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Invited Review Article

Environmental DNA (eDNA) technology: Fisheries and aquaculture perspectives

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Abstract

The use of environmental DNA (eDNA) technology has emerged as a pioneering tool in the fields of fisheries and aquaculture, offering novel approaches for the monitoring and management of aquatic ecosystems. This study explores the potential of eDNA technology usage in the research and management of aquatic ecosystems. The importance of this method is discussed in relation to a number of ecological scenarios, including the assessment of biodiversity, monitoring of fish populations and pathogens, early detection of invasive fish species, and evaluation of water quality. Additionally, it addresses the challenges and obstacles that arise in the utilization of eDNA and discusses the ethical considerations that should be taken into account in future applications.This can underscore itssignificance as a non-intrusive, economical, and exceptionally responsive tool for advancing sustainable fisheries and aquaculture practices. This comprehensive review provides an in-depth analysis of the several applications of eDNA technology within the domains of fisheries and aquaculture.

Keywords: Aquaculture, Aquatic ecosystems, Environmental DNA, Invasive fish, Pathogen detection

Highlights

- eDNA: Revolutionizing fisheries and aquaculture management with precision
- From biodiversity to ethics: eDNA's multifaceted role in aquatic sciences
- eDNA tech: Pioneering sustainable fisheries and ecosystem monitoring
- The power of eDNA: Transforming aquatic conservation efforts

INTRODUCTION

The concept of eDNA technology garnered more attention and progress in recent years, resulting in an increase in techniques for the recovery, amplification and sequencing of DNA from environmental materials. The utilisation of eDNA has emerged as a potent technique in contemporary ecological studies, facilitating the acquisition of information pertaining to species occurrence, population size and spatial distribution. These aspects were historically difficult to ascertain through conventional survey techniques. The initial groundwork in the domains of fisheries and aquaculture established the basis for the subsequent development of eDNA technology. Its non-intrusive characteristics and capacity to furnish valuable insights into aquatic ecosystems contributed to its recognition in this field. Through the examination of genetic material, researchers are able to get valuable insights pertaining to the existence, population size and general ecological well-being of many species.

To meet the world's growing fish demand and maintain aquaculture output and food biosecurity, pathogen detection methods must be developed (Mugimba *et al.*, 2021). Aquatic pathogens include bacteria, viruses, and fungi threaten worldwide aquaculture. Aquafarms need early diagnosis and health monitoring to prevent disease spread. Disease research and outbreak control are hampered by the volume of organ samples that must be processed (Sieber *et al.*, 2020). Target-specific primers can amplify the pathogenic agent's DNA, which can subsequently be detected by sequencing. The third generation of DNA sequencing (Liu *et al.*, 2020) has brought high-throughput genetic tools and eDNA technology as a bio-monitoring tool. eDNA technology is one of numerous advanced diagnostic methods that use genetic markers specific to the target taxon or taxa. DNA or RNA from nucleic acid (small DNA fragments) lost by a living entity can be detected in soil, water, ice, sediment, and air without isolating it (Díaz-Ferguson and Moyer, 2014; Thomsen and Willerslev, 2015). Due to DNA's greater stability than RNA, eDNA detection in the environment is easier and less laborious. In freezing, dry permafrost, DNA degrades over thousands of years (Willerslev *et al.*, 2003), but in temperate water, it takes weeks (Thomsen *et al.*, 2012). Standard eDNA sample composition tests include DNA extraction, purification,filtration, polymerase chain reaction (PCR) amplification and DNA sequencing are the main components of this approach.

Using eDNA for species identification is a great example of a technology innovation that opens up new

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ways to study and quantify biosphere change, recovery, and resilience (Thomsen *et al.*, 2012). Early monitoring methods improve precision and standardisation in identifying cryptic, inaccessible, and low-abundance organisms (Thomsen *et al.*, 2012). eDNA from ancient and modern materials can identify a single species or analyze entire ecosystems. This can also measure farmed fish, parasite, bacteria, fungi and other organism. As crucial as it is to detect pathogens, it is also important to determine if target pathogenic organisms breach food safety or water quality. eDNA research began in microbiology since culture-based methods greatly alter the natural range of bacteria (Thomsen and Willerslev, 2015). Species quantification is still the goal of eDNA research, and many authors have made success in aquatic organisms (Nathan *et al.*, 2014), but this method can easily identify harmful algae and pathogens.

After sediments were analyzed for ancient and contemporary animal and plant DNA, eDNA was obtained from a range of terrestrial and aquatic environmental samples to assess macro-organismal diversity. eDNA methods have helped researchers to investigate ancient habitats and monitor contemporary biodiversity in terrestrial and marine ecosystems (Thomsen and Willerslev, 2015). After discharge, eDNA may persist in the environment, be picked up by organic and inorganic particles, decay, or be altered by bacteria. There was a correlation between species abundance, body size and DNA release and degradation. Few abiotic factors impact eDNA stability, including oxygen concentration, nuclease activity, pH, conductivity, UV light, and temperature (Shapiro, 2008). Alvarez *et al.* (1996) suggest researching target eDNA fragment size since smaller fragments survive for weeks or years.

Potentially harmful organisms in the environment and near aquaculture facilities must be regularly monitored with eDNA metabarcoding to prevent economic losses due to infections. The development of technology has made it simpler to identify various living beings without negatively affecting either the environment or the organisms themselves (Bohara *et al.*, 2022). Antibiotic resistance genes (ARGs) are highly concerned due to the intensive use of antibiotics in aquaculture species to prevent and cure bacterial diseases. So, this review shows how the scientific community is constantly increasing the use of eDNA surveys as an advantageous tool in aquaculture perspectives.

