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A Systematic Scoping Review on the Current Applications of Environmental DNA (eDNA)

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Abstract

Environmental DNA (eDNA) are DNA shredded by an organism to its surrounding, which presents a non-invasive means to detect organisms of interests or to assess the biodiversity of the specific environment. Applications of eDNA is rapidly growing but a systematic review on the breath and depth of the applications of eDNA has not been carried out. Here, we present a systematic scoping review of the current applications of eDNA up to July 31, 2021, using Google Scholar and PubMed as source databases. 159 articles were identified, and 54 articles were included in this review. Our analysis suggests 10 themes of applications; namely, (a) detecting rare, cryptic or endangered species, (b) detecting bacterial and parasitic pathogens/disease outbreaks, (c) invasive species detection, (d) biodiversity characterisation and biomonitoring, (e) spawning ecology, (f) management of fisheries, (h) hatchery management/selective breeding application, (i) forensic/forensic ecology, (j) crop cultivation and soil fertility, and (k) anthropogenic effects on biodiversity.

Keywords: *Systematic Review; Scoping Review; Environmental DNA; Google Scholar; PubMed; PRISM*

Introduction

Environmental DNA (eDNA) is defined as short DNA fragments obtained directly from environmental samples such as water, air, or sediment that an organism leaves behind in an ecosystem [1]. eDNA is derived from a wide variety of sources including shed intestinal and skin cells, mucus, saliva, gametes, secretions, faeces, urine, carcasses, pollen, and microorganisms. Over the past decade, it has been increasingly clear that the analysis of eDNA from natural environments can reveal information on the occurrence of targeted organisms with high efficiency and sensitivity without destructive sampling [2]. Unlike invasive, laborious, time-consuming, and expensive traditional biodiversity assessment methods which rely on physical identification of species by visual surveys and counting of individuals, the advent of eDNA can overcome these challenges and revolutionize numerous scientific disciplines such as phylogenetics, conservation biology, and ecology in several ways. Firstly, eDNA techniques are rapid, efficient, and cost-effective, enabling monitoring of the dynamics of species, populations, and communities, and to map their geographic distribution over long time and across large spatial scales [3]. Secondly, eDNA sampling is non-destructive, and non-invasive, resulting in no significant damage to the target species or its habitats unlike traditional methods such as marine bottom trawls, electrofishing, and rotenone poisoning [4,5]. Thirdly, eDNA possess incredible sensitivity for achieving high detection probabilities for rare, cryptic, and elusive species, even at relatively low densities [6,7]. Fourthly, eDNA enables the early detection of biological invasions and timely eradication before full establishment [8,9]. Lastly, eDNA analyses potentially

offers a broad taxonomic breadth, allowing simultaneous biodiversity assessments of a wide variety of taxa [10]. Thus, the adoption of eDNA techniques creates an unprecedented opportunity to advance biodiversity monitoring and environmental management.

Review is the primary means to summarize and synthesize the knowledge from primary literature, which can be seen as periodic pitstops in the advancement of science. Existing reviews on eDNA narrative reviews and traditional literature reviews [11,12], with no systematic review of eDNA applications to date. Narrative reviews lack a rigorously pre-specified eligibility criteria and a systematic search strategy [13]; thus, a systematic review is superior in evidence-based practice and considered one of the highest levels of evidence [14]. However, the current applications of eDNA had not been systematically reviewed. Hence, this study aims to provide a map of the current applications of eDNA. Munn., *et al*. [15] argue that a scoping review can be used map out evidence in a field. Furthermore, when performed systematically; a scoping review (then more appropriately known as systematic scoping review) can provide boundaries of a field and a precursor to full systematic review especially if a systematic review had not been performed [16]; which is in the case of eDNA applications. Therefore, we aim to undertake a systematic scoping review on the applications and current potential of eDNA using search carried out on July 31, 2021.

Methods

Search strategy

A literature search was undertaken on July 31, 2021, to locate studies examining the numerous present-day and potential applications of eDNA along with its associated limitations and assumptions with each application. Google Scholar (https://scholar.google.com/) and PubMed (https://pubmed.ncbi.nlm.nih.gov/) were sufficient for systematic reviews [17]. Google Scholar was searched using "allintitle: application eDNA" and "allintitle: applications eDNA" as search terms separately while PubMed was searched using ("applications" AND "eDNA") as search term.

Inclusion and exclusion criteria

The following exclusion criteria were applied: (A) articles which are duplicated based on title and author names were removed; (B) articles with no access to full-text articles were removed, resulting in only full text articles; (C) articles with no mention of "eDNA" or "environmental DNA" within the main text was removed; (D) articles that were not in written in English language were removed; (E) articles that are not published in journals or conferences were removed; (F) secondary research consisting of systematic reviews, narrative reviews and meta-analyses are removed; (G) articles on methodological processes such as eDNA bioinformatic processing software or sampling procedures. Articles remaining after the exclusion criteria were included in this review.

Data extraction strategy and synthesis strategy

A qualitative studies data extraction was performed. An evidence summary table and review matrix were used. Data extracted from studies were title, authors, journal, eDNA source, taxon studied, environment, methods, results, and application and categorized. A subsequent narrative synthesis of the extracted data was performed.

Results and Discussion

Analysis of search results

A hit is defined as an atomic result from a search and the number of hits from a search refers to the number of results returned. A total of 159 hits were returned from both Google Scholar and PubMed; of which, 112 articles and 27 articles were obtained respectively (Figure 1). 153 hits are non-duplicates within the set (Exclusion Criterion A); of which, 141 have full-text articles (Exclusion Criterion B). Within

the full-text articles, 102 articles mention of "eDNA" or "environmental DNA" within the main text (Exclusion Criterion C); of which 101 articles are written in English language (Exclusion Criterion D). Within the English articles, 96 articles are journal or conference articles (Exclusion Criterion E). Within the journal or conference articles, 62 articles are primary research (Exclusion Criterion F). Within the primary research articles, 54 articles are on eDNA application-based articles. Based on the above criteria, a total of 54 articles are used in this review.

