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ORIGINAL ARTICLE

Beyond species detection—leveraging environmental DNA and environmental RNA to push beyond presence/absence applications

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1 | INTRODUCTION

Environmental DNA (eDNA) has unequivocally revolutionized the science of species detection and identification. Ficetola et al. (2008) first demonstrated the potential application of eDNA for the detection of aquatic macroorganisms. The application of genetic tools to detect (and quantify) macroorganismal eDNA has seen subsequent widespread use by researchers and burgeoning adoption by conservation managers (Bruce et al., 2021; Jerde et al., 2013; Jerde, 2019; Sepulveda et al., 2020). For aquatic macroorganisms, the capture and analysis of eDNA represents a cost-effective means to detect rare and/or invasive species and quantify community composition (Cristescu & Hebert, 2018; Jerde, 2019; Jerde et al., 2013; Taberlet et al., 2012), often outperforming traditional approaches (Boivin-Delisle et al., 2021; Sard et al., 2019; Sigsgaard et al., 2015; Spear et al., 2015). More recently, the toolbox of molecular methods to identify organisms from environmental samples has been expanded to include the analysis of environmental RNA (eRNA; Pochon et al., 2017; von Ammon et al., 2019).

However, novel research has demonstrated that eDNA and eRNA could provide additional genetic and ecological information beyond species detection inferences. The articles within this Special Issue focus on five important areas of emergent research that illustrate the potential utility of eDNA/eRNA beyond presence/absence applications. First, an improved understanding of "eDNA dynamics" (production, transportation, degradation, etc.) is crucial for the accurate interpretation of quantitative eDNA signals. Such considerations are particularly important for the application of eDNA to infer metrics of organism abundance, a second key theme tackled by several studies within this Special Issue. Environmental DNA has also been identified as a potential source of population-level genetic information, highlighting its potential utility to quantify genetic diversity in natural populations. The distinct properties of eRNA enable its application to differentiate conspecific organisms (e.g., living or dead organisms, life-history stages, and physiology); an improved understanding of eRNA dynamics is thus critical for advancing its application in ecology and the study of biodiversity. Finally, several studies demonstrate the application of eDNA and eRNA to test broad ecological or biological inferences beyond characterizing community composition, including quantifying functional diversity, assessing species' life-history events, and its potential application for ecological impact assessments. Collectively, the articles herein highlight the rapid pace of development of eDNA science in the last 15 years and illustrate the potential breadth and flexibility of environmental nucleic acids (eNAs) as tools to study ecology and biodiversity.

2 | eDNA DYNAMICS IN NATURAL ECOSYSTEMS

The quantitative interpretation of eDNA signals in natural ecosystems requires the consideration of the processes responsible for the release of eDNA by organisms, its state in the environment, transport, deposition, and degradation processes, and its spatial distribution. These factors were originally summarized as the "ecology of eDNA" (Barnes & Turner, 2016) and more recently as the "nature of eDNA" (Beentjes et al., 2019) or "eDNA dynamics" (Lacoursière-Roussel & Deiner, 2021; Mauvisseau et al., 2022). To this date, there is no overarching framework that can account for the effects of all these processes on the distribution of eDNA in nature. The development of such a model is, nevertheless, critical for correctly interpreting quantitative eDNA data, particularly as it applies to relating eDNA signals to spatial distribution, metrics of organism abundance, and life-history events (e.g., spawning). However, the patchwork of case studies delivering data points for the eventual calibration of such a model is constantly growing, with several relevant publications in this Special Issue making notable contributions. Suter et al. (2023) quantify Antarctic krill (*Euphausia superba*) eDNA via qPCR and measure its degradation for different fragment lengths of the 16S gene. Based on the comparably faster degradation of the longest fragment, they can thus discern signals obtained from "recent" versus "older" eDNA. This approach is successfully applied to samples collected in the Southern Ocean along a 4800 km long transect. Additionally, the authors show that Antarctic krill eDNA is unlikely to be detected via metabarcoding if a sample was classified as containing "older" eDNA with the qPCR assays. Scriver et al. (2023) review eDNA and eRNA persistence with a focus on potential applications of these processes to coastal marine biosecurity monitoring. They provide a standardized summary of previously measured decay rates and the factors influencing them in marine environments as a basis for accurately modeling eDNA and eRNA persistence in the future. Albeit the core focus of Brys et al. (2023) is on the improved detection and quantification of eDNA signals in duplex reactions, the ddPCR amplitude data generated in their study potentially indicate that relatively small, unlinked DNA fragments might be the basis for the majority of amplifications, thus supporting the negative relation between fragment length and abundance detected by Suter et al. (2023).