Overview of eDNA technology

Collecting samples, extracting DNA, amplifying it, and sequencing it are all foundational steps in eDNA

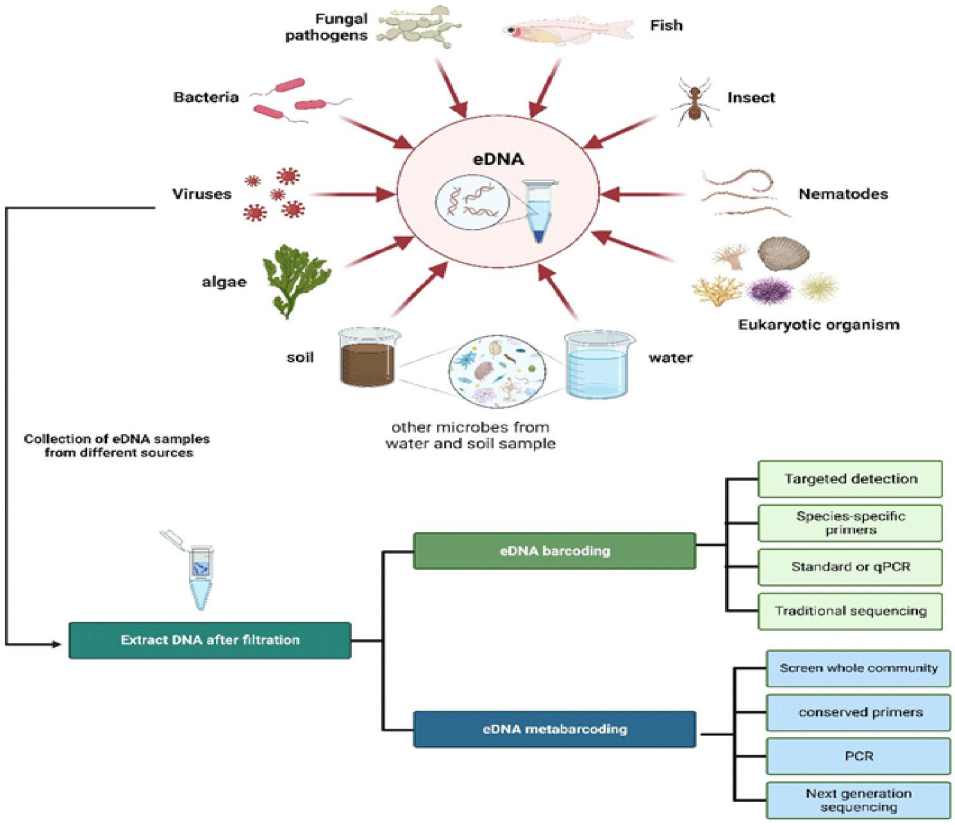

Fig. 1. Overview of the sequential processes involved in environmental DNA (eDNA) technology

technology (Fig. 1). First, soil and water samples are taken from the area of interest. Next, specific procedures are used to extract the DNA from these samples with the goal of isolating and purifying the eDNA. The DNA sections serving as genetic markers for the species of interest are subsequently amplified using PCR. High-throughput sequencing and other modern sequencing technologies have made it possible to analyze many genetic markers in a single sample. DNA sequences are compared to databases to determine what kinds of species live in the area being studied. A quantitative study can show how common or rare each species is. Due to the sensitivity of eDNA technology, it is possible to find uncommon or cryptic species that would otherwise go undetected by more conventional survey techniques. In addition, eDNA investigations can track changes in species diversity in real time because of its high temporal resolution.

Assessment of biodiversity, conservation biology, and monitoring of aquatic ecosystems are just few of the ecological fields that might benefit from eDNA technology. It helps scientists monitor the whereabouts of threatened species, find the carriers of disease, and evaluate the effects of human interference with natural environments. However, there are still obstacles to overcome, such as contamination, degradation, and differences in eDNA shedding rates. To perfect procedures, standardize methods, and set up stringent quality controls, further study is needed. However, the vast volume of sea water promotes dilution and dispersion of the eDNA, making detection more difficult than in freshwater ecosystems (Díaz-Ferguson and Moyer, 2014). The quantity of eDNA in aquatic ecosystems is highly dependent on the diversity of species present and the rate of degradation by biotic and abiotic factors. Diet and trophic investigations can be carried out using eDNA fragments or metabarcoding techniques without the need to see or identify prey in the stomach or faces by employing stomach contents as target DNA (Zarzoso-Lacoste *et al.*, 2013).

Application of eDNA technology in fisheries and aquaculture

eDNA is fish DNA lost in the environment, such as skin, scales, feces, gametes, dead cells, etc. Understanding aquatic species diversity and distribution is crucial to managing and monitoring aquatic habitats. Jo *et al.* (2019) suggest it helps discover spatial variations in coastal ecosystem species communities. Numerous aquatic creatures are hidden under the water; therefore, their accessibility is being studied (Ardura *et al.*, 2015). As eDNA technology advances, it can be used to examine a wider aquatic environment and easily identify creatures (Hänfling *et al.*, 2016). As monitoring this resource under climate change is crucial, eDNA allows rapid monitoring of vast ecosystems like the ocean and its bottom distribution, which is rich in biodiversity. Since it detects eDNA in the water, not the fish, it may give a false positive if the source water contains the parasite. DNA metabarcoding may help explain freshwater plankton community structure and dispersion. Microorganisms and macroalgae inhibit harmful algal blooms through biological processes that need further study using eDNA analysis. Researchers can also evaluate aquaculture ARGs prevalence by focusing on certain genes. To accurately detect species in aquaculture, this technology must be purified, amplified, bioinformatics analyzed, DNA extracted, and sampled. Standardization of methods is essential for data comparability and dependability, and eDNA technology raises ethical and regulatory issues for responsible management, including privacy, data sharing, and species discovery. This section covered aquaculture applications of eDNA technology.

Detection of fish pathogens: The risk of infection and disease spread is higher in fish farms than in natural habitats because farmed animals are often kept in high density and are subject to constant stress and a variety of pollutants. So, the use of eDNA technology for the detection of fish diseases has emerged as a game-changing approach to aquatic health monitoring and disease management. Bacteria, viruses, and parasites are some of the fish diseases that can have devastating effects on both wild and farmed fish populations. The release of genetic material from bacteria into the extracellular media has been documented by Bohara *et al.*(2022).