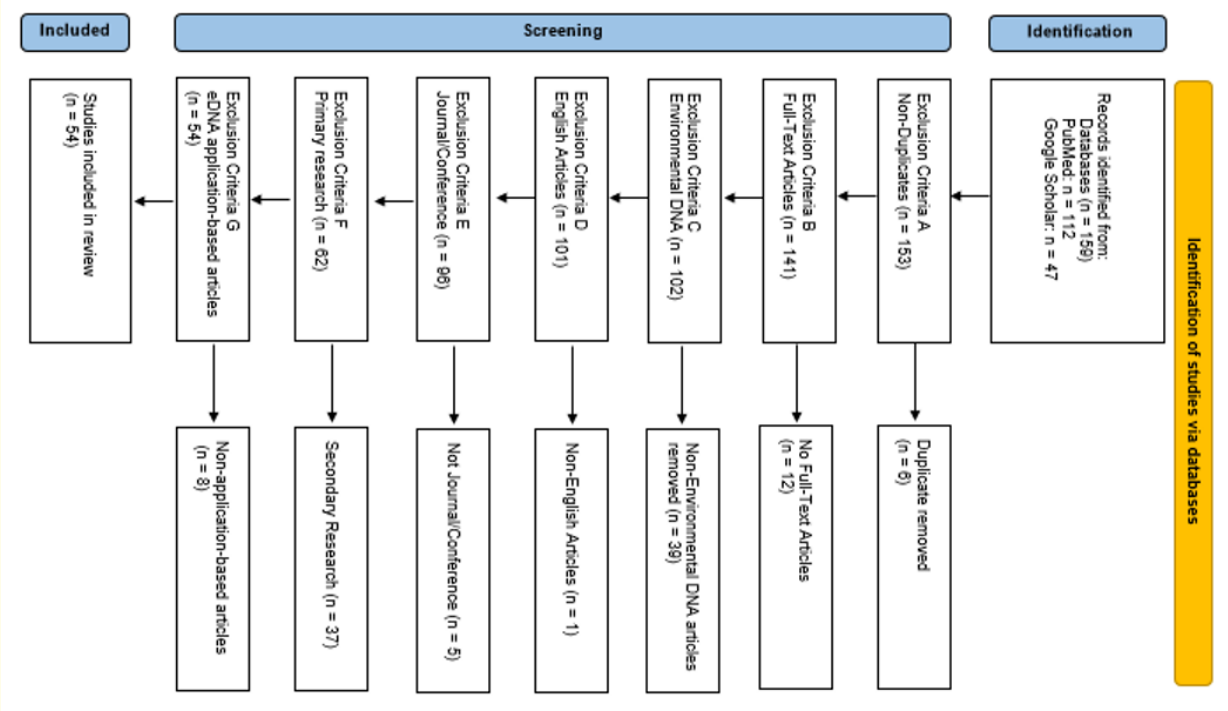

Figure 1: *Flow of Hits Through Exclusion Criteria.*

Overall workflow and methodologies of eDNA studies

The general workflow for eDNA-based studies is environmental sampling (ice cores, soil/sediment core samples, freshwater/seawater samples), DNA extraction, polymerase chain reaction (PCR) amplification of extracted DNA using universal or species-specific primers (i.e., conventional PCR, quantitative PCR (qPCR), digital droplet PCR (ddPCR) and subsequent DNA sequencing of amplicons (i.e. Sanger sequencing, high-throughput next-generation sequencing (NGS)); bioinformatic data-processing (i.e., error trimming, sequence sorting, and identification pipelines leading to various taxonomic level or molecular operational taxonomic units (MOTUs)).

Barcoding and metabarcoding are the 2 main broad approaches used in eDNA-based studies that have garnered a great deal of interest [18]. The primary distinction between barcoding and metabarcoding is that barcoding employs species-specific primers to detect DNA fragments from a single species from an environmental sample [19] while metabarcoding uses universal primers to simultaneously detect millions of DNA fragments from the widest possible coverage of multiple taxa across different trophic levels [20].

Current eDNA applications. The primary eDNA studies obtained after the filtering of hits through the exclusion criteria can be classified into 10 themes (Table 1). Biodiversity characterization and biomonitoring top the charts with 37% of the studies. However, there are also substantial limitations (Table 2).

Applications	Number of articles	References
Detecting rare, cryptic or endangered species	12	[11,21-31]
Detection of bacterial and parasitic pathogens/Disease outbreaks	8	[30,32-38]
Invasive species detection	11	[31,32,39-47]
Biodiversity characterisation and biomonitoring	20	[12,21,34,48-64]
Spawning ecology	1	[40]
Management of fisheries	2	[65, 66]
Hatchery management/selective breeding application	1	[67]
Forensic/forensic ecology	2	[68]
Crop cultivation and soil fertility	1	[69]
Anthropogenic effects on biodiversity	2	[70, 71]

Table 1: *Thematic Classification of Primary Environmental DNA (eDNA) Studies.*

Application	Limitations	Potential solution(s)
Detecting rare, cryptic or endangered species	Laboratory sample contamination	Include negative controls samples [21] Decontamination protocols at each stage autoclaving or sterilization with 10% sodium hypochlorite solution [27]
	Species-specific seasonal variability	Deployment of replicates in field sampling and in the laboratory [29]
	PCR inhibitors	Purification protocols before PCR amplification [28]
Detection of bacterial and parasitic pathogens/ Disease outbreaks	Incomplete reference databases	Increase barcode efforts [36] Use targeted species- specific organisms with molecular probes [38]
Invasive species detection	Difficulties in quantifying abundance and biomass	Quantify the relationship between eDNA release and biotic, and abiotic factors [26]
Biodiversity characterisation and biomonitoring	Difficulties in quantifying abundance and biomass	Investigate the multiple environmental factors on the efficiency of eDNA detection [61] Investigate the transport and degradation rates of eDNA within fluvial systems [61]
	Variability of sampling effort across studies and potential sampling bias	Standardization of sampling protocols [53] Increase sampling volume and frequency [53]
	PCR inhibitors	Analyse multiple eDNA extracts per sample [49] Inclusion of positive controls [49]
	Insufficient sampling efforts	Increasing the number of samples per locality [53]
	Low eDNA quantity	Increasing the number of PCR cycles [60]
	Natural environmental contamination	Perform simultaneous traditional assessment of community composition to compare with eDNA results [51]
	Imperfect sampling of eDNA and false detection	Estimate detection rates using occupancy models [50]

