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The future of biodiversity monitoring and conservation utilizing environmental DNA

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1 | INTRODUCTION

Technology is often looked to as a means to an end. The explosion in use of environmental DNA (eDNA) for species detection is a great example of a technological discovery that is fueling new possibilities to study and quantify the state of change, but also the recovery and resiliency of the biosphere. The articles herein contribute to *The Future of Biodiversity Monitoring and Conservation Utilizing Environmental DNA*, by tackling methodological advances and validation, shedding light on the ecology of DNA in different contexts, revealing the use of eDNA to observe behavior of species and unravel diversity of large and hyperdiverse ecosystems. On the applied side, several contributions demonstrate the use of eDNA for detecting species invasions and restoration of populations after habitat restoration. Lastly, several contributions make efforts to reduce the learning curve for use of eDNA methods and inspire people about biodiversity through using eDNA surveys in education. Thus, this special issue shows the myriad of ways in which the scientific community is continually improving the utility of surveying eDNA for the purpose of measuring, monitoring, and understanding the biosphere.

2 | METHODOLOGICAL ADVANCEMENTS

At the forefront, researchers are moving beyond detection of species to assess population information such as abundance and testing the potential to derive functional information from environmental RNA. Measuring a population's abundance is fundamental to many conservation and management questions. Spear et al. (2021) make progress on using the quantities of eDNA measured from water samples of a culturally and economically important sportfish, walleye, from 22 lakes in Wisconsin. Their observed correlation is among the best yet in natural systems and compares with adult population abundance and biomass estimated from mark–recapture surveys. Thus, demonstrating that eDNA concentrations can have a strong relationship with abundance in a range of uncertainty similar to conventional methods. They urge, though, that demographic information is still unknown. But recent advances on studying environmental RNA may change this outlook. Tsuri et al. (2021) push forward the idea that specific tissues produce certain messenger RNAs, and that by typing them, we could identify the source of their production. By using tank experiments with zebrafish, they demonstrate detection from water of the specific mRNAs to gills, skin, and intestine. While this is a proof-of-concept study, many advances can be built on the technique of mRNA-typing of eRNA in the future to dig into not only presence and abundance, but even developmental state, stress, and likely other aspects of demography when informed by developmental biology.

Several advancements in the special issue also continue to build on our established methods with the goal to improve accuracy, sensitivity, and better sampling design for specific habitats. For single species, Williams et al. (2021) compare a CRISPR-Cas assay for eDNA detection against the standard qPCR approach for the Atlantic salmon across two watersheds in northern Canada. Both detection methods showed agreement, with only a few sites not in agreement and with opposite results. Advantages for

CRISPR-Cas assays for eDNA detection are they are done with isothermal reactions and are not as likely to be inhibited as with qPCR assays. The latter of which could reduce the need for highly time-consuming DNA purification methods. These advantages are positive for more rapid and onsite detection abilities that can be useful in many management contexts.

For biomonitoring whole communities, many challenges are faced and Leese et al. (2021), derived a method for improving target detection for benthic macroinvertebrates used in water quality assessment. They first sequenced water samples in a river system with primers that more generally amplify eDNA from both prokaryotic and eukaryotic diversity to derive knowledge of the total eDNA in their system and then developed specific primers to target the desired groups while maximizing the differences to this site-specific nontarget diversity. They showed that amplification of non-target diversity could be substantially reduced using this method and recovered taxa exceeded that detected base on morphological identification. Lastly, detection methods are only as good as their samples and Carraro et al. (2021) used modeling to improve the choice of how to optimally sample eDNA from a river system. Basing the model's predictions in hydrological terms, they showed that different sampling strategies are needed depending on the distribution of the species in the river's catchment and that eDNA decay rates are important for model predictions. Having a greater understanding of the two can better guide the sampling effort needed and likely improve detection of species.

3 | METHOD VALIDATION

For making eDNA analysis a practical biodiversity monitoring tool, comparison of detection sensitivity with conventional monitoring techniques is fundamental. Afzali et al. (2021) conducted a trawling survey on the demersal fish community in the Estuary and Gulf of Saint-Lawrence, Canada, and compared the results with that of eDNA metabarcoding. Both methods produced about a 50% consensus in the detected species and the relative abundances estimated were significantly correlated with each other. Boivin-Delisle et al. (2021) compared the results of a gillnet fish survey in the Rupert River, Canada, and found eDNA metabarcoding detected a larger number of fish species. Moreover, the read number of each species detected by eDNA metabarcoding had a significant correlation with the fish captured for several fish species. Polanco Fernández et al. (2021) conducted an underwater visual census for fish in two bays near Santa Marta, Colombia, and found that eDNA metabarcoding detected a wider range of taxonomic groups than the visual surveys with higher efficiency of eDNA metabarcoding in detecting species with smaller body sizes and those inhabiting deeper water. Overall, it was found that eDNA metabarcoding can provide monitoring results that are comparable to, or more sensitive than, a variety of conventional monitoring methods.

