



Review

Aquatic environmental DNA: A review of the macro-organismal biomonitoring revolution



Miwa Takahashi ^{a,b,*}, Mattia Saccò ^{a,*¹}, Joshua H. Kestel ^a, Georgia Nester ^a, Matthew A. Campbell ^a, Mieke van der Heyde ^a, Matthew J. Heydenrych ^{a,c}, David J. Juszakiewicz ^a, Paul Nevill ^a, Kathryn L. Dawkins ^a, Cindy Bessey ^d, Kristen Fernandes ^a, Haylea Miller ^b, Matthew Power ^a, Mahsa Mousavi-Derazmahalleh ^a, Joshua P. Newton ^a, Nicole E. White ^a, Zoe T. Richards ^a, Morten E. Allentoft ^{a,e,*²}

^a Trace and Environmental DNA (TrEnD) Lab, School of Molecular and Life Sciences, Curtin University, Kent St, Bentley, WA 6102, Australia

^b Commonwealth Scientific and Industrial Research Organization, Indian Oceans Marine Research Centre, Enviroeconomics Future Science Platform, Crawley, Western Australia, Australia

^c Jarman Laboratory, Indian Ocean Marine Research Centre, School of Biological Sciences, University of Western Australia, Australia

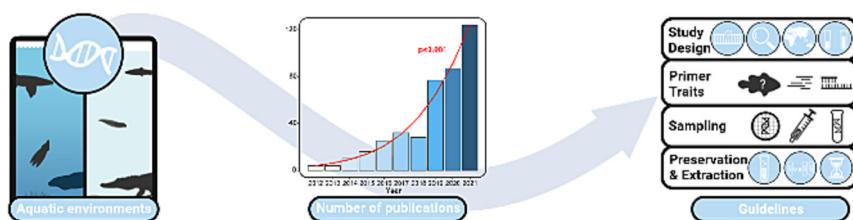
^d Commonwealth Scientific and Industrial Research Organization, Indian Oceans Marine Research Centre, Oceans and Atmosphere, Crawley, Western Australia, Australia

^e Lundbeck Foundation GeoGenetics Centre, Globe Institute, University of Copenhagen, Copenhagen, Denmark

HIGHLIGHTS

- 407 manuscripts were reviewed to examine the last decade of aquatic eDNA research.
- Temporal trends in methods, major achievements, and knowledge gaps are identified.
- Despite attempt to standardise protocols, eDNA is becoming more method-diverse.
- We propose 17 key considerations to guide eDNA study design and method selection.
- We compile a list of 663 PCR primers with relevance for aquatic eDNA research.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Abasiofiok Mark Ibekwe

Keywords:
eDNA
Biodiversity
Standardisation
Primers
Freshwater
Marine

ABSTRACT

Environmental DNA (eDNA) is the fastest growing biomonitoring tool fuelled by two key features: time efficiency and sensitivity. Technological advancements allow rapid biodiversity detection at both species and community levels with increasing accuracy. Concurrently, there has been a global demand to standardise eDNA methods, but this is only possible with an in-depth overview of the technological advancements and a discussion of the pros and cons of available methods. We therefore conducted a systematic literature review of 407 peer-reviewed papers on aquatic eDNA published between 2012 and 2021. We observed a gradual increase in the annual number of publications from four (2012) to 28 (2018), followed by a rapid growth to 124 publications in 2021. This was mirrored by a tremendous diversification of methods in all aspects of the eDNA workflow. For example, in 2012 only freezing was applied to preserve filter samples, whereas we recorded 12 different preservation methods in the 2021 literature. Despite an ongoing standardisation debate in the eDNA community, the field is seemingly moving fast in the opposite direction and we discuss the reasons and implications. Moreover, by compiling the largest PCR-primer database to date, we provide information on 522 and 141 published species-specific and metabarcoding primers targeting a wide range of aquatic organisms. This works as a user-friendly 'distillation' of primer information that was hitherto scattered across hundreds

* Corresponding authors.

** Correspondence to: M. E. Allentoft, Trace and Environmental DNA (TrEnD) Lab, School of Molecular and Life Sciences, Curtin University, Kent St, Bentley, WA, 6102, Australia.

E-mail addresses: miwa.takahashi@csiro.au (M. Takahashi), mattia.sacco@curtin.edu.au (M. Saccò), morten.allentoft@curtin.edu.au (M.E. Allentoft).

¹ Co-first author.

of papers, but the list also reflects which taxa are commonly studied with eDNA technology in aquatic environments such as fish and amphibians, and reveals that groups such as corals, plankton and algae are under-studied. Efforts to improve sampling and extraction methods, primer specificity and reference databases are crucial to capture these ecologically important taxa in future eDNA biomonitoring surveys. In a rapidly diversifying field, this review synthesises aquatic eDNA procedures and can guide eDNA users towards best practice.

Contents

1. Introduction	2
2. Materials and methods	4
2.1. Literature survey and data extraction	4
2.2. Data visualization and statistical analyses	4
3. Overview of Scopus data.	4
4. eDNA sampling and laboratory procedures	6
4.1. Sampling	6
4.1.1. Volume	6
4.1.2. Field replication	6
4.2. Water sample preservation (pre-DNA collection)	8
4.3. DNA collection	8
4.3.1. Filtration method	8
4.3.2. Filter type	8
4.3.3. Filter pore size	9
4.4. Preservation (post-DNA collection)	10
4.5. DNA extraction	11
4.6. Negative controls	11
4.7. Primers	12
5. Discussion	13
5.1. Methods for aquatic eDNA analysis: what to use and when	13
5.2. Most recent developments and future research avenues	14
5.3. To standardise or not to standardise, that is the eDNA-related question!	16
5.4. Knowledge gaps	16
6. Conclusions	17
CRediT authorship contribution statement	17
Data availability	17
Declaration of competing interest	17
Acknowledgements	17
Funding	18
References	18

1. Introduction

Biological monitoring (biomonitoring) is pivotal to characterizing biological diversity (e.g., van der Heyde et al., 2020; Vivien et al., 2015), assessing the ecological status of ecosystems (e.g., Andreasen et al., 2001; Vivien et al., 2015), investigating environmental contamination (e.g., Golden and Rattner, 2003; Zhou et al., 2008), and detecting the presence of invasive species (e.g., Guareschi et al., 2021; Pirtle et al., 2021), among many other applications. Over the last two decades, an endless repertoire of bioindicators, targeting a broad range of taxonomic groups from microbes to vertebrates, have been studied across many types of terrestrial and aquatic environments (Burger, 2006 and references therein). The investigation of species composition over space and time has consistently improved our understanding of ecosystems worldwide and their conservation, restoration and sustainability, including sophisticated ecological modelling for inferring future environmental scenarios (e.g., Schuwirth et al., 2019; Adam et al., 2021). In the face of escalating climate change, the generation of high-quality biomonitoring data represents the backbone of effective conservational, political, societal and cultural agendas to ensure a habitable and ecologically thriving planet (Turner et al., 2020).

Water bodies, including inland water ecosystems, oceans and estuaries, constitute the major units of living environments, covering approximately 71 % of Earth's surface and hosting the vast majority of biodiversity worldwide. Water acts as a medium and vector for biota but also for contaminants; it is not surprising that particular attention has always been directed towards developing and refining biomonitoring tools in the

aquatic biome. The first written record of a water pollution assessment based on biotic observations dates back to the cradle of Western civilisation and was made by Aristotle in 350 B.C (Thienemann, 1912 as cited in Moog et al., 2018). This remarkable observation led to the suggestion that ecological assessments of river quality are possibly older than the science of ecology itself (Moog et al., 2018). However, it was only during the second half of the 19th century that aquatic biomonitoring was consolidated as an established science (Hassall, 1850; Cohn, 1853), particularly promoted by Forbes (1887) who invented the biological community concept. Since then, some monitoring procedures have been widely applied due to their reliability (e.g., using nets and traps to collect organisms) in capturing a representative snapshot of the biodiversity hosted in a given habitat or ecosystem. Concurrently, rapid technological advancements have resulted in a vast range of alternative techniques often employed in combination with conventional protocols. Examples of such "next-generation" aquatic biomonitoring tools developed in the last twenty years include acoustic sensors (e.g., Føré et al., 2017), robotic samplers (e.g., Schwarzbach et al., 2014), and underwater video systems (Cappo et al., 2004). In particular, one approach to biomonitoring has gained tremendous momentum in recent years: the detection, analysis and characterization of DNA shed by living organisms in the environment, conventionally known as environmental DNA (eDNA) (Ogden, 2022).

The original and commonly accepted concept of eDNA refers to the total pool of DNA isolated from environmental samples (Pawlowski et al., 2020a and references therein), with suggestion that eDNA can be classified into two types: organismal and extra-organismal DNA (Rodríguez-Ezpeleta

et al., 2021 and references therein), representing whole organisms (i.e., microbes) and shed tissue/DNA (i.e., from macro-organisms), respectively.

Originally developed, tested and applied in the mid-1980s for the detection of bacteria in marine sediments (Ogram et al., 1987), aquatic eDNA applications only started to get attention in the early 2000s through studies on fecal pollution in freshwater systems targeting both prokaryotic (Layton et al., 2006) and eukaryotic communities (Martellini et al., 2005) (Fig. 1). The late 2000s and early 2010s saw a burst – mainly driven by the emergence of high-throughput sequencing – in conceptual, analytical and technological developments that allowed aquatic eDNA methods to be employed for the first time to detect invasive species in freshwater (Ficetola et al., 2008; Jerde et al., 2011) and to monitor marine mammals (Thomsen et al., 2012) (Fig. 1). Shortly after these ground-breaking studies, 2012 marked the year of the first comprehensive literature reviews (Taberlet et al., 2012; Yoccoz, 2012), consolidating the term “eDNA” (Miaud et al., 2012), and the introduction of further methodological developments for assessing fish biomass and improving eDNA detection probability (Takahara et al., 2012) (Fig. 1). In the decade that followed, eDNA analyses have been developed, tested and applied in almost every type of aquatic (and terrestrial) ecosystem (Beng and Corlett, 2020 and references therein) - including subterranean environments (Saccò et al., 2022 and references therein), Antarctic geothermal sites (Fraser et al., 2018), coral reefs (Richards et al., 2022) and deep oceans (McClenaghan et al., 2020). These eDNA developments have recently been described as a “quiet revolution transforming conservation”, fostering enormous benefits for biomonitoring and all its derived disciplines over the last decade. Environmental DNA biomonitoring has the potential to become one of the most effective baselining tools for assessing the impact of the numerous anthropogenic and non-anthropogenic stressors that our planet is facing.

The rapid global expansion of eDNA-based biomonitoring approaches has been accompanied by the invention of multiple new eDNA sampling techniques and laboratory procedures (Rees et al., 2014) to investigate biodiversity in aquatic ecosystems. For every new technique developed, there is a critical need to optimise protocols to fit numerous different conditions

(e.g., remote areas, turbid water, high salinity, etc. See Buxton et al., 2021). This constant tweaking and optimization of methods will broaden the applicability and reliability of eDNA approaches and maintain the momentum of this innovative field. However, with new eDNA methods being developed at a fast pace, there is also the risk of losing or devaluating common methodological standards which are often hailed as an essential condition for enabling cross-study datamining (Goldberg et al., 2016). While advancements towards universal standards in eDNA would potentially ensure its use as a tool for obtaining ecological insights at a global scale, it is crucial that a call for standardizations must not stall the current innovative momentum of this field.

Given the dramatic increase in aquatic eDNA publications over the past decade, this systematic literature review aims to examine the methodological developments in one of the fastest-growing research fields in biology. This review is timely for three main reasons. First, as per every process characterised by exponential growth, there is a real need to establish procedural benchmarks for the generation of accurate and reliable data and to identify the limitations of the technology. Second, eDNA analyses are increasingly becoming an applied science used in numerous different biomonitoring contexts by a vast number of end-users from many different disciplines and backgrounds. A concise review of the available methods and their pros and cons is therefore vital in framing baseline technical and conceptual aspects for the broader audience of non-molecular biologists. Third, in a time of global environmental change it is more important than ever that biomonitoring can accurately capture those changes with sufficient sensitivity and reliability. Hence, the comparison of methods and summary of state-of-the-art techniques and applications we review here represents an essential consolidation of our current knowledge before moving aquatic eDNA technology into the future.

By acknowledging 2012 as a cornerstone year in the short but intense history of eDNA, our systematic review treats this as a 10-year ‘anniversary’ and focuses on methodological developments over that period of time. By using 2022 as a compass between the past and future, we look back at the lessons learned over the last decade, discuss the current state-of-the-art technologies, and outline a “wish list” for future advancements of eDNA

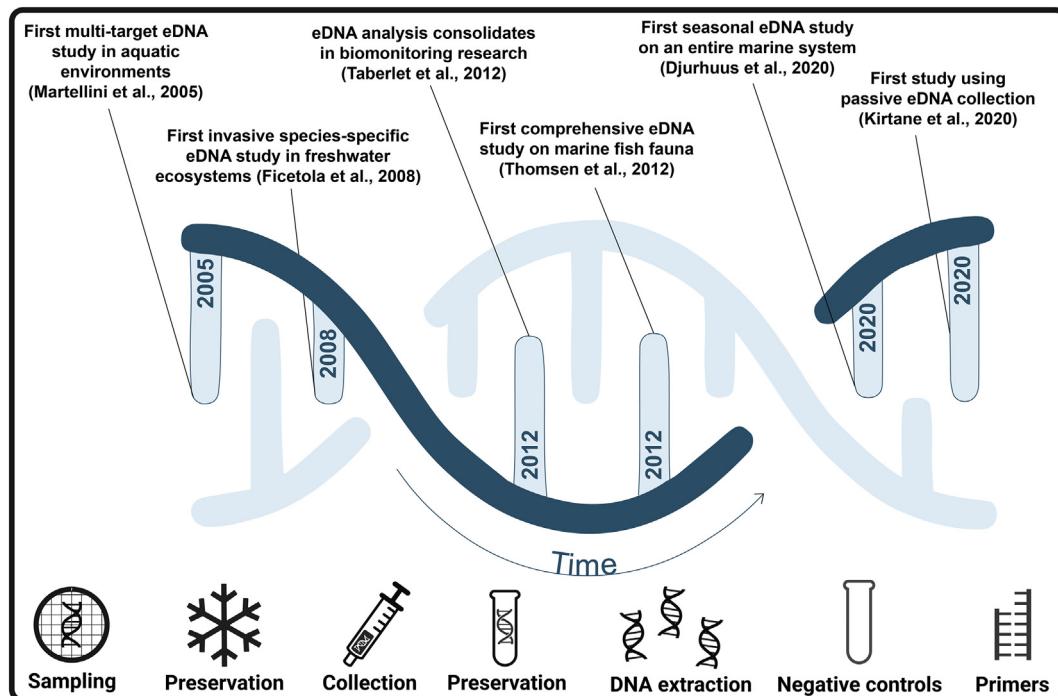


Fig. 1. Simplified timeline of six of the key studies shaping the recent history of eDNA research in aquatic environments, and below: graphical description of the seven primary topics (sampling, preservation method (pre-DNA collection), collection of DNA (i.e., filtering), preservation (post-DNA collection), DNA extraction, negative controls, primers) comprising this literature review. Please refer to e.g., Diaz-Ferguson and Moyer (2014), and Senapati et al. (2019) for more detailed historical examinations of aquatic eDNA research.

as an aquatic biomonitoring tool for macro-organisms (organisms that can be visualized without a microscope, as in Minamoto, 2022). A total of 407 manuscripts were screened (keyword ‘environmental DNA’ or ‘eDNA’ in Scopus) targeting seven key components of aquatic eDNA pre-sequencing work: i) water sampling, ii) preservation (pre-DNA collection), iii) DNA collection (i.e., filtration), iv) preservation (post-DNA collection), v) DNA extractions, vi) negative controls, and vii) PCR primers (Fig. 1). In addition to our decade of review scrutiny (2012–2021), we provide a broad overview of the aquatic eDNA work carried out in 2022 and up to the 19th of January 2023 – 155 studies including research manuscripts, reviews and perspectives. While we acknowledge the equal importance of both the pre- and post-sequencing phases for a successful and robust eDNA study, because of space constraints, we focused on the most fundamental steps preceding DNA sequencing, rather than developments in sequencing, bioinformatics and data analyses which are reviewed elsewhere.

Conceived with the objective to look back, treasure the lessons learned, and discuss the future perspectives of this discipline, this review gives a loud voice to the once “quiet eDNA revolution” that is currently changing how we investigate biodiversity in aquatic ecosystems. As part of this overview of recent aquatic eDNA history, we identify and compare the key methodologies for each step. We discuss aspects such as the value of standardisation, optimisation of protocols, field-specific and taxaspecific guidelines, and identify the most problematic knowledge gaps. The intent is to provide a comprehensive overview of empirically founded eDNA guidelines to the broader research field of aquatic biodiversity monitoring.

2. Materials and methods

2.1. Literature survey and data extraction

A systematic literature survey was conducted to identify methodologies applied in aquatic eDNA studies on macroorganisms. Peer-reviewed papers published between the 1st of January 2012 and the 31st of December 2021 containing “environmental DNA” or “eDNA” in the title, abstract or keywords were searched using the Scopus citation database on the 23rd of January 2022. The Scopus database was chosen because it offers greater coverage for the subjects relevant to the life sciences (Mongeon and Paul-Hus, 2016; Vera-Baceta et al., 2019). To reduce the initial large number of publications (exceeding 50,000) and address our particular aim, we excluded subject areas such as immunology, pharmacology, chemistry, chemical engineering and neuroscience, as well as conference papers, book chapters and notes. We also limited our search to articles written in English. These search criteria reduced the number of matching publications to 885 which were then individually screened using the following additional criteria: (i) did the study use eDNA? (ii) was eDNA extracted from water samples? (iii) was the study conducted on macroorganisms? Publications not meeting all these criteria were excluded. Review papers were also removed from our quantitative analyses as one of our primary purposes was to conduct statistical analyses on field and laboratory methods. With these constraints in place, 407 publications were included in the review (Table S1). For each of these publications, we systematically recorded the following information: study country and region, aquatic system (i.e., marine, freshwater, brackish), study taxa, water volume per sample, number of sample replicates, DNA collection method (i.e., filtration), filter type and pore size, preservation method (pre- and post-DNA collection), DNA extraction kit/method, identification method (i.e., species-specific detection, metabarcoding), types of negative controls, and PCR primers.

2.2. Data visualization and statistical analyses

Analyses and data visualization were undertaken with RStudio (v.2022.02.3, <http://www.rstudio.com/>; R Core Team, 2020). When multiple methods were used in a study, each method was counted independently for the summary statistics and analyses. Several studies recorded several different water volumes in their sampling (i.e., different volumes were

collected at different sampling events or locations), while other studies only provided a range of the sampled volumes. When several different volumes were listed, we counted all volumes independently for our analyses. When only the range of volumes was listed, the middle value (median) within that range was recorded in our data. Generalised linear models (package ‘vegan’ and ‘labdsv’) were used to examine whether the number of methods under each category (i.e., sampling, extraction) changed significantly during the study period due to method diversification. We used binomial generalised linear regression models (package ‘vegan’ and ‘labdsv’) to examine the temporal patterns in the number of publications using each method. To show overall trends in eDNA aquatic monitoring, a Sankey diagram was created using SankeyMatic and RawGraphs (Mauri et al., 2017), and spatial illustrations were made in RStudio using Natural Earth data.

3. Overview of Scopus data

We identified a total of 407 aquatic eDNA studies with the given search criteria across the decade (2012–2021) covered by our review. The vast majority (84.2 %) of studies targeted three continents: Asia, Europe and North America (Fig. 2), with a marked prevalence of environments investigated in the Northern hemisphere. The majority of studies within these three regions, together with Africa, Australia and Antarctica, focused on species-specific approaches, while metabarcoding studies were more prevalent in South America.

The number of publications using water as a source of eDNA has grown steadily since 2012. A dramatic increase took place in 2019, when the number was almost triple that of 2018, and an additional spike occurred in 2021 (Fig. 3 and Fig. S1). Studies in freshwater systems have dominated the number of publications since the first application of this method, comprising 64 % of studies in comparison to 34 % in marine systems (Fig. 3 and Fig. S2a). However, the proportions of studies in marine systems significantly increased over the decade, nearly tripling between 2017 and 2019 (from 12.5 to 32.5 %) (Fig. 3 and Fig. S2b). Most studies were conducted in natural systems (80 %) throughout the study period of 2012–2021, with studies in both marine and freshwater tanks and mesocosms (e.g., Kelly et al., 2014; Sassoubre et al., 2016; Sansom and Sassoubre, 2017) becoming less frequent over time (32 % of studies in 2014 to 15 % of studies in 2021; Fig. 3 and Fig. S3). This is potentially due to a decreased focus on baseline methodological development and experimentation, in favour of in-situ method validations for surveying aquatic biodiversity in natural environments.

To date, single-species eDNA detection methods like quantitative PCR (qPCR) (e.g., Ratsch et al., 2020) and digital PCR (e.g., digital droplet PCR - ddPCR) (e.g., Capo et al., 2020) have been used more frequently (61 % of studies) than multispecies metabarcoding-based studies of community composition (39 %) (e.g., West et al., 2020; Fig. S4a). However, 2021 saw the annual number of publications using metabarcoding (69) exceed the number of those using single-species detection methods (57; Fig. S4b). Interestingly, while species-specific approaches nearly doubled metabarcoding in freshwater systems, metabarcoding was considerably more common in marine systems (Fig. S5). Only two studies (Pinfield et al., 2019; Roesma et al., 2021) that we reviewed have used shotgun sequencing of the entire metagenome (0.5 %), despite this approach allowing for comprehensive sequencing of all genes from organisms present in a sample. The main issue with using this approach for community composition monitoring, is the small proportion of total reads assigned to focal taxa because of the inundated number of Bacteria and Archaea reads (Tringe et al., 2005). Another novel but still underused technique is the hybridisation capture, a target enrichment method alternative to shotgun sequencing for obtaining DNA data from samples with high nontarget content (Sigsgaard et al., 2020). Once combined with eDNA sampling, this approach hosts great potential for future population genomic research (Jensen et al., 2021 and references therein). Indeed, more reference genomes for target taxa, and improved DNA extraction and library construction methods, may lead to more eDNA studies implementing this method (Parducci et al., 2019).