Some bacteria extrude damaged DNA and manufacture new DNA in water to survive, whereas others lyse their cells and release their DNA (Battista, 1997). Rainwater and groundwater intrusion may transfer the fungus to surrounding lakes and rivers, but cannot be traced accurately because fungal DNA is less studied than bacterial DNA (Martins *et al.*, 2010). Traditionally, fish were collected and euthanized before being examined for endoparasites, which was time-consuming and cruel. However, eDNA technology has made this process more efficient. Several studies (Taengphu *et al.*, 2022) have shown that eDNA or eRNA may detect viral DNA and RNA (Ip *et al.*, 2023). The number of different bacteria varies with temperature, but eDNA can detect

Table 1. Fish diseases identified by using eDNA technology

Disease	Pathogens detected	Source	Country	References
Viral pathogens	Red seabream iridovirus	Seawater	Singapore	Minamoto *et al.*, 2009; Ip *et al.*, 2023
	Nervous necrosis virus	Seawater	Singapore	Ip *et al.*, 2023
	Cyprinus herpes virus (CyHV-3)	Lake, Pond, River	Japan	Minamoto *et al.*, 2009
	Tilapia tilapine virus	Pond water	Thailand	Taengphu *et al.*, 2022
	Ranavirus	Pond water	France	Miaud *et al.*, 2019
	Scale drop disease virus	Pond water	Singapore	Kiat *et al.*, 2023
Bacterial pathogens	*Renibacterium salmoninarum*	River and pond water	Sweden	Persson *et al.*, 2022
	Aeromonas spp.	River	Korea	Fong *et al.*, 2016
	Flavobacterium psychrophilum	River	Japan	Tenma *et al.*, 2021
	Yersinia ruckeri	Recirculatory aquaculture system (RAS)	Norway	Lewin *et al.*, 2020
Fungal pathogens	*Saprolegnia parasitica*	River	France	Rocchi *et al.*, 2017
Parasitic pathogens	Myxosporean	River	Spain	Lisnerová *et al.*, 2023
	Chilodonella hexasticha	Pond water	Australia	Gomes *et al.*, 2017
	Pseudoloma neurophilia	RAS	United States	Schuster *et al.*, 2023
	Gyrodactylus salaris	River and pond water	Norway	Rusch *et al.*, 2018
	Schistocephalus solidus	Seawater	Canada	Berger and Aubin-Horth, 2018
	Nematode and Platyhelminths	Lakes	New Zealand	Thomas *et al.*, 2022
	Dactylogyrus spp.	Fish consignment	Australia	Trujillo-González *et al.*, 2019
	Myxobolus cerebralis	River	California	Richey *et al.*, 2018
	Tetracapsuloides bryosalmonae	River	Switzerland	Carraro *et al.*, 2018
	Ceratonova shasta	River	California	Richey *et al.*, 2020
	Neoparamoeba perurans	Seawater	Australia	Bridle *et al.*, 2010
	Cryptocaryon irritans	Marine fish farm	Hong Kong	Tsang *et al.*, 2021
	Sphaerothecum destruens	River and pond water	Great Britain	Sana *et al.*, 2018

it. Table 1 lists several fish diseases found by this method.

Invasive and time-consuming approaches often used to identify these infections may be stressful for fish and prevent early diagnosis. In order to identify fish diseases quickly and accurately, eDNA technology provides a non-invasive, extremely sensitive alternative. eDNA-based virus detection works on the premise that infected fish leak genetic material into the environment (Kawato *et al.*, 2021). Researchers can now easily isolate the eDNA, and allows for a comprehensive assessment of the range of pathogens present in each body of water or aquaculture facility. This eDNA monitoring reduces stress during surveillance while providing a more thorough evaluation of disease prevalence than conventional sample approaches.

Eutrophication and algae blooms control: Eutrophication, which harms aquatic ecosystems, is caused by algal blooms due to addition of excess nutrients into water body. To address this problem eDNA

technology is helpful. Human activities and nutrient imports have been linked to eutrophication, which may degrade water quality, diminish biodiversity, and pose risks to human health. Genetic material discharged by organisms is used for in-depth species monitoring without direct observation. When it comes to controlling algal blooms, eDNA can help by seeing the first symptoms of a bloom-forming and determining which species are most prevalent. Timely treatments, such altering nutrient inputs or deploying targeted algicides, are made possible by timely information. Safeguarding water quality and the delicate balance of aquatic habitats, eDNA technology provides a non-intrusive, cost-effective solution to monitoring and mitigating eutrophication and algal blooms. In the 1990s, eDNA techniques were used to track phytoplankton blooms and evaluate the impact of environmental factors on bacterial community biomass (Bailiff and Karl, 1991).

We can use species compositions to find ecological indicators to reduce aquatic eutrophication and HABs in freshwater and marine habitats. Due to their toxicity and quick development, harmful algal blooms, induced by phytoplankton, hypoxia, or a lack of local flora and fauna (Smith and Schindler, 2009), destroy aquatic food webs (Wiegand and Pflugmacher, 2005). Algicidal chemical and biological approaches for plankton management are time-consuming, expensive, and only used when algae are visible (He *et al.*, 2012). Their presence has already degraded water quality by lowering oxygen levels and restricting nutrient cycling and light (Paerl and Otten, 2013), but eDNA can detect them at extremely low levels and allow early treatment. Imai *et al.* (2010) found that cyanobacteria toxins harm animals, plants, and humans. Thus, rapid eDNA advances should help monitor and avoid marine HABs. Lowering N and P inputs in too-fertile aquatic systems can stop eutrophication and HABs. eDNA could locate microorganisms that efficiently absorb and change nutrients like N and P in waterbodies to reduce their concentration (Liu *et al.*, 2020). Identifying bacteria that suppress toxic algal growth is crucial. Species diversity and environmental characteristics like pH, N, and P must be monitored throughout time to determine microbe preferences. Early warning systems and HAB prevention can be achieved by purposely encouraging such bacteria to multiply before an epidemic.

DNA content in coastal waters has been shown to correspond with the spatial and temporal fluctuations of bacterial and phytoplankton populations during blooming episodes (Bailiffand Karl, 1991). Combining eDNA procedures with additional approaches, such as physical and chemical studies, may provide more complete information.

