(44):11615–11620. https://doi.org/10.1073/pnas.1710262114

Levi T, Allen JM, Bell D, Joyce J, Russell JR, Tallmon DA, Vulstek SC, Yang C, Yu DW (2018) Environmental DNA for the enumeration and management of Pacific salmon. Mol Ecol Resour. https://doi.org/10.1111/1755-0998.12987

Li F, Peng Y, Fang W, Altermatt F, Xie Y, Yang J, Zhang X (2018) Application of environmental DNA metabarcoding for predicting anthropogenic pollution in rivers. Environ Sci Technol 52(20):11708–11719

Lim NKM, Tay YC, Srivathsan A, Tan JWT, Kwik JTB, Baloğlu B, Meier R, Yeo DCJ (2016) Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. R Soc Open Sci 3(11):160635. https://doi.org/10.1098/rsos.160635

Lindahl T (1993) Instability and decay of the primary structure of DNA. Nature 362(6422):709–715

Linnarsson S (2010) Recent advances in DNA sequencing methods—general principles of sample preparation. Exp Cell Res 316(8):1339–1343. https://doi.org/10.1016/j.yexcr.2010.02.036

Liu D, Nishida M, Takahashi T, Asakawa S (2018) Transcription of mcrA gene decreases upon prolonged non-flooding period in a methanogenic archaeal community of a paddy-upland rotational field soil. Microb Ecol 75(3):751–760. https://doi.org/10.1007/s00248-017-1063-2

Mächler E, Deiner K, Steinmann P, Altermatt F (2014) Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. Freshw Sci 33(4):1174–1183

Madison JT, Holley RW (1965) The presence of 5,6-dihydrouridylic acid in yeast "soluble" ribonucleic acid. Biochem Biophys Res Commun 18(2):153–157

Matsuhashi S, Doi H, Fujiwara A, Watanabe S, Minamoto T (2016) Evaluation of the environmental DNA method for estimating distribution and biomass of submerged aquatic plants. PLoS ONE 11(6):e0156217. https://doi.org/10.1371/journal.pone.0156217

Mauvisseau Q, Burian A, Gibson C, Brys R, Ramsey A, Sweet M (2019) Influence of accuracy, repeatability and detection probability in the reliability of species-specific eDNA based approaches. Sci Rep 9(1):580

Mohiuddin M, Schellhorn H (2015) Spatial and temporal dynamics of virus occurrence in two freshwater lakes captured through metagenomic analysis. Front Microbiol. https://doi.org/10.3389/fmicb.2015.00960

Ogram A, Sayler GS, Barkay T (1987) The extraction and purification of microbial DNA from sediments. J Microbiol Methods 7(2–3):57–66

Oh S-Y, Fong JJ, Park MS, Chang L, Lim YW (2014) Identifying airborne fungi in Seoul, Korea using metagenomics. J Microbiol 52(6):465–472

Pace NR (1985) Analyzing natural microbial populations by rRNA sequences. ASM News 51:4–12

Pansu J, Giguet-Covex C, Ficetola GF, Gielly L, Boyer F, Zinger L, Arnaud F, Poulenard J, Taberlet P, Choler P (2015) Reconstructing long-term human impacts on plant communities: an ecological approach based on lake sediment DNA. Mol Ecol 24(7):1485–1498. https://doi.org/10.1111/mec.13136

Parducci L, Bennett KD, Ficetola GF, Alsos IG, Suyama Y, Wood JR, Pedersen MW (2017) Ancient plant DNA in lake sediments. New Phytol 214(3):924–942. https://doi.org/10.1111/nph.14470

Parro V, Moreno-Paz M, González-Toril E (2007) Analysis of environmental transcriptomes by DNA microarrays. Environ Microbiol 9(2):453–464

Parsons KM, Everett M, Dahlheim M, Park L (2018) Water, water everywhere: environmental DNA can unlock population structure in elusive marine species. R Soc Open Sci 5(8):180537. https://doi.org/10.1098/rsos.180537

Paul JH, Kellogg CA, Jiang SC (1996) Viruses and DNA in marine environments. In: Colwell RR, Simidu U, Ohwada K (eds) Microbial diversity in time and space. Springer, Boston, pp 115–124. https://doi.org/10.1007/978-0-585-34046-3_14

Pereira HM, Leadley PW, Proença V, Alkemade R, Scharlemann JPW, Fernandez-Manjarrés JF, Araújo MB, Balvanera P, Biggs R, Cheung WWL, Chini L, Cooper HD, Gilman EL, Guénette S, Hurtt GC, Huntington HP, Mace GM, Oberdorff T, Revenga C, Rodrigues P, Scholes RJ, Sumaila UR, Walpole M (2010) Scenarios for global biodiversity in the 21st century. Science 330(6010):1496–1501. https://doi.org/10.1126/science.1196624

Pikitch EK (2018) A tool for finding rare marine species. Science 360(6394):1180–1182. https://doi.org/10.1126/science.aao3787

