CSIRO PUBLISHING

SPECIAL ISSUE

*Marine and Freshwater Research*, 2023, **74**, i–v
https://doi.org/10.1071/MF23045

Editorial

# Environmental (e)DNA in the aquatic sciences: the CATG is now well and truly out of the bag

*Anthony A. Chariton* AB

[A]School of Natural Sciences, Wallumattagal Campus, Macquarie University, Darug Nation, NSW 2113, Australia.
[B]Corresponding author. Email: anthony.chariton@mq.edu.au

Received 7 March 2023, accepted 12 March 2023, published online 27 March 2023

Environmental DNA (eDNA) can broadly be defined as the extraction of genomic DNA from multiple organisms from an environmental matrix (Tablerlet *et al.* 2012). This can include approaches that focus on extracting the DNA of taxa presently residing within the sampled matrix, e.g. micro- and meiofauna within a sediment; or the collection of degraded fragments of DNA that persist within the environment, e.g. fish shed within a water body. The latter is akin to a forensic assessment at a crime scene, where DNA of the perpetrator may still remain despite their lack of presence. Although the capacity to extract DNA from environmental samples has been around for many decades, it was the advent of high-throughput sequencing – enabling numerous taxa to be sequenced simultaneously from a single sample – combined with growing and extensive online sequence repositories, and access to high performance computing, that have been the main drivers for eDNA-based research.

Subsequent to the extraction of the DNA, several approaches can be used to obtain taxonomic information from an eDNA sample. In cases where only a few *a priori* known taxa are targeted (e.g. detecting a specific alien or endangered fish species), quantitative polymerase chain reaction (qPCR) is commonly used (e.g. Hinlo *et al.* 2017). By contrast, where numerous taxa (often thousands) of unknown and of mixed composition are being examined (e.g. benthic microbial communities), metabarcoding is the dominant approach. Traditionally, metabarcoding studies use small and taxonomically informative amplified sequences of targeted regions derived from PCRs (polymerase chain reactions). The pooled products, whether from numerous samples or various targeted loci and genes, are then collectively sequenced and subjected to bio-informatic pipelines that clean the data and assign taxonomy. Metagenomics, a PCR-free approach that sequences and assembles random DNA fragments, also known as 'shot-gun sequencing', can also be used. Presently, this approach is more bioinformatically challenging than metabarcoding, and most commonly applied to microbial studies, as they are the major constituent of most environmental samples (Wilcox *et al.* 2018). However, with the advancement of capture-based approaches, which enable the DNA of taxonomic groups of interest within a sample to be enriched prior to shot-gun sequencing (Wilcox *et al.* 2018; Seeber *et al.* 2019), there will undoubtedly be a future shift towards PCR-free approaches for routine eDNA research and monitoring. For a thorough understanding of all aspects of environmental DNA research, including approaches, challenges and applications, readers are referred to Taberlet *et al.* (2018).

Arguably, there are few fields in recent decades that have had such a rapid and profound impact on ecology as eDNA. Today, eDNA has gained considerable global popularity as an ecological tool, encompassing all levels of biodiversity from microbes to mega-fauna and across all terrestrial and aquatic biomes. Its applications are broad, ranging from the detection of invasive species (Dougherty *et al.* 2016), dietary studies (Shehzad *et al.* 2012), to non-invasive approaches for indirectly detecting mammals by their DNA within blood-sucking invertebrates (e.g. leeches) (Schnell *et al.* 2015), to the monitoring and assessment of aquatic ecosystems (Chariton *et al.* 2015; Laroche *et al.* 2016). Aquatic ecologists were among the earliest pioneers and adopters of eDNA-based approaches (Ficetola *et al.* 2008; Deagle *et al.* 2009; Chariton *et al.* 2010; Hajibabaei *et al.* 2011). Today, eDNA-based approaches are being applied routinely around the world (Cordier *et al.* 2021), as evidenced by the European Union's DNAquaNet, which aims to develop and apply eDNA-based approaches for monitoring Europe's aquatic systems (Leese *et al.* 2016).

One of the most exciting aspects of eDNA research is the capacity to obtain a vast array of ecological information from the same samples. For example, one researcher might examine the microbial component of a water sample; the same sample can be interrogated by others to detect fish or obtain phytoplankton composition. Although there is a need to take into consideration the experimental design of the initial study and its influence on subsequent interpretations (Zinger *et al.* 2019), the ability to 're-fish' ecological data from the same samples not only highlights one of the unique attributes of eDNA-based approaches but also emphasises the need for biobanking (Jarman *et al.* 2018) and the sharing of eDNA samples, which in a vast majority of cases are collected using public funds. Encouraging these approaches will not only enable researchers to reuse the samples for retrospective analyses, which are critical for monitoring anthropogenic impacts on the Earth's biomes, but also opens opportunities for the samples to be utilised to explore questions completely unrelated to their initial collection purpose.