Spawning ecology	Inhibition of eDNA amplification	Use inhibition-reducing assays & Purification protocols before PCR amplification [40]
	Eggs exhibit variability in eDNA shedding rates	Quantification of shedding rate in controlled environments [40]
Management of fisheries	Lack of ecological information	Conduct eDNA and traditional surveys simultaneously [65]
Hatchery management/ selective breeding application	Little evidence to support the use of eDNA analyses in hatchery-based selective breeding	More empirical validations [67]
Forensic/ forensic ecology	mistakes in public databases entries of sequence repository	Universal standards for data collection and sequence curation and interpretation [58]
Crop cultivation and soil fertility	Little evidence to support the use of eDNA analyses in crop growth	More empirical validations [69]
Anthropogenic effects on biodiversity	Morphological information on taxa affected is unavailable	Conduct eDNA and traditional surveys simultaneously [70]

Table 2: *Limitations in eDNA Analysis and Potential Means of Reducing Limitations.*

Theme 1. Detecting rare, cryptic or endangered species

Detection and monitoring of elusive, rare and endangered species are challenging due to being unable to physically locate them and their small population size. Conventional surveys are labour-intensive, invasive and suffer from low detection rates of endangered species. Therefore, the development and validation of techniques such as eDNA analysis are in great demand in the management and conservation of these vulnerable species. A non-invasive eDNA method can provide absence/presence data, species distribution and even estimates of population size, will be of great use in conservation biology involving the conservation and management of these species [31].

eDNA analyses has been used to infer presence of endangered taxa in freshwater ecosystems. For instance, eDNA barcoding has been used as a quick and inexpensive tool to detect presence and distribution data of the great crested newt, an amphibian of conservation concern [11]. This is supported Cooper., *et al.* [24] which demonstrate the detection of dwarf sawfish (*Pristis clavata*) using a species-specific qPCR assay in a location coincide with the known habitat use of this endangered species. Other positive detection of presence of endangered taxas in freshwater ecosystems using eDNA barcoding are white-clawed crayfish [22,29,30], black warrior waterdog (*Necturus alabamensis*), and the flattened musk turtle (*Sternotherus depressus*) [26]. Additionally, eDNA metagenomics analysis has helped in the study of entire communities, rare/cryptic species, vulnerable species without a priori knowledge of taxons in a habitat. This makes it an ideal tool to work on distribution censuses of many taxa. An example using eDNA metabarcoding of water samples detected over 200 native taxa of which vulnerable and endangered species; such as, catfish (*Pseudoplatystoma magdaleniatum)* and Antillean manatee (*Trichechus manatus*) were detected [27]. Interestingly, another study has shown consistent findings comparing eDNA metabarcoding and capture methods detected species of high conservation importance that were never sampled by capture techniques, including native European shads (*Alosa spp.*) [21].

eDNA surveys have shown to be valuable in detecting rare and cryptic species in freshwater systems. However, few studies have been conducted in a marine environment, especially on invertebrates. In the ocean, eDNA is affected by multiple factors including distribution by currents and tides, dilution, mixing, sources of inhibition, and faster degradation rates relative to freshwater systems. One pilot study done utilised genus-specific primers were used to amplify red abalone (*Haliotis spp.*) eDNA in tanks and coastal waters. This revealed

eDNA in tanks from small abalone was detectable throughout a large volume and field validation also detected presence of abalone in oceans, but weaker bands were observed due to inhibitory substances [28]. Thus, eDNA is a promising tool to detect the presence of cryptic and endangered species in the ocean.

Recently, eDNA analyses has been employed to infer presence of endangered taxa in terrestrial ecosystems. An innovative approach using eDNA PCR-based assay that samples water drawn from the water-filled phytotelmata of the giant bromeliad and detected the presence of elusive and critically endangered Trinidad golden tree frog (*Phytotriades auratus*) [23]. However, studies on other taxa and in terrestrial environments for conservation are scarce. Thus, further studies are warranted for the monitoring of rare and endangered species in terrestrial environments.

Moreover, eDNA analyses has shown promise in monitoring imperiled freshwater species around management interventions such as invasive species eradications, barriers or relocated populations. A pilot eDNA study suggests that recent management interventions for Shasta crayfish (*Pacifastacus fortis*) can be effective as detection of *P. fortis* eDNA at a lake where the status of this species was uncertain and extirpation of signal crayfish (*Pacifastacus leniusculus*) in recent years was performed [25]. However, the utilisation of eDNA methods have several drawbacks such as sample contamination, species-specific seasonal variability, and PCR inhibitors. Contamination occurs when exogenous DNA gets mixed with DNA relevant to the research, consequently, resulting in false positive detections and subsequent misinterpretation of results. Contamination in the field occurs with repeated use of the same field equipment (e.g., corers, filters, gloves) across different sampling sites without thorough sterilization [25]. In the lab, contamination may occur when remnant DNA from previous molecular experiments (e.g., DNA extraction, amplification, library preparation and sequencing) spreads into new samples [24]. To reduce the risk of contamination, negative controls samples (field controls and extraction blanks) should be included at each step and decontamination protocols at each stage should be implemented such autoclaving or sterilization with 10% sodium hypochlorite solution [21,27].

In addition, seasonal changes in environmental conditions and species activity can influence eDNA concentrations. Substantial variations in detection probability observed over time, results in risk of false negatives during period with lower species activity, or an increase in specific seasonal conditions. A number of avenues exist to reduce false negatives including judicious deployment of replicates in field sampling and in the laboratory. Design sampling strategy to periods of highest species activity, and least weather extremes. If sampling in less reliable time periods or conditions, increase sample replication number. Troth., *et al*. [29] demonstrate that one replicate was sufficient to accurately demonstrate the species presence/absence in laboratory samples, while three replicates were required for field samples. This corroborates well with de Souza., *et al.* [26] suggesting that season strongly affected eDNA detection probability for both species, with *N. alabamensis* having higher eDNA detection probabilities during the cool season and *S. depressus* have higher eDNA detection probabilities during the warm season. These results illustrate the influence of organismal behaviour or activity on eDNA detection in the environment and identify an important role for ecological data on seasonal activity or behaviour of focal organisms in designing eDNA monitoring programs.