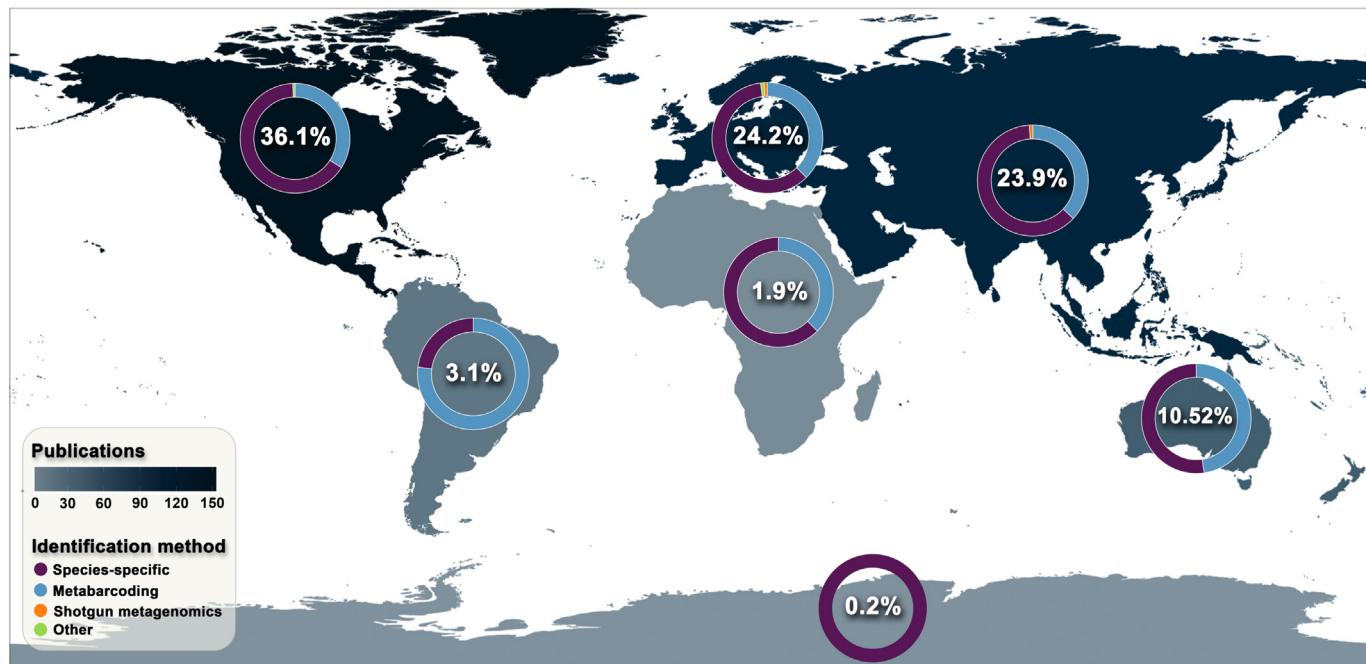


Fig. 2. Map illustrating the proportions (%) of publications targeting aquatic ecosystems across seven continents - Asia, Africa, North America, South America, Antarctica, Europe and Australia. The identification methods (species-specific, metabarcoding, shotgun metagenomics and other (SNP type genotyping and specific to genetically modified animal)) per region are indicated in the pie/donut graphs. The studies are assigned to country and continent as by where the fieldwork was conducted, as specified in the reviewed papers.

Fish taxa have been studied most frequently (48 % of studies) (Fig. S6), likely because of their commercial value as a food source for both humans and animals, vital functional roles in aquatic ecosystems (e.g., nutrient cyclers), and importance as indicators of ecosystem health (e.g., Lynch et al., 2016; Miya et al., 2022). Amphibians were the next most studied taxa (9 %), which is unsurprising considering at least one-third of amphibian species are at risk of extinction (Stuart et al., 2004) and there is a great demand for sensitive biomonitoring tools to inform conservation efforts. Marine mammals (6 %) and molluscs (6 %), were also the focus of some studies in the literature (Fig. S6), possibly because of their charismatic nature and role as keystone species (marine mammals and reptiles; Pinfield

et al., 2019), or because of their invasive nature (molluscs; Clusa et al., 2017).

There were surprisingly few studies on corals (Cnidaria, 0.7 %; Fig. S6), despite their global significance as reef and habitat builders, and the increasing threat that global warming presents to coral communities (i.e., 50 % of global coral cover has declined since the 1950s, Eddy et al., 2021). Studies on fish, amphibians and mammals dominated the early aquatic eDNA studies, and continue to do so. However, there has been a consistent increase in the diversity of organisms studied since 2014, with studies on plants, polychaetes, arthropods, fungi, nematodes and decapods all emerging after 2018–2019 (Figs. S6 and S7).

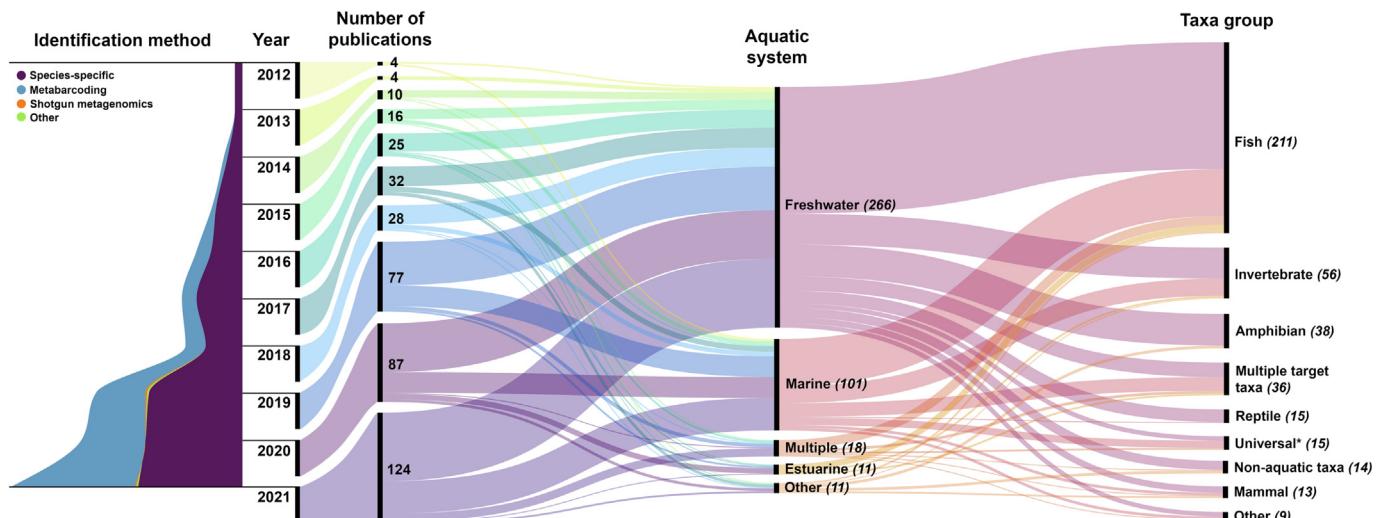


Fig. 3. A Sankey diagram showing the number of publications, types of aquatic systems and taxa groups found from the Scopus literature search with queries “environmental DNA” or “eDNA” between 2012 and 2021. On the left, four eDNA identification methods (species-specific, metabarcoding, shotgun metagenomics and other) were found in the search, with this diagram illustrating the trends in number of publications, aquatic system, and target taxa between 2012 and 2021 for each method. For visualization purposes, categories under the label “Taxa group” are a composite of different target groups specified in Fig. S6.

The use of eDNA in aquatic systems has demonstrated success in monitoring community composition (e.g., Djurhuus et al., 2020; West et al., 2020), population dynamics (e.g., Carim et al., 2017), trophic interactions and diet (e.g., Saccò et al., 2020; D'Alessandro and Mariani, 2021), and detecting rare, endangered, and invasive species (e.g., Pukk et al., 2021).

4. eDNA sampling and laboratory procedures

4.1. Sampling

4.1.1. Volume

By its nature, eDNA is characterised by considerable variation in concentration based on the abundance and shedding rate of the target organism, and the rate of degradation in the environment (Barnes and Turner, 2016). Logically, the larger the volume of water sampled, the greater the proportion of local biodiversity will be detected; however, there is a trade off as sampling large volumes can be logistically and mechanically challenging. While the volume of water sampled for aquatic eDNA studies sets the stage for all downstream processes, the amount of water filtered varies considerably between studies. Early eDNA studies commonly used small volumes (<100 mL) (Fig. 4a1 and a2), but the development of various more efficient DNA filtration methods has now made it feasible to sample and filter >50 L at a time (Cilleros et al., 2019) (see 4.3. DNA Collection section for further details). Over the period 2012–2021, a third (33.5 %) of all relevant studies used 500–1000 mL (majority 1000 mL) (Figs. 4a1 and 6a). This volume can be relatively quickly processed using different filtration methods (i.e., pump, syringe) and has been demonstrated as sufficient to detect a representative range of taxa in marine (e.g., Andruszkiewicz et al., 2017; Gold et al., 2021) and freshwater ecosystems (e.g., Janosik and Johnston, 2015; Feng et al., 2020). However, defaulting to this small volume would be risky and may be more related to its accessibility than its suitability for eDNA biomonitoring.

Filtering larger volumes of water has been shown to increase the amount of eDNA collected in both lab-controlled environments (Mirimin et al., 2020) and field conditions (Muha et al., 2019; Ahn, 2020). Increasing the volume is particularly important when the DNA concentration of the target taxa is expected to be low. For example, because pelagic animal biomass declines exponentially with water depth, Laroche et al. (2020) used greater volumes of water to sample for eDNA at depth (5 L) than at the surface (1 L). Larger than usual pore sizes are also employed to filter larger volumes of water, with the downside of a decrease in DNA recovery (e.g., Majaneva et al., 2018, and see 4.3.3. Filter pore size for further details).

Importantly, enhancing the sensitivity of an eDNA survey by increasing sample volume may be desirable when the impact associated with reporting a false negative result is high, such as in many biosecurity contexts. However, sampling greater volumes of water can be challenging. Filters often become clogged with sediment (or plankton/algae), reducing the volume that can be filtered and potentially resulting in considerable variability between replicates such as the 200–1000 mL range reported in Eichmiller et al. (2014) or the 1.6–10 L reported in Wittwer et al. (2019). A standard method to mitigate this issue includes prefiltering the water using filters with larger pore sizes (Takasaki et al., 2021), mesh (Brandt et al., 2021), or nets (Zaiko et al., 2015). While prefiltering can reduce variability between samples (Takasaki et al., 2021), there is a risk of lowering the DNA yield and impacting the number of species detected in downstream analyses (Majaneva et al., 2018). Also, this extra step requires additional handling of the sample, which may introduce contamination. Whether introducing prefiltering or not, sampling large volumes requires more equipment including specialized pumps such as the Mark II inDepth eDNA samplers (Applied Genomics, Brixham, UK), which can pump 50 L over a 25-h period (Mirimin et al., 2021). Alternatively, an in-situ pump, such as the Serial Autonomous Larval Sampler (SALSA), can be used to prefilter with a 20 µm nylon mesh and can be programmed to filter for hours, resulting in sample volumes of ~6000 L (Brandt et al., 2021). This additional equipment may not be accessible or practical in remote

locations. This is why Muha et al. (2019) recommended obtaining 100 mL precipitated samples in the field although 2 L filtered samples yielded higher DNA concentration. The 100 mL samples were obtained with minimal handling and easy to store and transport, and there was no difference in qPCR success rate between the two volumes in this particular study. Another strategy is to obtain multiple smaller samples (i.e., employ multiple filters), which are merged at the DNA extraction stage and treated as one sample replicate in the downstream procedures (Capo et al., 2020; Hunter et al., 2019). In summary, sampling scheme and water volume must be determined on a case-by-case scenario, ideally governed by a pilot study that examines the feasibility of sampling different volumes and testing the impact on qPCR detection accuracy and/or the reported biodiversity measures.

4.1.2. Field replication

In a field that is essentially based on determining presence/absence of certain taxa from low copy number DNA there is an inherent risk of obtaining results that are not representative of the sampled environment. This is why replication is a recurrent theme throughout most stages of the eDNA workflow. Technical replication is often introduced in the post-DNA collection stage, most commonly through the use of PCR replicates (e.g., Davis et al., 2018; Harper et al., 2018). While helpful to account for stochasticity in PCR amplification (Piggott, 2016; Kelly et al., 2019), these are not true replicates of the sampled environment because they would still be based on the same DNA extract. Therefore, adjustment of the replication level is essential (Ficetola et al., 2016), and pre-biological inference steps such as i) run occupancy models scanning for false negatives, ii) evaluate thoroughly the level of replication, and iii) remove false positives unconfirmed by several PCR runs, are highly recommended (Ficetola et al., 2015). False positives can also arise from index or tag jumping during sequencing, and this is reviewed elsewhere (e.g., Schnell et al., 2015; Ushio et al., 2022).

In many cases field replication, the number of individually collected samples at the same study site, is crucial to examine the extent of cross sample variation. Generally, replicate sampling will very often increase the species richness detected through eDNA metabarcoding, as demonstrated by species accumulation curves in many studies (e.g., West et al., 2020; Macher et al., 2021; Mena et al., 2021), although this increase can also be largely disproportional (Macher et al., 2021). The early aquatic eDNA studies (2012–2014) tended to have a low number of replicates or less per sampling point (1–3, but see Thomsen et al., 2012 for an exception). After 2014, more studies introduced four or more replicates, but by 2021, still >60 % of the studies only used three replicates or less (Fig. 4b1 and b2). The diversity detected in replicate samples collected simultaneously and in the same conditions can vary considerably, and even six replicates (30 L each) can underestimate local diversity (Stauffer et al., 2021). Hence, a high risk of false negatives and/or unrepresentative samples is a major challenge in eDNA biomonitoring. To mitigate this, some studies employ the use of subsamples, smaller samples of water collected over an area and then pooled and homogenized, to create a single composite sample that may be more representative of a given system (Kamoroff and Goldberg, 2018; Mena et al., 2021). The use of subsamples is very common in the field of soil microbiology because of the high levels of heterogeneity and may become more common in aquatic eDNA surveys in the future, especially where the goal is to characterize diversity in a large body of water (Miaud et al., 2019). Using this method, Mena et al. (2021) detected more terrestrial Amazonian mammal species using aquatic eDNA than mist nets, pitfall traps, and camera traps.

To what extent replication is required to accurately capture the local biodiversity is highly dependent on the sample volume (Cantera et al., 2019), the concentration of target taxa (Furlan et al., 2016), and the type of ecosystem sampled (Bylemans et al., 2018b). For example, increasing the number of samples from one to 18 resulted in a growth of species detection of 24.8 % for fish/lamprey, 68.9 % for mammals and 77.3 % for birds (Macher et al., 2021). Cantera et al. (2019) detected 87 % of the expected fauna with a single 34 L sample of tropical freshwater. Bylemans et al.

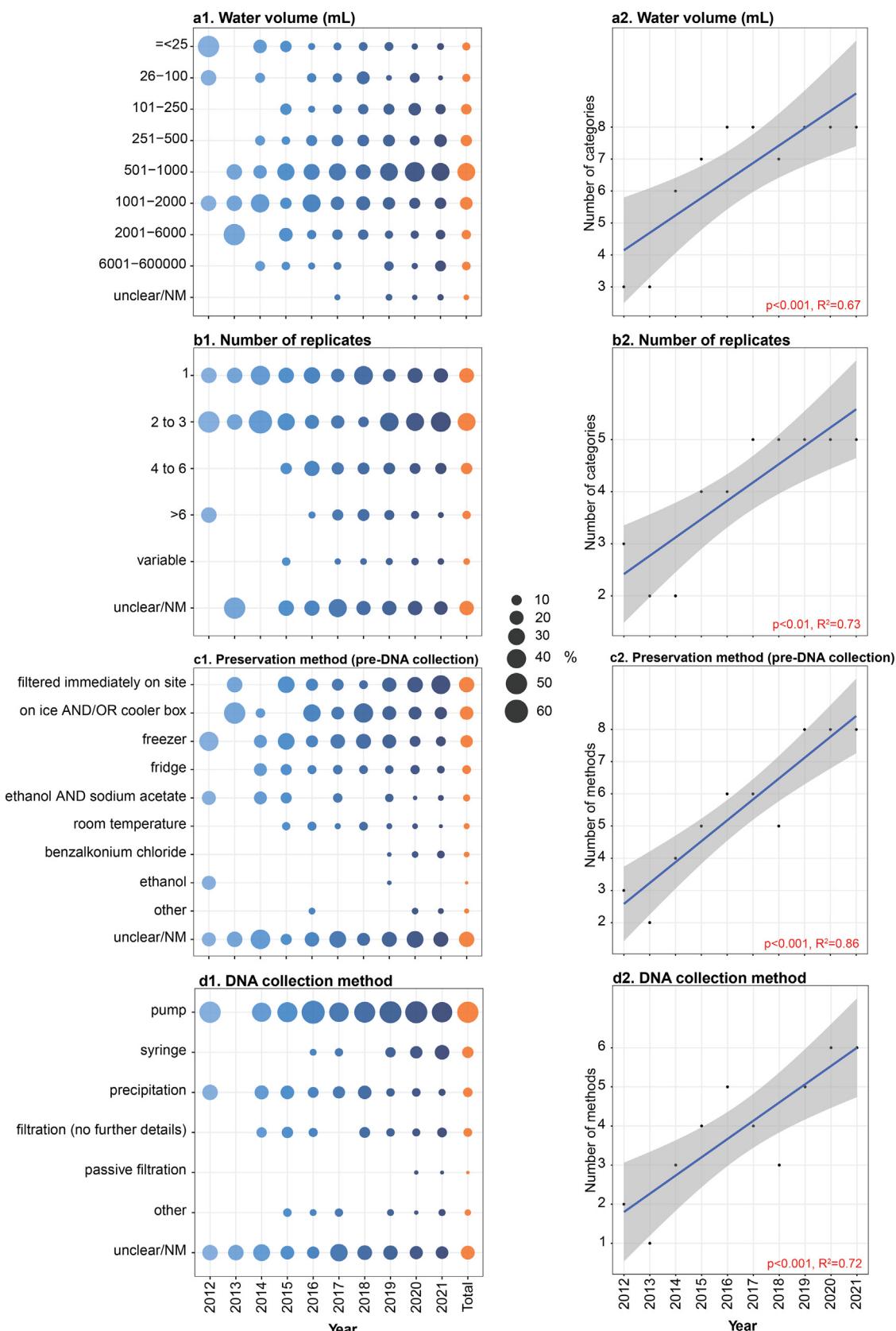


Fig. 4. Bubble plots for the first four factors targeted in the review (water volume, number of replicates, preservation method (pre-DNA collection) and DNA collection method) showing the proportion of studies across the targeted interval of time (2012–2021) (a1, b1, c1, d1) and linear regressions with R^2 values and p -values (a2, b2, c2, d2). The solid blue line was fitted using a generalised linear model, and the grey areas indicate the 95 % confidence intervals. Number of methods in Y axes of Fig. 4a2 and Fig. 4b2 refers to the number of different categories of water volumes ($\leq 25, 26\text{--}100, 101\text{--}250$, etc.) and sample replicates (1, 2 to 3, 4 to 6, etc.) employed in the reviewed manuscripts. NM, not mentioned.

(2018b) noted that relatively modest replication is needed at upstream and higher altitudes due to the lack of between-sample variation compared to further downstream. Furlan et al. (2019) calculated that to achieve a 95 % certainty of the absence of a single breeding pair of the invasive European carp in a lake, they would need to obtain 11,600 samples, because the required level of replicates increases exponentially as the concentration of DNA decreases. This clearly exemplifies one of the limitations in eDNA monitoring, but by thoroughly assessing the sampling effort required to detect a target, given the sensitivity of an eDNA survey, it can inform practitioners of the feasibility and reliability of such surveys (Furlan et al., 2019).

In general, a single eDNA sample may be able to detect more species than a conventional survey sample due to its higher sensitivity to detect cryptic species as well as the organisms that were previously at a sampling site or at adjacent area, resulting in the lower replicate requirement and higher cost-efficiency of eDNA (e.g., Smart et al., 2016; Beng and Corlett, 2020). However, given the multiple factors influencing the eDNA sensitivity as exemplified above, comparison of required level of replicates and cost-efficiency between eDNA and conventional surveys is also confounded by such factors and not straightforward. In conclusion, there is no universal rules-of-thumb, and a priori assessment of the required replication level, or at the minimum, the assessment of accumulation curves and implication of potential false negatives, are essential for each eDNA study.

4.2. Water sample preservation (pre-DNA collection)

Following water sample collection, DNA will continue to degrade by microbial activity (Kumar et al., 2020), exogenous enzymes and by spontaneous chemical reactions (e.g., hydrolytic, oxidative) (Lindahl, 1993). Due to this continued degradation, samples require careful preservation to accurately capture the biological genetic diversity at the time of collection. Filtering on-site is often preferable because filters occupy far less space than large water volumes, and the filters can easily be transferred to a buffer (e.g., DNA shield) that aims to stabilize the eDNA fragments. This explains why the strategy of immediate on-site water filtering has increased significantly since 2012 in the reviewed studies ($p < 0.001$; Fig. 6b). However, on-site filtration might not be feasible in some cases, such as when intensive sampling is conducted over a short time period or when the water is difficult to filter because of high turbidity or salinity (Harper et al., 2019a, b). Such challenges have led research into minimising the degradation of eDNA in a water sample prior to filtration. These methods include chilling (e.g., Bylemans et al., 2018a; Lafferty et al., 2021), freezing (e.g., Coulter et al., 2019; Harper et al., 2019a, b), or adding a preservative to the sample, such as ethanol (e.g., Foote et al., 2012; Williams et al., 2017) (Fig. 4c1 and c2). We observe that the number of studies using freezing or adding a preservative directly to water samples has significantly decreased over time ($p < 0.01$; Fig. 6b) and may have been replaced by on-site filtering. The decrease in studies using these preservation methods can also be attributed to challenges with transporting hazardous preservatives like flammable ethanol to the sampling location, as well as the freeze/thaw process degrading the DNA molecules (Chung et al., 2017). More recently, the use of benzalkonium chloride as an antimicrobial agent has been shown to dramatically suppress the degradation of eDNA (Takahara et al., 2020) and our review documents an increased use since 2019 (Fig. 6b). Many of the reviewed studies indicated that keeping collected water samples at room temperature, storing in a fridge or on ice were becoming more favourable strategies (Fig. 4c1). Pulling a water sample out of its environment is unlikely to dramatically increase DNA degradation, but unlike the situation in the natural environment, the degraded eDNA in a water sample will not be replenished and therefore it is highly advisable that it is filtered within 24 h of collection (e.g., Boivin-Delisle et al., 2021). Several studies (e.g., Hinlo et al., 2017; Holman et al., 2022) have observed a significant decrease in DNA copy numbers from 24 to 48 h in aquatic samples regardless of storage method. Overall, the reviewed studies have indicated that water samples should be either filtered immediately on-site or within 24 h. If water samples are to be stored longer, refrigeration may be a better option than freezing for short-term storage (i.e., 3–5 days) (Hinlo et al.,

2017). If being stored longer than 5 days, samples can then be frozen (e.g., Julian et al., 2019) or a preservative added to deactivate DNases and prevent bacterial growth (e.g., Williams et al., 2017). A comprehensive systematic study testing how various water preservation methods may affect downstream qPCR results and biodiversity measures in eDNA samples seems highly relevant.