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This Special Issue of *Marine and Freshwater Research* highlights some of the diversity of eDNA research within the aquatic sciences, including its methods, applications and utilisation. West *et al.* (2023, this issue) examined the potential for eDNA to provide occurrence data on tropical aquatic reptiles. To date, the collection of data associated with this group of reptiles has been biased towards the saltwater crocodile (*Crocodylus porosus*), resulting in data deficiencies in most species, including several species of sea snake and turtle. As stated by the authors, robust spatio-temporal records on the occurrence of species are pivotal for determining their conservation status, as well as for understanding any potential threats, e.g. bycatch from fishing. However, given the diversity of Australia's aquatic and semi-aquatic reptiles (>90 species), obtaining such information using traditional means is unviable. Using eDNA metabarcoding targeting a region of the *16S* mitochondrial gene, the authors were able to detect nine taxa. This included marine species, such as flatback and green turtles in a marine site in Western Australia (Roebuck Bay), and freshwater and semi-aquatic taxa in Cooktown (Queensland), an environment subjected to both freshwater and marine influences. Interestingly, the Cooktown samples detected an Indo-Australian water snake from the family Homalopsidae, which could not be matched to any known genus in the database, suggesting a potentially undescribed species or genus within the region. However, both saltwater crocodiles and sea snakes were not detected by the eDNA assay, despite their visual presence at a number of sites. These and other possible false negative detections may have been due to a number of reasons, including the lack of skin shedding of the species, thereby resulting in very low concentrations of labile DNA in the water bodies, inefficiencies or limitations of the assay, or an artefact of the amount of collection water. Despite the need for refinement, as required with any novel approach, this study highlights the potential to obtain occurrence data on often over-looked taxa from eDNA samples traditionally used for other means, e.g. fish studies, and supports the need for future studies to employ a greater suite of primers to truly capture the diversity of life within samples (Ficetola and Taberlet 2023).

Pollitt *et al.* (2023, this issue) further extends the idea of repurposing water samples, in this case from groundwaters, to extract novel and ecologically important data. In this paper the authors explore the possibility of using eDNA data for determining whether a tree is using groundwater. Given that groundwater is often the only reliable source of water in arid and semi-arid Australia, with this resource being extracted at an increasing and arguably unsustainable rate, there are concerns for both subterranean environments that are reliant on groundwater, as well as trees that require access to this resource, especially during periods of protracted drought. There are comparatively few remaining stands of native trees within the Murray–Darling Basin, with the region being heavily cleared for agriculture. Unfortunately, many of the remaining trees are stressed, with several species in decline (Ngugi *et al.* 2022). In order to appropriately manage groundwater-dependent ecosystems, both those above and below ground, it is pivotal to understand the reliance of different tree species on groundwater. In this paper, the authors provide a framework for incorporating eDNA as a line of evidence for groundwater use by trees. This includes conceptualising how tree eDNA enters the system; proposing what a 'real' signal might look like compared to contamination from detritus material; as well as suggesting several potential genes and regions. When combined with other eDNA data obtained from groundwaters (e.g. prokaryotic and eukaryote composition), the inclusion of information about groundwater use by trees will provide environmental managers with a greater understanding of the role of groundwater systems, and the interaction between above and below ground components.

Lopes *et al.* (2023, this issue) are looking for amphibious unicorns, or more specifically, surveying Brazilian bromeliads with the aim of detecting three very rare species of frog, including one that has not been observed for over 100 years. The authors sampled accumulated waters from tank bromeliads from the Brazilian Atlantic Forest. Given the rarity of the targeted species and to attenuate false positives, 12 PCR replicates (plus controls and blanks) were performed on each sample. Unfortunately, the three targeted species were not detected; however, the authors successfully identified the DNA of one tribe, two genera and nine amphibian species. The authors emphasise the importance of eDNA as a non-invasive tool, with the findings illustrating the need to sample microhabitats as well as other substrates, e.g. rivers and ponds, in order to gain a better of understanding of Brazil's immense and tragically declining amphibian diversity.

Although sequencing costs have markedly come down since the advent of high-throughput sequencing, extraction costs remain high. This not only constrains the number of samples that are collected in any given study, therefore potentially hindering the experimental design and robustness of the results, but can also be the limiting factor on whether an eDNA study will be performed or not. Furthermore, commercial kits are for the most part designed to use only a small amount of starting material (generally less than 1 g), requiring a sub-sample to be taken from a larger homogenised sample. This can have huge implications for sampling, and can lead to samples that are not representative of the system being sampled, or bias towards smaller taxa. This is particularly the case in sediments, where the composition of data derived from eDNA is primarily composed of micro- and meiofauna, despite our understanding of the ecological condition of aquatic systems being predominantly predicated by the knowledge derived from macrobenthos (Chariton *et al.* 2015). To both circumvent the costs and starting volume requirements of commercial kits, Zinger *et al.* (2016) demonstrated the use of a cheap and efficient phosphate-buffer approach for extracting extra-cellular eDNA (extDNA) from rainforest soils. In Pansu *et al.* (2023, this issue), they compare the extDNA approach from Zinger *et al.* (2016) with the total DNA (totalDNA) extracted using a common commercial kit from a range of sediments targeting several genes. In general, the authors found that both approaches produced similar concentrations of DNA and measurements of diversity, with the sediment type playing a more important role than the extraction protocol. Some differences were observed between the two approaches; however, each had their trade-offs. Although this may partially hinder direct comparisons between datasets collected with the two different approaches, it by no means negates the use of either. In summary, the authors provide compelling evidence that the phosphate extraction approach is viable for biomonitoring sediments, especially given its capacity to easily sample large volumes of sediment, as done in traditional

macrofauna surveys. Furthermore, reducing the costs of extractions makes eDNA benthic surveys more cost-effective, potentially aiding in their broader adoption.