Fish biodiversity monitoring: Fish species were detected by direct observation to determine their distributions, patterns, and abundances (Thomsen *et al.*, 2012). However, these methods are laborious and expensive, so new molecular methods for aquatic organism detection have emerged. There are many ways to use eDNA in conservation biology. Fish biodiversity monitoring, analysis, and conservation are greatly improved by eDNA technology. Monitoring of fish populations, species ranges, and habitat changes is necessary. These analyses inform ecosystem status and conservation efforts. Studying fish populations helps researchers conserve, identify threatened species, and assess environmental stressors. Genetic analysis and eDNA sampling offer novel biodiversity assessment methods. This innovative method allows non-intrusive sampling of aquatic ecosystems to complete fish population studies and also estimates population. This knowledge aids fisheries management, habitat degradation assessments, and conservation. eDNA technology makes fish biodiversity research fast, cheap, and scalable, preserving aquatic ecosystems and fish populations for future generations. Maintaining healthy fisheries, food supplies, and aquatic ecosystems' delicate balance requires protecting fish populations. Researchers examined biological life events like spawning and larval settling in fish and shrimp to determine when and where a species' life history event occurred (Díaz-Ferguson and Moyer, 2014). eDNA and water quality assessments of planktonic species may help researchers develop technologies with the data.

Because short DNA sequences can persist for long periods in cold environments with reduced exposure or no light, ancient eDNA in sediments, ice cores, and other environmental sources can help scientists reconstruct community structure and historical ecological processes (Willerslev *et al.*, 2003). Most of Earth's species haven't been formally described by scientists, compounding this problem. Since fish eDNA was 8-1,800 times more concentrated in sediment than water and surficial sediments than surface water, sediment was used to identify fish species (Turner *et al.*, 2015). Directly collecting and observing many species or samples is impossible. Many closely related species lack morphological distinguishing features, making visual differentiation difficult (Ko *et al.*, 2013). Monitoring species and populations provides reliable distribution patterns and population size estimates, which helps conserve biodiversity (Thomsen and Willerslev, 2015).

As eDNA metabarcoding detected a broader range of taxonomic groups than the visual surveys (Afzali *et al.*, 2021), it was found that eDNA metabarcoding can provide monitoring results that are comparable to, or

more sensitive than, a variety of conventional monitoring methods. The fauna and vegetation of springs in the Mojave Desert of California, USA, were detected using three different eDNA metabarcoding primer sets by Palacios Mejia *et al.* (2021). Székely *et al.* (2021) took water samples from the ocean to look for bowhead whales using a recently developed real-time PCR technique. To better forecast geographic and temporal biodiversity trends, eDNA-based techniques are likely to transition in the future from single-marker assessments of species or groups to meta-genomic surveys of whole ecosystems. The biological, geological, and environmental sciences may all benefit from these developments (Thomsen and Willerslev, 2015).

Ecology and environment: eDNA technology is a game-changer in the fields of ecology and environmental management since it provides a fast, non-invasive way to measure biodiversity and monitor ecosystem vitality. It includes the collection of genetic material that animals shed into the environment, which reveals information on the existence, distribution, and interactions of different species. This enables scientists to keep an eye on sensitive or inaccessible environments without causing any damage. Its accuracy and scalability make it a powerful resource for monitoring ecosystems and predicting future changes. By facilitating sustainable practices that protect biodiversity and environmental integrity, eDNA technology enables a proactive strategy for managing ecosystems.

This method was used to determine habitat connectivity for fish migration between the sea and river (Yamanaka and Minamoto, 2016) and to easily identify the presence or absence of the targeted species during seasonal migration pattern identification to maintain fish diversity and river ecosystem functions. Tsuji and Shibata (2021) demonstrated how their eDNA method can study fish reproductive biology. It could be useful for assessing river-artificial barrier effects before and after construction (Yamanaka and Minamoto, 2016). Using eDNA analysis to predict community composition (Minamoto *et al.*, 2012), quantify species abundance (Pilliod *et al*., 2013), and detect aquatic species has advanced rapidly. Higher species extinction rates than pre-human times (Barnosky *et al.*, 2011) threaten human health and the planet's future (Diaz *et al*., 2006). Long-term data can provide innovative conservation and management guidelines for aquatic ecosystems. Better aquatic ecosystem management would improve freshwater and saltwater biodiversity and quality. These traits would boost biodiversity, the economy, and health (Liu *et al.*, 2020).

Early detection of invasive species: This technique greatly improves the early identification of invasive species. Rapid and sensitive identification of species that cannot be directly seen is made possible by eDNA via the collection of genetic material shed into the environment. This non-invasive method improves monitoring efficiency and accuracy, both of which are essential for preventing further harm from incursions. Rare and elusive species may be found with the use of eDNA, as an invasive species and the effects of environmental changes. Even in difficult settings, eDNA enables more thorough collection. It's useful for spotting invasive species in their early stages when they're often hard to notice using conventional approaches. The ability to implement quick reaction techniques is greatly enhanced by early discovery, which helps to minimize additional ecological harm. According to Yamanaka and Minamoto (2016), eDNA technology is an excellent tool for fish monitoring since it enables proactive management and protects native ecosystems from the detrimental consequences of alien species.

Locating an invasive alien species' range is the first step in addressing the difficulties it causes. However, it might be challenging to research species in aquatic habitats (Fujiwara *et al.*, 2016). The abundance of food in their new settings has allowed many invasive fish species to rapidly expand their biomass via breeding. These fish may eventually displace local species by outcompeting them for food and other resources. It has been claimed that eDNA detection may be used to distinguish between fish species in water (Minamoto *et al.*, 2012). The introduction of invasive alien species may have negative consequences on native ecosystems and fisheries, contributing to the worldwide loss of biodiversity that has been identified as a pressing issue. With this technology, invading species may be found in their early stages by tracing their genetic footprints in water bodies. In North America, eDNA has been shown to be an effective detection method for invasive species in freshwater systems by researchers Jerde *et al.* (2011). Asian carp (*Hypophthalmichthys molitrix* and *H. nobilis*) were the primary research subjects in this investigation of their detection methods. When problems are identified quickly, solutions may be implemented to slow or stop their growth. Assessing ecosystem health may be done indirectly via the use of eDNA to track shifts in community makeup and declines in species diversity.