Theme 2: Detection of bacterial, parasitic pathogens and disease outbreaks

eDNA monitoring is likely to have a major impact on the ability of aquaculture industry producers and their regulators to detect the presence and abundance of pathogens in the environment and around aquaculture sites. However, aquaculture industries are largely affected by bacterial and parasitic disease outbreaks and often resulting in large mortalities and huge economic loss in farmed fishes. Traditional diagnosis of infections relies on microscopy and histological techniques that less sensitive, time-consuming and impose significant handling stress [33]. Thus, eDNA methods provide a sensitive and non-lethal method of detecting pathogenic microorganisms at early stages of infections in habitats as host discharges or living environments contain pathogen eDNA [33]. Identification at early stages of infections enables rapid decisions are to mitigate losses from disease outbreaks where early treatment is vital [36]. For example, Peters.,

et al. [36] demonstrate using eDNA metabarcoding and high-throughput sequencing of the 18S SSU rDNA v9 region, identification of presence of two parasites (*Lepeophtheirus salmonis* and *Paramoeba perurans*) to species level, whereas the microalgae species *Prymnesium parvum*, *Pseudo-nitzschia seriata*, and *Pseudo-nitzschia delicatissima* could be assigned correctly to the genus level in salmonid aquaculture. This is consistent with Jørgensen., *et al*. [33] who demonstrate that eDNA from the parasite *Bonamia ostreae* can be detected in seawater from tanks containing infected oysters following an incubation period of 6 days. This suggests eDNA metabarcoding is a non-lethal diagnostic method which offers significant potential in the monitoring of infectious species harmful to aquaculture.

eDNA monitoring in natural ecosystems have also been applied for the conservation and management of imperilled wild species to curb further population decline from infections. For instance, Troth., *et al.* [30] demonstrate the detection of crayfish plague in a UK river using eDNA methods, illustrating a positive relationship between endangered, white-clawed crayfish and crayfish plague and reflect a disease outbreak within the crayfish population. More recently, the first robotic environmental sample processor successfully detects the eDNA of human and fish pathogens; such as, waterborne protozoa, *Tetracapsuloides bryosalmonae* and *Scomber japonicas*; at river locations [32]. Shahraki., *et al*. [35] utilise high throughput eDNA metabarcoding and detects *Acinetobacter*, *Burkholderia*, *Legionella* (genus with potential pathogenicity) in environmental samples.

In addition, an eDNA approach can include the detection of insect vectors of zoonotic diseases, which are often difficult to detect using conventional surveying techniques. The higher detection rate of sensitive eDNA molecular assays allows for the early detection of vectors and critical for vector eradication and reduction of transmission and infections rate of diseases. Many insects function as vectors for disease transmission, for example, it is well-known that *Anopheles* mosquitoes are vectors for the transmission of malaria through bites and introduction of *Plasmodium* species into the bloodstream of the human host. To illustrate this, Odero [38] demonstrates using eDNA analyses in the detection and quantification of *Anopheles* mosquito larvae in laboratory condition was successful but undetectable in field studies. Other studies have shown consistent findings in fishes and detected disease vectors such as *Fredericella sultana* and its parasite *Tetracapsuloides bryosalmonae*, which are implicated in proliferative kidney disease in salmonids in natural river habitats [34] and *Culicoides* species that are vectors for livestock causing epizootic hemorrhagic disease and African horse sickness [37].

However, challenges are being faced for implementing eDNA methods in detecting pathogens and disease outbreaks. One such difficulty is a limited database from Barcode of Life Data System (BOLD) for the BIN matches obtained in eDNA samples. This creates a knowledge gap on metazoans and unicellular species and limits the capability to sequence unknown samples and species misidentifications as it is not recognised in the database [37]. Reference sequences for taxonomic assignment are only available for one or a few genes for most species and the targeted marker regions (e.g., 18S rDNA) cannot accurately resolve most groups to species or higher taxonomic levels due to incompleteness of reference sequence databases [36,37]. Consequently, eDNA studies are often interpreted using molecular operational taxonomic units (MOTUs) or higher taxonomic ranks (genus, family, order) instead of binomial species names [36]. In future work, targeted specific organisms with tailored molecular probes can help improve global species resolution and differentiation [38]. Additionally, reference databases will need to be expanded by further analysis, sequencing, and traditional taxonomic identification by expert taxonomists to include identifiable sequence data for all target biodiversity for establishment of a comprehensive reference database.

Theme 3. Invasive species detection

In recent years, the application of eDNA molecular method for identifying the introduction of invasive species has risen. Alien invasive species (AIS) are a major threat to biodiversity resulting in biodiversity loss, displacement of native species, and modifies food web structures and ecosystem processes [39]. They may out-compete native species, act as predators or hybridize with native species and can function as a vector for exotic diseases [41]. Severe economic loss attributable to AIS have also been documented in the tourism, aquaculture, and other industry sectors. Overall, invasive species cause billions of dollars of damage and losses to the economy of the United

States [40]. There are numerous examples, worldwide, of AIS causing biodiversity and economic loss. For example, consistent findings with well-known AIS species resulting in biodiversity and economic loss; including silver and bighead carp [40], rusty crayfish [39], and several agricultural pests [44].

eDNA analyses has shown to be a useful tool for detection of presence/absence of AIS. Due to eDNA methods incredible sensitivity and higher detection probabilities, eDNA methods can contribute to rapid detection of AIS in early stages of invasions when AIS are in low densities which is hard to detect using traditional methods. Thus, eDNA methods provides an early warning of the presence of an AIS, and rapid control responses are implemented for successful prevention of AIS establishment, and to mitigate the negative impacts on native biodiversity. Preliminary field eDNA studies demonstrated the use of eDNA barcoding to detect presence of AIS such as fishes, insects, and crustaceans. eDNA barcoding has been used to detect presence/absence of bighead carps; including silver carp, bighead carp [40], bluegill sunfish [42], round goby [43], brown marmorated stink bug [44], grass carp [46], rusty crayfish [41], Mozambique tilapia [47], and golden mussels [31]. These studies utilise eDNA barcoding – a method of taxon identification using a standardized DNA region. This involves eDNA extraction, PCR amplification of mitochondrial DNA (mtDNA) regions; such as, Cytb, 12S, or 18S; of the target species, DNA sequencing, bioinformatic analysis for taxonomic identification. Recently, the robotic environmental sample processor automates the collection and preservation of eDNA water samples for more rapid execution of *in situ* eDNA analyses, which provides reliable timing information on presence of invasive *Dreissena spp* [32]. As a result, eDNA analyses proved to be a sensitive tool for rapid and accurate species identification and detection of presence or absence of invasive species. This ability of eDNA analyses prevents further spread and establishment of AIS, mitigating the ecological impacts of biological invasions, and swift implementation of eradication strategies [44].

