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ORIGINAL ARTICLE

Introduction to special issue: Advancing disease ecology through eDNA monitoring of infectious agents

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Abstract

This special issue focuses on the applications of environmental DNA (eDNA) sequencing for the detection and monitoring of disease-causing agents, including viruses, bacteria, protozoans, myxozoans, fungi, trematodes, and arthropods. We explore the impact of eDNA technologies, such as metabarcoding and qPCR, in understanding the dynamics of pathogens in various environments as well as their implications for conservation, biosecurity, and veterinary and agricultural health under the "One Health" framework. This issue addresses how molecular sequencing provides innovative solutions to the challenges faced by conventional parasite and pathogen detection methods, enabling a more comprehensive understanding of the spatiotemporal dynamics of disease agents. Finally, we discuss the challenges in eDNA applications, such as primer development and taxonomic resolution, and the opportunities for future research in advancing eDNA methodologies for infectious disease studies. This issue highlights the growing importance of eDNA surveillance in understanding and managing the health of ecosystems and at-risk species.

1 | INTRODUCTION

In this special issue, the focus is on the detection and monitoring of disease-causing agents. The body of work herein applies both targeted species approaches (qPCR, ddPCR) and metabarcoding approaches (e.g., amplicons for specific taxonomic groups) to detect viruses, bacteria, protozoans, myxozoans, fungi, trematodes, and arthropods. Study motivations range from disentangling ecological interactions to tool development and investigation of important agricultural, food security, and health challenges. Further, we discuss the opportunities and limitations relevant for sustained and improved use of DNA detection methods for monitoring parasites and pathogens. The exemplary research in this special issue demonstrates applications of eDNA technologies in the directions of the "One Health" unifying approach to balance and manage the health of people, animals, and the environment (Cleaveland et al., 2017; One Health High-Level Expert Panel (OHHLEP) et al., 2022).

2 | ADVANCES OF eDNA DETECTION METHODS

In the past 15 years, there has been a surge in research on the sampling and detection of environmental DNA (eDNA) from nearly every sample type, including water (Ficetola et al., 2008), soil (Yoccoz et al., 2012), surfaces (Valentin et al., 2018), and air (Després et al., 2007), with hundreds of papers now published annually. This body of work has resulted in many proof-of-concept examples showing that the detection of DNA is possible and powerful for knowing what species are where (Deiner et al., 2017).

Further advances in application have allowed for a more robust understanding of when and how eDNA detections are useful for routine monitoring applications. For example, some studies have been able to move from presence absence in detection to a unified model for accurately estimating population abundance (Yates et al., 2023) and measuring the temporal (Levi et al., 2019) and spatial inference of presence in a land- or sea-scape (Allan et al., 2021; Carraro et al., 2020). Thus, eDNA biodiversity monitoring is moving from proof-of-concept to implementation across a broad set of applications. In this special issue, we provide a collection of research applying eDNA methodologies to the study of disease-causing agents.

3 | CONVENTIONAL METHODS TO DETECT DISEASE-CAUSING AGENTS, AND HOW eDNA SEQUENCING OFFERS POTENTIAL SOLUTIONS

Disease-causing agents range in size from miniscule viroids (single-stranded, circular RNA molecules that infect plants) to visible with the naked eye (i.e., whale tapeworms can be over 30 m). Disease-causing pathogens and parasites can also be directly inaccessible, persisting, and replicating within difficult-to-access organs within hosts. Conventional detection techniques have adapted to these challenges to identify pathogens and parasites. Expertise in morphological features is often needed for microscopic identification, or specific and sensitive molecular methods can be developed for low-titer pathogens. Oftentimes, significant optimization is required for pathogen detection from single hosts, and techniques are not always transferable to other systems. Other barriers to successful detection include time-intensive collection methods, the large number of targeted hosts needed, and timing (e.g., mass shedding coordinated to facilitate life cycle transmission and/or reproduction). Extensive host surveys and a range of biological sample types are often required to detect pathogens and parasites that reside in specific areas. Samples can range from noninvasive (fecal samples), invasive (blood spots), to lethal (necropsies). However, parasites and pathogens must be transmitted between hosts, which may involve an extended period of time in the environment.