Water quality monitoring: The prosperity of fishing and farming in water requires pristine aquatic habitats. By measuring the existence and quantity of indicator species, eDNA may be used to keep tabs on water quality.

Lake water quality can be measured by phytoplankton biomass and species composition (Chen *et al.*, 2017). Pollution, habitat degradation, and other environmental stresses may all be deduced from variations in eDNA signals. Parameters of water quality such as temperature, oxygen content, pH, and pollution levels must be kept optimal for aquatic life to thrive. Overall fish productivity may be hampered by poor water quality due to stress, sickness, and decreased reproduction. Specifically, changes in species variety might impact the distribution of submerged macrophytes, water quality (Didham *et al.*, 2005), and the general dynamics of an ecosystem. Therefore, eDNA may prove to be a valuable resource in future risk-based decision making for natural resources and environmental impact assessments (Veldhoen *et al*., 2012).

Diatom has high protein and fat content, which aids in the development of fish and shrimp, and this is also used to monitor the water quality. Miyata *et al*. (2022) evaluated a diatom eDNA-based approach and employed eDNA/eRNA from algae and arthropods to assess biodiversity and water quality. Sedimentary eDNA was employed by Sun *et al*. (2021) to make connection between reservoir water quality and long-term microbiological profiles, andobserved bacterial communities of sediments provided a historical window into the effects that human activities had on water quality.

Detecting antibiotic resistance genes using DNA: The efficacy of antibiotics used in animal treatment may be diminished if bacteria in aquatic settings acquire the ability to transmit ARGs to native bacterial populations. The widespread use of antibiotics in aquaculture contributes to a worldwide health crisis known as antibiotic resistance (AR). Aquatic and terrestrial ecosystems are equally impacted by the development of AR due to the horizontal transmission of ARGs across bacteria in aquaculture settings. The health of aquatic creatures may be impacted, and the delicate equilibrium of microbial communities in aquatic environments may be upset if these genes are introduced. There may be serious ecological and public health consequences if antibiotic-resistant bacteria are accidentally released into the environment. Comparison of clinical, agricultural, or eDNA sequences to collections of reference AMR sequences is the backbone of AMR surveillance and genome analysis, underscoring the significance of well-curated AMR databases (McArthur and Tsang, 2017).

It has been suggested that the acquisition of genes from several sources may lead to multidrug resistance in certain bacteria (Saavedra *et al.*, 2018). Possible mechanisms for the emergence of AMR include spontaneous mutations and the horizontal transfer of genes from resistant bacteria through transformation, transduction, and conjugation. Transformation involves the transfer of cell-free DNA to bacteria that are receptive or competent; transduction involves the use of bacteriophages; and conjugation involves the transfer of mobile genetic elements like plasmids through direct cell-to-cell contact. Antimicrobial usage may provide a selection pressure that favours the survival and spread of drug-resistant microorganisms. Water use in irrigation, animal farms, and aquaculture all pose risks for spreading AMR. This is because water may carry pathogens from one location to another. In addition, long-distance travellers like seagulls and other wild animals are known to spread resistant bacteria to new ecosystems.

Aquaculture facilities and their surrounding water bodies may be sampled for eDNA analysis, eliminating the requirement for time-consuming and laborious culture-based approaches for detecting antibiotic-resistant bacteria and ARGs. AMR research in surface waters may utilize molecular methods that evaluate the DNA of a bacterium for related ARGs (Chang *et al.*, 2017). This is because environmental bacteria might represent key resistance reservoirs, and their culture is also not straightforward; thus, they evade detection. By analyzing the retrieved eDNA using molecular tools like PCR or metagenomic approaches, scientists get insight into the prevalence, diversity, and dynamics of antibiotic-resistant populations. qPCR experiments directed at individual ARGs that also provide information on the prevalence of resistance genes in aquaculture settings. As shown by Chang *et al.* (2017), qPCR data may be used as a reliable substitute for ARG transferability. Depending on the DNA extraction method, qPCR may reveal the abundance of the targeted ARGs in diverse genetic settings, such as live bacteria, mobile DNA fragments like MGEs (Mobile genetic element), and "free" ambient DNA, i.e., extracellular DNA (Eramo*et al.*, 2019).

Future directions

Future advances in fisheries and aquaculture with eDNA technology are likely to continue. More research is needed to perfect the technique, increase detection sensitivity, and expand uses. Academics and industry must collaborate to maximize eDNA's potential. Future species identification will require optimizing molecular tests and verifying positive samples, both eDNA-related challenges. Optimizing eDNA for disease diagnosis is still difficult, and to ensure that eDNA comes from active infection but not from discarded DNA fragments. For accurate results, sample collection, processing, and analysis must minimize contamination. Validation and

standardization of eDNA-based assays are improving their reliability. Another way to reduce eDNA false positive ambiguity is to Sanger sequence every positive eDNA.

This technique can only provide information on organisms discovered from genetic material, so reference DNA databases may limit its use for certain species, and we needed species-specific primer sets so that we could identify other species also. If eDNA analysis technologies improve, metabarcoding with universal primers, it can estimate fish biomass and biodiversity. These advances will help us protect endangered species and eradicate invasive ones. Deep-sea community composition is unknown due to the rarity of hadal ecosystem studies, so this method allows us to identify deep oceanic aquatic fauna.

Aquaculture and host species repatriation may benefit from eDNA infection testing to stop infectious agents from spreading to wild fish populations. Thus, Sana *et al.* (2018) addressed a major fisheries conservation issue. eDNA tools need more work before they can fully replace conventional pathogen abundance detection methods (Rusch *et al.*, 2018), but researchers say they will cut costs, improve surveillance, and speed up risk mitigation if they return positive.

Conclusion

Fisheries and aquaculture research and management are changing rapidly due to eDNA technology. Its use in biodiversity assessment, pathogen screening, fish population monitoring, HAB detection, invasive species identification, and water quality monitoring shows its forging potential. As genetic methods are being refined with the advancement of genomic technologies, eDNA can help to promote sustainable practices and protect aquatic ecosystems. Sustainable methods are needed to reduce aquaculture ARG transfer risks. This includes prudent antibiotic use, probiotics, vaccinations, and strict biosecurity. Using eDNA to monitor ARGs may help develop and evaluate preventative measures. Due to its wide coverage, eDNA technology for fish pathogen identification is a paradigm shift in aquatic disease monitoring, protecting both wild and cultivated fish populations. Despite its ability to reliably detect target species, eDNA's use in the real world is limited by its inability to accurately assess the prevalence of non-targeted agents in the environment.