High-throughput sequencing metabarcoding studies also provide bio-surveillance presence/absence data of AIS but at a broader scale than eDNA barcoding. Such a protocol increases effectiveness at detecting numerous AIS within a single sample. For example, Young at al. [45] demonstrate using a high-throughput sequencing metabarcoding approach detected presence of four agricultural invasive species in a single sample – *Vitisiella brevicauda*, *Sciaphilus asperatus*, *Empoasca fabae*, and *Liposcelis decolor*. Moreover, the same approach has identified introduction vectors of anthropogenic pathways for spread of AIS. Pochon., *et al.* [39] demonstrated using similar methods to detect 2 invasive species in bilge water that has contributed to secondary spread of marine AIS.

Due to the advancement of genetic surveillance tools, the scientific focus has shifted to quantification of the genetic signal produced by target organisms to link eDNA concentration to species abundance or biomass. However, to date, results of most eDNA studies have been interpreted as presence/absence qualitative information. The assumption of eDNA abundance quantification is aquatic vertebrates release eDNA into the water as in proportion with their biomass. Thus, eDNA can be used to estimate species biomass by measuring the number of eDNA copies in a water sample. Preliminary eDNA quantification studies' results from controlled laboratory environments; such as, aquaria and mesocosms; suggesting that eDNA can also be used to estimate population abundance [43] or total biomass [42,43].

However, field studies in aquatic environment have shown conflicting results regarding eDNA quantities and biomass/relative population abundance. For example, Erickson., *et al.* [40] demonstrated positive correlation in quantity of eDNA correlates with big-headed carp densities. Findings from Dougherty et al. [41] contradict previous results found in the literature and demonstrate that eDNA copy number in water samples failed to correlate well with relative abundance of *Orconectes rusticus*. Thus, eDNA correlation with species abundance or biomass is variable, weak, and contentious in field studies. This is attributed to quantities of eDNA released from invasive species under controlled laboratory conditions may not accurately represent field conditions. High amounts of variability also exist in the amount of eDNA released from individual fish which is affected by biotic factors such as age and abiotic factors such as diet, temperature, pH, microbial activity, and UVB radiation [26]. For example, Maruyama., *et al*. [42] demonstrate that eDNA release rate per fish body weight was slightly higher in the juvenile group than in the adult group. Additionally, Robson., *et al*. [47] demonstrated high temperatures (35°C) significantly increase fish eDNA shedding rates.

Thus, such factors may bias the link between biomass and eDNA concentrations. The current understanding of different environmental conditions on eDNA concentration is limited but this information is critical for the utilization of eDNA methods to estimate with species biomass and densities of invasive species. More empirical studies are needed to support the relationship between eDNA concentration and species abundance in natural environments. Moreover, more research is needed to establish the effect of environmental factors on the degradation of eDNA in natural ecosystems.

Theme 4. Biodiversity characterisation and biomonitoring

The majority of eDNA studies have targeted aquatic environments, especially in freshwater habitats. In freshwater systems, eDNA is now used as a bioassessment tool in both single-species qPCR-based studies in lentic and lotic environments. In lotic systems which include water flowing bodies such as creeks, rivers and streams, the concentration of eDNA and its detectability are not only dependent on production and degradation rates but also on dilution, transport through the river network, deposition and resuspension [56]. Variable detection success of targeted taxa is observed. A pilot study tested for downstream detection of eDNA for two invertebrate species, *Daphnia longispina* and *Unio tumidus*, to confirm presence of each species' eDNA in the river [12]. This suggests that eDNA can persist over relatively large distances in a natural river system, approximately 20km downstream of alpine lakes where they can be present [12].

Recently, emphasis shifts from species-level approaches to communities and ecosystem-level investigations. This shift is based on the analysis of a single generalist marker targeting a larger taxonomic group (eDNA metabarcoding) have proven useful for in sequencing-based eDNA metabarcoding community assessments and inventories. For instance, Cantera., *et al.* [54] employ eDNA metabarcoding in streams and rivers. A total of 279 species occurrences were detected in the six sites with an average of 67% and 87% of the expected richness in rivers and streams respectively. A similar approach identifies DNA from 15 fish species, 17 mammalian species, 8 avian species, 15 arthropod species, one turtle and one salamander [60]. This is consistent with Antognazza., *et al*. [21] who made comparisons between the species identified as present by eDNA metabarcoding versus long-term data available from fisheries monitoring data based on capture methods. This reveals 15 and 9 species detected by metabarcoding and fish surveys respectively demonstrating eDNA metabarcoding increased sensitivity and detection rate [21]. Moreover, eDNA metabarcoding detected species of high conservation importance that were never sampled by capture techniques, including native European shads.

Additionally, an eDNA metabarcoding approach was used and showed the species composition obtained using eDNA is comparable to historical data of traditional sampling session [55]. Comparisons between eDNA metabarcoding and capture methods shows that the detected fish communities were similar between the two methods with an overlap of 70% [49].

Expanding the scope from species occupancy and community composition data, quantification of species densities in freshwater systems have been attempted with mixed results. Carraro., *et al*. [34] developed a general framework to reconstruct distribution and abundance of *Fredericella sultana* based on eDNA concentrations and hydro-geomorphological features to reveal successful spatially explicit abundance estimates of species distribution. Other studies demonstrate positive correlations between metrics of species density and eDNA concentration in different aquatic organisms [55]. However, Chambert., *et al*. [51] assess a negative binomial model in mesocosms and natural setting to infer animal density from eDNA. A negative binomial model is useful for predicting count-based data and a discrete probability distribution that models the number of successes in a sequence of independent and identically distributed Bernoulli trials before a specified number of failures occurs. Although eDNA provide accurate abundance estimates in mesocosms, greater imprecision was found in the field data with less precise density estimates [51]. The effect of multiple environmental factors on the efficiency of eDNA detection (e.g., pH, temperature, UV, PCR inhibitors, organic materials) has been poorly investigated, but largely affects eDNA transport, degradation rates and eDNA concentrations in the environment. A pilot study compared eDNA degradation rates of intracellular and extracellular eDNA in an aquarium and reveal that the half-life of intracellular eDNA is longer than the half-life of extracellular eDNA [61]. Pivotal to this is the need for increased knowledge on the transport and degradation rate of eDNA within fluvial systems.

