

Recent advances in environmental DNA-based biodiversity assessment and conservation

1 | INTRODUCTION

Knowledge of species distribution across space and time is critical to ecological conservation and environmental management at the local, regional and global scales (Albert et al., 2021). Traditional morphology-based surveys on either single-celled protists or larger animals and plants are time-consuming and largely expert-dependent (Baird & Hajibabaei, 2012; Liu et al., 2017; Yang et al., 2017). Recently, there has been considerable interest in the detection of environmental DNA (eDNA) fragments to allow species identification and monitoring within different environments, including soil, sediment, water, snow or air (Abdullah et al., 2021; Rees et al., 2014; Xie et al., 2018). The eDNA analysis can be used to detect common, endangered, invasive or rare species (Liu et al., 2019; Sepulveda et al., 2020), and provide a potent tool for elucidating mechanistic insights into ecological and evolutionary processes (Baird & Hajibabaei, 2012; Bohmann et al., 2014; Pawlowski et al., 2021). In past decades, eDNA metabarcoding has been increasingly used to study the present and past biodiversity from population to community levels, and eDNA-based surveys have revolutionized studies in ecology and biodiversity sciences, particularly in aquatic ecosystems (Euclide et al., 2021; Valentini et al., 2016).

The significance of various human activities has resulted in multiple interacting environmental stressors in all types of ecosystems (Pukk et al., 2021; Yang et al., 2022). Such stressors, including global climate change, invasive species, chemical pollution and habitat loss, have led to biodiversity crises and threatened the human sustainability and ecosystem health (Osathanunkul & Minamoto, 2021; Yang et al., 2017). Comprehensive biodiversity assessment and conservation management are prerequisites for addressing these significant challenges in the Anthropocene (Mace et al., 2012; Sepulveda et al., 2020). Indeed, effective biodiversity assessment and conservation management require a deep understanding of organisms' geographical distributions and their respective roles in ecosystem processes and services (Mo et al., 2021; West et al., 2021). However, researchers and conservation managers have encountered numerous obstacles in answering these fundamental and applied research questions at the local, regional and global scales.

The aim of this special issue—Environmental DNA-based biodiversity assessment and conservation—was to provide a selection

of studies that highlight the utility and diversity of eDNA-based research for biodiversity assessment and conservation management within marine and freshwater ecosystems. This special issue includes 12 articles that advance our knowledge of eDNA. Together, these studies deliver compelling evidence for successful applications of eDNA-based surveys in aquatic ecosystems in the Anthropocene.

2 | THIS ISSUE

2.1 | Methodological and technical advances

Six papers in this issue are focused on different technological aspects of environmental DNA, including sampling, sequence reference libraries and DNA markers. Sampling and DNA extraction are key steps in the analysis of environmental DNA (Liu et al., 2019). Curtis et al. (2021) investigated the role of water flow on eDNA concentrations and subsequent effects on the detectability of an invasive freshwater clam (*Corbicula fluminea*) in streams across seasons. Their results indicated that higher stream flows decreased eDNA concentrations and that stream floods increased the rate of false negatives (i.e. non-detections) at locations where the target organism was relatively common in autumn. These results also highlight that eDNA applications for environmental management and conservation can be highly sensitive to the abiotic and biotic context of field sampling in lotic ecosystems. Wang et al. (2021) compared eDNA metabarcoding results between classical kick-net-based samples and water samples. Interestingly, they found that water eDNA-based sampling exhibited more exact sequence variants (ESVs) than kick-net-ethanol-based sampling (2,866 vs. 2,406), but fewer macroinvertebrate ESVs (381 vs. 481). Further, kick-net-based metabarcoding was more consistent with morphological identification compared with water eDNA-based metabarcoding (24.24% vs. 17.63%).

Reliable DNA barcode reference libraries are critical for accurate species identification and biodiversity monitoring based on eDNA. Lin et al. (2021) analysed the cytochrome c oxidase subunit 1 (COI) DNA barcodes from 298 individuals of *Tanytarsus* non-biting midges, representing 56 morphospecies including several cryptic species from China. This study was an important step to build a comprehensive DNA barcode library for chironomids,