4.3. DNA collection

4.3.1. Filtration method

eDNA is continuously shed by organisms into the aquatic ecosystems they inhabit. The shed DNA may come in the form of tissue, excreted cells, eggs or larvae, faeces, or genetic material released from deceased individuals (Thomsen and Willerslev, 2015). As the number of eDNA studies has steadily increased over the period 2012–2021, so too has the variety of strategies for eDNA capture, yet the most common method remains active filtration through a membrane using a pumping mechanism (Figs. 4d1, d2 and 6c). Active filtering varies from using a vacuum or peristaltic pump in a laboratory setting with collected water samples, to in-situ field sampling using enclosed filters (e.g., Sterivex™) and battery-operated pumps, all of which enable a pre-determined volume of water to be used for consistent sampling (Rees et al., 2014; Tsuji et al., 2019). Over recent years there has also been a significant increase ($p < 0.01$) in studies using syringes to manually force water through a filter membrane (Fig. 6c). Syringes are easy to use in the field for both professionals and non-experts with minimal contamination risk when used with enclosed filters, although they are commonly used for sampling smaller water volumes (≤ 1 L). Because it is easy to use, the syringe method seems to be the method of choice in citizen science initiatives (Miya et al., 2022). Ethanol precipitation of eDNA works by adding sodium acetate and ethanol to small water samples (≤ 1 L but typically 15 mL), which is later centrifuged and the supernatant discarded (Ficetola et al., 2008; Deiner et al., 2015). The proportion of studies using the precipitation method in aquatic eDNA contexts has decreased significantly over the period 2012–2021 ($p < 0.01$, Fig. 6c), possibly because it only accommodates small water volume samples and is only useful in situations where target species eDNA concentrations are high. Other less frequently used methods, including centrifugation and ultrafiltration, are also primarily suited to smaller water volumes. In contrast, active filtration can accommodate any volume but is most often used for larger water volumes (typically ≥ 1 L) that improve eDNA species detection (Bessey et al., 2020). Because active filtration can be time consuming and membranes can become blocked by particulates in highly turbid waters, more recently researchers have started exploring other approaches. Examples include using in-situ remotely deployed sampling instruments that automate filtering (McQuillan and Robidart, 2017), high-volume sampling using tow nets (Schabacker et al., 2020), passive collection using various membrane materials submerged at the site (Kirtane et al., 2020; Bessey et al., 2021), or by recovering eDNA from the tissues of marine organisms, such as sponges and mussels, which naturally filter large water volumes per day (Mariani et al., 2019). While some of these alternative approaches require additional or expensive equipment, others such as passive eDNA collection holds great potential because they are rapid, low-cost, and easily deployable in many contexts (Bessey et al., 2022; Chen et al., 2022) and have already been applied to reconstruct fish composition from the nets of commercial trawlers (Maiello et al., 2022).

4.3.2. Filter type

As the number of eDNA collection strategies has increased, so too has the type of filter membranes deployed, with glass fibre, cellulose papers, and other synthetic thermoplastic membranes all frequently being used for eDNA collection (Figs. 5a and 6d). Glass fibre filters are manufactured from borosilicate and combine fast flow rate with high retention of fine particles. They are hydrophilic and generally have a higher ability to load particulates into the fibrous matrix (loading capacity) than a cellulose filter of similar retention. Cellulose filter papers are produced with cellulose acetate, cellulose nitrate (also known as nitrocellulose; NC), and mixed

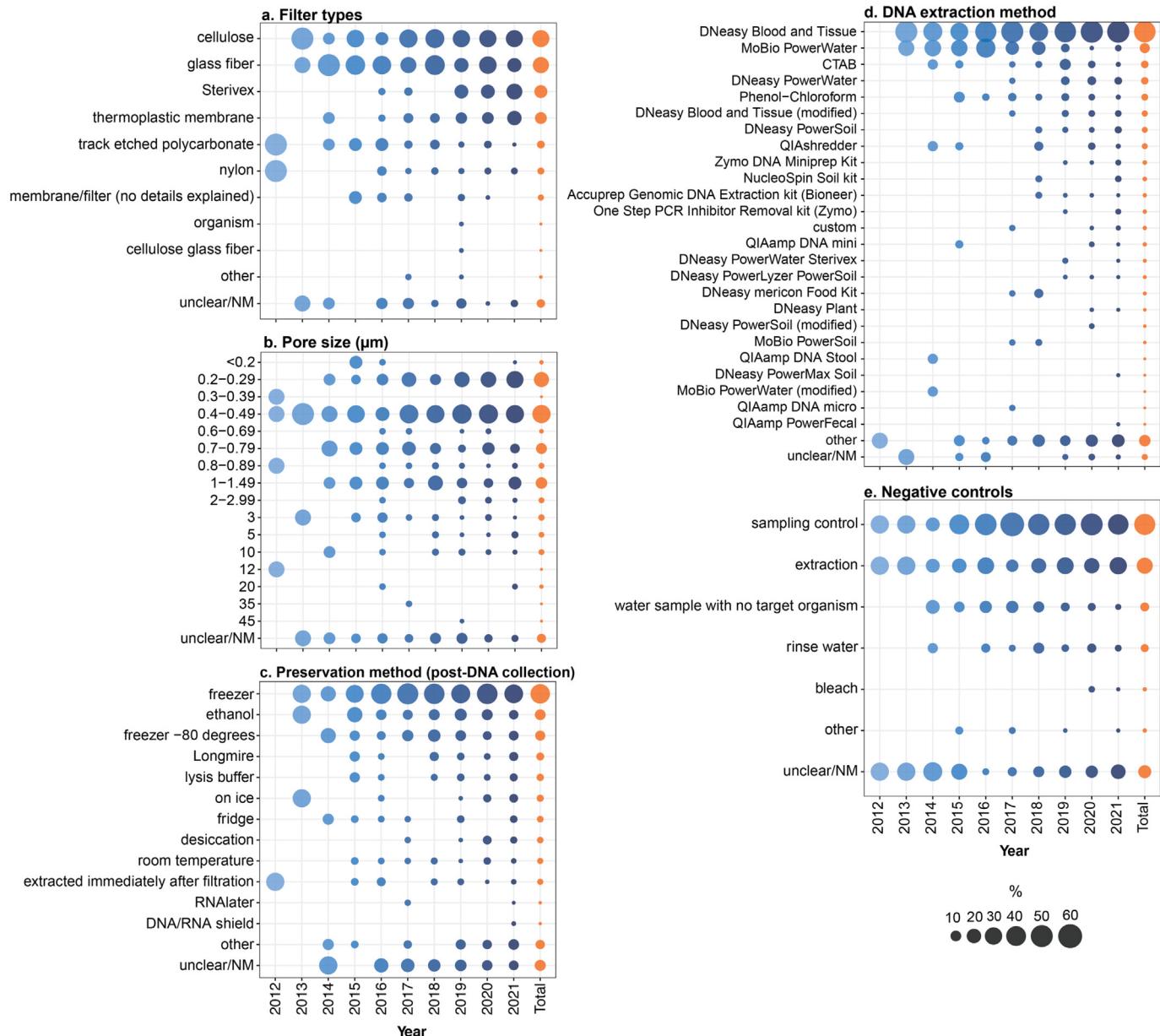


Fig. 5. Bubble plots for the last five factors targeted in the review (filter types, poser size (μm) preservation method (post-DNA collection), DNA extraction method and negative controls) showing the proportion of studies across the targeted interval of time (2012–2021) (a, b, c, d, e). Linear regression plots showing the significant increases in the number of methods throughout the studied period can be found in Fig. S8. NM, not mentioned.

cellulose esters (MCE) and are relatively inexpensive, hydrophilic, but are more delicate and can tear easily. Other synthetic thermoplastic membranes are composed of either polysulfone, polyethersulfone (PES, PESU), polyvinylidene fluoride (PVDF), polycarbonate (track-etched; PCTE) or polyamide (nylon) and are used because of their thermal stability, chemical resistance, and mechanical strength (toughness), but may require chemical modification to overcome hydrophobic surface qualities which can cause biofouling effects. Mechanically tougher filter materials may be more appropriate if passive eDNA methods are deployed, especially in turbid or turbulent environments, such as in-situ collection from trawl nets (Maiello et al., 2022). Track-etched polycarbonate membranes have an absolute pore size because they have a top to bottom hole through the membrane. Enclosed capsule thermoplastic filters (Sterivex™) are increasingly used ($p < 0.001$, Fig. 6d) because they minimise contamination issues in the field and easily allow for the addition of a preservation buffer, enabling room temperature storage for weeks (Spens et al., 2017), but are considerably more expensive than other membranes (e.g., \$1.50 and \$15.00 AUD

for one cellulose filter and Sterivex respectively). Although each filter material has unique characteristics that will affect the total eDNA concentration obtained, a wide variety of materials (including cotton, hemp, granular activated carbon, clay, hydroxyapatite and chitosan covered membranes) (Kirtane et al., 2020; Bessey et al., 2022; Verdier et al., 2022), can be successfully employed, yet their effectiveness may be specific to target taxa or filtration method. For example, Capo et al. (2020) found that Sterivex recovered the highest fish eDNA concentrations compared to mixed cellulose ester or glass fibre filters, whereas Djurhuus et al. (2017) found that nitrocellulose recovered the highest eDNA concentrations for a variety of taxa (microorganisms, phytoplankton and vertebrates) compared to thermoplastic membranes.

4.3.3. Filter pore size

A wide variety of filter pore sizes - from $<0.2 \mu\text{m}$ up to $45 \mu\text{m}$ - have been employed for eDNA collection over recent years, although the large majority used are $<1.5 \mu\text{m}$ (Figs. 5b). The most frequently and consistently

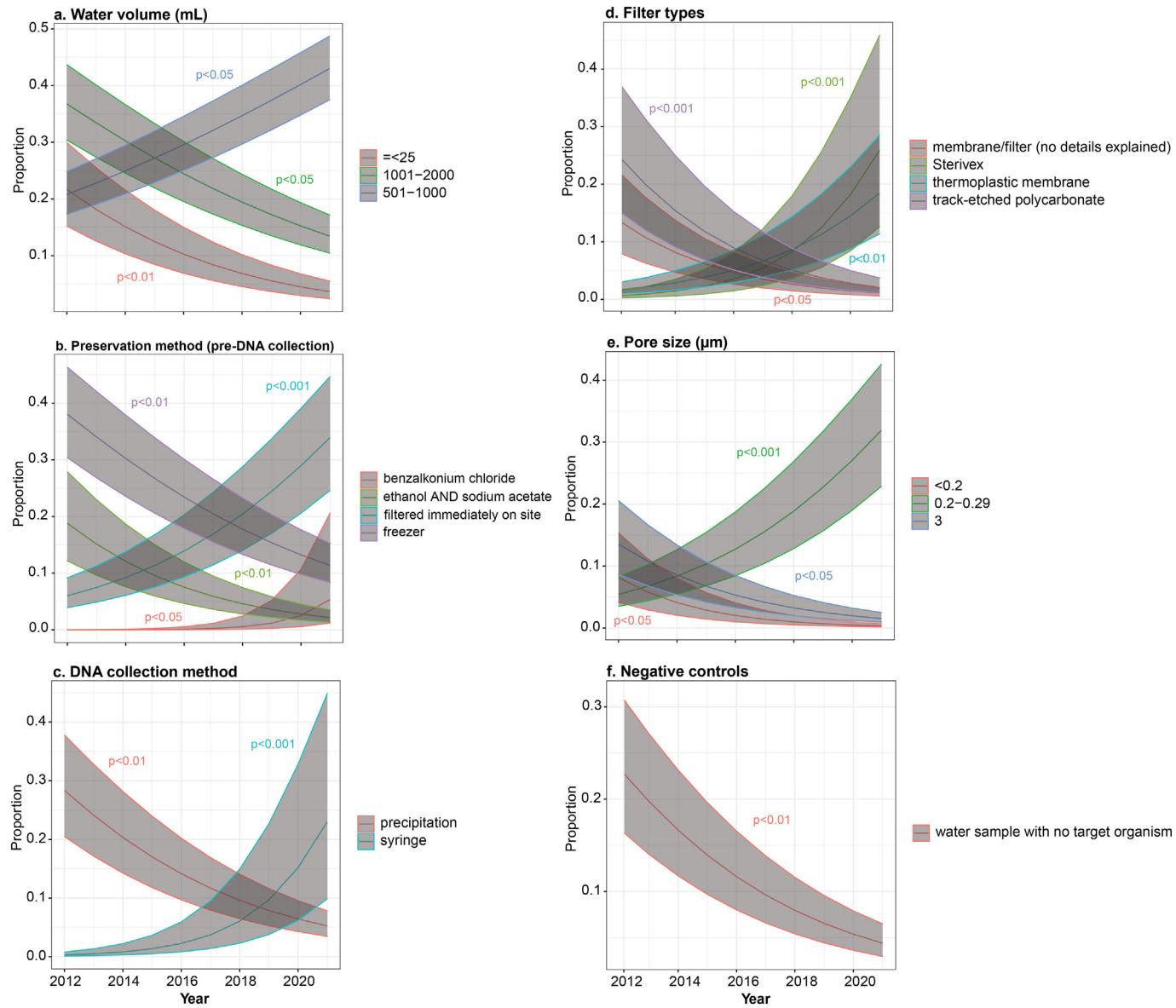


Fig. 6. The temporal trends in the relative proportion of eDNA publications focusing on water volume (a), preservation method (pre-DNA collection) (b), DNA collection method (c), filter types (d), pore size (e) and negative controls (f) published between 2012 and 2021. Solid lines are fitted using binomial generalised linear regression models, and grey areas indicated the 95 % confidence intervals (*p*-values of each model are shown in each plot). Only regression models revealing statistically significant trends are shown.

used pore size over the period 2012–2021 is between 0.40 and 0.49 μm (mainly 0.45 μm). Other frequently used pore size ranges are 0.20 to 0.29 μm , 0.70 to 0.79 μm , and 1.0 to 1.49 μm . The 0.45 μm pore size filter has been used successfully in studies covering a myriad of different target taxa including nematodes, mussels, oysters, crayfish and corals, amphibians, reptiles, fish and mammals. Nevertheless, these small pore size filters are prone to clogging in turbid waters or those with high levels of suspended particulate matter. Some studies prefilter water using larger pore sizes, allowing for an increased volume of water to be sampled.

The optimal pore size will be study and site specific, with studies showing no consistent patterns in target taxa retention efficiency. For example, while some studies have shown that 0.45 μm and 0.22 μm pore size better retain particles binding fish eDNA molecules than 1.2 μm (Capo et al., 2020; Miya et al., 2016; Turner et al., 2014), others recommend the use of 0.8 μm or larger for turbid, eutrophic or high fish density environments because of the trade-off between filtration efficiency and species detection (Takahara et al., 2012; Lacoursière-Roussel et al., 2016; Li et al., 2018). Still, other studies suggest that $>20 \mu\text{m}$ filters can capture nearly four

times more mitochondrial fish DNA than 10 μm filters, indicating that mitochondria can occur in large aggregates of biological material (Cooper et al., 2022).

4.4. Preservation (post-DNA collection)

As mentioned in previous sections, the decay of DNA is heavily influenced by temperature, and to prevent degradation and contamination one of the commonly used approaches for preservation of filtered DNA is keeping filter papers in cold storage, such as ice, fridge, -20°C and -80°C freezers (Friebertshauser et al., 2019; Alexander et al., 2020; Broadhurst et al., 2021). Our data also shows that the use of cold storage system, in particular freezers, has been consistently the most popular method (Fig. 5c). However, access to a cold chain of storage is not always feasible, such as in remote areas, resulting in the diversification of preservation methods at ambient temperature over the period 2012–2021 ($p < 0.001$, Fig. S8c).

In the period 2012–2021, high-grade ethanol (95–100 %) has also been used in preservation of eDNA samples (Fig. 5c) (i.e., Zaiko et al.,

2015; Goldberg et al., 2018; Pukk et al., 2021). Nevertheless, as it is in the category of dangerous goods due to its toxic inflammable nature, its transportation will often require special shipping permits (Marquina et al., 2021). To tackle the challenge of transporting the preservation material into remote areas, a partially biodegradable self-preserving filter – eDNA filter housing – was created by Thomas et al. (2019) : it automatically preserves eDNA via desiccation and does not require any additives to be carried along in the field. Additionally, silica beads have also been successfully used for desiccation of filter-immobilized eDNA as a lightweight, non-toxic, cost effective and portable alternative to ethanol preservation (Wilcox et al., 2020; Allison et al., 2021; Plante et al., 2021).

Addition of lysis buffers has proven to successfully preserve eDNA filters at ambient temperature. Among various lysis buffers, Longmire's solution (Renshaw et al., 2015; Everett and Park, 2018; Jacobs-Palmer et al., 2021; Lafferty et al., 2021) has been the most popular one. Buffers, such as STE sterile buffer (Valdivia-Carrillo et al., 2021), ATL (Qiagen) (Czachur et al., 2022; Seymour et al., 2020; Wacker et al., 2019) and CL1 conservation buffer (SPYGEN) (Coutant et al., 2021) have also been used successfully. In addition to those above, in recent years other preservatives, such as DNA/RNA Shield (David et al., 2021; Madduppa et al., 2021) and RNAlater Stabilization Solution (Thermo Fisher Scientific, Waltham, MA, USA), an aqueous non-toxic tissue storage reagent (Sengupta et al., 2019; Ahn, 2020), are becoming more common.

Multiple studies have compared different methods commonly used in preservation of eDNA post collection (see Table S2) but most of these are limited to comparing just of a few methods. Moreover, as discussed by Majaneva et al. (2018), performance of the same method could vary in different ecosystems due to differences in physical and chemical characteristics of the environments. Therefore, a comprehensive study encompassing all available methods in various ecosystems is required to shed more light in this area. Additionally, while performance of a lysis buffer preservative has been compared with other methods, a direct comparison of the performance between different lysis buffers is missing.

4.5. DNA extraction

DNA extraction methods vary in effectiveness across different cell types and sample substrates, making protocol choice a critical consideration when designing an eDNA study (Bruce et al., 2021). The most commonly used kit-based extraction methods were the commercially available DNeasy Blood & Tissue (DNeasy B&T) and MoBioPowerWater (PowerWater) kits from Qiagen (used in 46 % and 18 % of the reviewed publications, respectively, Fig. 5d). Various in-house formulations of phenol-chloroform-isoamyl alcohol (PCI) and acetyltrimethyl ammonium bromide (CTAB) were less commonly used, 3 % and 4 %, respectively. PCI and CTAB are inexpensive but involve hazardous chemicals and lengthy protocols (Kumar et al., 2020). Commercial kits are usually more expensive but are generally easier to use, plus they have the additional benefits of not including harmful reagents and they allow for automation and, thus, higher throughput (Hinlo et al., 2017). The use of commercial kits may not be suitable for limited budgets, but comparative studies of extraction kits/methods have indicated that the CTAB method (Lin et al., 2019) and AxyPrep DNA Gel Extraction Kit (Chen et al., 2020) show similar extraction efficiencies, offering a low-cost alternative. From 2019 to 2021, DNeasy B&T was used in almost half of the reviewed publications. Other extraction kits have been used (e.g., DNeasy PowerSoil, Zymo DNA Miniprep kit), and the tendency or the DNeasy B&T protocol to be modified to improve DNA extraction yields has increased in recent years (Fig. 5d). Modifications included the usage of different purification columns, such as QiaShredder (Hobbs et al., 2019) or Zymo (Hunter et al., 2019) columns; increasing the volume of cell lysis solution and Proteinase K during the incubation (e.g., Stat et al., 2019; Kawato et al., 2021; Ellis et al., 2022); and incubating overnight (e.g., Min et al., 2021). Furthermore, the PowerSoil kit from Qiagen, which includes specific inhibitor removal stages, has gained interest as it can outperform DNeasy B&T and PowerWater kits when applied to water samples from turbid environments such as marshes (Kumar et al., 2020;

Neice and McRae, 2021) which typically capture substances such as humic acids that can inhibit PCR amplification. Some researchers consider that there should be a universal DNA extraction method for environmental samples (Hermans et al., 2018). However, others consider that an inhibitor-removal step should only be introduced if necessary, as removing PCR inhibition from DNA extracts without compromising DNA yield can be difficult (Hunter et al., 2019). Overall, the efficiency, reliability, and comparability of an extraction protocol, its cost and ease to use must be carefully considered before starting a project.

4.6. Negative controls

Negative controls are essential in eDNA studies, but the field is still lacking a consensus on what type of negative controls should be included to provide the required rigour. While the use of negative controls in PCR is well established, methods used to detect contamination that may occur prior to this step vary widely. Negative control types from the literature were summarised into the following categories: i) sampling control (clean water exposed at a site or filtered with samples), ii) no-target control (water collected from a location known not to have target species or water collected from tank prior to the introduction of target species), iii) bleach control (bleach used to clean filtration equipment), iv) rinse water control (water used to clean filtration equipment after bleaching), v) extraction control (pure water processed alongside actual samples), and vi) and negative controls not detailed (controls were carried out but specifications were not provided).

Negative controls are essential at every point of the workflow to detect the source and stage of contamination (Goldberg et al., 2016; Abbott et al., 2021; Hutchins et al., 2022). Additionally, consistent reporting of the controls used is equally important for study evaluation and replicability (Goldberg et al., 2016; Abbott et al., 2021). However, findings from this and other reviews suggest minimal convergence over time regarding the application of negative controls despite extensive guidance provided in the literature concerning their use. Of the reviewed studies, 49 % included a sampling control, 27 % included an extraction control and all other control categories occurred at <10 % (Fig. 5e). Previous reviews have highlighted a discrepancy between the almost ubiquitous use of negative controls in the lab, with the absence of appropriate sampling and/or no-target controls (Dickie et al., 2018; Sepulveda et al., 2020). Our findings concur with this result, because 16 % of the reviewed studies did not detail the use of any category of negative control (Fig. 5e).

Undoubtedly, many studies that experience systemic contamination are not published, making it difficult to determine things such as (i) how frequently wide-reaching contamination occurs, (ii) particular taxa that are more likely to be contaminants, (iii) the most common point where contamination enters the workflow, and (iv) how to minimise and/or deal with contamination when it occurs. The field would greatly benefit from contamination results being made publicly available.

Several literature reviews investigating the use of negative controls in eDNA studies (e.g., Sepulveda et al., 2020) found that where negative controls produced unexpected amplification, studies targeting a particular organism would often provide a rationale for ignoring amplification as low-level background noise. In contrast, metabarcoding studies used this information to delimit a detection threshold “*above which is considered significant or provided rationale for why the unexpected amplifications did not affect results*” (Sepulveda et al., 2020). Other metabarcoding strategies involve retaining only sequences produced by multiple PCR replicates and/or removing rare sequences (like doubletons or singletons) from sequence read counts. Conversely, in qPCR studies any amplification observed in negative controls requires that the results for all associated samples should be discarded (Goldberg et al., 2016). It should be noted, however, that many studies do not report how negative control contamination is accounted for and/or they do not provide adequate information to determine which type of negative control is referred to throughout the experiment (Dickie et al., 2018; Sepulveda et al., 2020).

Interestingly, as demonstrated in a study by Furlan et al. (2020), while sterile lab practices are often cited as mitigating contamination risks, intensive bioinformatic filtering processes are required post-data generation to ensure both field and laboratory contaminants are satisfactorily removed. In their study, the contaminants *Homo sapiens* and *Canis lupus* were detected in negative control samples and could be removed from the dataset under the assumption that these occurred due to human-induced contamination. However, other instances of environmental (e.g., food products or livestock such as cows, pigs, and chickens) and/or laboratory-based contamination (e.g., Tasmanian Devil from a co-occurring lab project) identified through bioinformatic processes were not consistently detected in sampling or laboratory controls. This demonstrates the necessity for using negative control samples alongside rigorous sterile lab procedures and careful bioinformatic interpretations.

4.7. Primers

The choice of PCR primers is one of the crucial steps in any eDNA study design as it will define the type of data to be analysed (Alberdi et al., 2018). With the primary goal to provide a free open access source of information on this crucial aspect, we compiled the largest PCR primer database to date, providing information on 522 single-species and 141 metabarcoding primers assays used in aquatic eDNA research (see Tables S3 and S4). We also listed the most frequently used metabarcoding primers over the period 2012–2021 (Table 1). This database provides a ‘distillation’ of primer information that was hitherto scattered across hundreds of papers. It can be used to guide eDNA users towards optimal primer choice for further studies, and prevent re-developing primers that already exist but are not well-known.