The sampled water volume, a crucial aspect for efficient species detection, has been empirically variable (ranging from few centilitres to tens of litres). This results in a high variability of sampling effort across studies and potential sampling bias, making comparisons challenging and raising uncertainties about the completeness of eDNA inventories. For instance, Cantera., *et al.* [54] demonstrate that sampling 34 litres of water detected more than 64% of the expected fish fauna compared to 17 litres of water. Likewise, Cornman., *et al.* [53] compare eDNA profiles from composite water samples aggregated over three hours with grab water samples and estimated higher biodiversity richness in composite samples compared grab samples. Thus, improvement and standardization of sampling protocols with increased sampling volume and higher frequency from composite water sampling is likely to enhance biodiversity detection rate and facilitate cross-study comparability.

The main problem encountered are rates of false positives and false negatives. False-negative detections are mainly due to low eDNA quantity [60], PCR inhibitors [49], or insufficient sampling efforts [53]. False negatives can be significantly reduced by increasing analytical efforts, for instance, increasing the number of samples per locality, analysing multiple DNA extracts per sample, increasing the number of PCR cycles, and the inclusion of positive controls. False-positive detections can be attributed to contamination [21]. Contamination of the studied system by external sources of eDNA, such as sewage effluent and animal excrements or legacy eDNA from dead organisms [56]. Simultaneous traditional assessment of community composition and eDNA results can be compared to estimate the incidence of false-positive detections.

Relatively less work has been completed on freshwater plants. However, a pilot study has utilised eDNA metabarcoding of lake sediments to analyse plant species composition compared to vegetation surveys [52]. The study detects numerous plant species and demonstrates higher sensitivity and detection rate of plant species than traditional vegetation surveys [52]. However, more evidence is required to validate the use of eDNA metabarcoding for vegetation.

The deep ocean is the largest biome on Earth and faces increasing anthropogenic pressures from climate change and commercial fisheries. Sustainable management of this expansive habitat is impeded by the poor understanding of its inhabitants and the difficulties in monitoring marine environments. eDNA analyses provide a viable solution to the above problem as a bioassessment tool to generate high-resolution biodiversity data; such as, species richness [50], community compositions [59], and assemblage diversity [57].

Although the use of eDNA to detect species is significantly more challenging in marine environments than in freshwater due to greater dilution, increased mixing, and higher salinity, some studies have detected wildlife in oceanic settings. For example, Singer., *et al*. [59] use eDNA metabarcoding of seawater to elucidate comprehensive biodiversity analysis via ultra-deep patterned flow cell technology. They discover 80 identifiable families; such as, metazoan families, marine mammals, and bony fishes. This agrees favorably with McClenaghan., *et al.* [50] who utilize eDNA metabarcoding of deep seawater to elucidate deep-sea fish diversity in the Labrador Sea and identifies a total of 21 fish families, 23 genera, and 15 species with 7 rare deep-water fishes. Therefore, an eDNA approach has facilitated biomonitoring of a broad range of taxa in marine environments.

Despite having shown promising application in marine biomonitoring, eDNA is prone to imperfect sampling and false detection, which can occur at various project stages, including field collection, sample storage, molecular analysis, and bioinformatics workflows [48,50]. Consequently, false positives and false negatives are common, leading to biased estimates of species richness and individual species occupancy. Occupancy models enable the calculation of detection probabilities from each taxon sequenced and are designed to work with presence-absence data. More recently, the application of multi-species, multi-scale occupancy models to metabarcoding data have improved the accuracy and predictive ability of occupancy models compared to single-species models to account for imperfect detection [50].

Terrestrial animals require water and other nutrient sources to survive. As such, eDNA originating from terrestrial species can be collected and analysed from these sources in terrestrial ecosystems to determine species use. It is hypothesized when terrestrial species drinks from a water source, DNA from its saliva and mouth tissues is shed and can be used as a tool for species identification [64]. Assessing species presence using eDNA in seclusive, difficult to survey habitats such as forests, saltlicks or waterholes could be a viable alternative or augmentation to traditional study methods.

A 2017 study use eDNA metabarcoding to characterize the mammalian community utilizing forest ponds in Japan, from which they were able to detect several common mammals from the area. Not all common mammals were detected; however, as pond use is dependent on individual need and likely varies greatly over space and time [64]. This is consistent with Furlan., *et al.* [63] who use eDNA metabarcoding to detect vertebrate species at waterholes in Australia's arid zone and revealed presence of both aquatic and terrestrial taxa. A larger scale study by Mena., *et al.* [62] utilise eDNA metabarcoding in comparison with traditional methods (e.g., camera trapping, grids, mist nets, pitfall trapping) for assessing Amazonian mammal communities. Results show eDNA metabarcoding recorded 47% of the total recorded species; thus, recording more taxa than traditional methods and with less effort and cost [62]. Recently, Clare., *et al.* [58] use an innovative approach by collection of air samples and subsequent eDNA analyses, suggesting that air can be a viable substrate for eDNA collection from animal and humans in terrestrial environments. Thus, an eDNA approach has shown to be promising for habitat terrestrial biodiversity surveys.

Theme 5. Spawning ecology

Most aquatic animals, except for aquatic mammals and reptiles, reproduce through the process of spawning. Spawning is defined as the process of releasing eggs and sperm into the environment. Identifying areas for spawning, as well as the spatial extent of spawning activities, is not only vital for the effective conservation of vulnerable species but for effective control and management of invasive species [21]. In aquatic species such as bighead carps, specific hydrological conditions must be met to induce spawning and specific spatial and hydrologic conditions to allow egg and larval development.

Erickson., *et al.* [40] compare drifting egg surveillance and telemetry of acoustically tagged bighead carps to qPCR survey to examine relationships among bighead carps' movement, spawning and eDNA. The results demonstrate relationships among water discharge, eDNA and individual bighead carp movement that evaluated mass spawning events, suggesting promising application of eDNA in identification of aquatic spawning populations.