eDNA-based approaches have the capacity to obtain compositional information at previously unobtainable levels, with this being particularly the case for microbial communities. This provides an opportunity to gain a far greater insight into how human activities are affecting our aquatic ecosystems. There is growing evidence to show that microbial communities are equally, and in some cases, more sensitive to pollutants than macrobenthic invertebrates (Sun *et al.* 2012; Gardham *et al.* 2014; Sutcliffe *et al.* 2019). However, there are numerous challenges in extrapolating this information into routine monitoring programs. Most notably, it still remains unclear what a microbial community should look like in a healthy system. This is in marked contrast to macrofaunal studies, where specific metrics such as diversity or the abundances of particular taxa likely reflects the condition of the environment. Secondly, composition may vary across space and time, limiting the use of indicator taxa to specific regions or times. Consequently, to date, most eDNA-bioassessment surveys are singular events that look at correlative patterns in composition versus abiotic variables, with condition being determined by the correlative strength between measured stressors and composition. A number of approaches have been developed to address these limitations, including bacterial-based indices (Aylagas *et al.* 2017), as well as machine-learning based approaches for classifying communities (Cordier *et al.* 2017; Frühe *et al.* 2021). In this issue, Codello *et al.* (2023), look at a different approach for measuring ecological stress by the use of co-occurrence networks. Co-occurrence networks are produced by examining the correlative patterns between individuals and how to they are integrated as a community (Faust and Raes 2012). It is founded on the premise that organisms do not live in isolation, and it is both their presence and interactions that drive key processes (e.g. trophic and functional) and ultimately supports and maintains biodiversity. In their paper, the authors provide readers with an overview of co-occurrence networks. They then highlight metrics and properties associated with networks that may potentially be used as indicators of stress in aquatic ecosystems. Codello *et al.* emphasised that, despite their potential, co-occurrence networks are still rarely applied as biomonitoring tool. This is likely due to a number of reasons including costs; sequencing bias; differences in methodologies; a lack of empirical data; and the challenge of distilling the information into a format that is easily useable by environmental managers and decision-makers. However, as emphasised in this article, there is a critical need for eDNA bioassessment and biomonitoring to look beyond composition in order to truly understand how communities are affected by both natural and anthropogenic stressors.

Although the papers in the Special Issue only provide a tiny snippet of eDNA research across the aquatic sciences, they undoubtedly highlight that eDNA research extends far beyond the detection of fish and monitoring of benthos. As with all approaches, eDNA-based tools are not void of limitations or misuse. Indeed, eDNA-derived data are simply data, and in common with all other data, these have pros and cons, limitations and sensitivities. From my experience, there has been an insatiable need from end-users and many researchers to make eDNA data directly comparable to those that have been traditionally obtained (e.g. macrobenthic surveys). This is reflected in the endless requests for quantifiable data from complex assemblages, when in reality this is unattainable. This is not due to the limitations of sequencing or bioinformatics, but rather due to the inherent characteristics of the samples. Within deeply complex community samples, some taxa will have more copies of the targeted genes than others; some taxa will be bigger than others; some taxa will have eggs or larvae connected to them; some will have other taxa within their guts; and some may have cells that grow larger as they age. A more-constructive approach is to determine whether eDNA-based approaches suit your ecological questions or provide more robust data to support decision-making within a specific time-frame and budget. This should include taking into consideration the ability to collect samples in a non-invasive manner; the capacity to detect cryptic and rare biota; and the potential to biobank and repurpose samples, especially given that collection is generally the greatest cost. As outlined by Codello *et al.* (2023, this issue), there is a need to re-evaluate how we look at ecological systems. This is particularly pertinent as we face the increasing influence of climate change and human-associated activities. As scientists and managers we need to truly understand how biomes are connected and the connections within them, and how changes in composition will affect ecosystems as a whole. It is here that eDNA research can truly complement other approaches.

## Data availability

Data sharing is not applicable as no new data were generated or analysed during this study.

## Conflicts of interest

A. Chariton is an editor for *Marine and Freshwater Research* and is a co-author on three of the papers within this Special Issue, but did not at any stage have editor-level access to this or those three manuscripts while they were in peer review, as is the standard practice when handling manuscripts submitted by an editor to this journal. *Marine and Freshwater Research* encourages its editors to publish in the journal and they are kept totally separate from the decision-making processes for their manuscripts. The authors have no further conflicts of interest to declare.

## Declaration of funding

This research did not receive any specific funding.

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