MiFish-U was the most commonly used metabarcoding assay ($N = 47$), followed by 12S-V5-a ($N = 17$) and Teleo ($N = 16$), all of which were primarily used to target fish taxa eDNA (Table 1). Together, these accounted for 67 % of all instances of metabarcoding assays found in this systematic review. Such assays have proven especially useful for detecting unknown marine pests (e.g., Alam et al., 2020), and exploratory studies where little is known about the taxa or diversity present (e.g., Valsecchi, 2021). The popularity of these assays is also likely driven by the expanding reference databases developed for the chosen target barcode regions (similar to the preference for COI assays for insects; see Elbrecht et al., 2016; Polanco et al., 2021).

Our primer database also highlights well-studied and under-studied taxa. The most frequently targeted taxa in species-specific studies were

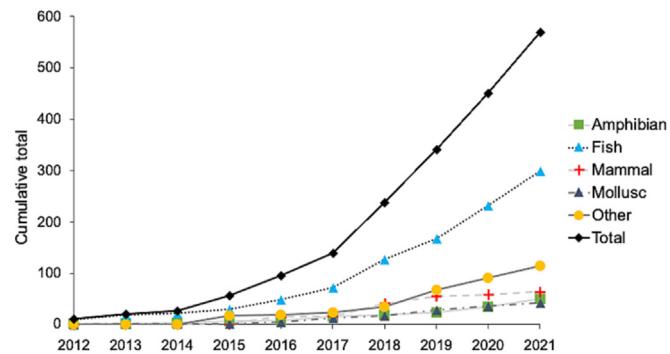


Fig. 7. Cumulative number of species-specific primers developed between 2012 and 2021. “Other” category includes algae, coral, eukaryotes, molluscs, plants, mammals, fungi, metazoans, reptiles and sponges.

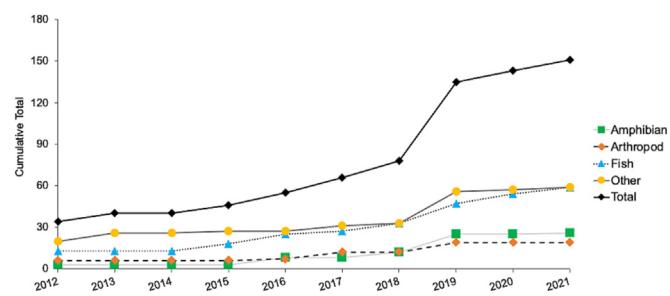


Fig. 8. Cumulative number of metabarcoding primers developed between 2012 and 2021. “Other” category includes algae, coral, eukaryotes, molluscs, plants, mammals, fungi, metazoans, reptiles and sponges.

fish (52 %), mammals (11 %) and amphibians (9 %) (Fig. 7). The most commonly targeted taxa in metabarcoding studies were fish (39 %), amphibians (18 %) and arthropods (12 %) (Fig. 8). The bias towards fish is a reflection of their commercial and ecosystem value (e.g., regulation of food web dynamics; e.g., Lynch et al., 2016). The frequent targeting of amphibian species by both approaches is unsurprising given that this group faces a high extinction risk (Stuart et al., 2004) and are thus the target of intense biomonitoring in many regions. Mammals and arthropods were also found to be well-studied in species-specific and metabarcoding studies, respectively.

Table 1

List of the 10 most commonly used metabarcoding primers in aquatic eDNA research within our given search criteria. The number of publications column excludes the original reference. Please refer to Tables S3 and S4 for the full list of primers considered in this review. Primers which do not explicitly specify forward or reverse direction in their name are shown as ‘(F)’ and ‘(R)’.

Target taxa	Primer name	5' to 3' end	Original reference	Target region	Amplicon size, bp	Number of publications
Fish	MiFish-U-F	GTCGGTAAACACTCGTGCCAGC	Miya et al., 2015	12S	163–185	47
	MiFish-U-R	CATAGTGGGTATCTAACCCAGTTG				
Vertebrate	12S-V5 (F)	ACTGGATTAGATACCCC	Riaz et al., 2011	12S	85–117	17
	12S-V5 (R)	TAGAACAGCTCTCTAG				
Fish	teleo_F	ACACCGCCGTCACCTCT	Valentini et al., 2016	12S	Under 100	16
	teleo_R	CTTCGGTACACTTACCATG				
Metazoan	mlCOLintF	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al., 2013	COI	313	13
	jgHCO2198	TAIACYTCIGGRTGICCRAARAAYCA	Geller et al., 2013			
Sharks/Rays	MiFish-E-F	GTITGGTAAATCTGGTCCAGC	Miya et al., 2015	12S	170–185	9
	MiFish-E-R	CATA GTGGGTATCTAACCTAGTTG				
Fish	Fish16SF/D	GACCTTATGGAGCTTAGAC	Berry et al., 2017	16S	Approx. 200	8
	16s2R (degenerate)	CGCTGTATCCCTADRGTAACT	Deagle et al., 2007			
Vertebrate	12S-V5 (F)	TAGAACAGGCTCTCTAG	Riaz et al., 2011	12S	73–110	4
	12S-V5 (R)	TTAGATACCCCACTATGC				
Fish	Tele02 (F)	AAACTCGTGCCAGCCACC	Taberlet et al., 2012	12S	129–167	4
	Tele02 (R)	GGGTATCTAACCCAGTTG				
Vertebrate	L14735	AAAAACCAACCGTTATTCAACTA	Burgener and Hübner, 1998	Cytb	464	3
	H15149	GCCCTCAGAATGATAATTGTCTCA				
Metazoan	mlCOLintF	GGWACWGGWTGAACWGTWTAYCCYCC	Leray et al., 2013	COI	313	3
	dgHCO-2198	TAAACTCAGGGTGACCAAARAAYCA	Meyer, 2003			

The former perhaps because of their charismatic nature and role as key-stone species (e.g., Pinfield et al., 2019), and the latter because of their key functional role in aquatic ecosystems (e.g., nutrient cyclers; e.g., Phillips et al., 2013).

Although the range of aquatic organisms that have been studied using eDNA has increased since 2014 (Fig. 7), some groups and taxa with ecologically significant functions have had surprisingly few primers developed. For instance, no metabarcoding assays and only one species-specific assay has been developed for parasitic aquatic organisms such as nematodes. This represents a significant omission from the current aquatic eDNA primer library given that 33 % of the known nematode genera are estimated to parasitise marine and freshwater vertebrates (Anderson, 2000). Further, parasites such as nematodes can lead to economic losses due to increased mortality and treatment costs, of for example fish (Anderson, 2000; Ajah et al., 2020). Similarly, sea sponges, although recognised as critical for aquatic habitat supply and nutrient cycling (Bell, 2008; Vad et al., 2018), have only two associated metabarcoding assays (Table S4). The absence of primers targeting these groups reduces our capacity to monitor certain ecologically highly important taxa and will slow the development of comprehensive reference sequence databases (Lim et al., 2016). Until these molecular tool kits are expanded, management decisions for either emerging parasites for aquaculture or sponge habitat loss from human activities (see Bell et al., 2015) will be reliant on manual methods (i.e., visual surveys) and limited reference databases, potentially increasing the opportunity for mismanagement and further economic losses.

The numbers of available species-specific and metabarcoding primers have increased markedly since 2012 (Figs. 7 and 8) as the potential of eDNA technology has gained greater attention (Taberlet et al., 2012). The number of annual eDNA publications has increased exponentially (Fig. S1), and a parallel increase in primers is evident. The majority (83 %) of species-specific primers was developed between 2017 and 2021 (Fig. 7) while 85 % of the metabarcoding assays were developed between 2012 and 2019 (Fig. 8). Nonetheless, this pace of development and designing of new assays is likely to slow as current primers and corresponding reference sequence databases are trialled in a variety of habitats and ecological contexts to determine their reliability and limitations.

Combinations of assays are needed to maximise the diversity captured with water samples. eDNA surveys can miss certain taxa (i.e., small-bodied or cryptic) if assays are too broad as they are likely to amplify more abundant DNA at early PCR cycling stages and less abundant DNA are masked, leading to incomplete community descriptions (Nester et al., 2020; Valdivia-Carrillo et al., 2021). Instead, combinations of metabarcoding and species-specific assays can be used, described as the ‘needle versus haystack’ approach (Saccò et al., 2022). Here, the ‘haystack’ generates preliminary taxonomic data using existing metabarcoding assays, which amplify both known and unknown sequences, from a broad range of taxa (generally identified to the genus or family level; e.g., Miya et al., 2015). Any unknown sequences detected can potentially be verified with additional traditional surveys, linking visual observations to genetically-unknown taxa present in the water sample (Kelly et al., 2017). If these taxa are of interest, they can then be targeted with the ‘needle’ approach with species-specific assays (Saccò et al., 2022). The development of primers for these metabarcoding and single-species approaches often uses commercial software packages. Our review suggests that Mesquite (Maddison and Maddison, 2018), ecoPrimers (Riaz et al., 2011), BioEdit (Hall, 1999) and PrimerMiner (Elbrecht and Leese, 2017) were the most common software packages. The in silico, in vivo and in vitro approaches used to test and verify these assays are reviewed in De Brauwer et al. (2022).

Although eDNA work is generally considered time efficient and relatively inexpensive, new methodological and developmental approaches such as ddPCR or lateral flow devices (Thomas et al., 2020; Doyle and Uthicke, 2021) are emerging, which may improve the efficiency and cost-effectiveness of eDNA even further. The incorporation of two or more assays in the same PCR reaction, known as multiplexing or ‘primer cocktails’, can help overcome the general bias of any one specific assay for

taxonomically diverse samples while simultaneously minimising the workload required when using two or more individual assays (Ivanova et al., 2007; Becker et al., 2011). Multiplexing has been shown to reliably identify fish taxa from muscle tissue (see Ivanova et al., 2007) and aid in seafood labelling (see Shehata et al., 2019). Unfortunately, the use of multiplexing remains limited in aquatic eDNA contexts (<10 % of the 141 metabarcoding assays, see Table S4). This underutilisation likely reflects the often-lengthy development times for multiplexing, to identify common PCR conditions that work for multiple primers together, and to evaluate the extent of co-inhibition and primer dimer formation (see Jennings, 2016). As such, multiplexing may prove most useful when combined with traditional barcoding of tissue samples to rapidly generate custom reference databases required for the sequence identification phase in eDNA studies (Vences et al., 2012; Prosser et al., 2013).

5. Discussion

In response to increased pollution, habitat loss, invasive species and overall degradation of entire aquatic ecosystems linked to for example climate change, efficient and accurate biomonitoring surveys are becoming increasingly important (Hampton et al., 2013). As a result, there is a need to develop and improve tools that can expand aquatic ecosystem monitoring capabilities, and molecular methods are among the most promising tools (Pawlowski et al., 2021). The utility of sequencing eDNA for the monitoring of aquatic ecosystems has been widely and increasingly demonstrated over the last decade, and when carefully applied, eDNA methods are now sufficiently advanced to study the taxonomic composition of aquatic communities and thus complement traditional surveying methods which can be more invasive and/or time demanding (Rees et al., 2014). However, with new eDNA data released at an incredible rate and new methodologies presented every month, we find ourselves in the middle of an exponential ‘explosion’ of eDNA outputs. Therefore, it seems crucial to pause for a moment and ponder on the (recent) past, present and future of this technology.

The immense potential for eDNA applications has led to a rapid expansion of the variety of laboratory methods developed and trialled over the period 2012–2021. A key observation coming out of our review is that the laboratory methods used varied extensively among the reviewed studies, and also diversified dramatically across the time span targeted. For instance, samples were collected in a range of water volumes, with 500 mL to 1000 mL being the most widely used, with one to more than six replicates taken at each sampling point (however, by 2021 60 % of the studies used three replicates or less). In addition, the number of preservation techniques increased, as well as the proportion of studies filtering water on site (alternatively to freezing). Active filtering through a membrane using either a vacuum or peristaltic pump was the most common DNA collection method documented, but the use of a syringe for DNA collection is increasing in popularity. We also recorded the use of multiple filter pore sizes, with pore sizes of 0.45 µm and 0.2 µm being the most commonly used (usually stored at –20 °C) and the DNeasy Blood and Tissue kit was the most commonly used extraction method. Unfortunately, no consensus in terms of the reporting (if any) of negative control was evident. For both metabarcoding and species-specific assays, fish taxa were overwhelmingly the most targeted in the papers reviewed. Below we will focus the discussion of our findings into 17 important aspects of the methodological approach for eDNA studies, pinpoint knowledge gaps and explore some of the most prominent research avenues that the field might experience in the near future.

5.1. Methods for aquatic eDNA analysis: what to use and when

Our data identified the most commonly used methods in each sampling and laboratory step, which are usually well-established and recommended as standard procedures, sometimes published as regional guidelines (e.g., Australia and New Zealand – De Brauwer et al., 2022; Japan – Minamoto et al., 2021). In many cases, however, they are not necessarily

the optimal approaches. For instance, filtering water samples with electronic pumps and preserving filter papers in the freezer - the most common approach - can be logistically challenging when collecting samples in remote areas with limited infrastructure. Manually filtering water with a syringe and preserving any eDNA deposited on the filter in a lysis buffer at ambient temperature is one way to overcome these constraints. Syringe filtration with enclosed filters provides further advantages such as low costs (not requiring electronic pumps) and minimal contamination risks, making it ideal for sampling by non-experts (e.g., local communities in citizen science programs or rangers in monitoring programs). However, there are disadvantages associated with the syringe method, including limited volumes that can be filtered, and limited replicate number, which is potentially further reduced when water is turbid because the filters are clogging. Passive filtration - the collection of eDNA through submerged filters - may provide the solution to the above issues (Bessey et al., 2021), although this approach was only recently presented and further testing in a range of aquatic systems using different submersion times and sampling materials is required.

As evident from this review there is no 'one size fits all' approach to aquatic eDNA analyses, and aspects of survey design including sample replicate number, water volumes, preservation and extraction methods should all be carefully considered when defining study-specific goals (Fig. 9). This is particularly important when high levels of accuracy are required (i.e., detecting high impact invasive species or conservation priority species), and/or when setting up long-term monitoring programs.

We suggest that there are six key factors to consider when establishing the number of replicates and water volume in the basic study design (Fig. 9):

- i) Characteristics of target taxa and/or community that will determine the concentration of target eDNA (i.e., abundance, diversity, behaviour, assumed DNA shedding rates) and environmental factors (i.e., water temperature, UV exposure, water movement).
- ii) Variability of DNA concentration over space/time at a sampling site - higher variability should foster more replicates to counter the risk of sampling error.
- iii) Water turbidity - if turbid, sampling high volumes of water is often not feasible, hence sampling smaller volumes across more filters is recommended.
- iv) Levels of required accuracy for study purposes - i.e., high accuracy is required to detect the presence or absence of high impact pest species for legal and management purposes, whereas more uncertainty is acceptable in a community outreach program for education purposes.
- v) When assessing biodiversity, a pilot study should be conducted to assess minimum replicate requirements - species accumulation curves should be also examined and reported, and careful interpretation of results is required if replicates are limited. Published work on similar taxa or in similar environments can be used to support decision making when project resources preclude pilot studies.
- vi) Using the guidelines above, adapt the spatial and temporal scales of the project to match the available budget without compromising the science.

The seven key factors to consider when determining the sampling, preservation and extraction methods are (Fig. 9):

- i) Distance from sampling sites to filtration location - carrying battery-operated pumps to sampling sites or water samples to the filtration location can be difficult or impossible, in which case syringe sampling or passive filtration is recommended.
- ii) Time from water sampling to filtration - cooling or chemical preservation of water samples is required if not filtered immediately. If pre-filtering preservation is required for >24 h, then water samples can be frozen, yet it is recognised that repeated freeze-thaw cycles significantly degrade DNA (Chung et al., 2017).
- iii) The number of replicates and water volume (see above), which defines the feasibility of doing on-site filtration and which filtration methods to use.

- iv) Experience level of sampling personnel - if non-experts are involved (i.e., citizen scientists, rangers), syringes with enclosed filters are recommended for ease of use and minimisation of DNA contamination.
- v) Time from filtration to extraction, which defines the optimal preservation methods after DNA collection. When possible, minimising the interval between these two stages is highly recommended for enhancing the quality of the environmental DNA targeted in the sequencing phase.
- vi) Water turbidity - if turbid, water volume needs to be reduced (see above), or apply filtration methods alternative to pumps or syringes such as passive filtration. DNA extraction methods with PCR-inhibitor removal are recommended.
- vii) Available resources - i.e., syringe filtration and custom extraction protocols are cheaper than purchasing electronic pumps and commercial kits, hence recommended when a projects budget is limited.

Finally, the four key factors for selecting primers (see our primer database for further assistance, Tables S3 and S4) and negative controls for each study are (Fig. 9):

- i) Targeted taxa - carefully select primers that match the project goals and have a demonstrated capability to detect the species of interest, whether this is broad scale metabarcoding or species-specific.
- ii) The coverage rates of the reference database - check that the target species or community has sufficient database coverage allowing sequence query and identification at the accuracy level required for the study purpose.
- iii) Target fragment length - if the target DNA concentration in the sample is assumed to be poor then it can be beneficial to select (or re-develop) primers targeting shorter inserts. A pilot study can reveal if this is required.
- iv) Negative controls - reliable options (ideally used in combination) include sampling, extraction and rinse water controls.

5.2. Most recent developments and future research avenues

We have focused this systematic review paper on the developments within the first decade after the transforming 2012 publications. However, reflecting briefly on the most recent (post 2021) developments, we note that a total of 131 research manuscripts on aquatic eDNA were published between the 1st of January 2022 and the 19th of January 2023. The year 2022 saw a minimal decrease (- 1 manuscript) in the number of published research articles compared to 2021, which is in contrast to the exponential increase we documented from 2018 to 2021 (Fig. S1). This may be coincidental, linked to the impact of COVID-19 on research outputs (Abramo et al., 2022), or it may also reflect that aquatic eDNA research is still at a transitional stage and perhaps towards an early stage of maturity (Schenekar, 2022), despite becoming increasingly applied in conservation efforts related to marine (Hinz et al., 2022) and freshwater ecosystems (Huang et al., 2022). Interestingly, while the vast majority of the 2022/2023 papers focused on marine environments, previously underexplored habitats such as tree hole water (Mullin et al., 2022), caves (Hashemzadeh Segherloo et al., 2022) and hypersaline lakes (Campbell et al., 2023) were also targeted recently with eDNA approaches. In addition, macroscale studies at country level (e.g., Andoh et al., 2022; King et al., 2022), together with investigations targeting major infrastructure - e.g., the world's largest constructed water diversion (Wang et al., 2022) - consolidated in the literature. New software (e.g., Jeunen et al., 2022), R packages (e.g., Espe et al., 2022) and workflow pipelines (e.g., Thompson et al., 2022) were also tested and released recently, with machine learning approaches gaining popularity in the field (Kimura et al., 2022; Kronenberger et al., 2022).

One of the most surprising findings in recent literature is perhaps the vast number of aquatic eDNA literature reviews and perspectives during

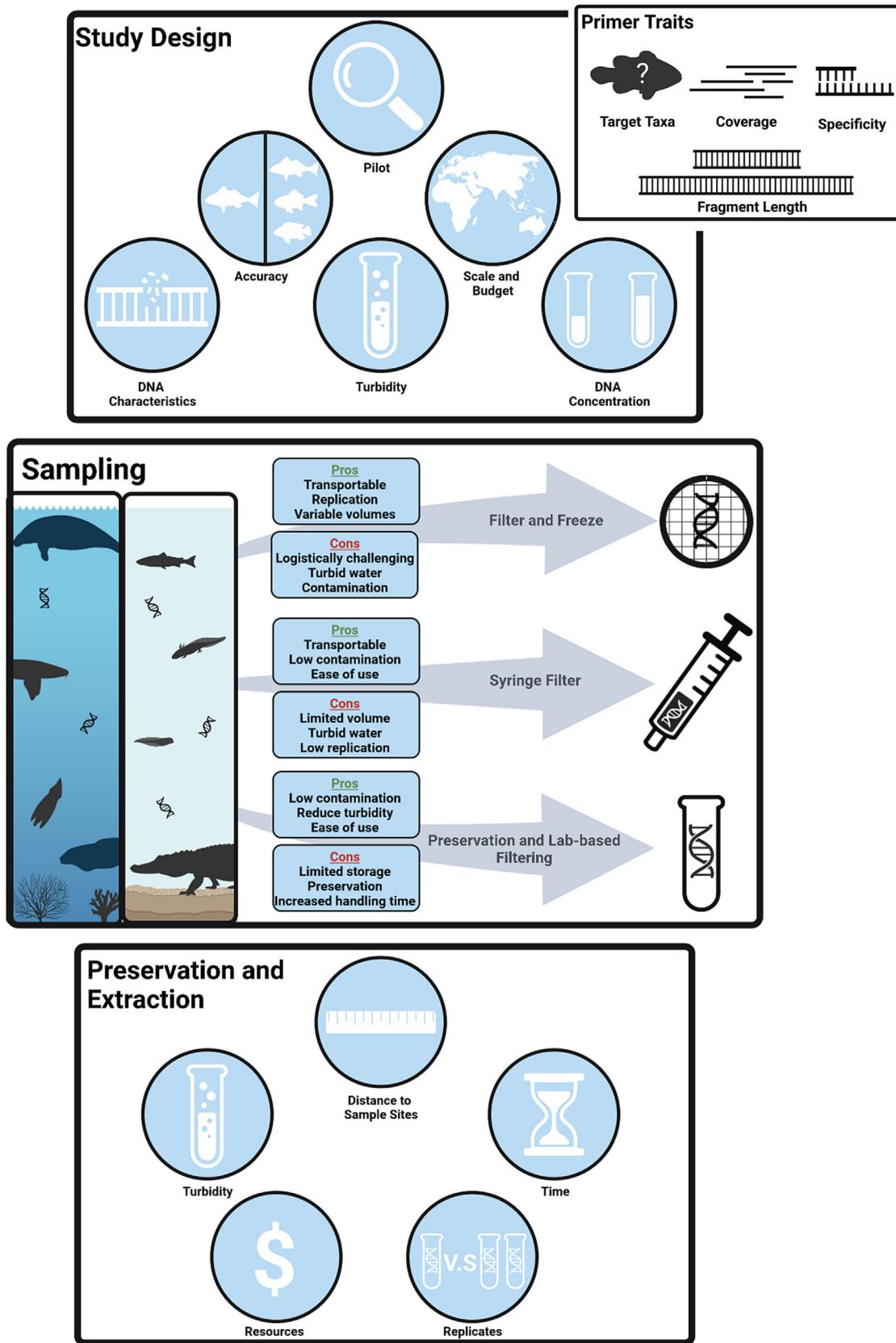


Fig. 9. A summary of conceptual and practical guidelines for the overall study design (top), sampling (middle), and preservation and DNA extraction (bottom).

this time (24 in total between the 24th of January 2022 and the 19th of January 2023). In brief, reviews targeting all the main aquatic biomes were published: marine (Suarez-Bregua et al., 2022), freshwater (Schenekar,

2022), estuaries (Nagarajan et al., 2022) and groundwater (Saccò et al., 2022). Similar to research manuscripts, perspectives and reviews principally focused on native fish (Yao et al., 2022; Xiong et al., 2022) but also

amphibians (Moss et al., 2022), plants (Pollitt et al., 2022) and invasive species more broadly were investigated (e.g., Sengupta et al., 2022). While this may seem excessive, it is likely reflecting a field that develops very rapidly across and thus has an urgent need to continually consolidate a very fast-growing pool of knowledge.