However, a relationship between eDNA quantities and egg densities was not established [40]. This was attributed to 2 main limitations in eDNA detection. Eggs may exhibit variability in eDNA shedding rates which has not been tested. PCR Inhibitors, such as humic acid and algae, present in eDNA samples may prevent amplification even if eDNA from the target species is present. PCR inhibitors have different mechanism of inhibition but they generally exert their effect through interaction to DNA or interference with thermostable DNA polymerases. Consequently, sensitivity of eDNA assays is decreased and lead to false negative results. Thus, robust matrix-based protocols for purification of nucleic acids before PCR are essential.

Theme 6. Management of fisheries

The successful management of commercial fisheries relies on the estimation the abundance and distribution of fish stocks. Estimating the abundance of fish is critical for the management of fisheries which relies on accurate assessment of population status to maximize yield without overharvesting populations. In contrast to the costly and personnel-intensive netting and electroshocking required by traditional mark–recapture surveys and trawl survey to capture individuals, eDNA surveys only require the collection of environmental samples (e.g., water) from the field.

Salter., *et al.* [65] use a quantitative eDNA survey of Atlantic cod (*Gadus morhua*) in marine fisheries in comparison with a standardized demersal trawl survey. The results show detection of eDNA originating from Atlantic cod was highly concordant (80%) with trawl catches. Additional findings revealed significantly positive correlations between the regional integrals of Atlantic cod biomass and eDNA quantities (R-square = 0.79, p-value = 0.003) and between sampling effort-normalised Catch Per Unit Effort (CPUE) and eDNA concentrations (R-square = 0.71, p-value = 0.008). These findings correlate favourably with Spear et al. [66] demonstrate a significant, positive relationship between traditional estimates of adult walleye (*Sander vitreus*), an economically important sportfish, in inland fisheries populations and biomass and eDNA concentration (R-square = 0.81; n = 22).

A limitation of eDNA analyses in conservation biology is current eDNA-based studies only report presence/absence and/or recent occupancy. However, information on the ecological status of target organisms, including organism size, the sex and age ratio, body condition (sick or healthy), and activity (breeding or non-breeding) cannot be obtained using standard eDNA methods, but are crucial for making informed management and conservation decisions [48,65]. For instance, some species of commercial importance such as Atlantic cod are subjected to numerous threats such as overfishing, habitat loss, climate change and invasive species which can severely affect population dynamics in fisheries [65]. Thus, knowledge of ecological status in commercial species is critical for effective management of their population. Although eDNA metabarcoding is unable to fully evaluate ecological status of endangered taxa, a solution is to integrate eDNA metabarcoding into the traditional monitoring framework. By doing so, effective acquisition of population dynamics and ecological status to guide restoration efforts, maintain stock sustainability in fisheries and pre-empt fishery collapse. Altogether, an integration of conventional and novel techniques appears to be the next step in conservation and management of aquatic species in commercial fisheries.

Theme 7. Hatchery management/selective breeding application

eDNA analyses has been used in hatchery and selective breeding programs applicable to aquatic organism to improve economically important traits such as growth and disease resistance by 10% - 15% per generation [67]. Current DNA sampling techniques in aquaculture hatchery-based production involve the use of invasive tissue sampling and internal fluid needle aspirates. These methods result in valuable broodstock mortality, physiological stress responses, and loss of revenue. eDNA isolation potentially offers a non-invasive approach to tissue sampling for parentage assignment and advanced marker-assisted or genomic selection strategies. Holman., *et al*. [67] compare the results of tissue and eDNA derived SNP genotype calls using a PCR-based genotyping platform in European flat oysters (*Ostrea edulis*) and found > 99% correct SNP genotype calls from eDNA in comparison with tissue sampling. Consequently, collection of eDNA can be used to accurately genotype bivalve molluscs and potentially other aquatic taxa. However, eDNA analyses in hatchery-based selective breeding is still in its infancy and more studies are needed to demonstrate the utilisation of eDNA-based analyses in hatchery-based selective breeding applications.

Theme 8. Forensic/forensic ecology

eDNA metabarcoding is increasingly applied in ecological studies, including studies with the primary purpose of criminal investigation, in which eDNA from soil or air can be used to pair samples, reveal environmental sample provenance/origin and geographic location. Environmental samples are encountered as trace evidence in criminal cases to convict criminals or exonerate the wrongly accused. Environmental samples are compared to samples from known locations such as a crime scene; thereby, establishing a link between the suspect or a victim in a crime scene. A recent 2019 study in Denmark collected 130 soil samples and eDNA metabarcoding with PCR amplification of genetic marker regions and massive parallel sequencing on the Illumina MiSeq platform by testing 2 mock crime scenes. Results demonstrate that eDNA data performed well in predicting environmental gradients (R-square > 0.81) [68]. However, results also show eDNA data to discriminate between habitat types was variable, with high accuracy for certain forest types and low accuracy for heathland, which was poorly predicted. Geographic region was also less accurately predicted by eDNA [68]. Thus, the application of eDNA

analyses in forensic ecology to determine sample provenance is still in its early stages, more empirical studies are needed to validate its use in criminal investigations.

A substantial downside from preliminary studies have highlighted interpretation of automated annotated OTU from biases and mistakes in widely used public databases entries of sequence repository (International Nucleotide Sequence Database, GenBank, EMBL, and DDBJ). For real case forensic work, sequence annotations (and not merely the OTU composition) to infer environmental sample origin should ideally rely on a dedicated reference database of sequences from relevant regional plants, and only unambiguous species matches should be used in the ecological inference to ensure validity, accuracy, and reproducibility. Applying universal standards for data collection and sequence curation and interpretation are essential to ensure that the dedicated database adheres to international rules and the latest technologies.

Additionally, a recent 2021 study use PCR amplification of eDNA from air sampling targeting 12S and 16S mitochondrial DNA with HTS [58], suggesting a strong potential to use air as a source of eDNA in forensic investigations. Thus, the application of air eDNA sampling can recover forensic traces of recent criminal activity even when no physical traces (such as blood or hair) have been left, for non-invasive DNA collection or in forensic anthropology.