Temperature has also consistently emerged as a critical parameter related to both the production and decay of eDNA (Jo et al., 2019). A number of studies in this Special Issue add to the growing consensus that temperature is a particularly important variable to consider when interpreting quantitative eDNA signals. Bourque et al. (2023), Morrison et al. (2023), and Gaudet-Boulay et al. (2023) demonstrate the importance of the effect of temperature when relating quantitative eDNA signals to metrics of organism abundance (see discussion below), and Jo et al. (2023) similarly highlight the effect of temperature on eDNA and eRNA degradation, particularly when comparing the ratio of eDNA to eRNA.

3 | INFERRING ORGANISM ABUNDANCE FROM QUANTITATIVE eDNA DATA

Estimating abundance from quantitative eDNA signals remains a major goal at the forefront of eDNA research. While studies in natural ecosystems have consistently demonstrated positive correlations between quantitative eDNA data and metrics of organism abundance (Lamb et al., 2019; Rourke et al., 2021; Yates et al., 2019), controlled laboratory experiments are critical to understanding the functional links between organism abundance and eDNA concentrations. Bourque et al. (2023) demonstrate that extra-organismal eDNA concentrations in large-scale experimental mesocosms track *Daphnia* abundance and biomass across space and time, albeit with variable time lag. They further illustrate the importance of integrating eDNA dynamics when interpreting quantitative eDNA signals, as eDNA concentrations across mesocosms were negatively correlated with temperature and algal density. Their study is additionally notable for utilizing an invertebrate as a focal study species, given the strong representation of fish in the eDNA/abundance literature (Rourke et al., 2021).

Moving to natural ecosystems, Morrison et al. (2023) demonstrate the importance of seasonal environmental conditions on Atlantic salmon (*Salmo salar*) eDNA concentrations and their relationship with organism abundance in a riverine system in New Brunswick (Canada). As discussed above, they also find an important role of temperature on trends in eDNA concentrations across time, although Spring eDNA concentrations near the outflow of their riverine study system were most strongly impacted by fluctuations in the abundance of migratory life-story stages of Atlantic salmon.

Similarly, Gaudet-Boulay et al. (2023) compare brook trout eDNA concentrations to Brook Trout (*Salvelinus fontinalis*) fisheries data collected from 30 lakes in Quebec. Employing a systematic design to estimate mean brook trout eDNA concentrations per lake, they observe that eDNA concentrations were generally positively correlated with several fisheries abundance indicators (e.g., fish harvested/ha and fish harvest per unit effort), although the strength of the correlation varied across years. As with Morrison et al. (2023) and Bourque et al. (2023), the authors similarly find an effect of temperature on lake eDNA concentrations, although its effect again exhibited variability across years likely associated with the timing of sample collection.

While the previous studies focused on eDNA/abundance relationships among populations within a single species, Skelton et al. (2023) and Yates et al. (2023) examine relationships between quantitative metabarcoding data and *inter*specific abundance. Skelton et al. (2023) examine relationships between metabarcoding read count and fish species abundance in a garden pond treated with the piscicide rotenone. Although the authors observe that read count data were positively correlated with species abundance, they note that variance in read count among spatial replicates may exhibit a more reliable relationship with organism abundance because biases in detection/amplification of eDNA from dominant taxa can strongly

affect correlations with total read numbers. Skelton et al. (2023) also find that read count data was more closely related to allometrically corrected biomass data (i.e., reflecting total surface area) compared with the numerical abundance of species.

Yates et al. (2023) extend metabolic theory to model eDNA production, reanalyzing data from Stoeckle et al. (2021) to investigate allometric relationships between eDNA metabarcoding read count data and traditional metrics of abundance in a northwest Atlantic coastal ecosystem. They estimate that the value of the allometric scaling coefficient in this system was 0.77 (0.64–0.92) for bony fishes, following hypothesized relationships based on metabolic/physiological allometric scaling rates. To facilitate the examination of the effect of allometry on eDNA/abundance relationships in other study systems, Yates et al. (2023) also published an online tool (https://nationalgenomicscenter.shinyapps.io/InterspecificASM/) in which users can upload their own eDNA/abundance data to explore how changing the value of the assumed "metabolic scaling coefficient" strengthens or weakens such relationships.

Finally, Brys et al. (2023) highlight the importance of methodological considerations when analyzing and interpreting quantitative eDNA signals in natural ecosystems. Generating accurate and precise estimates of eDNA concentrations in natural ecosystems is crucial for facilitating the inference of abundance from quantitative eDNA data, and Brys et al. (2023) show that utilizing multiple markers substantially improved both the accuracy and precision of quantitative estimates using digital PCR analysis, particularly at low eDNA concentrations.