Some of these recent reviews are highlighting eDNA metabarcoding as an innovative monitoring technique capable of detecting multiple species in a non-invasive, efficient and often semi-quantifiable way (e.g., Antognazza et al., 2021; Minamoto, 2022 and references therein; Luo et al., 2023). Richards et al. (2022) suggests that if upscaled, eDNA approaches can potentially transform our understanding of how human and climate impacts affect biodiversity across global, regional and local scales.

With the current momentum of eDNA as a biomonitoring tool, the future prospects are vast. Indeed, further development of current novel but underused techniques/approaches, such as 3D-printed samplers (Verdier et al., 2022), autonomous sampling (Truelove et al., 2022), gravity filtration (Oka et al., 2022), and use of mock communities (Coghlan et al., 2021) host great potential for the advancement of the research field. While the detection of fish has dominated aquatic eDNA research to date (Figs. 7, 8 and Fig. S6), future research efforts are likely to expand into less commercially-important taxa of high ecosystem and/or conservational importance such as corals, macroinvertebrates or amphibians. Furthermore, the ability to obtain entire metagenomes (Pinfield et al., 2019; Roesma et al., 2021), population genetic information (see Adams et al., 2019) or the potential uses of environmental RNA (eRNA; Miyata et al., 2021; Tsuri et al., 2021; Veilleux et al., 2021) provides exciting new research opportunities. Where eDNA represents fragmented genomes ‘floating around’ in the environment, eRNA represents only the expressed parts of those genomes (the transcriptome) – and this expression is influenced by both internal and external factors (i.e., stressors) experienced by the organism. Because RNA decays faster than DNA, eRNA should hypothetically reflect how the genome is being expressed in that particular environment, at that particular time. Combination of eDNA/eRNA-based approaches have been proved successfully in targeting aquatic parasites (Mérou et al., 2020), pathogens (Amarasiri et al., 2021), with further evidence that eRNA could outperform eDNA as an ecotoxicological tool assessing impact of marine pollution (Greco et al., 2022). Hence, with an eRNA component included, eDNA biomonitoring is likely to become a tool for assessing ecosystem health in real-time (Veilleux et al., 2021).

Further technological advancements will be essential for the elimination of many issues related to the sampling process. For example, purpose-built, portable sampling equipment such as fully integrated backpack water sampling systems (Smith-Root eDNA Sampler) allow for increased efficiency and replicability of eDNA sample collection while also reducing contamination due i.e., less exposure to the surrounding environment while sampling (Thomas et al., 2018; Pope et al., 2020). The autonomous acquisition and preservation of eDNA either with in-situ subsurface automated samplers (e.g., Brandt et al., 2021; Formel et al., 2021) or in combination with unmanned aerial vehicles (UAV; e.g., Doi et al., 2017) or autonomous underwater vehicles (AUV; e.g., Yamahara et al., 2019) is also gaining momentum. Advancements in this area would result in increased standardisation of the eDNA collection process, but also allow for thorough and repeated sampling in difficult to access locations (e.g., deep sea floor), while reducing the current time and effort often spent in the field both collecting and filtering samples.

As with any advancing field, a continual optimization, validation, and comparison of aquatic eDNA methods is essential to ensure accurate and reliable data. It is also critical to identify the limitations of the technology, such as limited application for quantitative analyses (i.e., abundance, size, life stage of organisms) and misclassification and/or lower taxonomic resolution due to incomplete reference database. eDNA can be considered as an additional tool to the existing set of biomonitoring approaches developed over the last century when these limitations are critical for study purposes (Gold et al., 2021).

While this review highlights the large number of methods already available, protocols to fit numerous different environmental conditions and taxa

must continue to be developed in order for the field to advance. In doing so, this will ensure that researchers, industry, and government bodies can utilize eDNA biomonitoring to enhance our understanding of the ecology of aquatic species and ultimately develop more informed management strategies for preserving whole aquatic ecosystems.

5.3. To standardise or not to standardise, that is the eDNA-related question!

Provided the various advantages of eDNA as a biomonitoring tool compared to conventional methods, and eDNA being successfully applied in increasing number of studies, the next question we naturally come across is “can we standardise eDNA protocols?”. Standardised protocols can provide numerous benefits such as enabling cross-study data comparison and thus studies at larger temporal and spatial scales. They can also serve as a “beginner’s guide” for non-eDNA experts to easily and correctly implement eDNA components in their studies. Indeed, efforts to implement standardisation and best practice guides for eDNA workflows have already been made at a regional level (Pawlowski et al., 2020b; Bruce et al., 2021; Minamoto et al., 2021; De Brauwer et al., 2022), however these guides are not universally recognised or implemented.

In contrast, the tremendous diversification of eDNA methods revealed in our review suggests the challenges to implement universal standardised guidelines. Arguably, this is partly explained by differences in physical and chemical properties across environments (Pawlowski et al., 2020b), requiring that experimental designs and protocols are continually adjusted to accommodate the study system and the scientific questions (Taberlet et al., 2018). This aspect makes standardisation difficult because there is no true ‘optimal’ methodology or primer set that could be employed universally. Another explanation may be linked to the innovative nature of an emerging field characterised by constant methodological advances that pushes the limits for biodiversity detection. In that sense it is crucial that much-needed standardisation efforts do not hamper future methodological developments, both paths should be pursued with equal ambition. It is therefore important to consider where or how standardisation could be implemented while allowing methodological development to be continued.

A flexible approach is recommended to address such challenges. If a standard was to be implemented, best practice guides developed on a case-by-case basis depending on the study organism, purpose, environment or available infrastructure may be appropriate, with requirements to revisit the protocols regularly (De Brauwer et al., 2022). Furthermore, the comparability of eDNA data would be greatly improved through the standards for data formatting and curation (Berry et al., 2021). This will require a consistent data framework containing detailed information about the methods used to collect the data, supported by the appropriate infrastructure and potentially biobanking (Jarman et al., 2018). There may also be scope for developing mathematical models that could normalise or rarefy results based on how eDNA data was collected, for example, based on water volume, filter pore size, and/or sequencing method.

5.4. Knowledge gaps

eDNA based surveys of aquatic ecosystems are still in a developing stage, but the method holds great future promise. However, as an emerging monitoring technique that is a step-change from conventional survey methods, we highlight four major knowledge gaps identified through our review analysis. First, most method comparison studies were restricted to a few methods under small ranges of conditions (i.e., habitats, targeted taxa), and in most cases significant interactions between the tested factors were identified (Table S2). Therefore, whether the study results are applicable in other systems, conditions, and/or taxa remains unknown.

Second, this is a young research field and the validation and optimisation of many recently developed methods are still in a preliminary stage. For instance, Bessey et al. (2021) developed and validated a passive filtration method that requires minimal time and equipment to collect a large number of replicates, even from turbid water, providing a promising alternative to active filtration. However, in order to effectively and reliably

apply the method to large scale studies such as long-term monitoring projects, an assessment of its efficiency in different environments with different taxa is required - including an evaluation of submerge time and capturing material. Similarly, while freezing filter paper was the most common preservation method, more recent studies have also demonstrated effective filter preservation in lysis buffer at ambient temperature (e.g., Everett and Park, 2018; Jacobs-Palmer et al., 2021; Lafferty et al., 2021). The latter approach provides several advantages including no cooling systems required during storage and transport, minimal DNA degradation by avoiding repeated freeze-thaw cycles, and a quicker process to a downstream DNA extraction step. However, there are various types of lysis buffers available (see Section 4.4. Preservation (post DNA filtration) for further details), but studies that thoroughly compare the capacity of these buffers under different eDNA-related conditions are warranted. A comprehensive study testing the capacity of all available sampling and preservation methods under a variety of environmental conditions would be extremely valuable to this field.

Third, in our review we recognised dozens of studies missing detailed method description, assessment of species accumulation curves, and reporting of sequences found in negative controls. Without data from negative controls for instance, it becomes impossible to determine (i) if wide-reaching DNA contamination occurs, (ii) which taxa that are more likely to be contaminants, (iii) where contamination enters the workflow, and (iv) how to best minimise and/or correct analytically for contamination when it occurs. Such information is critically important not only for the validation of the study in question, but also for baselining methods and sampling schemes in future studies. We would highly recommend that all eDNA-based publications provide detailed descriptions of data from negative control samples. Such consistent reporting would help fill this crucial knowledge gap.

Fourth, our review data including the primer databases identified the most commonly studied taxa (i.e., fish, amphibians) and pointed towards taxa that are rarely studied in aquatic eDNA publications. The reason why specific groups are under-studied may be due to limited commercial interest, lack of robust primers (i.e., corals) and/or low DNA shedding rates making them difficult to detect via eDNA (i.e., sea snakes). Despite the many benefits for incorporating eDNA technologies into biodiversity assessments of coral reefs, there were surprisingly few studies on hard corals, soft corals and sponges (but see Nichols and Marko, 2019; Alexander et al., 2020; West et al., 2022; Dugal et al., 2022; Ip et al., 2022). eDNA-based methods provide promising opportunities for monitoring of signals that are correlated with reproductive activity, spawning species and monitoring coral biodiversity (Alexander et al., 2020; Dugal et al., 2022; Ip et al., 2022). Other understudied aquatic groups that could highly benefit from the application of eDNA techniques from water samples are invertebrates (Harper et al., 2019a, b; Uchida et al., 2020), plants (Pollitt et al., 2022) and also semi-aquatic avian species (Ushio et al., 2018), among others. Increased efforts to improve primer specificity, reference database and efficiency to detect low concentration DNA are required to enhance the robustness of eDNA approaches for such under-studied, yet ecologically important taxa.

6. Conclusions

Keck et al. (2022) recently concluded that eDNA methods to date are “*sufficiently advanced to study the composition of fish communities and replace more invasive traditional methods*”. While conventional biodiversity surveys are still irreplaceable in many environmental and taxonomic contexts, eDNA biomonitoring is here to stay. In fact, eDNA applicability will only grow as new, improved methods become available, rigorous standards are implemented, and reference databases become more exhaustive. Our review has documented an explosion of eDNA studies accompanied by a remarkable diversification of methods over just ten years. While these trends will almost certainly continue, the credibility of this research field will rely on the scientific community to convene on certain standardisation cornerstones to ensure the generation of robust and representative data.

Here we have proposed 17 key factors to consider when implementing aquatic eDNA research – some being direct recommendations while others being more conceptual considerations. We have also pinpointed research gaps and future research avenues that will help evolve the research field. Indeed, given the current human-induced global biodiversity crisis and climate change, eDNA approaches can represent some of the basic tools to document those changes in real time which is an important first step towards the conservation of ecosystems more broadly (Pawlowski et al., 2020b). As stated by Taberlet et al., 2012 “*...future technological advances should lead to better coverage of sequence information and less biased data for optimal applicability ... this approach opens up many possibilities today for the applicability of DNA information in ecology*”. Now, eleven years later, the eDNA field has experienced innumerable technological advances, and many of those “possibilities” are effective applications.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.scitotenv.2023.162322>.

CRediT authorship contribution statement

Miwa Takahashi: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Mattia Saccò:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Visualization, Writing – original draft, Writing – review & editing. **Joshua H. Kestel:** Data curation, Formal analysis, Investigation, Visualization, Writing – original draft. **Georgia Nester:** Data curation, Formal analysis, Investigation, Visualization, Writing – review & editing. **Matthew A. Campbell:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Mieke van der Heyde:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Matthew J. Heydenrych:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **David J. Juszkiewicz:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Paul Nevill:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Kathryn L. Dawkins:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Cindy Bessey:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Kristen Fernandes:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Haylea Miller:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Matthew Power:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Mahsa Mousavi-Derazmahalleh:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Joshua P. Newton:** Data curation, Investigation, Methodology, Validation, Writing – original draft. **Nicole E. White:** Writing – review & editing. **Zoe T. Richards:** Writing – review & editing. **Morten E. Allentoft:** Conceptualization, Investigation, Resources, Supervision, Writing – review & editing.

Data availability

All the data used in the article is available in the Supplementary Tables S1, S2, S3 and S4

Declaration of competing interest

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

Acknowledgements

We acknowledge the traditional owners of the land on which the research presented was undertaken and pay our respects to Elders past, present and emerging. We acknowledge Prof. Mike Bunce and Prof. Euan Harvey for greatly contributing to the development, advancement, and contextualization of aquatic eDNA research at TrEnD Lab and internationally over the last 15 years.

Funding

We thank Curtin University (School of Molecular and Life Science and Research Office at Curtin University) for supporting M.E.A and research in TrEnD Lab. M.S. and N.E.W acknowledge support from the BHP-Curtin alliance within the framework of the “eDNA for Global Environment Studies (eDGES)” programme. M.v.H was supported by the Australian Research Council (Linkage Project LP190100555), and M.T. and K.L.D were supported by eDNA Frontiers at Curtin University.

References

- Abbott, C., Coulson, M., Gagné, N., Lacoursière-Roussel, A., Parent, G.J., Bajno, R., Dietrich, C., May-McNally, S., 2021. Guidance on the use of targeted environmental DNA (eDNA) analysis for the management of aquatic invasive species and species at risk. DFO Can. Sci. Advis. Sec. Res. Doc. 2021/019. iv + 42 p <https://waves-vagues.dfo-mpo.gc.ca/library-bibliothèque/40960791.pdf>.
- Abramo, G., D'Angelo, C.A., Mele, I., 2022. Impact of Covid-19 on research output by gender across countries. *Scientific Reports* 1–16.
- Adam, A.A., Garcia, R.A., Galaiduk, R., Tomlinson, S., Radford, B., Thomas, L., Richards, Z.T., 2021. Diminishing potential for tropical reefs to function as coral diversity strongholds under climate change conditions. *Divers. Distrib.* 27, 2245–2261. <https://doi.org/10.1111/ddi.13400>.
- Adams, C.M., Knapp, M., Gemmell, N.J., Jeunen, G.J., Bunce, M., Lamare, M.D., Taylor, H.R., 2019. Beyond biodiversity: can environmental DNA (eDNA) cut it as a population genetics tool? *Genes* 10, 192. <https://doi.org/10.3390/genes10030192>.
- Ahn, H., 2020. Variation of Japanese eel eDNA in sequentially changing conditions and in different sample volumes. *J. Fish Biol.* 97, 1238–1241. <https://doi.org/10.1111/jfb.14460>.
- Ajah, P.O., Ita, E.O.B., Allison, N.L., 2020. Characterization of nematode infestation on *Parachanna obscura* (Gunther, 1861) (Channidae) and infection in the blood. *Aquac. Stud.* 20. https://doi.org/10.4194/2618-6381-v20_1_07.
- Alam, M.J., Kim, N.K., Andriyono, S., Choi, H., Lee, J.H., Kim, H.W., 2020. Assessment of fish biodiversity in four Korean rivers using environmental DNA metabarcoding. *PeerJ* 8, e9508. <https://doi.org/10.7717/peerj.9508>.
- Alberdi, A., Aizpurua, O., Gilbert, M.T.P., Bohmann, K., 2018. Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods Ecol. Evol.* 9, 134–147. <https://doi.org/10.1111/2041-210X.12849>.
- Alexander, J.B., Bunce, M., White, N., Wilkinson, S.P., Adam, A.A.S., Berry, T., Stat, M., Thomas, L., Newman, S.J., Dugal, L., Richards, Z.T., 2020. Development of a multi-assay approach for monitoring coral diversity using eDNA metabarcoding. *Coral Reefs* 39, 159–171. <https://doi.org/10.1007/s00338-019-01875-9>.
- Allison, M.J., Round, J.M., Bergman, L.C., Mirabzadeh, A., Allen, H., Weir, A., Helbing, C.C., 2021. The effect of silica desiccation under different storage conditions on filter-immobilized environmental DNA. *BMC Res. Notes* 14, 106. <https://doi.org/10.1186/s13104-021-05530-x>.
- Amarasiri, M., Furukawa, T., Nakajima, F., Sei, K., 2021. Pathogens and disease vectors/hosts monitoring in aquatic environments: potential of using eDNA/eRNA based approach. *Sci. Total Environ.* 796, 148810.
- Anderson, R.C., 2000. Nematode Parasites of Vertebrates. Their Development and Transmission. second ed. CABI Publishing, Wallingford, UK.
- Andoh, K., Hidano, A., Sakamoto, Y., Sawai, K., Arai, N., Suda, Y., Mine, J., Oka, T., 2022. Current research and future directions for realizing the ideal One-Health approach: a summary of key-informant interviews in Japan and a literature review. *One Health* 16, 100468.
- Andreasen, J.K., O'Neill, R.V., Noss, R., Slosser, N.C., 2001. Considerations for the development of a terrestrial index of ecological integrity. *Ecol. Indic.* 1, 21–35. [https://doi.org/10.1016/S1470-160X\(01\)00007-3](https://doi.org/10.1016/S1470-160X(01)00007-3).
- Andruszkiewicz, E.A., Starks, H.A., Chavez, F.P., Sassoubre, L.M., Block, B.A., Boehm, A.B., 2017. Biomonitoring of marine vertebrates in Monterey Bay using eDNA metabarcoding. *PLoS One* 12, e0176343. <https://doi.org/10.1371/journal.pone.0176343>.
- Antognazza, C.M., Britton, R.J., Read, D.S., Goodall, T., Mantouratou, A., De Santis, V., Davies, P., Aaphramian, M., Franklin, E., Hardouin, E.A., Andreou, D., 2021. Application of eDNA metabarcoding in a fragmented lowland river: spatial and methodological comparison of fish species composition. *Environ. DNA* 3, 458–471.
- Barnes, M.A., Turner, C.R., 2016. The ecology of environmental DNA and implications for conservation genetics. *Conserv. Gen.* 17, 1–17.
- Becker, S., Hanner, R., Steinke, D., 2011. Five years of FISH-BOL: grief status report. *Mitochondrial DNA* 22, 3–9. <https://doi.org/10.3109/19401736.2010.535528>.
- Bell, J.J., 2008. The functional roles of marine sponges. *Estuar. Coast. Shelf Sci.* 79, 341–353. <https://doi.org/10.1016/j.ecss.2008.05.002>.
- Bell, J.J., McGrath, E., Biggerstaff, A., Bates, T., Cárdenas, C.A., Bennett, H., 2015. Global conservation status of sponges. *Conserv. Biol.* 29, 42–53. <https://doi.org/10.1111/cobi.12447>.
- Beng, K.C., Corlett, R.T., 2020. Applications of environmental DNA (eDNA) in ecology and conservation: opportunities, challenges and prospects. *Biodivers. Conserv.* 29, 2089–2121. <https://doi.org/10.1007/s10531-020-01980-0>.
- Berry, T.E., Osterrieder, S.K., Murray, D.C., Coghlan, M.L., Richardson, A.J., Grealy, A.K., Stat, M., Bejder, L., Bunce, M., 2017. DNA metabarcoding for diet analysis and biodiversity: a case study using the endangered Australian sea lion (*Neophoca cinerea*). *Ecol. Evol.* 7, 5435–5453. <https://doi.org/10.1002/ece3.3123>.
- Berry, O., Jarman, S., Bissett, A., Hope, M., Paepke, C., Bessey, C., Schwartz, M.K., Hale, J., Bunce, M., 2021. Making environmental DNA (eDNA) biodiversity records globally accessible. *Environ. DNA* 3, 699–705. <https://doi.org/10.1002/edn3.173>.
- Bessey, C., Jarman, S.N., Berry, O., Olsen, Y.S., Bunce, M., Simpson, T., Power, M., McLaughlin, J., Edgar, G.J., Keesing, J., 2020. Maximizing fish detection with eDNA metabarcoding. *Environ. DNA* 2, 493–504. <https://doi.org/10.1002/edn3.74>.
- Bessey, C., Neil Jarman, S., Simpson, T., Miller, H., Stewart, T., Kenneth Keesing, J., Berry, O., 2021. Passive eDNA collection enhances aquatic biodiversity analysis. *Commun. Biol.* 4, 236. <https://doi.org/10.1038/s42003-021-01760-8>.
- Bessey, C., Gao, Y., Truong, Y.B., Miller, H., Jarman, S.N., Berry, O., 2022. Comparison of materials for rapid passive collection of environmental DNA. *Mol. Ecol. Resour.* 22, 2559–2572. <https://doi.org/10.1111/1755-0998.13640>.
- Boivin-Delisle, D., Laporte, M., Burton, F., Dion, R., Normandeau, E., Bernatchez, L., 2021. Using environmental DNA for biomonitoring of freshwater fish communities: comparison with established gillnet surveys in a boreal hydroelectric impoundment. *Environ. DNA* 3, 105–120. <https://doi.org/10.1002/edn3.135>.
- Brandt, M.I., Pradillon, F., Trouche, B., Henry, N., Liatard-Haag, C., Cambon-Bonavita, M.-A., Cueff-Gauchard, V., Wincker, P., Belsier, C., Poulaire, J., Arnaud-Haond, S., Zeppilli, D., 2021. Evaluating sediment and water sampling methods for the estimation of deep-sea biodiversity using environmental DNA. *Sci. Rep.* 11, 7856. <https://doi.org/10.1038/s41598-021-86396-8>.
- Broadhurst, H.A., Gregory, L.M., Bleakley, E.K., Perkins, J.C., Lavin, J.V., Bolton, P., Browett, S.S., Howe, C.V., Singleton, N., Tansley, D., Sales, N.G., McDevitt, A.D., 2021. Mapping differences in mammalian distributions and diversity using environmental DNA from rivers. *Sci. Total Environ.* 801, 149724. <https://doi.org/10.1016/j.scitotenv.2021.149724>.
- Bruce, K., Blackman, R., Bourlat, S.J., Hellström, A.M., Bakker, J., Bista, I., Bohmann, K., Bouchez, A., Brys, R., Clark, K., Elbrecht, V., Fazi, S., Fonseca, V., Häneling, B., Leese, F., Mächler, E., Mahon, A.R., Meissner, K., Panksep, K., Pawłowski, J., Schmidt Yáñez, P., Seymour, M., Thalinger, B., Valentini, A., Woodcock, P., Traugott, M., Vasselon, V., Deiner, K., 2021. A Practical Guide to DNA-based Methods for Biodiversity Assessment. Pensoft Publishers, Sofia, Bulgaria <https://doi.org/10.3897/ab.e68634>.
- Burgener, M., Hübner, P., 1998. Mitochondrial DNA enrichment for species identification and evolutionary analysis. *Z. Lebensm. Unters. Forsch. A* 207, 261–263. <https://doi.org/10.1007/s002170050329>.
- Burger, J., 2006. Bioindicators: a review of their use in the environmental literature 1970–2005. *Environ. Bioindic.* 1, 136–144. <https://doi.org/10.1080/15555270600701540>.
- Buxton, A., Matechou, E., Griffin, J., Diana, A., Griffiths, R.A., 2021. Optimising sampling and analysis protocols in environmental DNA studies. *Sci. Rep.* 11, 11637. <https://doi.org/10.1038/s41598-021-91166-7>.
- Bylemans, J., Gleeson, D.M., Hardy, C.M., Furlan, E., 2018a. Toward an ecoregion scale evaluation of eDNA metabarcoding primers: a case study for the freshwater fish biodiversity of the Murray-Darling basin (Australia). *Ecol. Evol.* 8, 8697–8712. <https://doi.org/10.1002/ee.3487>.
- Bylemans, J., Gleeson, D.M., Linternmans, M., Hardy, C.M., Beitzel, M., Gilligan, D.M., Furlan, E.M., 2018b. Monitoring riverine fish communities through eDNA metabarcoding: determining optimal sampling strategies along an altitudinal and biodiversity gradient. *Metabarcod. Metagenom.* 2, e30457. <https://doi.org/10.3897/mbmg.2.30457>.
- Campbell, M.A., Laini, A., White, N.E., Saccò, M., 2023. When nets meet environmental DNA metabarcoding: integrative approach to unveil invertebrate community patterns of hypersaline lakes. *J. Oceanol. Limn.* <https://doi.org/10.1007/s00343-022-2151-9>.
- Cantera, I., Cilleros, K., Valentini, A., Cerdán, A., Dejean, T., Iríbar, A., Taberlet, P., Vigouroux, R., Brosse, S., 2019. Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers. *Sci. Rep.* 9, 3085. <https://doi.org/10.1038/s41598-019-39399-5>.
- Capo, E., Spong, G., Königsson, H., Byström, P., 2020. Effects of filtration methods and water volume on the quantification of brown trout (*Salmo trutta*) and Arctic char (*Salvelinus alpinus*) eDNA concentrations via droplet digital PCR. *Environ. DNA* 2, 152–160. <https://doi.org/10.1002/edn3.52>.
- Cappo, M., Speare, P., De'ath, G., 2004. Comparison of baited remote underwater video stations (BRUVS) and prawn (shrimp) trawls for assessments of fish biodiversity in inter-reef areas of the Great Barrier Reef Marine Park. *J. Exp. Mar. Biol. Ecol.* 302, 123–152. <https://doi.org/10.1016/j.jembe.2003.10.006>.
- Carim, K.J., Dysthe, J.C., Young, M.K., McKelvey, K.S., Schwartz, M.K., 2017. A noninvasive tool to assess the distribution of Pacific lamprey (*Entosphenus tridentatus*) in the Columbia River basin. *PLoS One* 12, e0169334. <https://doi.org/10.1371/journal.pone.0169334>.
- Chen, Z., Minamoto, T., Lin, L., Gao, T., 2020. An optional low-cost method of extracting environmental DNA macro-organisms from filter membranes in large scale eDNA surveys. *Pak. J. Zool.* 53. <https://doi.org/10.17582/journal.pjz/20190118100108>.
- Chen, X., Kong, Y., Zhang, S., Zhao, J., Li, S., Yao, M., 2022. Comparative evaluation of common materials as passive samplers of environmental DNA. *Environ. Sci. Technol.* 56, 10798–10807. <https://doi.org/10.1021/acs.est.2c02506>.
- Chung, W.J., Cui, Y., Chen, C.S., Wei, W.H., Chang, R.S., Shu, W.Y., Hsu, I.C., 2017. Freezing shortens the lifetime of DNA molecules under tension. *J. Biol. Phys.* 43, 511–524. <https://doi.org/10.1007/s10867-017-9466-3>.
- Cilleros, K., Valentini, A., Allard, L., Dejean, T., Etienne, R., Grenouillet, G., Iríbar, A., Taberlet, P., Vigouroux, R., Brosse, S., 2019. Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): a test with Guianan freshwater fishes. *Mol. Ecol. Resour.* 19, 27–46. <https://doi.org/10.1111/1755-0998.12900>.
- Clusa, L., Miralles, L., Basanta, A., Escot, C., García-Vázquez, E., 2017. eDNA for detection of five highly invasive molluscs. A case study in urban rivers from the Iberian Peninsula. *PLoS One* 12, e0188126. <https://doi.org/10.1371/journal.pone.0188126>.
- Coghlan, S.A., Currier, C.A., Freeland, J., Morris, T.J., Wilson, C.C., 2021. Community eDNA metabarcoding as a detection tool for documenting freshwater mussel (*Unionidae*) species assemblages. *Environ. DNA* 3, 1172–1191.
- Cohn, F., 1853. Über lebende organismen im trinkwasser. *Günsbergs Z. Für Klin. Med.* 4, 229–237.