Using molecular methods to survey parasites and pathogens from environmental samples has the potential to complement conventional methods and ameliorate some of the aforementioned challenges. Environmental DNA sequencing methods can improve our understanding of the spatiotemporal dynamics of pathogens and parasites across a diversity of systems by enabling more geographically widespread sampling (Jahan et al., 2023; Sieber et al., 2023; Young et al., 2023). The high frequency of surveillance with eDNA can lead to both the rapid update of genetic databases and increased effectiveness of conservation and management plans.

4 | LEVERAGING eDNA FOR ADVANCING UNDERSTANDINGS OF INFECTIOUS DISEASES RELEVANT FOR CONSERVATION

With ongoing anthropogenic changes to the climate and environment, there is a growing need to incorporate parasites and pathogens into assessments of wildlife and ecosystem health. Several examples in this issue highlight the ability to use both targeted and metabarcoding approaches to monitor the presence and potential impact of parasites and pathogens on terrestrial and aquatic hosts across large geographic scales. In particular, aquatic pathogens have been the subject of disease outbreaks in wildlife of both ecological and economic concern, such as crayfish, amphibians, and fish. Sieber et al. (2023) leveraged qPCR of eDNA to generate a risk map of four aquatic pathogens (the crayfish pathogen *Aphanomyces astaci*, the amphibian pathogen *Batrachochytrium dendrobatidis* (*Bd*), and the fish pathogens *Saprolegnia parasitica* and *Tetracapsuloides bryosalmonae*) across 280 sites in Switzerland. In comparison, only 45 sites had been monitored in the past 15 years for these pathogens within Switzerland using conventional methods. While the detection of the chytrid fungus, *Bd*, was rarely observed, qPCR revealed the widespread presence of all three other pathogens. In some cases, patterns of detection and co-occurrence were reflective of preferred host habitats as well as the timing of host reproduction.

In addition to monitoring the distributions of pathogens across large aquatic systems, DNA sequencing has also been applied to monitor the pathogen and mammal diversity of Côte d'Ivoire. Jahan et al. (2023) highlighted a major obstacle to using traditional camera trap methods to monitor wildlife diseases was that only animals with extremely visible disease symptoms were observed. Using DNA obtained from flies (Families: Calliphoridae and Sarcophagidae), an emerging form of anthrax caused by *Bacillus cereus biovar anthracis* (Bcbva) was tracked along a forest-to-village gradient in the Taï National Park. Positive detections of Bcbva were associated with higher rates of fly and mammal diversity, which occurred in forested areas. By taking advantage of when nonvector animals come into contact with animals or their by-products, Jahan et al. (2023) revealed new phylogenetic diversity of Bcbva and generated co-occurrence data on the rapidly changing geographic ranges of both host and pathogen.

Young et al. (2023) added an extra dimension to a large-scale metabarcoding project by assessing whether diet facilitated protozoan infection of the European turtle dove, *Streptopelia turtur*. They surveyed multiple sites in Senegal, Hungary, and France to determine if supplemental feeding sites spread the protozoan parasite, *Trichomonas gallinae*. Because supplemental feeding sites have previously been implicated in the horizontal transmission of *T. gallinae* in species of conservation concern, Young et al. (2023) expected to find a correlation between the proportion of cultivated seeds in the diet and *T. gallinae* infection. While there was no consistent association between diet and infection status, this study highlights the

potential of eDNA to gain insights into the transmission of disease-causing organisms through diet ecology. In addition to horizontal transmission, trophic transmission is a common method used by helminth parasites, and elucidating life cycles in the natural environment often presents a daunting task. By simultaneously targeting the host, diet, and parasite, DNA sequencing methods are poised to help narrow the list of candidate intermediate hosts, solve unknown life cycles, and further determine the influence of diet ecology on host–parasite interactions.

5 | APPLICATIONS OF eDNA SEQUENCING FOR VETERINARY AND AGRICULTURAL MONITORING

Pathogen surveillance is crucial for the identification of locations and time periods that are at risk for outbreaks and can be targeted for interventions. Successful surveillance relies on sufficient sampling coverage and sensitive diagnostics but is often limited by resources or access to technological advancements. In this special issue, several studies have applied eDNA approaches to improve monitoring of pathogens and important hosts, with the eventual aim of providing rapid and unbiased surveillance methods.