4 | ENVIRONMENTAL DNA AS A SOURCE OF POPULATION-LEVEL GENETIC INFORMATION

eDNA studies have traditionally relied on identifying just one or two genetic markers in an environmental sample to infer the presence (or absence) of species in an area. Each eDNA sample, however, will contain a multitude of DNA fragments or even entire genomes spanning numerous individuals and species (Bohmann et al., 2014; Jo et al., 2022). This vast collection of genetic content has the potential to offer detailed information on current population structure as well as species' evolutionary history (Adams et al., 2019; Sigsgaard et al., 2020). This Special Issue presents several examples where the scientific community is expanding the utility of eDNA surveys to extract population genetic information for the purposes of monitoring and understanding populations.

Two research articles use vastly different approaches to demonstrate how population genetic data can be extracted from eDNA samples. Tsuji et al. (2023) investigate haplotypic variation between two migratory groups of the Ayu (*Plecoglossus altivelis*), a commercially important fisheries resource, identifying genetic structure associated with migration patterns and geographical distance. The authors provide a prime illustration of how population genetic data obtained from eDNA samples increase our understanding of large-scale genetic structure and thus also improve fisheries management. Meanwhile, Danabalan et al. (2023) advance the field by recovering (near) complete mammalian mitochondrial genomes using hybridization capture on invertebrate-derived DNA (iDNA) extracted from leeches and flies. The authors demonstrated the utility of these reconstructed mitogenomes for phylogenomic analyses to explore population genomic characteristics.

While these articles highlight potential approaches to extract population genetic data from eDNA samples, a systematic review by Couton et al. (2023) identifies four main limitations of using eDNA for population genetics. These include (i) difficulties in retrieving species-specific datasets, (ii) limited potential accuracy in allele frequency estimates, (iii) loss of individual genetic information, and (iv) the absence of phenotypic and demographic information. While some of these limitations are inherent, others may be overcome through technological or methodological improvements. For example, research by Adams et al. (2023) and Wakimura et al. (2023) provides valuable insight into the issues of sensitivity and reliability of population genetic data obtained from eDNA samples and suggests methods for improvement.

Comparisons between population genetic data obtained from eDNA and traditional tissue sampling can help identify genetic variation that may otherwise be missed in eDNA samples. This was explored by Adams et al. (2023) in their study investigating haplotypic variation in Blackfoot Pāua (*Haliotis iris*), a species of abalone. The authors demonstrate the potential for eDNA sampling to uncover common haplotypic variation but concluded that rare (<5%) haplotypes were seldom recovered. To increase the detection of rare haplotypes, the authors recommend sampling multiple populations since haplotypes that may be rare at one site could be common at another.

Meanwhile, Wakimura et al. (2023) recognize that obtaining detailed genetic information from eDNA samples requires overcoming several challenges to successfully target and extract low-abundance DNA markers in a reliable and repeatable manner. The authors make progress on this by developing a high throughput sequencing approach to accurately discriminate chimeric and erroneous sequences without limiting the detection of rare haplotypes. They apply this approach to infer historic population expansions and contractions of an endangered fish (*Gnathopogon caerulescens*) in different locations. In doing so, they demonstrate the applicability of reliable population genetic eDNA data to the conservation and management of fish resources and endangered species.

5 | UNDERSTANDING eRNA DYNAMICS

The distinct properties of eRNA could lend itself to novel applications that are not possible to achieve with eDNA. Environmental RNA is produced through transcription, which only occurs in living cells, and eRNA thus degrades much more quickly relative to eDNA

(Wood et al., 2020; Marshall et al., 2021); as a result, eRNA may better reflect the temporal or spatial signal of living organisms compared with eDNA (Pochon et al., 2017; Cristescu, 2019). Similarly, because eRNA is only produced from actively transcribed genes, it may enable researchers to distinguish between conspecifics based on their physiological state (Tsuri et al., 2020; Yates et al., 2021). However, eRNA research is in its infancy relative to eDNA; foundational work on eRNA dynamics under different conditions is important to advancing further research and applications.

Jo et al. (2023) employ a factorial experimental design to evaluate Zebrafish (*Danio rerio*) mitochondrial and nuclear eDNA and eRNA degradation rates using qPCR under different temperature and pH conditions, finding that high temperatures increased degradation of both eNAs but that alkaline conditions increased only eRNA degradation, with no overall impact of genomic region on decay. Nevertheless, they demonstrate that eRNA was readily detectable and that the ratio of eDNA to eRNA increased over time, implying that eRNA may be a promising method for quantifying "living" biotic assemblages.

Kagzi et al. (2023) similarly employ metabarcoding to quantify *Arthropoda* community diversity after the removal of live organisms in seminatural mesocosms using eDNA and eRNA from both nuclear and mitochondrial markers. Contrary to Jo et al. (2023), they do not observe a consistent difference in degradation between eDNA and eRNA markers, although degradation was highly variability across eNA type and genomic source. They conclude that the comparability of estimates of community diversity recovered from different eNAs may be marker and context-specific, highlighting the need for further comparative research and careful consideration during application.