- Cooper, M.K., Villacorta-Rath, C., Burrows, D., Jerry, D.R., Carr, L., Barnett, A., Huvveneers, C., Simpfendorfer, C.A., 2022. Practical eDNA sampling methods inferred from particle size distribution and comparison of capture techniques for a critically endangered elasmobranch. *Environ. DNA* 4, 1011–1023. <https://doi.org/10.1002/edn3.279>.
- Coulter, D.P., Wang, P., Coulter, A.A., Van Susteren, G.E., Eichmiller, J.J., Garvey, J.E., Sorensen, P.W., 2019. Nonlinear relationship between silver carp density and their eDNA concentration in a large river. *PLoS One* 14, e0218823. <https://doi.org/10.1371/journal.pone.0218823>.
- Coutant, O., Richard-Hansen, C., de Thoisy, B., Decotte, J., Valentini, A., Dejean, T., Vigouroux, R., Murienne, J., Brosse, S., 2021. Amazonian mammal monitoring using aquatic environmental DNA. *Mol. Ecol. Resour.* 21, 1875–1888. <https://doi.org/10.1111/1755-0998.13393>.
- Czachur, M.V., Seymour, M., Creer, S., Heyden, S., 2022. Novel insights into marine fish biodiversity across a pronounced environmental gradient using replicated environmental DNA analyses. *Environ. DNA* 4, 181–190. <https://doi.org/10.1002/edn3.238>.
- D'Alessandro, S., Mariani, S., 2021. Sifting environmental DNA metabarcoding data sets for rapid reconstruction of marine food webs. *Fish Fish.* 22, 822–833. <https://doi.org/10.1111/faf.12553>.
- David, B.O., Fake, D.R., Hicks, A.S., Wilkinson, S.P., Bunce, M., Smith, J.S., West, D.W., Collins, K.E., Gleeson, D.M., 2021. Sucked in by eDNA – a promising tool for complementing riverine assessment of freshwater fish communities in aotearoa New Zealand. *N. Z. J. Zool.* 48, 217–244. <https://doi.org/10.1080/03014223.2021.1905672>.
- Davis, A.J., Williams, K.E., Snow, N.P., Pepin, K.M., Piaggio, A.J., 2018. Accounting for observation processes across multiple levels of uncertainty improves inference of species distributions and guides adaptive sampling of environmental DNA. *Ecol. Evol.* 8, 10879–10892. <https://doi.org/10.1002/ece3.4552>.
- De Brauwer, M., Chariton, A., Clarke, L.J., Cooper, M.K., DiBattista, J., Furlan, E., Giblot-Ducray, D., Gleeson, D., Harford, A., Herbert, S., 2022. Environmental DNA protocol development guide for biomonitoring. Natl. eDNA Ref. Cent. Canberra. <https://research.csiro.au/environmentics/wp-content/uploads/sites/187/2022/08/Environmental-DNA-protocol-development-guide-for-biomonitoring.pdf>.
- Deagle, B.E., Gales, N.J., Evans, K., Jarman, S.N., Robinson, S., Trebilco, R., Hindell, M.A., 2007. Studying seabird diet through genetic analysis of faeces: a case study on macaroni penguins (*Eudyptes chrysolophus*). *PLoS One* 2, e831. <https://doi.org/10.1371/journal.pone.0000831>.
- Deinier, K., Walser, J.-C., Mächler, E., Altermatt, F., 2015. Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA. *Biol. Conserv.* 183, 53–63. <https://doi.org/10.1016/j.biocon.2014.11.018>.
- Díaz-Ferguson, E.E., Moyer, G.R., 2014. History, applications, methodological issues and perspectives for the use environmental DNA (eDNA) in marine and freshwater environments. *Rev. Biol. Trop.* 62, 1273–1284.
- Dickie, I.A., Boyer, S., Buckley, H.L., Duncan, R.P., Gardner, P.P., Hogg, I.D., Holdaway, R.J., Lear, G., Makiola, A., Morales, S.E., Powell, J.R., Weaver, L., 2018. Towards robust and repeatable sampling methods in eDNA - based studies. *Mol. Ecol. Resour.* 18, 940–952. <https://doi.org/10.1111/1755-0998.12907>.
- Djurhuus, A., Port, J., Closek, C.J., Yamahara, K.M., Romero-Maraccini, O., Walz, K.R., Goldsmith, D.B., Michisaki, R., Breitbart, M., Boehm, A.B., Chavez, F.P., 2017. Evaluation of filtration and DNA extraction methods for environmental DNA biodiversity assessments across multiple trophic levels. *Front. Mar. Sci.* 4, 314. <https://doi.org/10.3389/fmars.2017.00314>.
- Djurhuus, A., Closek, C.J., Kelly, R.P., Pitz, K.J., Michisaki, R.P., Starks, H.A., Walz, K.R., Andruszkiewicz, E.A., Olesin, E., Hubbard, K., Montes, E., 2020. Environmental DNA reveals seasonal shifts and potential interactions in a marine community. *Nat. comm* 11, 254.
- Doi, H., Akamatsu, Y., Watanabe, Y., Goto, M., Inui, R., Katano, I., Nagano, M., Takahara, T., Minamoto, T., 2017. Water sampling for environmental DNA surveys by using an unmanned aerial vehicle. *Limnol. Oceanogr. Methods* 15, 939–944. <https://doi.org/10.1002/lim3.10214>.
- Doyle, J., Uthicke, S., 2021. Sensitive environmental DNA detection via lateral flow assay (dipstick)—A case study on corallivorous crown-of-thorns sea star (*Acanthaster cf. solaris*) detection. *Environ. DNA* 3, 323–342. <https://doi.org/10.1002/edn3.123>.
- Dugal, L., Thomas, L., Wilkinson, S.P., Richards, Z.T., Alexander, J.B., Adam, A.A.S., Kennington, W.J., Jarman, S., Ryan, N.M., Bunce, M., Gilmour, J.P., 2022. Coral monitoring in Northwest Australia with environmental DNA metabarcoding using a curated reference database for optimized detection. *Environ. DNA* 4, 63–76. <https://doi.org/10.1002/edn3.199>.
- Eddy, T.D., Lam, V.W.Y., Reygondeau, G., Cisneros-Montemayor, A.M., Greer, K., Palomares, M.L.D., Bruno, J.F., Ota, Y., Cheung, W.W.L., 2021. Global decline in capacity of coral reefs to provide ecosystem services. *One Earth* 4, 1278–1285. <https://doi.org/10.1016/j.oneear.2021.08.016>.
- Eichmiller, J.J., Bajer, P.G., Sorensen, P.W., 2014. The relationship between the distribution of common carp and their environmental DNA in a small lake. *PLoS One* 9, e112611. <https://doi.org/10.1371/journal.pone.0112611>.
- Elbrecht, V., Leese, F., 2017. PrimerMiner: an R package for development and in silico validation of 1406 DNA metabarcoding primers. *Methods Ecol. Evol.* 8, 622–626. <https://doi.org/10.1111/2041-1407210X.12687>.
- Elbrecht, V., Taberlet, P., Dejean, T., Valentini, A., Usseglio-Polatera, P., Beisel, J.-N., Coissac, E., Boyer, F., Leese, F., 2016. Testing the potential of a ribosomal 16S marker for DNA metabarcoding of insects. *PeerJ* 4, e1966. <https://doi.org/10.7717/peerj.1966>.
- Ellis, M.R., Clark, Z.S.R., Tremblay, E.A., Brown, M.S., Matthews, T.G., Pocklington, J.B., Stafford-Bell, R.E., Bott, N.J., Nai, Y.H., Miller, A.D., Sherman, C.D.H., 2022. Detecting marine pests using environmental DNA and biophysical models. *Sci. Total Environ.* 816, 151666. <https://doi.org/10.1016/j.scitotenv.2021.151666>.
- Espe, M.B., Johnston, M., Blankenship, S.M., Dean, C.A., Bowen, M.D., Schultz, A., Schumer, G., 2022. The Artemis package for environmental DNA analysis in R. *Environ. DNA* 4, 523–532.
- Everett, M.V., Park, L.K., 2018. Exploring deep-water coral communities using environmental DNA. *Deep Sea Res. Part II Top. Stud. Oceanogr.* 150, 229–241. <https://doi.org/10.1016/j.dsr2.2017.09.008>.
- Feng, W., Bulté, G., Lougheed, S.C., 2020. Environmental DNA surveys help to identify winter hibernacula of a temperate freshwater turtle. *Environ. DNA* 2, 200–209. <https://doi.org/10.1002/edn3.58>.
- Ficetola, G.F., Miaud, C., Pompanon, F., Taberlet, P., 2008. Species detection using environmental DNA from water samples. *Biol. Lett.* 4, 423–425. <https://doi.org/10.1098/rsbl.2008.0118>.
- Ficetola, G.F., Pansu, J., Bonin, A., Coissac, E., Giguet-Covex, C., De Barba, M., Gielly, L., Lopes, C.M., Boyer, F., Pompanon, F., Rayé, G., 2015. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Mol. Ecol. Res.* 15, 543–556.
- Ficetola, G.F., Taberlet, P., Coissac, E., 2016. How to limit false positives in environmental DNA and metabarcoding? *Mol. Ecol. Res.* 3, 604–607.
- Foote, A.D., Thomsen, P.F., Sveegaard, S., Wahlberg, M., Kielgast, J., Kyhn, L.A., Salling, A.B., Galatius, A., Orlando, L., Gilbert, M.T.P., 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PLoS One* 7, e41781. <https://doi.org/10.1371/journal.pone.0041781>.
- Forbes, S.A., 1887. The lake as a microcosm. *Bull. Peoria. Sei. Assn.* (reprinted 1925). *Bull. Ill. State. Nat. Hist. Surv.* 15, 537–550. <https://doi.org/10.21900/j.inhs.v15.303>.
- Føre, M., Frank, K., Dempster, T., Alfredsen, J.A., Høy, E., 2017. Biomonitoring using tagged sentinel fish and acoustic telemetry in commercial salmon aquaculture: a feasibility study. *Aquac. Eng.* 78, 163–172. <https://doi.org/10.1016/j.aquaeng.2017.07.004>.
- Formel, N., Enochs, I.C., Sinigalliano, C., Anderson, S.R., Thompson, L.R., 2021. Subsurface automated samplers for eDNA (SASe) for biological monitoring and research. *HardwareX* 10, e00239. <https://doi.org/10.1016/j.johx.2021.e00239>.
- Fraser, C.I., Connell, L., Lee, C.K., Cary, S.C., 2018. Evidence of plant and animal communities at exposed and subglacial (cave) geothermal sites in Antarctica. *Polar Biol.* 41, 417–421. <https://doi.org/10.1007/s00300-017-2198-9>.
- Friebertshauser, R., Shollenberger, K., Janosik, A., Garner, J.T., Johnston, C., 2019. The effect of bivalve filtration on eDNA-based detection of aquatic organisms. *PLoS One* 14, e0222830. <https://doi.org/10.1371/journal.pone.0222830>.
- Furlan, E.M., Gleeson, D., Hardy, C.M., Duncan, R.P., 2016. A framework for estimating the sensitivity of eDNA surveys. *Mol. Ecol. Resour.* 16, 641–654. <https://doi.org/10.1111/1755-0998.12483>.
- Furlan, E.M., Gleeson, D., Wisniewski, C., Yick, J., Duncan, R.P., 2019. eDNA surveys to detect species at very low densities: a case study of European carp eradication in Tasmania, Australia. *J. Appl. Ecol.* 56, 2505–2517. <https://doi.org/10.1111/1365-2664.13485>.
- Furlan, E.M., Davis, J., Duncan, R.P., 2020. Identifying error and accurately interpreting environmental DNA metabarcoding results: a case study to detect vertebrates at arid zone waterholes. *Mol. Ecol. Resour.* 20, 1259–1276. <https://doi.org/10.1111/1755-0998.13170>.
- Geller, J., Meyer, C., Parker, M., Hawk, H., 2013. Redesign of PCR primers for mitochondrial cytochrome c oxidase subunit I for marine invertebrates and application in all-taxa biotic surveys. *Mol. Ecol. Resour.* 13, 851–861. <https://doi.org/10.1111/1755-0998.12138>.
- Gold, Z., Sprague, J., Kushner, D.J., Zerecero Marin, E., Barber, P.H., 2021. eDNA metabarcoding as a biomonitoring tool for marine protected areas. *PLoS One* 16, e0238557. <https://doi.org/10.1371/journal.pone.0238557>.
- Goldberg, C.S., Turner, C.R., Deiner, K., Klymus, K.E., Thomsen, P.F., Murphy, M.A., Spear, S.F., McKee, A., Oyler-McCance, S.J., Cormann, R.S., Laramie, M.B., Mahon, A.R., Lance, R.F., Pilliod, D.S., Strickler, K.M., Waits, L.P., Fremier, A.K., Takahara, T., Herder, J.E., Taberlet, P., 2016. Critical considerations for the application of environmental DNA methods to detect aquatic species. *Methods Ecol. Evol.* 7, 1299–1307. <https://doi.org/10.1111/2041-210X.12595>.
- Goldberg, C.S., Strickler, K.M., Fremier, A.K., 2018. Degradation and dispersion limit environmental DNA detection of rare amphibians in wetlands: increasing efficacy of sampling designs. *Sci. Total Environ.* 633, 695–703. <https://doi.org/10.1016/j.scitotenv.2018.02.295>.
- Golden, N.H., Rattner, B.A., 2003. Ranking Terrestrial vertebrate species for utility in biomonitoring and vulnerability to environmental contaminants. In: Ware, G.W. (Ed.), *Reviews of Environmental Contamination and Toxicology. Reviews of Environmental Contamination and Toxicology*. vol 176. Springer, New York, NY https://doi.org/10.1007/978-1-4899-7283-5_2.
- Greco, M., Lejzerowicz, F., Reo, E., Caruso, A., Maccotta, A., Coccioni, R., Pawłowski, J., Frontalini, F., 2022. Environmental RNA outperforms eDNA metabarcoding in assessing impact of marine pollution: a chromium-spiked mesocosm test. *Chemistry* 298, 134239.
- Guareschi, S., Laini, A., England, J., Johns, T., Winter, M., Wood, P.J., 2021. Invasive species influence macroinvertebrate biomonitoring tools and functional diversity in British rivers. *J. Appl. Ecol.* 58, 135–147. <https://doi.org/10.1111/1365-2664.13795>.
- Hall, T.A., 1999. BioEdit: a user-friendly biological sequence alignment editor and analysis program for windows 95/98/NT. *Nucleic Acids Symp. Ser.* 41, 95–98.
- Hampton, S.E., Strasser, C.A., Tewksbury, J.J., Gram, W.K., Budden, A.E., Batcheller, A.L., Duke, C.S., Porter, J.H., 2013. Big data and the future of ecology. *Front. Ecol. Environ.* 11, 156–162. <https://doi.org/10.1890/120103>.
- Harper, L.R., Lawson Handley, L., Hahn, C., Boonham, N., Rees, H.C., Gough, K.C., Lewis, E., Adams, I.P., Brotherton, P., Phillips, S., Häfling, B., 2018. Needle in a haystack? A comparison of eDNA metabarcoding and targeted qPCR for detection of the great crested newt (*Triturus cristatus*). *Ecol. Evol.* 8, 6330–6341. <https://doi.org/10.1002/ece3.4013>.
- Harper, L.R., Buxton, A.S., Rees, H.C., Bruce, K., Brys, R., Halfmaerten, D., Read, D.S., Watson, H.V., Sayer, C.D., Jones, E.P., Priestley, V., Mächler, E., Múria, C., Garcés-Pastor, S., Medupin, C., Burgess, K., Benson, G., Boonham, N., Griffiths, R.A., Lawson Handley, L., Häfling, B., 2019. Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiologia* 826, 25–41. <https://doi.org/10.1007/s10750-018-3750-5>.