In agricultural settings, treatment is often reserved for infected individuals or populations. Trematode infections of livestock result in millions of lost income due to growth stunting and milk production losses, and effective treatment is hindered by a rise in antihelminthic resistance. Jones et al. (2022) demonstrate that eDNA improves the detection of areas at risk for *Fasciola hepatica* infection. By screening water samples for snail intermediate host DNA via ddPCR and qPCR assays, researchers identified more locations with snails compared to standard snail survey methods. The higher sensitivity and ability to quantify abundance fed into more robust statistical models to identify environmental features predictive of snail populations. They were also able to identify parasite DNA in a subset of the sites, but they emphasized that this may not translate to infective stages. This study highlights the potential of eDNA for more sensitive monitoring of vectors and highlights that inferences on infection risk cannot be made directly from these findings (Jones et al., 2022).

Honeybees provide essential pollinator services globally but are increasingly threatened by a myriad of parasites and pathogens. Early detection of these threats is essential for the treatment and management of colony health. In this special issue, Boardman et al. (2023) evaluate how different sources of eDNA can be used to quantify a diversity of arthropod, bacterial, and fungal species affecting honey bees. Sources of eDNA were either wiped with swabs or sprayed/washed surfaces to obtain eDNA, which was derived from areas inside hives (hive entrance reducer, frame of a drawn comb, etc.), hive tools, and the surrounding environment (soil, pond water, plant litter). Most sample sources yielded eDNA, and there were no major differences in the detection of target agents between swabbing and washing when both could be done in a similar manner. Invertebrate, bacterial, and fungal targets were enriched and submitted for sequencing. The source of eDNA impacted the detection of certain organisms, highlighting the importance of robust sampling protocols. Sources inside the hive provided detailed pathogen community data but were not practical for large-scale surveillance. Interestingly, hive tools provided sufficient eDNA and yielded a high diversity of arthropod and microbial communities; this may prove to be a useful monitoring target, but longer term studies are required (Boardman et al., 2023). Consistent with other studies in this issue (and the field), eDNA surveys detected more species than were visually observed, again highlighting these methods for high sensitivity.

6 | USING eDNA TO IMPROVE BIOSECURITY

Fungal and viral pathogens have had considerable economic impacts within the agricultural sector. Metagenomic approaches offer a solution to detect a diverse array of phytopathogens that hinder the efforts of global food biosecurity. While an increasing number of studies have shown success in testing the high-throughput sequencing methods for phytopathogen and aquaculture detections, there remains the need to balance between capturing broad taxonomic groups and identifying specific target pathogens within mixed samples.

Trollip et al. (2022) developed a modular, metabarcoding protocol to improve the species-level detection of fungal phytopathogens. The Internal Transcribed Spacer (ITS) region has become the de facto barcode for use in fungal taxonomic identification of environmentally derived sequences. Yet, the ITS region consistently underperforms for several taxa of biosecurity importance due to complications of intra-species and intra-isolate variation. Trollip et al. (2022) tested a protocol for rapidly and accurately detecting pathogens of biosecurity importance when present in mixed DNA samples at low concentrations. They used *Ophiostomatales* (*Ascomycota*: *Sordariomycetes*) as a model taxon and evaluated five barcoding loci: ITS1, ITS2, the large ribosomal subunit (LSU), translation elongation factor 1-alpha (TEF1α), and calmodulin (CAL). A dual approach using ITS1 and TEF1α performed the best when applied to mock communities. Trollip and colleagues have provided a reliable molecular diagnostic tool with the ability to capture both broad and specific phytopathogenic groups. When applied with knowledge about the system, this rigorous framework can be applied to new targets by tailoring the various primer sets, improving the ability to detect fungal phytopathogens.

Plant viruses negatively affect food sustainability by reducing the yield and quality of crops, threatening economic losses in communities worldwide. Lopez-Roblero et al. (2023) used a metabarcoding approach to determine the diversity of viral phytopathogens in freshwater, wastewater, and sludge in a southern tropical region of Mexico. They detected 15 different phytopathogenic viruses within the family Virgaviridae and the genus *Tobamovirus*. Further phylogenetic analysis of the detected viruses revealed that the strains infecting the legume and gourd families may have originated from

locally cultivated produce, whereas strains infecting the nightshade family may have derived from imported foods to Mexico. Biosecurity concerns remain as the freshwater and reclaimed wastewater used in agriculture in Mexico are highly contaminated. Discerning the identities and origins of viral pathogens that threaten agricultural production are the first steps in developing mitigation plans to prevent further spread.