Pochon X, Zaiko A, Fletcher LM, Laroche O, Wood SA (2017) Wanted dead or alive? Using metabarcoding of environmental DNA and RNA to distinguish living assemblages for biosecurity applications. PLoS ONE 12(11):e0187636. https://doi.org/10.1371/journal.pone.0187636

Prosser CM, Hedgpeth BM (2018) Effects of bioturbation on environmental DNA migration through soil media. PLoS ONE 13(4):e0196430. https://doi.org/10.1371/journal.pone.0196430

Ramírez GA, Jørgensen SL, Zhao R, D'Hondt S (2018) Minimal influence of extracellular DNA on molecular surveys of marine sedimentary communities. Front Microbiol. https://doi.org/10.3389/fmicb.2018.02969

Rees HC, Bishop K, Middleditch DJ, Patmore JRM, Maddison BC, Gough KC (2014a) The application of eDNA for monitoring of the Great Crested Newt in the UK. Ecol Evol 4(21):4023–4032. https://doi.org/10.1002/ece3.1272

Rees HC, Maddison BC, Middleditch DJ, Patmore JR, Gough KC (2014b) The detection of aquatic animal species using environmental DNA—a review of eDNA as a survey tool in ecology. J Appl Ecol 51(5):1450–1459

Rees HC, Baker CA, Gardner DS, Maddison BC, Gough KC (2017) The detection of great crested newts year round via environmental DNA analysis. BMC Res Notes 10(1):327. https://doi.org/10.1186/s13104-017-2657-y

Roussel J-M, Paillisson J-M, Tréguier A, Petit E (2015) The downside of eDNA as a survey tool in water bodies. J Appl Ecol 52(4):823–826. https://doi.org/10.1111/1365-2664.12428

Ruppert KM, Kline RJ, Rahman MS (2019) Past, present, and future perspectives of environmental DNA (eDNA) metabarcoding: a systematic review in methods, monitoring, and applications of global eDNA. Glob Ecol Conserv 17:e00547

Sansom BJ, Sassoubre LM (2017) Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. Environ Sci Technol 51(24):14244–14253. https://doi.org/10.1021/acs.est.7b05199

Sassoubre LM, Yamahara KM, Gardner LD, Block BA, Boehm AB (2016) Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. Environ Sci Technol 50(19):10456–10464. https://doi.org/10.1021/acs.est.6b03114

Sawaya NA, Djurhuus A, Closek CJ, Hepner M, Olesin E, Visser L, Kelble C, Hubbard K, Breitbart M (2019) Assessing eukaryotic biodiversity in the Florida Keys National Marine Sanctuary through environmental DNA metabarcoding. Ecol Evol 9(3):1029–1040. https://doi.org/10.1002/ece3.4742

Schmidt TM, DeLong EF, Pace NR (1991) Analysis of a marine picoplankton community by 16S rRNA gene cloning and sequencing. J Bacteriol 173(14):4371–4378. https://doi.org/10.1128/jb.173.14.4371-4378.1991

Shaw JLA, Clarke LJ, Wedderburn SD, Barnes TC, Weyrich LS, Cooper A (2016) Comparison of environmental DNA metabarcoding and conventional fish survey methods in a river system. Biol Conserv 197:131–138. https://doi.org/10.1016/j.biocon.2016.03.010

Shaw JLA, Weyrich LS, Hallegraeff G, Cooper A (2019) Retrospective eDNA assessment of potentially harmful algae in historical ship ballast tank and marine port sediments. Mol Ecol. https://doi.org/10.1111/mec.15055

Shogren AJ, Tank JL, Andruszkiewicz E, Olds B, Mahon AR, Jerde CL, Bolster D (2017) Controls on eDNA movement in streams: transport, retention, and resuspension. Sci Rep 7(1):5065. https://doi.org/10.1038/s41598-017-05223-1

Simpson TJS, Dias PJ, Snow M, Muñoz J, Berry T (2017) Real-time PCR detection of *Didemnum perlucidum* (Monniot, 1983) and *Didemnum vexillum* (Kott, 2002) in an applied routine marine biosecurity context. Mol Ecol Resour 17(3):443–453. https://doi.org/10.1111/1755-0998.12581

Soininen EM, Valentini A, Coissac E, Miquel C, Gielly L, Brochmann C, Brysting AK, Sønstebø JH, Ims RA, Yoccoz NG (2009) Analysing diet of small herbivores: the efficiency of DNA barcoding coupled with high-throughput pyrosequencing for deciphering the composition of complex plant mixtures. Front Zool 6(1):16

Song JW, Small MJ, Casman EA (2017) Making sense of the noise: the effect of hydrology on silver carp eDNA detection in the Chicago area waterway system. Sci Total Environ 605–606:713–720. https://doi.org/10.1016/j.scitotenv.2017.06.255

Stat M, Huggett MJ, Bernasconi R, DiBattista JD, Berry TE, Newman SJ, Harvey ES, Bunce M (2017) Ecosystem biomonitoring with eDNA: metabarcoding across the tree of life in a tropical marine environment. Sci Rep 7(1):12240. https://doi.org/10.1038/s41598-017-12501-5