- Harper, L.R., Buxton, A.S., Rees, H.C., Bruce, K., Brys, R., Halfmaerten, D., Read, D.S., Watson, H.V., Sayer, C.D., Jones, E.P., Priestley, V., 2019. Prospects and challenges of environmental DNA (eDNA) monitoring in freshwater ponds. *Hydrobiol.* 826, 25–41.
- Hashemzadeh Segherloo, I., Tabatabaei, S.N., Abdolah-Mousavi, E., Hernandez, C., Normandea, E., Laporte, M., Boyle, B., Amiri, M., GhaedRahmati, N., Hallerman, E., Bernatchez, L., 2022. eDNA metabarcoding as a means to assess distribution of subterranean fish communities: Iranian blind cave fishes as a case study. *Environ. DNA* 4, 402–416.
- Hassall, A.H., 1850. A microscopic examination of the water supplied to the inhabitants of London and the suburban districts. Samuel Highley, London. www.biodiversitylibrary.org/item/305137.
- Hermans, S.M., Buckley, H.L., Lear, G., 2018. Optimal extraction methods for the simultaneous analysis of DNA from diverse organisms and sample types. *Mol. Ecol. Resour.* 18, 557–569. <https://doi.org/10.1111/1755-0998.12762>.
- Hinlo, R., Gleeson, D., Linternans, M., Furlan, E., 2017. Methods to maximise recovery of environmental DNA from water samples. *PLoS One* 12, e0179251. <https://doi.org/10.1371/journal.pone.0179251>.
- Hinz, S., Coston-Guarini, J., Marnane, M., Guarini, J.M., 2022. Evaluating eDNA for use within marine environmental impact assessments. *J. Mar. Sci. Eng.* 10, 375.
- Hobbs, J., Round, J.M., Allison, M.J., Helbing, C.C., 2019. Expansion of the known distribution of the coastal tailed frog, *Ascaphus truei*, in British Columbia, Canada, using robust eDNA detection methods. *PLoS One* 14, e0213849. <https://doi.org/10.1371/journal.pone.0213849>.
- Holman, L.E., Cheng, Y., Rius, M., 2022. How does eDNA decay affect metabarcoding experiments? *Environ. DNA* 4, 108–116. <https://doi.org/10.1002/edn.3201>.
- Huang, S., Yoshitake, K., Watabe, S., Asakawa, S., 2022. Environmental DNA study on aquatic ecosystem monitoring and management: recent advances and prospects. *J. Environ. Manag.* 323, 116310.
- Hunter, M.E., Ferrante, J.A., Meigs-Friend, G., Ulmer, A., 2019. Improving eDNA yield and inhibitor reduction through increased water volumes and multi-filter isolation techniques. *Sci. Rep.* 9, 5259. <https://doi.org/10.1038/s41598-019-40977-w>.
- Hutchins, P.R., Simantel, L.N., Sepulveda, A.J., 2022. Time to get real with qPCR controls: the frequency of sample contamination and the informative power of negative controls in environmental DNA studies. *Mol. Ecol. Resour.* 22, 1319–1329. <https://doi.org/10.1111/1755-0998.13549>.
- Ip, Y.C.A., Chang, J.J.M., Tun, K.P.P., Meier, R., Huang, D., 2022. Multispecies environmental DNA metabarcoding sheds light on annual coral spawning events. *Mol. Ecol. molec.* 16621. <https://doi.org/10.1111/mec.16621>.
- Ivanova, N.V., Zemlak, T.S., Hanner, R.H., Hebert, P.D.N., 2007. Universal primer cocktails for fish DNA barcoding. *Mol. Ecol. Notes* 7, 544–548. <https://doi.org/10.1111/j.1471-8286.2007.01748.x>.
- Jacobs-Palmer, E., Gallego, R., Cribari, K., Keller, A.G., Kelly, R.P., 2021. Environmental DNA metabarcoding for simultaneous monitoring and ecological assessment of many harmful algae. *Front. Ecol. Evol.* 9, 612107. <https://doi.org/10.3389/fevo.2021.612107>.
- Janosik, A.M., Johnston, C.E., 2015. Environmental DNA as an effective tool for detection of imperiled fishes. *Environ. Biol. Fish.* 98, 1889–1893. <https://doi.org/10.1007/s10641-015-0405-5>.
- Jarman, S.N., Berry, O., Bunce, M., 2018. The value of environmental DNA biobanking for long-term biomonitoring. *Nat. Ecol. Evol.* 2, 1192–1193. <https://doi.org/10.1038/s41559-018-0614-3>.
- Jennings, W.B., 2016. Phylogenomic Data Acquisition: Principles and Practice. CRC Press and Taylor & Francis, Boca Raton <https://doi.org/10.1201/9781315181431>.
- Jensen, M.R., Sigsgaard, E.E., Liu, S., Manica, A., Bach, S.S., Hansen, M.M., Möller, P.R., Thomsen, P.F., 2021. Genome-scale target capture of mitochondrial and nuclear environmental DNA from water samples. *Mol. Ecol. Res.* 21, 690–702.
- Jerde, C.L., Mahon, A.R., Chadderton, W.L., Lodge, D.M., 2011. "Sight-unseen" detection of rare aquatic species using environmental DNA: eDNA surveillance of rare aquatic species. *Conserv. Lett.* 4, 150–157. <https://doi.org/10.1111/j.1755-263X.2010.00158.x>.
- Jeunen, G.J., Dowle, E., Edgecombe, J., von Ammon, U., Gemmell, N.J., Cross, H., 2022. CRABS—a software program to generate curated reference databases for metabarcoding sequencing data. *Mol. Ecol. Res.* <https://doi.org/10.1111/1755-0998.13741>.
- Julian, J., Glenney, G., Rees, C., 2019. Evaluating observer bias and seasonal detection rates in amphibian pathogen eDNA collections by citizen scientists. *Dis. Aquat. Org.* 134, 15–24. <https://doi.org/10.3354/dao03357>.
- Kamoroff, C., Goldberg, C.S., 2018. An issue of life or death: using eDNA to detect viable individuals in wilderness restoration. *Freshw. Sci.* 37, 685–696. <https://doi.org/10.1086/699203>.
- Kawato, M., Yoshida, T., Miya, M., Tsuchida, S., Nagano, Y., Nomura, M., Yabuki, A., Fujiwara, Y., Fujikura, K., 2021. Optimization of environmental DNA extraction and amplification methods for metabarcoding of deep-sea fish. *MethodsX* 8, 101238. <https://doi.org/10.1016/j.mex.2021.101238>.
- Keck, F., Blackman, R.C., Bossart, R., Brantschen, J., Couton, M., Hürlemann, S., Kirschner, D., Locher, N., Zhang, H., Altermatt, F., 2022. Meta-analysis shows both congruence and complementarity of DNA and eDNA metabarcoding to traditional methods for biological community assessment. *Mol. Ecol.* 31, 1820–1835. <https://doi.org/10.1111/mec.16364>.
- Kelly, R.P., Port, J.A., Yamahara, K.M., Crowder, L.B., 2014. Using environmental DNA to census marine fishes in a large mesocosm. *PLoS One* 9, e86175. <https://doi.org/10.1371/journal.pone.0086175>.
- Kelly, R.P., Closek, C.J., O'Donnell, J.L., Kralj, J.E., Shelton, A.O., Samhour, J.F., 2017. Genetic and manual survey methods yield different and complementary views of an ecosystem. *Front. Mar. Sci.* 3. <https://doi.org/10.3389/fmars.2016.00283>.
- Kelly, R.P., Shelton, A.O., Gallego, R., 2019. Understanding PCR processes to draw meaningful conclusions from environmental DNA studies. *Sci. Rep.* 9, 12133. <https://doi.org/10.1038/s41598-019-48546-x>.
- Kimura, M., Yamamoto, H., Nakashima, Y., 2022. Application of machine learning to environmental DNA metabarcoding. *IEEE Access* 10, 101790–101794.
- King, A.C., Krieg, R., Weston, A., Zenker, A.K., 2022. Using eDNA to simultaneously detect the distribution of native and invasive crayfish within an entire country. *J. Environ. Manag.* 302, 113929.
- Kirtane, A., Atkinson, J.D., Sassoubre, L., 2020. Design and validation of passive environmental DNA samplers using granular activated carbon and montmorillonite clay. *Environ. Sci. Technol.* 54, 11961–11970. <https://doi.org/10.1021/acs.est.0c01863>.
- Kronenberger, J.A., Wilcox, T.M., Mason, D.H., Franklin, T.W., McKelvey, K.S., Young, M.K., Schwartz, M.K., 2022. eDNAAssay: a machine learning tool that accurately predicts qPCR cross-amplification. *Mol. Ecol. Res.* 22, 2994–3005.
- Kumar, G., Eble, J.E., Gaither, M.R., 2020. A practical guide to sample preservation and pre-PCR processing of aquatic environmental DNA. *Mol. Ecol. Resour.* 20, 29–39. <https://doi.org/10.1111/1755-0998.13107>.
- Lacoursière-Roussel, A., Rosabal, M., Bernatchez, L., 2016. Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Mol. Ecol. Resour.* 16, 1401–1414. <https://doi.org/10.1111/1755-0998.12522>.
- Lafferty, K.D., Garcia-Vedrenne, A.E., McLaughlin, J.P., Childress, J.N., Morse, M.F., Jerde, C.L., 2021. At Palmyra atoll, the fish-community environmental DNA signal changes across habitats but not with tides. *J. Fish Biol.* 98, 415–425. <https://doi.org/10.1111/jfb.14403>.
- Laroche, O., Kersten, O., Smith, C.R., Goetze, E., 2020. From sea surface to seafloor: a benthic allochthonous eDNA survey for the abyssal ocean. *Front. Mar. Sci.* 7, 682. <https://doi.org/10.3389/fmars.2020.00682>.
- Layton, A., McKay, L., Williams, D., Garrett, V., Gentry, R., Sayler, G., 2006. Development of *bacteroides* 16S rRNA gene TaqMan-based real-time PCR assays for estimation of total, human, and bovine fecal pollution in water. *Appl. Environ. Microbiol.* 72, 4214–4224. <https://doi.org/10.1128/AEM.01036-05>.
- Leray, M., Yang, J.Y., Meyer, C.P., Mills, S.C., Agudelo, N., Ranwez, V., Boehm, J.T., Machida, R.J., 2013. A new versatile primer set targeting a short fragment of the mitochondrial COI region for metabarcoding metazoan diversity: application for characterizing coral reef fish gut contents. *Front. Zool.* 10, 34. <https://doi.org/10.1186/1742-9994-10-34>.
- Li, J., Handley, L.J.L., Read, D.S., Häneling, B., 2018. The effect of filtration method on the efficiency of environmental DNA capture and quantification via metabarcoding. *Mol. Ecol. Resour.* 18, 1102–1114. <https://doi.org/10.1111/1755-0998.12899>.
- Lim, N.K.M., Tay, Y.C., Srivathsan, A., Tan, J.W.T., Kwik, J.T.B., Baloğlu, B., Meier, R., Yeo, D.C.J., 2016. Next-generation freshwater bioassessment: eDNA metabarcoding with a conserved metazoan primer reveals species-rich and reservoir-specific communities. *R. Soc. Open Sci.* 3, 160635. <https://doi.org/10.1098/rsos.160635>.
- Lin, M., Zhang, S., Yao, M., 2019. Effective detection of environmental DNA from the invasive american bullfrog. *Biol. Invasions* 21, 2255–2268. <https://doi.org/10.1007/s10530-019-01974-2>.
- Lindahl, T., 1993. Instability and decay of the primary structure of DNA. *Nature* 362, 709–715. <https://doi.org/10.1038/362709a0>.
- Luo, M., Ji, Y., Warton, D., Yu, D.W., 2023. Extracting abundance information from DNA-based data. *Mol. Ecol. Res.* 23, 174–189.
- Lynch, A.J., Cooke, S.J., Deines, A.M., Bower, S.D., Bunnell, D.B., Cowx, I.G., Nguyen, V.M., Nohner, J., Phouthavong, K., Riley, B., Rogers, M.W., Taylor, W.W., Woelmer, W., Youn, S.-J., Beard, T.D., 2016. The social, economic, and environmental importance of inland fish and fisheries. *Environ. Rev.* 24, 115–121. <https://doi.org/10.1139/er-2015-0064>.
- Macher, T.-H., Schütz, R., Arle, J., Beermann, A.J., Koschorreck, J., Leese, F., 2021. Beyond fish eDNA metabarcoding: field replicates disproportionately improve the detection of stream associated vertebrate species. *Metabarcod. Metagenom.* 5, e66557. <https://doi.org/10.3897/mbmg.5.66557>.
- Maddison, W.P., Maddison, D.R., 2018. Mesquite: a modular system for evolutionary analysis. Version 3.51. <http://www.mesquiteproject.org>.
- Madduppa, H., Cahyani, N.K.D., Anggoro, A.W., Subhan, B., Jefri, E., Sani, L.M.I., Arafat, D., Akbar, N., Bengen, D.G., 2021. eDNA metabarcoding illuminates species diversity and composition of three phyla (chordata, mollusca and echinodermata) across Indonesian coral reefs. *Biodivers. Conserv.* 30, 3087–3114. <https://doi.org/10.1007/s10531-021-02237-0>.
- Maiello, G., Talarico, L., Carpenteri, P., De Angelis, F., Franceschini, S., Harper, L.R., Neave, E.F., Rickards, O., Sbrana, A., Shum, P., Veltre, V., Mariani, S., Russo, T., 2022. Little samplers, big fleet: eDNA metabarcoding from commercial trawlers enhances ocean monitoring. *Fish. Res.* 249, 106259. <https://doi.org/10.1016/j.fishres.2022.106259>.
- Majaneva, M., Diserud, O.H., Eagle, S.H.C., Boström, E., Hajibabaei, M., Ekrem, T., 2018. Environmental DNA filtration techniques affect recovered biodiversity. *Sci. Rep.* 8, 4682. <https://doi.org/10.1038/s41598-018-23052-8>.
- Mariani, S., Baillie, C., Colosimo, G., Riesgo, A., 2019. Sponges as natural environmental DNA samplers. *Curr. Biol.* 29, R401–R402. <https://doi.org/10.1016/j.cub.2019.04.031>.
- Marquina, D., Buczek, M., Ronquist, F., Łukasik, P., 2021. The effect of ethanol concentration on the morphological and molecular preservation of insects for biodiversity studies. *PeerJ* 9, e10799. <https://doi.org/10.7717/peerj.10799>.
- Martellini, A., Payment, P., Villemur, R., 2005. Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water. *Water Res.* 39, 541–548. <https://doi.org/10.1016/j.watres.2004.11.012>.
- Mauri, M., Elli, T., Caviglia, G., Ubaldi, G., Azzi, M., 2017. RAWGraphs: a visualisation platform to create open outputs. Proceedings of the 12th Biannual Conference on Italian SIGCHI Chapter. ACM, New York, NY, USA <https://doi.org/10.1145/3125571.3125585> (p. 28:1–28:5).
- McClenaghan, B., Fahner, N., Cote, D., Chawarski, J., McCarthy, A., Rajabi, H., Singer, G., Hajibabaei, M., 2020. Harnessing the power of eDNA metabarcoding for the detection of deep-sea fishes. *PLoS One* 15, e0236540. <https://doi.org/10.1371/journal.pone.0236540>.
- McQuillan, J.S., Robidart, J.C., 2017. Molecular-biological sensing in aquatic environments: recent developments and emerging capabilities. *Curr. Opin. Biotechnol.* 45, 43–50. <https://doi.org/10.1016/j.copbio.2016.11.022>.
- Mena, J.L., Yagui, H., Tejeda, V., Bonifaz, E., Bellemain, E., Valentini, A., Tobler, M.W., Sánchez-Vendizú, P., Lyet, A., 2021. Environmental DNA metabarcoding as a useful

- tool for evaluating terrestrial mammal diversity in tropical forests. *Ecol. Appl.* 31. <https://doi.org/10.1002/eaap.2335>.
- Mérou, N., Lecadet, C., Pouvreau, S., Arzul, I., 2020. An eDNA/eRNA-based approach to investigate the life cycle of non-cultivable shellfish micro-parasites: the case of Bonamia ostreae, a parasite of the European flat oyster Ostrea edulis. *Microb. Biotechnol.* 13, 1807–1818.
- Meyer, C.P., 2003. Molecular systematics of cowries (Gastropoda: Cypraeidae) and diversification patterns in the tropics. *Biol. J. Linn. Soc.* 79, 401–459. <https://doi.org/10.1046/j.1095-8312.2003.00197.x>.
- Miaud, C., Taberlet, P., Dejean, T., Coissac, É., Miquel, C., Pompanon, F., Valentini, A., 2012. ADN «environnemental»: un saut méthodologique pour les inventaires de la biodiversité. *Sci. Eaux Terri.* 6, 92–95. <https://doi.org/10.3917/set.006.0092>.
- Miaud, C., Arnal, V., Poulain, M., Valentini, A., Dejean, T., 2019. eDNA increases the detectability of ranavirus infection in an alpine amphibian population. *Viruses* 11, 526. <https://doi.org/10.3390/v11060526>.
- Min, M.A., Barber, P.H., Gold, Z., 2021. MiSebastes: an eDNA metabarcoding primer set for rockfishes (genus *Sebastes*). *Conserv. Genet. Resour.* 13, 447–456. <https://doi.org/10.1007/s12686-021-01219-2>.
- Minamoto, T., 2022. Environmental DNA analysis for macro-organisms: species distribution and more. *DNA Res.* 29, dsac018. <https://doi.org/10.1093/dnares/dsac018>.
- Minamoto, T., Miya, M., Sado, T., Seino, S., Doi, H., Kondoh, M., Nakamura, K., Takahara, T., Yamamoto, S., Yamanaka, H., Araki, H., Iwasaki, W., Kasai, A., Masuda, R., Uchii, K., 2021. An illustrated manual for environmental DNA research: water sampling guidelines and experimental protocols. *Environ. DNA* 3, 8–13. <https://doi.org/10.1002/edn3.121>.
- Mirimin, L., Hickey, A., Barrett, D., DePaoite, F., Boschetti, S., Venkatesh, S., Graham, C.T., 2020. Environmental DNA detection of Arctic char (*Salvelinus alpinus*) in Irish lakes: development and application of a species-specific molecular assay. *Environ. DNA* 2, 221–233. <https://doi.org/10.1002/edn3.60>.
- Mirimin, L., Desmet, S., Romero, D.L., Fernandez, S.F., Miller, D.L., Mynott, S., Brincau, A.G., Stefanni, S., Berry, A., Gaughan, P., Aguzzi, J., 2021. Don't catch me if you can – using cabled observatories as multidisciplinary platforms for marine fish community monitoring: an in situ case study combining underwater video and environmental DNA data. *Sci. Total Environ.* 773, 145351. <https://doi.org/10.1016/j.scitotenv.2021.145351>.
- Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J.Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H., Kondoh, M., Iwasaki, W., 2015. MiFish: a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *R. Soc. Open Sci.* 2, 150088. <https://doi.org/10.1098/rsos.150088>.
- Miya, M., Minamoto, T., Yamanaka, H., Oka, S., Sato, K., Yamamoto, S., Sado, T., Doi, H., 2016. Use of a filter cartridge for filtration of water samples and extraction of environmental DNA. *J. Vis. Exp.* 54741. <https://doi.org/10.3791/54741>.
- Miya, M., Sado, T., Oka, S., Fukuchi, T., 2022. The use of citizen science in fish eDNA metabarcoding for evaluating regional biodiversity in a coastal marine region: a pilot study. *Metabarcod. Metagenom.* 6, e80444. <https://doi.org/10.3897/mbmg.6.80444>.
- Miyata, K., Inoue, Y., Amano, Y., Nishioka, T., Yamane, M., Kawaguchi, T., Morita, O., Honda, H., 2021. Fish environmental RNA enables precise ecological surveys with high positive predictivity. *Ecol. Indic.* 128, 107796. <https://doi.org/10.1016/j.ecolind.2021.107796>.
- Mongeon, P., Paul-Hus, A., 2016. The journal coverage of web of science and scopus: a comparative analysis. *Scientometrics* 106, 213–228. <https://doi.org/10.1007/s11192-015-1765-5>.
- Moog, O., Schmutz, S., Schwarzsinger, I., 2018. Biomonitoring and bioassessment. In: Schmutz, S., Sendzimir, J. (Eds.), *Riverine Ecosystem Management: Aquatic Ecology Series* 8. Springer, Cham, pp. 371–390. https://doi.org/10.1007/978-3-319-73250-3_19.
- Moss, W.E., Harper, L.R., Davis, M.A., Goldberg, C.S., Smith, M.M., Johnson, P.T., 2022. Navigating the trade-offs between environmental DNA and conventional field surveys for improved amphibian monitoring. *Ecosph.* 13, e3941.
- Muha, T.P., Robinson, C.V., Garcia de Leoniz, C., Consuegra, S., 2019. An optimised eDNA protocol for detecting fish in lentic and lotic freshwaters using a small water volume. *PLoS One* 14, e0219218. <https://doi.org/10.1371/journal.pone.0219218>.
- Mullin, K.E., Barata, I.M., Dawson, J., Orozco-torWengel, P., 2022. First extraction of eDNA from tree hole water to detect tree frogs: a simple field method piloted in Madagascar. *Cons. Gen. Res.* 14, 99–107.
- Nagarajan, R.P., Bedwell, M., Holmes, A.E., Sanches, T., Acuña, S., Baerwald, M., Barnes, M.A., Blankenship, S., Connan, R.E., Deiner, K., Gille, D., 2022. Environmental DNA methods for ecological monitoring and biodiversity assessment in estuaries. *Estuar. Coasts* 45, 2254–2273.
- Neice, A.A., McRae, S.B., 2021. An eDNA diagnostic test to detect a rare, secretive marsh bird. *Glob. Ecol. Conserv.* 27, e01529. <https://doi.org/10.1016/j.gecco.2021.e01529>.
- Nester, G.M., De Brauwer, M., Koziol, A., West, K.M., DiBattista, J.D., White, N.E., Power, M., Heydenrych, M.J., Harvey, E., Bunce, M., 2020. Development and evaluation of fish eDNA metabarcoding assays facilitate the detection of cryptic seahorse taxa (family: Syngnathidae). *Env. DNA* 2, 614–626.
- Nichols, P.K., Marko, P.B., 2019. Rapid assessment of coral cover from environmental DNA in Hawai'i. *Environ. DNA* 1, 40–53. <https://doi.org/10.1002/edn3.8>.
- Ogden, L.E., 2022. The emergence of eDNA: an interdisciplinary tool helps monitor biodiversity and health. *Bioscience* 72, 5–12. <https://doi.org/10.1093/biosci/biab120>.
- Ogram, A., Sayler, G.S., Barkay, T., 1987. The extraction and purification of microbial DNA from sediments. *J. Microbiol. Methods* 7, 57–66. [https://doi.org/10.1016/0167-7012\(87\)90025-X](https://doi.org/10.1016/0167-7012(87)90025-X).
- Oka, S.I., Miya, M., Sado, T., 2022. Gravity filtration of environmental DNA: a simple, fast, and power-free method. *MethodsX* 9, 101838.
- Parducci, L., Alsol, I.G., Unneberg, P., Pedersen, M.W., Han, L., Lammers, Y., Salonen, J.S., Välijanta, M.M., Slotte, T., Wohlforth, B., 2019. Shotgun environmental DNA, pollen, and macrofossil analysis of lateglacial lake sediments from southern Sweden. *Front. Ecol. Evol.* 7, 189. <https://doi.org/10.3389/fevo.2019.00189>.
- Pawlowski, J., Apothélo-Perré-Gentil, L., Altermatt, F., 2020. Environmental DNA: what's behind the term? Clarifying the terminology and recommendations for its future use in biomonitoring. *Mol. Ecol.* 29, 4258–4264. <https://doi.org/10.1111/mec.15643>.
- Pawlowski, J., Apothélo-Perré-Gentil, L., Mächler, E., Altermatt, F., 2020. Environmental DNA applications for biomonitoring and bioassessment in aquatic ecosystems. Guidelines, Environmental Studies. No. 2010. Federal Office for the Environment, Bern. <https://doi.org/10.5167/UZH-187800>.
- Pawlowski, J., Bonin, A., Boyer, F., Cordiner, T., Taberlet, P., 2021. Environmental DNA for biomonitoring. *Mol. Ecol.* 30, 2931–2936. <https://doi.org/10.1111/mec.16023>.
- Phillips, B.F., Wahle, R.A., Ward, T.J., 2013. Lobsters as part of marine ecosystems - a review. In: Phillips, B.F. (Ed.), *Lobsters: Biology, Management, Aquaculture and Fisheries*. John Wiley & Sons Ltd, Oxford, UK, pp. 1–35. <https://doi.org/10.1002/9781118517444.ch1>.
- Piggott, M.P., 2016. Evaluating the effects of laboratory protocols on eDNA detection probability for an endangered freshwater fish. *Ecol. Evol.* 6, 2739–2750. <https://doi.org/10.1002/ee3.2083>.
- Pinfield, R., Dillane, E., Runge, A.K.W., Evans, A., Mirimin, L., Niemann, J., Reed, T.E., Reid, D.G., Rogan, E., Samarra, F.I.P., Sigsgaard, E.E., Foote, A.D., 2019. False-negative detections from environmental DNA collected in the presence of large numbers of killer whales (*Orcinus orca*). *Environ. DNA* 1, 316–328. <https://doi.org/10.1002/edn3.32>.
- Pirtle, E.I., Rooyen, A.R., Maino, J., Weeks, A.R., Umina, P.A., 2021. A molecular method for biomonitoring of an exotic plant-pest: leafmining for environmental DNA. *Mol. Ecol.* 30, 4913–4925. <https://doi.org/10.1111/mec.16092>.
- Plante, F., Bourgault, P., Dubois, Y., Bernatchez, L., 2021. Environmental DNA as a detection and quantitative tool for stream-dwelling salamanders: a comparison with the traditional active search method. *Environ. DNA* 3, 1128–1141. <https://doi.org/10.1002/edn3.233>.
- Polanco, A.F., Richards, E., Flück, B., Valentini, A., Altermatt, F., Brosse, S., Walser, J., Eme, D., Marques, V., Manel, S., Albouy, C., Dejean, T., Pellissier, L., 2021. Comparing the performance of 12S mitochondrial primers for fish environmental DNA across ecosystems. *Environ. DNA* 3, 1113–1127. <https://doi.org/10.1002/edn3.232>.
- Pollitt, L., Korbel, K., Dabovic, J., Chariton, A., Hose, G.C., 2022. Can eDNA be an indicator of tree groundwater use? A perspective. *Mar. Freshw. Res.* <https://doi.org/10.1071/MF21293>.
- Pope, K.L., Goldberg, C.S., Nelson, N.L., Cummings, A.K., Seaborn, T., Piovina-Scott, J., 2020. Designing environmental DNA surveys in complex aquatic systems: backpack sampling for rare amphibians in Sierra Nevada meadows. *Aquat. Conserv. Mar. Freshwat. Ecosyst.* 30, 1975–1987. <https://doi.org/10.1002/aqc.3444>.
- Prosser, S.W.J., Velarde-Aguilar, M.G., León-Régagnon, V., Hebert, P.D.N., 2013. Advancing nematode barcoding: a primer cocktail for the cytochrome c oxidase subunit I gene from vertebrate parasitic nematodes. *Mol. Ecol. Resour.* 13, 1108–1115. <https://doi.org/10.1111/1755-0998.12082>.
- Pukk, L., Kanefsky, J., Heathman, A.L., Weise, E.M., Nathan, L.R., Herbst, S.J., Sard, N.M., Scribner, K.T., Robinson, J.D., 2021. eDNA metabarcoding in lakes to quantify influences of landscape features and human activity on aquatic invasive species prevalence and fish community diversity. *Divers. Distrib.* 27, 2016–2031. <https://doi.org/10.1111/ddi.13370>.
- R Core Team, 2020. R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. <https://www.R-project.org/>.
- Ratsch, R., Kingsbury, B.A., Jordan, M.A., 2020. Exploration of environmental DNA (eDNA) to detect Kirtland's snake (*Clonophis kirtlandii*). *Animals* 10, 1057. <https://doi.org/10.3390/ani10061057>.
- Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., Gough, K.C., 2014. Review: the detection of aquatic animal species using environmental DNA - a review of eDNA as a survey tool in ecology. *J. Appl. Ecol.* 51, 1450–1459. <https://doi.org/10.1111/1365-2664.12306>.
- Renshaw, M.A., Olds, B.P., Jerde, C.L., McVeigh, M.M., Lodge, D.M., 2015. The room temperature preservation of filtered environmental DNA samples and assimilation into a phenol-chloroform-isooamyl alcohol DNA extraction. *Mol. Ecol. Resour.* 15, 168–176. <https://doi.org/10.1111/1755-0998.12281>.
- Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., Coissac, E., 2011. ecoPrimers: inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic Acids Res.* 39. <https://doi.org/10.1093/nar/gkr732> e145–e145.
- Richards, Z.T., Stat, M., Heydenrych, M., DiBattista, J.D., 2022. Environmental DNA for biodiversity monitoring of coral reefs. In: van Oppen, M.J.H., Aranda Lastra, M. (Eds.), *Coral Reef Conservation and Restoration in the Omics Age, Coral Reefs of the World*. Springer International Publishing, Cham, pp. 203–224. https://doi.org/10.1007/978-3-031-07055-6_13.
- Rodriguez-Espeleta, N., Morissette, O., Bean, C.W., Manu, S., Banerjee, P., Lacoursière-Roussel, A., Beng, K.C., Alter, S.E., Roger, F., Holman, L.E., Deiner, K., 2021. Trade-offs between reducing complex terminology and producing accurate interpretations from environmental DNA: comment on "Environmental DNA: What's behind the term?" By Pawlowski et al., (2020). *Mol. Ecol.* 30, 4601–4605. <https://doi.org/10.1111/mec.15942>.
- Roesma, D.I., Djong, H.T., Janra, M.N., Aidil, D.R., 2021. Freshwater vertebrates monitoring in Maninjau Lake, West Sumatra, Indonesia using environmental DNA. *Biodiversitas J. Biol. Divers.* 22. <https://doi.org/10.13057/biodiv/d220543>.
- Saccò, M., Blyth, A.J., Humphreys, W.F., Cooper, S.J., Austin, A.D., Hyde, J., Mazumder, D., Hua, Q., White, N.E., Grice, K., 2020. Refining trophic dynamics through multi-factor Bayesian mixing models: a case study of subterranean beetles. *Ecol. and Evol.* 10, 8815–8826.
- Saccò, M., Guzik, M.T., van der Heyde, M., Nevill, P., Cooper, S.J.B., Austin, A.D., Coates, P.J., Allentoft, M.E., White, N.E., 2022. eDNA in subterranean ecosystems: applications, technical aspects, and future prospects. *Sci. Total Environ.* 820, 153223. <https://doi.org/10.1016/j.scitotenv.2022.153223>.
- Sansom, B.J., Sasseboure, L.M., 2017. Environmental DNA (eDNA) shedding and decay rates to model freshwater mussel eDNA transport in a river. *Environ. Sci. Technol.* 51, 14244–14253. <https://doi.org/10.1021/acs.est.7b05199>.