Theme 9. Crop cultivation and soil fertility

Soil microorganisms and their activities play important roles in those functions, especially various metabolic reactions in soil such as mineralization of soil organic nitrogen and decomposition of compost applied to soil, which support crop production and maintain the fertility of soil. eDNA analysis has been used to elucidate microbial community structures in paddy fields in order to understand the roles of microorganisms. A 2010 preliminary study amplified 16S rRNA and 18S rRNA gene fragments with PCR from eDNA extracted from the soils for rice cultivation and subjected to denaturing gradient gel electrophoresis (DGGE) [69]. They discovered microbial communities in paddy fields were not influenced by long-term application of organic matter but were affected by conversion to upland fields from paddy fields [69]. Thus, soil microbial communities differ in different soils and play important roles in productivity, sustainability of crop cultivation. However, the use of eDNA analyses in agriculture is still in its infancy and more studies are required to elucidate the applicability of eDNA analyses to determine soil microbial communities.

Theme 10. Anthropogenic effects on biodiversity

Anthropogenic stressors interact and are directly or indirectly impacting ecological status and organisms. For example, human industrial activities result in pollution of multiple ecosystems and death of organisms through the release of synthetic chemicals (i.e., DDT), heavy metals (i.e., Cu, Fe, Hg, Mn, Pb, and Zn) and oils spills. Thus, biomonitoring is essential for assessing the impacts of human disturbance at the multiple scales of ecosystems. Understanding the response of ecosystems to multiple anthropogenic stressors and identifying important stressors are prerequisites for conducting effective ecosystem restoration and biodiversity conservation.

Standard approaches employed to examine anthropogenic effects on biodiversity involves sorting and morphological identification of aquatic communities, which is time-consuming and demands high levels of taxonomic expertise. However, the high-throughput amplicon sequencing of environmental DNA (eDNA) has recently provided a viable option for biomonitoring in polluted areas, which purified from substrates such as sediment or water contains eDNA fragments originating from organisms present in that environment. Cavaliere., *et al*. [71] use PCR amplification of 18S rRNA gene eDNA metabarcoding and HTS in the heavily polluted Bangoli area and found significant differences (i.e., diversity and assemblage composition) in both morphological and molecular datasets of benthic foraminiferal communities were found between the relatively low- to unpolluted and the most polluted areas. This is consistent with another study by Fan., *et al.* [70]

which utilised eDNA metabarcoding with a support vector machine (SVM) model. This has revealed biodiversity indices such as Shannon Wiener index and Simpson index (i.e., species richness and evenness of community) tended to decrease with increasing disturbance from human activity from the upstream to the downstream regions, as reflected by increasing urban and industrial land use. Both Simpson's Diversity Index and Shannon-Wiener diversity index are measures of diversity which that combines species richness (the number of species in a given area) and the relative abundance of each species (evenness).

Moreover, both studies have shown consistent findings in comparison of eDNA metabarcoding with traditional morphological data [70,71]. These studies demonstrate eDNA metabarcoding provided more accurate predictions than morphological identification. This is attributed to some species and microscopic taxa which were neglected with the traditional morphological identification approaches but these taxa were detectable with eDNA metabarcoding since OTUs obtained from DNA sequencing analysed greater biodiversity information and even potential indicators of pollution.

Principal findings of this review

Some eDNA applications, as indicated in this study, have been accurately recognized in earlier narrative reviews. The preceding list of eDNA applications, on the other hand, is out of date and is largely focused on detecting aquatic species in aquatic environments. Examples of well-established eDNA-based applications include detection of rare and endangered species, detection of invasive species, biodiversity characterisation and biomonitoring. Since then, the rapid adoption of eDNA-based techniques facilitated by the ongoing revolution of sequencing technologies and preliminary success in aquatic environments have expanded to marine and terrestrial environments and detected a huge phylogenetic range of simple and complex species assemblages across trophic chains. This expanded the scope of eDNA applications to include new applications as summarised in this review such as monitoring of spawning events, management of fisheries, hatchery-based selective breeding, forensics, crop cultivation and soil fertility, and examining anthropogenic effects on biodiversity.

Interpretation of many eDNA studies is currently hindered due to the lack of comprehension of how different field and laboratory technique impact eDNA detection. Additionally, there is significant deal of ambiguity about the effects of biotic and abiotic conditions on the ecology of eDNA (its production, state, transport, and half-life within an environment). Furthermore, there are substantial constraints such as the challenge in extracting quantitative information (abundance data) from multispecies eDNA samples which is required for numerous applications and the lack of information on the developmental stage, sex, and size of individuals detected. Another barrier to data interpretation results from gaps in taxon coverage in reference libraries, so many sequences derived from eDNA analysis are unable to be assigned to their source taxon. Due to these shortcomings, the actual prevalence of false positives and false negatives is likely high. Furthermore, the lack of standardized protocols for eDNA sampling in the field and analysis in the lab makes studies insular and comparative inferences difficult.

Main strength and weakness of this review

The main strength of this review is it is the first systematic scoping review of eDNA applications covered in the literature. Unlike the several former eDNA-based reviews which consist of narrative reviews and traditional literature reviews, this review adopts a rigorously pre-specified eligibility criteria and a systematic search strategy in accordance with reporting guideline of Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) statement [72,73]. Therefore, this review is superior in evidence-based practice and one of the highest quality of evidence to answer eDNA-based research questions, policies, or practices. The main limitation of this review is the small number of databases used although it has been shown that a combination of Google Scholar and PubMed may be sufficient [17]. This is supported by Bramer., *et al.* [74] who suggest that a combination of Google Scholar and PubMed may be sufficient coverage for a systematic review.

Conclusion

We reviewed 54 out of 159 (34%) articles on the applications of eDNA and revealed 10 themes of applications; namely, (a) detecting rare, cryptic or endangered species, (b) detecting bacterial and parasitic pathogens/disease outbreaks, (c) invasive species detection, (d) biodiversity characterisation and biomonitoring, (e) spawning ecology, (f) management of fisheries, (h) hatchery management/selective breeding application, (i) forensic/forensic ecology, (j) crop cultivation and soil fertility, and (k) anthropogenic effects on biodiversity.

Supplementary Materials

Data files for this study can be downloaded at https://bit.ly/eDNA_SSR.

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Conflict of Interest

The authors declare no conflict of interest.

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