Suchan T, Talavera G, Sáez L, Ronikier M, Vila R (2019) Pollen metabarcoding as a tool for tracking long-distance insect migrations. Mol Ecol Resour 19(1):149–162

Taberlet P, Coissac E, Hajibabaei M, Rieseberg LH (2012) Environmental DNA. Mol Ecol 21(8):1789–1793. https://doi.org/10.1111/j.1365-294X.2012.05542.x

Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J (2016) NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44(14):6614–6624. https://doi.org/10.1093/nar/gkw569

Tessler M, Brugler MR, DeSalle R, Hersch R, Velho LFM, Segovia BT, Lansac-Toha FA, Lemke MJ (2017) A global eDNA comparison of freshwater bacterioplankton assemblages focusing on large-river floodplain lakes of Brazil. Microb Ecol 73(1):61–74. https://doi.org/10.1007/s00248-016-0834-5

Thomsen PF, Sigsgaard EE (2019) Environmental DNA metabarcoding of wild flowers reveals diverse communities of terrestrial arthropods. Ecol Evol 9(4):1665–1679. https://doi.org/10.1002/ece3.4809

Thomsen PF, Willerslev E (2015) Environmental DNA—an emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv 183:4–18. https://doi.org/10.1016/j.biocon.2014.11.019

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E (2012) Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol 21(11):2565–2573. https://doi.org/10.1111/j.1365-294X.2011.05418.x

Turner CR, Barnes MA, Xu CC, Jones SE, Jerde CL, Lodge DM (2014) Particle size distribution and optimal capture of aqueous macrobial eDNA. Methods Ecol Evol 5(7):676–684

Ushio M, Murata K, Sado T, Nishiumi I, Takeshita M, Iwasaki W, Miya M (2018) Demonstration of the potential of environmental DNA as a tool for the detection of avian species. Sci Rep 8(1):4493. https://doi.org/10.1038/s41598-018-22817-5

Valentini A, Taberlet P, Miaud C, Civade R, Herder J, Thomsen PF, Bellemain E, Besnard A, Coissac E, Boyer F, Gaboriaud C, Jean P, Poulet N, Roset N, Copp GH, Geniez P, Pont D, Argillier C, Baudoin J-M, Peroux T, Crivelli AJ, Olivier A, Acqueberge M, Le Brun M, Møller PR, Willerslev E, Dejean T (2016) Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. Mol Ecol 25(4):929–942. https://doi.org/10.1111/mec.13428

Van Dijk EL, Auger H, Jaszczyszyn Y, Thermes C (2014) Ten years of next-generation sequencing technology. Trends Genet 30(9):418–426

Wakelin SA, Cave VM, Dignam BE, D'Ath C, Tourna M, Condron LM, Zhou J, Van Nostrand JD, O'Callaghan M (2016) Analysis of soil eDNA functional genes: potential to increase profitability and sustainability of pastoral agriculture. N Z J Agric Res 59(4):333–350. https://doi.org/10.1080/00288233.2016.1209529

Watson JD, Crick FH (1953) Molecular structure of nucleic acids. Nature 171(4356):737–738

Weltz K, Lyle JM, Ovenden J, Morgan JAT, Moreno DA, Semmens JM (2017) Application of environmental DNA to

detect an endangered marine skate species in the wild. PLoS ONE 12(6):e0178124. https://doi.org/10.1371/journal.pone.0178124

Wilcox TM, McKelvey KS, Young MK, Jane SF, Lowe WH, Whiteley AR, Schwartz MK (2013) Robust detection of rare species using environmental DNA: the importance of primer specificity. PLoS ONE 8(3):e59520

Wilcox TM, McKelvey KS, Young MK, Lowe WH, Schwartz MK (2015) Environmental DNA particle size distribution from Brook Trout (*Salvelinus fontinalis*). Conserv Genet Resour 7(3):639–641

Woese CR (1987) Bacterial evolution. Microbiol Rev 51(2):221–271

Won N-I, Kim K-H, Kang JH, Park SR, Lee HJ (2017) Exploring the impacts of anthropogenic disturbance on seawater and sediment microbial communities in korean coastal waters using metagenomics analysis. Int J Environ Res Public Health 14(2):130

Xie Y, Zhang X, Yang J, Kim S, Hong S, Giesy JP, Yim UH, Shim WJ, Yu H, Khim JS (2018) eDNA-based bioassessment of coastal sediments impacted by an oil spill. Environ Pollut 238:739–748

Yang J, Jeppe K, Pettigrove V, Zhang X (2018) Environmental DNA metabarcoding supporting community assessment of environmental stressors in a field-based sediment microcosm study. Environ Sci Technol 52(24):14469–14479. https://doi.org/10.1021/acs.est.8b04903

Zallen DT (2003) Despite Franklin's work, Wilkins earned his Nobel. Nature 425:15. https://doi.org/10.1038/425015b