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REFERENCES

Afzali SF, Bourdages H, Laporte M, Mérot C, Normandeau *et al.*, 2021. Comparing environmental metabarcoding and trawling survey of demersal fish communities in the Gulf of St. Lawrence, Canada. Environ DNA, 3(1): 22-42, doi: 10.1002/edn3.111

Alvarez AJ, Yumet GM, Santiago CL and Toranzos GA, 1996. Stability of manipulated plasmid DNA in aquatic environments. Environ Toxicol, 11(2): 129-135, doi: 10.1002/(SICI)1098-2256(1996)11:2<129::AID-TOX8>3.0.CO;2-B

Ardura A, Zaiko A, Martinez JL, Samulioviene A, Semenova A *et al.*, 2015. eDNA and specific primers for early detection of invasive species- A case study on the bivalve *Rangia cuneata*, currently spreading in Europe. Mar Environ Res, 112: 48-55, doi: 10.1016/j.marenvres. 2015.09.013

Bailiff MD and Karl DM, 1991. Dissolved and particulate DNA dynamics during a spring bloom in the Antarctic Peninsula region, 1986–1987. Deep Sea Res Part I Oceanogr Res Pap, 38(8-9): 1077-1095, doi: 10.1016/0198-0149(91)90097-Y

Barnosky AD, Matzke N, Tomiya S, Wogan GO, Swartz B *et al.*, 2011. Has the Earth's sixth mass extinction already arrived. Nature, 471(7336): 51-57, doi: 10.1038/nature09678

Battista JR, 1997. Against all odds: The survival strategies of *Deinococcus radiodurans*. Annual review of microbiology, 51(1): 203-224, doi: 10.1146/annurev.micro.51.1.203

Berger CS and Aubin-Horth N, 2018. An eDNA-qPCR assay to detect the presence of the parasite *Schistocephalus solidus* inside its three spine stickleback host. J Exp Biol, 221(9): jeb178137, doi: 10.1242/jeb.178137

Bohara K, Yadav AK and Joshi P, 2022. Detection of fish pathogens in freshwater aquaculture using eDNA methods: A review, doi: 10.3390/d14121015

Bridle AR, Crosbie PBB, Cadoret K and Nowak BF, 2010. Rapid detection and quantification of *Neoparamoeba perurans* in the marine environment. Aquaculture, 309(1-4): 56-61, doi:10.1016/j.aquaculture.2010.09.018

Carraro L, Hartikainen H, Jokela J, Bertuzzo E and Rinaldo A, 2018. Estimating species distribution and abundance in

river networks using environmental DNA. Proc Natl Acad Sci, 115(46): 11724-11729, doi:10.1073/pnas.181384311

Chang PH, Juhrend B, Olson TM, Marrs CF and Wigginton KR, 2017. Degradation of extracellular antibiotic resistance genes with UV254 treatment. Environ Sci Technol, 51(11): 6185-6192, doi:10.1021/acs.est.7b01120

Chen K, Allen J and Lu J, 2017. Community structures of phytoplankton with emphasis on toxic cyanobacteria in an Ohio inland lake during bloom season. J Water Resource Prot, 9(11): 1, doi: 10.4236/jwarp.2017.911083

Diaz S, Fargione J, Chapin III FS and Tilman D, 2006. Biodiversity loss threatens human well-being. PLoS Biol, 4(8): e277, doi: 10.1371/journal.pbio.0040277

Díaz-Ferguson EE and Moyer GR, 2014. History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. Rev Biol Trop, 62(4): 1273-1284, doi: 10.15517/rbt.v62i4.13231

Didham RK, Tylianakis JM, Hutchison MA, Ewers RM and Gemmell NJ, 2005. Are invasive species the drivers of ecological change. Trends Ecol Evol, 20(9): 470-474, doi: 10.1016/j.tree.2005.07.006

Eramo A, Medina WRM and Fahrenfeld NL, 2019. Viability-based quantification of antibiotic resistance genes and human fecal markers in wastewater effluent and receiving waters. Sci Total Environ, 656: 495-502, doi: 10.1016/j.scitotenv.2018.11.325

Fong JJ, Cho HJ, Park MS and Lim YW, 2016. Evaluating seasonality and pathogenicity of Aeromonas in Korea using environmental DNA. Asian J Microbiol Biotechnol Environ Sci, 18(3): 605-613

Fujiwara A, Matsuhashi S, Doi H, Yamamoto S and Minamoto T, 2016. Use of environmental DNA to survey the distribution of an invasive submerged plant in ponds. Freshw Sci, 35(2): 748-754, doi: 10.1086/685882

Gomes GB, Hutson KS, Domingos JA, Chung C, Hayward S *et al.*, 2017. Use of environmental DNA (eDNA) and water quality data to predict protozoan parasites outbreaks in fish farms. Aquaculture, 479: 467-473, doi: 10.1016/j.aquaculture.2017.06.021

Hänfling B, Lawson Handley L, Read DS, Hahn C, Li J *et al.*, 2016. Environmental DNA metabarcoding of lake fish communities reflects long term data from established survey methods. Mol Ecol, 25(13): 3101-3119, doi: 10.1111/mec.13660

He X, Liu YL, Conklin A, Westrick J, Weavers LK *et al.*, 2016. Toxic cyanobacteria and drinking water: impacts, detection, and treatment. Harmful algae, 54: 174-193, doi: 10.1016/j.hal.2016.01.001

Imai I, Kido T, Yoshinaga I, Ohgi K and Nagai S, 2010. Isolation of Microcystis-killer bacterium *Agrobacterium vitis* from the biofilm on the surface of the water plant *Egeria densa*. Kalliopi A Pagou, 150: 150-152

Ip YCA, Chen J, Tan LY, Lau C, Chan YH *et al.*, 2023. Establishing environmental DNA and RNA protocols for the simultaneous detection of fish viruses from seawater. Environ DNA, 00: 1-14, doi: 10.1002/edn3.418