Scriver et al. (2023) again highlight the potential of eRNA to detect "living" assemblages but note that a myriad of factors can potentially impact eRNA degradation (e.g., environmental conditions, genomic source, taxon, and physiology of source organisms) and thus the interpretation of data generated from it.

6 | APPLYING eDNA OR eRNA TO TEST ECOLOGICAL OR BIOLOGICAL QUESTIONS BEYOND COMMUNITY COMPOSITION

While there is a high demand for practical applications of eDNA analyses for inferring ecological information such as behaviors and species interactions, there are some uncertainties regarding such usages. In this Special Issue, five studies presented cutting-edge usage of eDNA analyses in this context or assessed the variabilities potentially caused by the analyses in these applications.

Di Muri et al. (2023) assess the effectiveness of eDNA metabarcoding for inferring the spatiotemporal distribution of the spawning activity of Arctic char (*Salvelinus alpinus*) in Windermere, UK, and observe corresponding peaks of relative read numbers with conventional netting survey. They show that eDNA metabarcoding may accurately and efficiently define the spatial and temporal characteristics of fish spawning activity in lentic environments. Sutherland et al. (2023) conduct a unique trial using eRNA for taxonomic assignments of microbes and gene expression analysis at the same time. Water samples were collected in a shellfish hatchery in British Columbia, Canada, and they detected temporal changes in the microbial community composition and functional gene differences associated with abiotic factors and time. This broadens the application of eRNA analysis and enables the evaluation of environmental conditions based on the spatiotemporal distribution of transcribed functional genes in the focal ecosystem.

An interesting simulation on the variability of estimates of functional diversity for fish communities based on eDNA metabarcoding was carried out by Condachou et al. (2023). By employing eDNA analysis to infer species composition, they demonstrate that it may be possible to estimate the functional characteristics of a community in conjunction with another database containing functional attributes of the inhabiting organisms. The assessment of functional diversity, however, may be influenced by uncertainty in the taxonomic assignment of species due to variations in taxonomic granularity. Condachou et al. (2023) manipulate the uncertainty in the taxonomic assignment for fish species and found that misidentification of species within the same genus or family did not cause variability in functional diversity higher than 30% in comparison with a case with perfect taxonomic identification at the species level. This is a case study focusing on two sample rivers, but it raises warning flags for the general functional diversity estimation based on metabarcoding data.

Querejeta et al. (2023) assess the pollinated plants in sunflower crops in France by using metabarcoding of pollen loads from wild bees for plant species identification. The study's goal was unique in that they attempt to demonstrate the association between species traits of bees such as body size and sociality level and the taxonomic spectrum of pollinated plants. By researching pollinator–plant interactions and their context-dependent changes, it may be possible to better understand the functional value of wild bees in agroecosystems. Changes in species composition and their distributions are the measurable indices for Ecological Impact Assessments (EIA), and eDNA analysis would be an advantageous method for EIA. Coston-Guarini et al. (2023) explore the suitability of eDNA analysis for EIA by simulating the detection probability of eDNA of the target population with some factors causing variabilities such as population growth and the mobility of the population. Their results highlighted the need for testing sampling strategies and basic environmental factors to ensure reliable forecasting of eDNA reactivity when applying eDNA analysis for EIA.

7 | CONCLUSION

While eDNA and eRNA have proven effective for species detection, emergent research has clearly demonstrated that eDNA/eRNA represent a potential trove of additional genetic and

ecological information. The contributions in this Special Issue illustrate the rapid advance of our understanding of eDNA and eRNA in natural ecosystems, as well as the importance of incorporating the dynamic processes behind eDNA/eRNA release, distribution, and persistence in the environment into the ecological and genetic interpretation of eDNA and eRNA signals. Environmental DNA and environmental RNA demonstrate strong potential for applications that push beyond presence/absence inferences in natural ecosystems, and the articles represented in this collection are at the forefront of this knowledge boundary. While the practical implementation of eDNA/eRNA for such purposes is still in its infancy, the manuscripts in this issue highlight findings that simultaneously illustrate its potential promise and inherent limitations, while also identifying auspicious areas for further research. In 15 short years, the field of eDNA has moved from a proof-of-concept demonstration of aquatic species detection (Ficetola et al., 2008) to a rapidly expanding number of potential applications. The manuscripts in this Special Issue represent examples of the ground-breaking ways in which the scientific community is applying the analysis of eDNA and eRNA to advance the study of ecology, genetics, and conservation of biodiversity, setting the stage for another innovative 15 years of scientific discovery.

CONFLICT OF INTEREST STATEMENT

None

DATA AVAILABILITY STATEMENT

None

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