4 | ECOLOGY OF EDNA

To make accurate inferences in space and in time for the species studied, the behavior of DNA in the environment itself is especially needed when extra-organismal DNA is the source. River systems are particularly important to understand transport dynamics as the flow of water can almost immediately separate the DNA from its source individual. Thalinger et al. (2021) demonstrate using a caged fish experiment to study the lateral and longitudinal distribution of eDNA once released from its source across seasons. They show that detection of eDNA changes seasonally and that in order to make inferences in time for detection of species in the same location, hydrological conditions and species traits are important for determining the distribution of eDNA in the lotic environment. While most of the studies in the special issue focus on aquatic systems, Kunadiya et al. (2021) used spiked soil samples of DNA and RNA to study their persistence of an important agricultural soil-borne plant pathogen, *Phytophthora cinnamomi*. Detection of this species in both natural and horticulture settings is important for many crops including avocado, pineapple, peach, chestnut, and macadamia. They show that depending on soil type, DNA inoculated soil can persist for up to 378 days, whereas RNA persisted only for a short time in the order of 1–3 days. This shows that many detections of eDNA may not be indicative of the species presence, whereas eRNA would provide a more accurate inference that the species was recently present in the soil.

5 | SPECIES ECOLOGY

Moving beyond the detection of species occurrence and community description, an increasing number of studies are showcasing the potential advantages of applying eDNA methods to gain insights into the ecology of species. The special issue comprises two illustrative examples of such studies. The reproductive biology of species is an obvious ecological component which is crucial to understand, both from a fundamental and conservation management standpoint. For aquatic species, this is challenging as it most often requires laborious and time-consuming work. In their contribution, Tsuji and Shibata (2021) combined both experimental and field observations for monitoring and understanding spawning (reproductive) events by detecting spikes in eDNA concentration after spawning events in medaka (*Oryzias* sp.). Besides detecting a spike in eDNA concentration after spawning, they found that the magnitude of the eDNA spike depended on the number of spawning activities. As such, it demonstrates the usefulness of their eDNA approach as a practical tool for studying fish reproductive biology.

Another important facet of a species' ecology pertains to their feeding behaviors. Here, dietary plasticity is important to consider as this can be a key factor allowing species to cope with environmental changes. Yet, spatio-temporal variation in diet has not been investigated routinely, possibly because of logistical constraints. In their study, Tournayre et al. (2021) applied an

eDNA metabarcoding approach to analyze the prey content of nearly 2000 fecal samples of the insectivorous greater horseshoe bat (*Rhinolophus ferrumequinum*) for several maternal colonies in France. Besides describing the diet, they tested whether the landscape characteristics surrounding colonies at different times of the year influenced the diet diversity and composition. They showed that the diet of the species was much more diverse than reported in previous studies, and that it was composed of both a core diet shared by all the colonies and a secondary diet that varied among colonies. This study is a prime illustration of how eDNA metabarcoding can improve our knowledge on the dietary habits of an elusive species.

6 | BIODIVERSITY SURVEYS

Advancing the use of eDNA methods to measure both a broad species diversity, but also population-level information is exemplified in several contributions. For example, Ratcliffe et al. (2021) used eDNA metabarcoding in fish spawning areas in the Irish and Celtic seas. Concurrent larval fish sampling by netting produced equivalent estimates of species richness and diversity with eDNA metabarcoding with 75% agreement. Klymus et al. (2020) developed two metabarcoding assays for freshwater mussels for future monitoring of the diverse and unique mussel populations in Clinch River watershed in the southeastern United States. The assays detected 19 species including eight federally endangered species. Oka et al. (2021) applied eDNA metabarcoding on a fish community with remarkably high species richness in a coral reef lagoon in Okinawa, Japan. In this system, eDNA metabarcoding detected a larger number of species and also a clear difference in the fish communities between the reef's edge and the shore-side seagrass bed even in a small lagoon, suggesting habitat segregation. Palacios Mejia et al. (2021) used three eDNA metabarcoding primer sets to detect the fauna and flora from water and sediments from Mojave Desert springs in California, USA. They successfully detected a variety of taxonomic groups, but the agreement of the species composition with the previous field survey data varied among the groups, suggesting further improvements to eDNA sampling or method choices are likely required in these habitats for complete detection. Székely et al. (2021) sampled seawater in Disko Bay, West Greenland, for detecting bowhead whales using a newly developed species-specific real-time PCR assay. While detection was observed, the assay enabled them to amplify the mtDNA control region and when Sanger sequenced, they could detect haplotypes. They also observed that water samples formed a "footprint," where the whale just started diving, marked higher success in the detection compared to the other transect water samples. These demonstrations move eDNA analysis forward further as a powerful noninvasive sampling method for applied usage with higher reliability in a diversity of systems and across the branches of the tree of life.