Jerde CL, Mahon AR, Chadderton WL and Lodge DM, 2011. "Sight unseen" detection of rare aquatic species using environmental DNA. Conserv Lett, 4(2): 150-157, doi: 10.1111/j.1755-263X.2010.00158.x

Jo H, Kim DK, Park K and Kwak IS, 2019. Discrimination of spatial distribution of aquatic organisms in a coastal ecosystem using eDNA. Appl Sci, 9(17): 3450, doi: 10.3390/app9173450

Kawato Y, Mekata T, Inada M and Ito T, 2021. Application of environmental DNA for monitoring Red Sea bream Iridovirus at a fish farm. Microbiol Spectr, 9(2): e00796-21, doi: 10.1128/Spectrum.00796-21

Kiat EOJ, Nair T, Sobana M, Hann NT, Domingos JA *et al.*, 2023. Identification of scale drop disease virus based on environment DNA in an aquaculture facility of Singapore. Aquaculture, 563: 738993, doi: 10.1016/j.aquaculture.2022.738993

Ko HL, Wang YT, Chiu TS, Lee MA, Leu MY *et al.*, 2013. Evaluating the accuracy of morphological identification of larval fishes by applying DNA barcoding. PLoS One, 8(1): e53451, doi: 10.1371/journal.pone.0053451

Lewin AS, Haugen T, Netzer R, Tøndervik A, Dahle SW *et al.*, 2020. Multiplex droplet digital PCR assay for detection of *Flavobacterium psychrophilum* and *Yersinia ruckeri* in Norwegian aquaculture. J Microbiol Methods, 177: 106044, doi: 10.1016/j.mimet.2020.106044

Lisnerová M, Holzer A, Blabolil P and Fiala I, 2023. Evaluation and optimization of an eDNA metabarcoding assay for detection of freshwater myxozoan communities. Environ DNA, 5(2): 312-325, doi: 10.1002/edn3.380

Liu Q, Zhang Y, Wu H, Liu F, Peng W *et al.*, 2020. A review and perspective of eDNA application to eutrophication and HAB control in freshwater and marine ecosystems. Microorganisms, 8(3): 417, doi: 10.3390/microorganisms8030417

Martins M, Uppuluri P, Thomas DP, Cleary IA, Henriques M *et al.*, 2010. Presence of extracellular DNA in the *Candida albicans* biofilm matrix and its contribution to biofilms. Mycopathologia, 169, 323-331, doi: 10.1007/s11046-009-9264-y

McArthur AG and Tsang KK, 2017. Antimicrobial resistance surveillance in the genomic age. Ann NY Acad Sci, 1388(1): 78-91, doi: 10.1111/nyas.13289

Miaud C, Arnal V, Poulain M, Valentini A and Dejean T, 2019. eDNA increases the detectability of ranavirus infection in an alpine amphibian population. Viruses, 11(6): 526, doi: 10.3390/v11060526

Minamoto T, Honjo MN, Uchii K, Yamanaka H, Suzuki AA *et al.*, 2009. Detection of cyprinid herpesvirus 3 DNA in river water during and after an outbreak. Vet Microbiol, 135(3-4): 261-266, doi: 10.1016/j.vetmic.2008.09.081

Minamoto T, Yamanaka H, Takahara T, Honjo MN and Kawabata ZI, 2012. Surveillance of fish species composition using environmental DNA. Limnology, 13: 193-197, doi: 10.1007/s10201-011-0362-4

Miyata K, Inoue Y, Amano Y, Nishioka T, Nagaike T *et al.*, 2022. Comparative environmental RNA and DNA metabarcoding analysis of river algae and arthropods for

ecological surveys and water quality assessment. Sci Rep, 12(1): 19828, doi: 10.1038/s41598-022-23888-1

Mugimba KK, Byarugaba DK, Mutoloki S, Evensen Ø and Munang'andu HM, 2021. Challenges and solutions to viral diseases of finfish in marine aquaculture. Pathogens, 10(6): 673, doi: 10.3390/pathogens10060673

Nathan LM, Simmons M, Wegleitner BJ, Jerde CL and Mahon AR, 2014. Quantifying environmental DNA signals for aquatic invasive species across multiple detection platforms. Environ Sci Technol, 48(21): 12800-12806, doi: 10.1021/es5034052

Paerl HW and Otten TG, 2013. Harmful cyanobacterial blooms: causes, consequences and controls. Microb Ecol, 65: 995-1010, doi: 10.1007/s00248-012-0159-y

Palacios Mejia M, Curd E, Edalati K, Renshaw MA, Dunn R *et al.*, 2021. The utility of environmental DNA from sediment and water samples for recovery of observed plant and animal species from four Mojave Desert springs. Environ DNA, 3(1): 214-230, doi: 10.1002/edn3.161

Persson DB, Aspán A, Hysing P, Blomkvist E, Jansson E *et al.*, 2022. Assessing the presence and spread of *Renibacterium salmoninarum* between farmed and wild fish in Sweden. J Fish Dis, 45(5): 613-621, doi: 10.1111/jfd.13586

Pilliod DS, Goldberg CS, Arkle RS and Waits LP, 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. Can J Fish Aquat Sci, 70(8): 1123-1130, doi: 10.1139/cjfas-2013-0047

Richey CA, Kenelty KV, Hopkins KVS, Stevens BN, Martínez-López B *et al.*, 2020. Validation of environmental DNA sampling for determination of *Ceratonova shasta* (Cnidaria: Myxozoa) distribution in Plumas National Forest, CA. Parasitol Res, 119: 859-870, doi: 10.1007/s00436-019-06509-1

Richey CA, Kenelty KV, Van Stone Hopkins K, Stevens BN, Martínez López B *et al.*, 2018. Distribution and prevalence of *Myxobolus cerebralis* in postfire areas of Plumas National Forest: utility of environmental DNA sampling. J Aquat Anim Health, 30(2): 130-143, doi: 10.1002/aah.10014