- Sassoubre, L.M., Yamahara, K.M., Gardner, L.D., Block, B.A., Boehm, A.B., 2016. Quantification of environmental DNA (eDNA) shedding and decay rates for three marine fish. *Environ. Sci. Technol.* 50, 10456–10464. <https://doi.org/10.1021/acs.est.6b03114>.
- Schabacker, J.C., Amish, S.J., Ellis, B.K., Gardner, B., Miller, D.L., Rutledge, E.A., Sepulveda, A.J., Luikart, G., 2020. Increased eDNA detection sensitivity using a novel high-volume water sampling method. *Environ. DNA* 2, 244–251. <https://doi.org/10.1002/edn3.63>.
- Schenekar, T., 2022. The current state of eDNA research in freshwater ecosystems: are we shifting from the developmental phase to standard application in biomonitoring? *Hydrobiol.* 1–20.
- Schnell, I.B., Bohmann, K., Gilbert, M.T.P., 2015. Tag jumps illuminate reducing sequence-to-sample misidentifications in metabarcoding studies. *Mol. Ecol. Res.* 15, 289–1303.
- Schuwirth, N., Borgwardt, F., Domisch, S., Friedrichs, M., Kattwinkel, M., Kneis, D., Kuemmerlen, M., Langhans, S.D., Martínez-López, J., Vermeiren, P., 2019. How to make ecological models useful for environmental management. *Ecol. Model.* 411, 108784. <https://doi.org/10.1016/j.ecolmodel.2019.108784>.
- Schwarzbach, M., Laiacker, M., Mulero-Pazmany, M., Kondak, K., 2014. Remote water sampling using flying robots. 2014 International Conference on Unmanned Aircraft Systems (ICUAS). Presented at the 2014 International Conference on Unmanned Aircraft Systems (ICUAS), IEEE, Orlando, FL, USA, pp. 72–76 <https://doi.org/10.1109/ICUAS.2014.6842240>.
- Senapati, D., Bhattacharya, M., Kar, A., Chini, D.S., Das, B.K., Patra, B.C., 2019. Environmental DNA (eDNA): a promising biological survey tool for aquatic species detection. *Proc. Zool. Soc.* 72, 211–228.
- Sengupta, M.E., Hellström, M., Kariuki, H.C., Olsen, A., Thomsen, P.F., Mejer, H., Willerslev, E., Mwanje, M.T., Madsen, H., Kristensen, T.K., Stensgaard, A.-S., Vennervald, B.J., 2019. Environmental DNA for improved detection and environmental surveillance of schistosomiasis. *Proc. Natl. Acad. Sci.* 116, 8931–8940. <https://doi.org/10.1073/pnas.1815046116>.
- Sengupta, M.E., Lynggaard, C., Mukaratirwa, S., Vennervald, B.J., Stensgaard, A.S., 2022. Environmental DNA in human and veterinary parasitology-current applications and future prospects for monitoring and control. *Food Waterb. Paras.* 29, e00183.
- Sepulveda, A.J., Hutchins, P.R., Forstchen, M., McKeefry, M.N., Swigris, A.M., 2020. The elephant in the lab (and Field): contamination in aquatic environmental DNA studies. *Front. Ecol. Evol.* 8, 609973. <https://doi.org/10.3389/fevo.2020.609973>.
- Seymour, M., Edwards, F.K., Cosby, B.J., Kelly, M.G., de Bruyn, M., Carvalho, G.R., Creer, S., 2020. Executing multi-taxa eDNA ecological assessment via traditional metrics and interactive networks. *Sci. Total Environ.* 729, 138801. <https://doi.org/10.1016/j.scitotenv.2020.138801>.
- Shehata, H.R., Bourque, D., Steinke, D., Chen, S., Hanner, R., 2019. Survey of mislabelling across finfish supply chain reveals mislabelling both outside and within Canada. *Food Res. Int.* 121, 723–729. <https://doi.org/10.1016/j.foodres.2018.12.047>.
- Sigsgaard, E.E., Jensen, M.R., Winkelmann, I.E., Möller, P.R., Hansen, M.M., Thomsen, P.F., 2020. Population-level inferences from environmental DNA—Current status and future perspectives. *Evol. Appl.* 13, 245–262. <https://doi.org/10.1111/eva.12882>.
- Smart, A.S., Weeks, A.R., van Rooyen, A.R., Moore, A., McCarthy, M.A., Tingley, R., 2016. Assessing the cost-efficiency of environmental DNA sampling. *Methods Ecol. Evol.* 7, 1291–1298.
- Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S.T., Sigsgaard, E.E., Hellström, M., 2017. Comparison of capture and storage methods for aqueous microbial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods Ecol. Evol.* 8, 635–645. <https://doi.org/10.1111/210X.12683>.
- Stat, M., John, J., DiBattista, J.D., Newman, S.J., Bunce, M., Harvey, E.S., 2019. Combined use of eDNA metabarcoding and video surveillance for the assessment of fish biodiversity. *Cons. Biol.* 33, 196–205.
- Stauffer, S., Jucker, M., Keggan, T., Marques, V., Andrello, M., Bessudo, S., Cheutin, M., Borroto-Pérez, G.H., Richards, E., Dejean, T., Hocdé, R., Juvel, J., Ladino, F., Letessier, T.B., Loiseau, N., Maire, E., Mouillot, D., Mutis Martínezguerra, M., Manel, S., Polanco Fernández, A., Valentini, A., Velez, L., Albouy, C., Pellissier, L., Waldock, C., 2021. How many replicates to accurately estimate fish biodiversity using environmental DNA on coral reefs? *Ecol. Evol.* 11, 14630–14643. <https://doi.org/10.1002/ece3.8150>.
- Stuart, S.N., Chanson, J.S., Cox, N.A., Young, B.E., Rodrigues, A.S.L., Fischman, D.L., Waller, R.W., 2004. Status and trends of amphibian declines and extinctions worldwide. *Science* 306, 1783–1786. <https://doi.org/10.1126/science.1103538>.
- Suarez-Bregua, P., Álvarez-González, M., Parsons, K.M., Rotllant, J., Pierce, G.J., Saavedra, C., 2022. Environmental DNA (eDNA) for monitoring marine mammals: challenges and opportunities. *Front. Mar. Sci.* 1886. <https://doi.org/10.3389/fmars.2022.987774>.
- Taberlet, P., Coissac, E., Hajibabaei, M., Rieseberg, L.H., 2012. Environmental DNA. *Mol. Ecol.* 21, 1789–1793. <https://doi.org/10.1111/j.1365-294X.2012.05542.x>.
- Taberlet, P., Bonin, A., Zinger, L., Coissac, E., 2018. Environmental DNA: For Biodiversity Research and Monitoring. Oxford University Press <https://doi.org/10.1093/oso/9780198767220.001.0001>.
- Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., Kawabata, Z., 2012. Estimation of fish biomass using environmental DNA. *PLoS One* 7, e35868. <https://doi.org/10.1371/journal.pone.0035868>.
- Takahara, T., Taguchi, J., Yamagishi, S., Doi, H., Ogata, S., Yamanaka, H., Minamoto, T., 2020. Suppression of environmental DNA degradation in water samples associated with different storage temperature and period using benzalkonium chloride. *Limnol. Oceanogr. Methods* 18, 437–445. <https://doi.org/10.1002/lom3.10374>.
- Takasaki, K., Aihara, H., Imanaka, T., Matsudaira, T., Tsukahara, K., Usui, A., Osaki, S., Doi, H., 2021. Water pre-filtration methods to improve environmental DNA detection by real-time PCR and metabarcoding. *PLoS One* 16, e0250162. <https://doi.org/10.1371/journal.pone.0250162>.
- Thienemann, A., 1912. Aristoteles und die Abwasserbiologie. *Festschrift Medizinisch-Naturwissenschaftlichen Gesellschaft Münster. Commissionsverlag, Universitäts Buchhandlung Franz Coppenrath Münster*.
- Thomas, A.C., Howard, J., Nguyen, P.L., Seimon, T.A., Goldberg, C.S., 2018. eDNA sampler: a fully integrated environmental DNA sampling system. *Methods Ecol. Evol.* 9, 1379–1385. <https://doi.org/10.1111/210X.12994>.
- Thomas, A.C., Nguyen, P.L., Howard, J., Goldberg, C.S., 2019. A self-preserving, partially biodegradable eDNA filter. *Methods Ecol. Evol.* 10, 1136–1141. <https://doi.org/10.1111/210X.13212>.
- Thomas, A.C., Tank, S., Nguyen, P.L., Ponce, J., Sinnesael, M., Goldberg, C.S., 2020. A system for rapid eDNA detection of aquatic invasive species. *Environ. DNA* 2, 261–270. <https://doi.org/10.1002/edn3.25>.
- Thompson, L.R., Anderson, S.R., Den Uyl, P.A., Patin, N.V., Lim, S.J., Sanderson, G., Goodwin, K.D., 2022. Tourmaline: a containerized workflow for rapid and iterable amplicon sequence analysis using QIIME 2 and snakeake. *GigaScience* 11.
- Thomsen, P.F., Willerslev, E., 2015. Environmental DNA – an emerging tool in conservation for monitoring past and present biodiversity. *Biol. Conserv.* 183, 4–18. <https://doi.org/10.1016/j.biocon.2014.11.019>.
- Thomsen, P.F., Kielgast, J., Iversen, L.L., Möller, P.R., Rasmussen, M., Willerslev, E., 2012. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One* 7, e41732. <https://doi.org/10.1371/journal.pone.0041732>.
- Tringe, S.G., von Mering, C., Kobayashi, A., Salamov, A.A., Chen, K., Chang, H.W., Podar, M., Short, J.M., Mathur, E.J., Detter, J.C., Bork, P., Hugenholtz, P., Rubin, E.M., 2005. Comparative metagenomics of microbial communities. *Science* 308, 554–557. <https://doi.org/10.1126/science.1107851>.
- Truelove, N.K., Patin, N.V., Min, M., Pitz, K.J., Preston, C.M., Yamahara, K.M., Zhang, Y., Raanan, B.Y., Kieft, B., Hobson, B., Thompson, L.R., 2022. Expanding the temporal and spatial scales of environmental DNA research with autonomous sampling. *Environ. DNA* <https://doi.org/10.1002/edn3.299>.
- Tsuji, S., Takahara, T., Doi, H., Shibata, N., Yamanaka, H., 2019. The detection of aquatic macroorganisms using environmental DNA analysis—A review of methods for collection, extraction, and detection. *Environ. DNA* 1, 99–108. <https://doi.org/10.1002/edn3.21>.
- Tsuri, K., Ikeda, S., Hirohara, T., Shimada, Y., Minamoto, T., Yamanaka, H., 2021. Messenger RNA typing of environmental RNA (eRNA): a case study on zebrafish tank water with perspectives for the future development of eRNA analysis on aquatic vertebrates. *Environ. DNA* 3, 14–21. <https://doi.org/10.1002/edn3.169>.
- Turner, C.R., Barnes, M.A., Xu, C.C.Y., Jones, S.E., Jerde, C.L., Lodge, D.M., 2014. Particle size distribution and optimal capture of aqueous microbial eDNA. *Methods Ecol. Evol.* 5, 676–684. <https://doi.org/10.1111/2041-210X.12206>.
- Turner, M.G., Calder, W.J., Cumming, G.S., Hughes, T.P., Jentsch, A., LaDeau, S.L., Lenton, T.M., Shuman, B.N., Turetsky, M.R., Ratajczak, Z., Williams, J.W., Williams, A.P., Carpenter, S.R., 2020. Climate change, ecosystems and abrupt change: science priorities. *Philos. Trans. R. Soc. B. Biol. Sci.* 375, 20190105. <https://doi.org/10.1098/rstb.2019.0105>.
- Uchida, N., Kubota, K., Aita, S., Kazama, S., 2020. Aquatic insect community structure revealed by eDNA metabarcoding derives indices for environmental assessment. *PeerJ* 8, e9176.
- Ushio, M., Murata, K., Sado, T., Nishiumi, I., Takeshita, M., Iwasaki, W., Miya, M., 2018. Demonstration of the potential of environmental DNA as a tool for the detection of avian species. *Sci. Rep.* 8, 4493.
- Ushio, M., Furukawa, S., Murakami, H., Masuda, R., Nagano, A.J., 2022. An efficient early-pooling protocol for environmental DNA metabarcoding. *Environ. DNA* 4, 1212–1228.
- Vad, J., Kazanidis, G., Henry, L.-A., Jones, D.O.B., Tendal, O.S., Christiansen, S., Henry, T.B., Roberts, J.M., 2018. Potential impacts of offshore oil and gas activities on deep-sea sponges and the habitats they form. *Advances in Marine Biology*. Elsevier, pp. 33–60 <https://doi.org/10.1016/bs.amb.2018.01.001>.
- Valdivia-Carrillo, T., Rocha-Olivares, A., Reyes-Bonilla, H., Domínguez-Contreras, J.F., Munguía-Vega, A., 2021. Integrating eDNA metabarcoding and simultaneous underwater visual surveys to describe complex fish communities in a marine biodiversity hotspot. *Mol. Ecol. Resour.* 21, 1558–1574. <https://doi.org/10.1111/1755-0998.13375>.
- Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F., Gaboriaud, C., Jean, P., Poulet, N., Roset, N., Copp, G.H., Geniez, P., Pont, D., Argillier, C., Baudois, J.-M., Peroux, T., Crivelli, A.J., Olivier, A., Acqueberge, M., Le Brun, M., Möller, P.R., Willerslev, E., Dejean, T., 2016. Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Mol. Ecol.* 25, 929–942. <https://doi.org/10.1111/mec.13428>.
- Valsecchi, E., 2021. Scanning ferry routes: looking for eDNA traces of marine mammals and their prey. *ARPHA Conf. Abstr.* 4, e65448. <https://doi.org/10.3897/aca.e65448>.
- van der Heyde, M., Bunce, M., Wardell-Johnson, G., Fernandes, K., White, N.E., Nevill, P., 2020. Testing multiple substrates for terrestrial biodiversity monitoring using environmental DNA metabarcoding. *Mol. Ecol. Resour.* 20, 732–745. <https://doi.org/10.1111/1755-0998.13148>.
- Veilleux, H.D., Misutka, M.D., Glover, C.N., 2021. Environmental DNA and environmental RNA: current and prospective applications for biological monitoring. *Sci. Total Environ.* 782, 146891. <https://doi.org/10.1016/j.scitotenv.2021.146891>.
- Vences, M., Nagy, Z.T., Sonet, G., Verheyen, E., 2012. DNA barcoding amphibians and reptiles. In: Kress, W.J., Erickson, D.L. (Eds.), *DNA Barcodes: Methods and Protocols*. Humana Press, Totowa, NJ, pp. 79–107. https://doi.org/10.1007/978-1-61779-591-6_5.
- Vera-Baceta, M.-A., Thelwall, M., Kousha, K., 2019. Web of science and scopus language coverage. *Scientometrics* 121, 1803–1813. <https://doi.org/10.1007/s11192-019-03264-z>.
- Verdier, H., Konecny-Dupré, L., Marquette, C., Reveron, H., Tadier, S., Grémillard, L., Barthès, A., Datry, T., Bouchez, A., Lefèbvre, T., 2022. Passive sampling of environmental DNA in aquatic environments using 3D-printed hydroxyapatite samplers. *Mol. Ecol. Resour.* 22, 2158–2170. <https://doi.org/10.1111/1755-0998.13604>.
- Vivien, R., Wyler, S., Lafont, M., Pawłowski, J., 2015. Molecular barcoding of aquatic oligochaetes: implications for biomonitoring. *PLoS One* 10, e0125485. <https://doi.org/10.1371/journal.pone.0125485>.
- Wacker, S., Fossøy, F., Larsen, B.M., Brandsegg, H., Sivertsgård, R., Karlsson, S., 2019. Downstream transport and seasonal variation in freshwater pearl mussel (*Margaritifera*

- margaritifera) eDNA concentration. Environ. DNA 1, 64–73. <https://doi.org/10.1002/edn3.10>.
- Wang, H., Xia, Z., Li, S., MacIsaac, H.J., Zhan, A., 2022. What's coming eventually comes: a follow-up on an invader's spread by the world's largest water diversion in China. Biol. Invasions 1–5.
- West, K.M., Stat, M., Harvey, E.S., Skepper, C.L., DiBattista, J.D., Richards, Z.T., Travers, M.J., Newman, S.J., Bunce, M., 2020. eDNA metabarcoding survey reveals fine-scale coral reef community variation across a remote, tropical island ecosystem. Mol. Ecol. 29, 1069–1086. <https://doi.org/10.1111/mec.15382>.
- West, K.M., Adam, A.A., White, N., Robbins, W.D., Barrow, D., Lane, A.T., Richards, Z., 2022. The applicability of eDNA metabarcoding approaches for sessile benthic surveying in the Kimberley region, North-Western Australia. Environ. DNA 4, 34–49. <https://doi.org/10.1002/edn3.184>.
- Wilcox, T.M., McKelvey, K.S., Young, M.K., Engkjer, C., Lance, R.F., Lahr, A., Eby, L.A., Schwartz, M.K., 2020. Parallel, targeted analysis of environmental samples via high-throughput quantitative PCR. Environ. DNA 2, 544–553. <https://doi.org/10.1002/edn3.80>.
- Williams, K.E., Huyvaert, K.P., Piaggio, A.J., 2017. Clearing muddied waters: capture of environmental DNA from turbid waters. PLoS One 12, e0179282. <https://doi.org/10.1371/journal.pone.0179282>.
- Wittwer, C., Stoll, S., Thines, M., Nowak, C., 2019. eDNA-based crayfish plague detection as practical tool for biomonitoring and risk assessment of a. Astaci-positive crayfish populations. Biol. Invasions 21, 1075–1088. <https://doi.org/10.1007/s10530-018-1886-x>.
- Xiong, F., Shu, L., Gan, X., Zeng, H., He, S., Peng, Z., 2022. Methodology for fish biodiversity monitoring with environmental DNA metabarcoding: the primers, databases and bioinformatic pipelines. Water Biol. Sec. 1, 100007.
- Yamahara, K.M., Preston, C.M., Birch, J., Walz, K., Marin, R., Jensen, S., Pargett, D., Roman, B., Ussler, W., Zhang, Y., Ryan, J., Hobson, B., Kieft, B., Raanan, B., Goodwin, K.D., Chavez, F.P., Scholin, C., 2019. In situ autonomous acquisition and preservation of marine environmental DNA using an autonomous underwater vehicle. Front. Mar. Sci. 6, 373. <https://doi.org/10.3389/fmars.2019.00373>.
- Yao, M., Zhang, S., Lu, Q., Chen, X., Zhang, S.Y., Kong, Y., Zhao, J., 2022. Fishing for fish environmental DNA: ecological applications, methodological considerations, surveying designs, and ways forward. Mol. Ecol. 31, 5132–5164.
- Yoccoz, N.G., 2012. The future of environmental DNA in ecology. Mol. Ecol. 21, 2031–2038. <https://doi.org/10.1111/j.1365-294X.2012.05505.x>.
- Zaiko, A., Samoiloviene, A., Ardura, A., Garcia-Vazquez, E., 2015. Metabarcoding approach for nonindigenous species surveillance in marine coastal waters. Mar. Pollut. Bull. 100, 53–59. <https://doi.org/10.1016/j.marpolbul.2015.09.030>.
- Zhou, Q., Zhang, J., Fu, J., Shi, J., Jiang, G., 2008. Biomonitoring: an appealing tool for assessment of metal pollution in the aquatic ecosystem. Anal. Chim. Acta 606, 135–150. <https://doi.org/10.1016/j.aca.2007.11.018>.