7 | IMPACT AND RESTORATION ASSESSMENT

The application of eDNA detections to evaluate the success of restoration activities or management strategies is emerging because it provides a fast and potentially reliable way to monitor. To advance its use in this arena, Duda et al. (2021) used multiple species-specific real-time PCR assays to monitor over four years the fish distribution after a dam removal project in Elwha River, Washington, USA. Indigenous salmon species expanded their migrating area to the upstream side of removed dam sites whereas a non-native Brook Trout has been suggested not to expand its range due to the dam removal. Rasmussen et al. (2021) applied metabarcoding of fungi and arthropod communities in an experimental vineyard to analyze the correspondence with the management strategy among integrated, organic, and biodynamic. They could show that management-dependent biodiversity changes resulted in the agricultural fields. Pearman et al. (2021) tested eDNA metabarcoding for surveillance of marine nonindigenous species in harbors with intensive trading activities. They suggested that increased detection sensitivity was achieved by using multiple barcoding markers. The continuing accumulation of case studies such as these illustrate the ways in which eDNA detection methods can be applied to monitor biological communities' responses to anthropogenic activities and can contribute to increase the efficiency of restoration/management measures aiming to restore and conserve the biosphere.

8 | ACCESSIBILITY AND EDUCATION

The several complementary advantages of using eDNA detection of species compared with or in addition to conventional survey methods are now undeniable. Yet, the value of data being collected could be undermined by the current lack of standardization guidelines, which, if adopted, would allow for more rigor in comparing data from multiple monitoring sites at different points in time. Here, Minamoto et al. (2021) summarize this key point of the recently published "Environmental DNA Sampling and Experiment Manual," which was developed under the initiative of The eDNA Society established in Japan in 2018. In particular, the authors introduce the detailed methods for surveys and experiments that are described in this manual, including the selection of sampling sites, sampling methods, filtration methods, DNA extraction, species-specific detection by both qPCR and eDNA metabarcoding. Minamoto et al. (2021) also report how the manual will assist users in conducting standardized surveys and quality experiments and provides a basis for collecting comparable data. This manual is an example of an increasing number of national and international initiatives aiming to implement standardized methodologies for local monitoring. Leading to what is hopefully the next step of standardizing the key steps that may differ among the various protocols for the collection of comparable eDNA data from around the world.

In the context of the global biodiversity declines we are facing; it is becoming urgent and crucially important to better engage all people with the scale of the biodiversity crisis and an efficient way to achieve this is through community engagement in conservation initiatives. Having people of all ages and horizons participate in measuring biodiversity of their surroundings is an efficient means to create awareness. In their contribution, Hupało et al. (2021) explore the feasibility of sampling and then sequencing eDNA from water as means of rapidly surveying urban biodiversity for educational purposes in Norway. By identifying 435 taxa from 15 sites in only two days of sampling performed by two people, this study nicely illustrates the usefulness and relative ease of eDNA analysis for rapid biodiversity surveys and its value for educational purposes. The authors also convincingly show that when combined with openly available resources, the analysis of eDNA sequence data can be used as an educational tool to raise awareness about the importance of biodiversity.

9 | DEDICATION TO CAMERON R. TURNER

We want to honor the passing of a colleague and friend by dedicating this special issue to Dr. Cameron R. Turner. His exceptional contributions to the field of Environmental DNA are infused throughout the studies included in this special issue. We are all building off the instrumental framework summarized and put forth as the 'Ecology of eDNA' (Barnes & Turner, 2016) and the many important insights regarding the structure, state, and detection of eDNA for the improvement of managing biodiversity. His body of work is cited in many of the studies in this special issue as fundamental support for hypotheses, observations, and comparative results derived from his contributions and keen insights that have pushed the field forward. While he is no longer with us, his knowledge generated and shared will continue to live on in this way.

CONFLICT OF INTEREST

None declared.

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