Aquaculture is a growing and significant contributor to the global food supply. However, the industry is impacted by sporadic and devastating animal disease outbreaks, which cause significant animal welfare issues and massive economic losses. Viral infections have high mortality rates, and early and sensitive diagnostics are key for mitigating outbreaks. Environmental monitoring, wherein genetic material is concentrated to improve sensitivity, offers an option for monitoring populations. In this issue, Ip and colleagues (Ip et al., 2023) tested collection and extraction methodologies for detecting two serious fish pathogens: red seabream iridovirus (RSIV, dsDNA virus) and nervous necrosis virus (NNV, ssRNA virus). The study determined how variation in concentration methods (vacuum filtration, syringe filtration, and centrifugal ultrafiltration) of naturally occurring sea water and nucleic acid extraction methods (automated or manual spin column) affected recovery rates of known levels of virus spike-ins. eDNA and eRNA were quantified using real-time PCR with probes for each specific virus' DNA or cDNA. The recovery of NNV was highest with centrifugal ultrafiltration concentration. However, centrifugal filtration was not able to recover RSIV, and instead, vacuum filtration was most successful in recovering the DNA virus. Variation in filter pore size for each concentration method is likely to drive the yield differences between viruses in this study. This emphasizes that specific pathogen sizes should be considered when designing eDNA monitoring methods. Important next steps are determining the limit of detection in aquaculture systems and interpreting how eDNA/eRNA detection translates to active infections.

7 | CHALLENGES AND OPPORTUNITIES IN THE ADVANCEMENT OF eDNA SEQUENCING FOR INFECTIOUS DISEASE STUDIES

Molecular methods applied to disease studies are not without constraints that might limit their utility, and many of these constraints are highlighted by the papers in this special issue. Researchers used a variety of sampling and laboratory methods to detect and quantify infectious agents and hosts. Targeted approaches, such as those used to detect specific trematode species (Jones et al., 2022), can be fine-tuned to have high sensitivity and specificity. However, detection of DNA in environmental samples does not translate to transmission risk because not all parasite and pathogen life stages are infective. Future studies may be able to leverage environmental RNA specific to life stages to quantify transmission potential.

Another constraint is the difficulty of developing effective primers. Approaches that cast a wide taxonomic net, that is, arthropods, fungi, and bacteria (Boardman et al., 2023), are able to characterize entire communities. However, this requires several different metabarcoding primer sets and multiple enrichment steps. Because parasites and pathogens are characterized based on life history strategies rather than shared genetic histories, neither are monophyletic groups. Therefore, primer selection can be difficult when designing a multimarker sequencing approach. Considering the financial and time constraints as well as the tradeoffs between specificity and sensitivity amongst available primers is crucial when designing a multimarker sequencing approach for parasites and pathogens.

Particularly for metabarcoding studies, a lack of barcoded taxa deposited in online databases imposes a taxonomic limit of detectability (Trollip et al., 2022). As taxonomic resolution increases, detectability rises. In some cases, prior knowledge about the taxa likely to occur at the sampling location can make it possible to infer finer taxonomic detail than is available in databases, depending on the size of the system. Nonetheless, more taxonomy and systematics research will improve the resolution capabilities of such sequencing approaches.

Similar to free-living organisms, the ecology of parasites and pathogens (e.g., life cycle stage, seasonality, and species/host distribution changes) may bias occurrence detections. As discussed by Sieber et al. (2023), performing temporally repeated surveys will help account for the distribution dynamics of targeted parasites, pathogens, and their hosts. One final consideration that is frequently discussed amongst those who study free-living organisms with eDNA but not parasites and pathogens is the difficulty of moving from occurrence data (presence/absence) to biomass estimates (counts, burden densities, etc.). For example, life cycle stages may differentially influence detection rates, whereby juvenile or adult helminths actively shedding DNA may yield higher read abundances compared to embryo DNA that is tightly bound within an eggshell. Increased experimental studies measuring the correlations between DNA read abundance data and parasite or pathogen densities at various life stages stand to further advance the utility of eDNA sequencing methods to monitor infectious agents.

This special issue highlights recent advances in the use of environmental DNA surveillance for disease-causing parasites and pathogens. They further the applications in complex ecological backgrounds that play a role in the transmission of disease and give us new tools that go beyond our conventional methods for improved monitoring of the environment and species at risk. While much remains to be done, these studies showcase how and in which contexts these molecular methods can help us better understand the health of ecosystems.

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