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which are an important group for biomonitoring. In another article, Marques et al. (2021) developed a user-friendly web interface (i.e. GAPeDNA) that provides a global overview of genetic database completeness for a given fish taxon, based on different metabarcoding primer pairs across space and conservation status. This tool is flexible and can be expanded to other taxa and primers upon data availability. Vieira et al. (2021) evaluated the gaps in the availability of DNA sequences, including cytochrome c oxidase subunit I (COI) and 18S rRNA (18S) gene sequences, and their accuracy for macroinvertebrates inhabiting Macaronesia's shallow marine habitats. They demonstrated the reference DNA sequence libraries are still highly incomplete, non-indigenous species have higher levels of sequence completion than native species, and that native morphospecies have a high proportion of hidden or cryptic diversity. More than one-third of the species exhibited discordant records, with higher percentages in non-indigenous species than native species. These results suggest careful compilation, verification and annotation of available sequences are fundamental to assembling large curated and reliable reference libraries with rigorous species identifications by taxonomic experts.

The eDNA-based methods are becoming technically mature and have acceptable reliabilities in many cases; however, the lack of standardized efforts in method development and validation represents a critical obstacle of eDNA applications (Pawlowski et al., 2021). Xia et al. (2021) conducted a meta-analysis to test DNA marker sensitivity and found most studies used newly designed DNA markers, but researchers did not report the marker sensitivity and the limit of detection of non-indigenous or endangered species. These results may guide researchers in experimental design choices for environmental DNA-based studies in the near future.

2.2 | Biodiversity and distributions

Six papers in this issue are focused on the biodiversity assessment and conservation management. Environmental DNA metabarcoding provides a valuable and complementary survey technique in conservation and management (Sepulveda et al., 2020). One study indicated that a simple eDNA metabarcoding assessment using a high number of low-volume (50-ml) samples, centrifugation and a single gene (mitochondrial 12S gene) can describe the coarse fish community structure of freshwater lakes and rivers (Euclide et al., 2021). The authors also suggested that additional conventional sampling and environmental DNA sampling may be necessary for a complete diversity census. Osathanukul and Minamoto (2021) used eDNA-based approaches to qualitatively and quantitatively track the crocodile newt in Thailand. They found *Tylototriton uyenoi* is severely declining due to anthropogenic factors, thereby suggesting that eDNA-based methods could help in designing an effective conservation plan. In north-western Australia, West et al. (2021) applied metabarcoding of the mitochondrial 16S rRNA and COI genes to detect bony fish, elasmobranchs and aquatic reptiles from

71 mid-shelf, inshore, coastal and nearshore estuarine sites. Their eDNA metabarcoding was a highly sensitive detection tool that was able to discern fine-scale patterns of marine fishes across the large-scale oceanic region.







Pukk et al. (2021) collected eDNA samples from 22 Michigan lakes and sequenced two mitochondrial gene regions (12S and 16S rRNA) to quantify influences of drainage connectivity and human activity on aquatic invasive species prevalence. The detection probabilities were generally higher with eDNA than traditional fisheries gear. More importantly, incorporating eDNA metabarcoding as a supplement to traditional fisheries surveys will permit managers to identify greater numbers of taxa, including early detection of invasive species, with less field effort and fish mortality. Further, eDNA methods may facilitate management activities of aquatic invasive species.

Recently, with the rapid development of sequencing technology DNA-based experiments have become routinely used to study the microbial community in various ecosystems (Abdullah et al., 2021; Liu et al., 2019). Based on high-throughput sequencing and qPCR of anammox 16S rRNA gene, Liu et al. (2021) found diverse anammox bacteria in the aerobic water columns of the South China Sea, and *Ca. Brocadia* was the most dominant genus. This finding has improved our understanding of the distribution and survival strategies of anammox communities in an aerobic marine environment. In another study, Wu et al. (2021) investigated free-living (FL), nanoparticle-associated (NA) and microparticle-associated (MA) bacterial communities in the coastal South China Sea. They found the number of amplicon sequence variants (ASVs) increased from FL, NA to MA communities, and the majority of core ASVs was potential hydrocarbonoclastic bacteria. Interestingly, the homogeneous selection was most prominent in shaping FL communities, followed by NA and MA communities, whereas the stochastic processes exhibited the reverse pattern.

In the past two decades, publication titles with “environmental DNA” have increased dramatically from 3 in 2000 to 16 in 2010 and to 223 in 2020 (Web of Science, accessed on 1 September 2021). Therefore, by no means will this special issue provide a full picture of environmental DNA (eDNA)-based studies; rather, it can serve as a window to showcase the recent and global developments in environmental DNA-based biodiversity assessment and conservation management. We believe further substantial advance is foreseeable in the fields of eDNA-based studies because they are entering an exciting and rapidly accelerating era.

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