Rocchi S, Tisserant M, Valot B, Laboissière A, Frossard V *et al.*, 2017. Quantification of *Saprolegnia parasitica* in river water using real-time quantitative PCR: from massive fish mortality to tap drinking water. Int J Environ Health Res, 27(1): 1-10, doi: 10.1080/09603123.2016.1246653

Rusch JC, Hansen H, Strand DA, Markussen T, Hytterød S *et al.*, 2018. Catching the fish with the worm: A case study on eDNA detection of the monogenean parasite *Gyrodactylus salaris* and two of its hosts, Atlantic salmon (*Salmosalar*) and rainbow trout (*Oncorhynchus mykiss*). Parasite Vectors, 11(1): 1-12, doi: 10.1186/s13071-018-2916-3

Saavedra J, Grandón M, Villalobos-González J, Bohle H, Bustos P *et al.*, 2018. Isolation, functional characterization and transmissibility of p3PS10, a multidrug resistance plasmid of the fish pathogen *Piscirickettsia salmonis*. Front Microbiol, 9: 923, doi: 10.3389/fmicb.2018.00923

Sana S, Williams C, Hardouin EA, Blake A, Davison P *et al.*, 2018. Phylogenetic and environmental DNA insights into emerging aquatic parasites: implications for risk management. Int J Parasitol, 48(6): 473-481, doi: 10.1016/j.ijpara.2017.11.002

Schuster CJ, Murray KN, Sanders JL and Kent ML, 2023. Application of an eDNA assay for the detection of *Pseudoloma neurophilia* (Microsporidia) in zebrafish (*Danio rerio*) facilities. Aquaculture, 564: 739044, doi: 10.1016/j.aquaculture.2022.739044

Shapiro B, 2008. Engineered polymerases amplify the potential of ancient DNA. Trends Biotechnol, 26(6): 285-287, doi: 10.1016/j.tibtech.2008.03.005

Sieber N, Hartikainen H and Vorburger C, 2020. Validation of an eDNA-based method for the detection of wildlife pathogens in water. Dis Aquat Org, 141: 171-184, doi: 10.3354/dao03524

Smith VH and Schindler DW, 2009. Eutrophication science: where do we go from here. Trends Ecol Evol, 24(4): 201-207, doi: 10.1016/j.tree.2008.11.009

Sun J, Chen X, Yu J, Chen Z, Liu L *et al.*, 2021. Deciphering historical water-quality changes recorded in sediments using eDNA. Front Environ Sci, 9: 669582, doi: 10.3389/fenvs.2021.669582

Székely D, Corfixen NL, Mørch LL, Knudsen SW, McCarthy ML *et al.*, 2021. Environmental DNA captures the genetic diversity of bowhead whales (*Balaena mysticetus*) in West Greenland. Environ DNA, 3(1): 248-260, doi: 10.1002/edn3.176

Taengphu S, Kayansamruaj P, Kawato Y, Delamare-Deboutteville J, Mohan CV *et al.*, 2022. Concentration and quantification of Tilapia tilapinevirus from water using a simple iron flocculation coupled with probe-based RT-qPCR. Peer J, 10: e13157, doi: 10.7717/peerj.13157

Tenma H, Tsunekawa K, Fujiyoshi R, Takai H, Hirose M *et al.*, 2021. Spatiotemporal distribution of *Flavobacterium psychrophilum* and ayu *Plecoglossusa ltivelis* in rivers revealed by environmental DNA analysis. Fish Sci, 87: 321-330, doi: 10.1007/s12562-021-01510-z

Thomas LJ, Milotic M, Vaux F and Poulin R, 2022. Lurking in the water: testing eDNA metabarcoding as a tool for ecosystem-wide parasite detection. Parasitology, 149(2): 261-269, doi: 10.1017/S0031182021001840

Thomsen PF and Willerslev E, 2015. Environmental DNA- An emerging tool in conservation for monitoring past and present biodiversity. Biol Conserv, 183: 4-18, doi: 10.1016/j.biocon.2014.11.019

Thomsen PF, Kielgast JOS, Iversen LL, Wiuf C, Rasmussen M *et al.*, 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Mol Ecol, 21(11): 2565-2573, doi: 10.1111/j.1365-294X.2011.05418.x

Trujillo-González A, Edmunds RC, Becker JA and Hutson KS, 2019. Parasite detection in the ornamental fish trade using environmental DNA. Sci Rep, 9(1), 5173, doi: 10.1038/s41598-019-41517-2

Tsang HH, Domingos JA, Westaway JA, Kam MH, Huerlimann R *et al.*, 2021. Digital droplet PCR-based environmental DNA tool for monitoring *Cryptocaryon*

irritans in a marine fish farm from Hong Kong. Diversity, 13(8): 350, doi: 10.3390/d13080350

Tsuji S and Shibata N, 2021. Identifying spawning events in fish by observing a spike in environmental DNA concentration after spawning. Environ DNA, 3(1): 190-199, doi: 10.1002/edn3.153

Turner CR, Uy KL and Everhart RC, 2015. Fish environmental DNA is more concentrated in aquatic sediments than surface water. Biol Conserv, 183: 93-102, doi: 10.1016/j.biocon.2014.11.017

Veldhoen N, Ikonomou MG and Helbing CC, 2012. Molecular profiling of marine fauna: integration of omics with environmental assessment of the world's oceans. Ecotoxicol Environ Saf, 76: 23-38, doi: 10.1016/j.ecoenv.2011.10.005

Wiegand C and Pflugmacher S, 2005. Ecotoxicological effects of selected cyanobacterial secondary metabolites a short review. Toxicol Appl Pharmacol, 203(3): 201-218, doi: 10.1016/j.taap.2004.11.002

Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MTP *et al.*, 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. Science, 300(5620): 791-795, doi: 10.1126/science.1084114

Yamanaka H and Minamoto T, 2016. The use of environmental DNA of fishes as an efficient method of determining habitat connectivity. Ecol Indic, 62: 147-153, doi: 10.1016/j.ecolind.2015.11.022

Zarzoso Lacoste D, Corse E and Vidal E, 2013. Improving PCR detection of prey in molecular diet studies: importance of group specific primer set selection and extraction protocol performances. Mol Ecol Resour, 13(1): 117-127, doi: 10.1111/1755-0998.12029

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