Review

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Environmental DNA for wildlife biology and biodiversity monitoring

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Extraction and identification of DNA from an environmental sample has proven noteworthy recently in detecting and monitoring not only common species, but also those that are endangered, invasive, or elusive. Particular attributes of so-called environmental DNA (eDNA) analysis render it a potent tool for elucidating mechanistic insights in ecological and evolutionary processes. Foremost among these is an improved ability to explore ecosystem-level processes, the generation of quantitative indices for analyses of species, community diversity, and dynamics, and novel opportunities through the use of time-serial samples and unprecedented sensitivity for detecting rare or difficult-to-sample taxa. Although technical challenges remain, here we examine the current frontiers of eDNA, outline key aspects requiring improvement, and suggest future developments and innovations for research.

From sampling organisms to sampling environments

In 1966, the writers of *Star Trek* introduced intergalactic battles, alien invaders, and technology beyond the realm of reality. When the handheld Tricorder was used by Spock to test unexplored habitats, little did the writers know that the sci-fi technology to analyse an environment and its living components from a small sample would become a reality in just 50 Earth years. Free DNA molecules are ubiquitous, released from skin, mucous, saliva, sperm, secretions, eggs, faeces, urine, blood, root, leaves, fruit, pollen, and rotting bodies and are collectively referred to as eDNA (see Glossary) [1]. Any given environmental sample will contain myriad eDNA and the information contained therein is now accessible owing to advances in sample preparation and sequencing technology. Today, science fiction is becoming reality as a growing number of biologists are using eDNA for species detection and biomonitoring, circumventing, or at least alleviating, the need to sight or sample living organisms. Such approaches are also accelerating the rate of discovery, because no *a priori* information about the likely species found in a particular environment is required to identify those species. Those working on invasive species, community and ecosystem processes underpinning biodiversity and functional diversity, and wildlife and conservation biology are likely to benefit the most through adoption of eDNA techniques. Current barriers to the use of eDNA include the requirement for extensive training in molecular biology and

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Glossary

Amplicon: a fragment of DNA or RNA created by replication events or amplification, either naturally or artificially, through, for example, PCR.
Ancient DNA (aDNA): DNA extracted from specimens that have not been intentionally preserved for genetic analysis. Such samples are typically low quality and can include specimens from museum collections, archaeological finds, and subfossil remains of tissues or other DNA-containing sources (e.g., coprolites, hair).
Blocking primer: an oligonucleotide used to bind to DNA and overlap the primer-binding sites, so that amplification of the undesired species is prevented.
Chimera: sequences that arise during amplification combining DNA fragments from two or more individuals.
Environmental DNA (eDNA): trace DNA in samples such as water, soil, or faeces. eDNA is a mixture of potentially degraded DNA from many different organisms. It is important to note that this definition remains controversial due to the sampling of whole microorganisms that might appear in an environmental sample. Although metagenomic microbial studies might use environmental sampling, they cannot always be defined as true eDNA studies because some methods first isolate microorganisms from the environment before extracting DNA.
Metagenomics: sequencing of the total DNA extracted from a sample containing many different organisms.
Operational taxonomic unit (OTU): the taxonomic level of sampling defined by the researcher in a study; for example, individuals, populations, species, genera, or strains. OTUs are generated by comparing sequences against each other to form a distance matrix, followed by clustering groups of sequences with a specified amount of variability allowed within each OTU (e.g., [67]).
Second-generation sequencing: sequencing technologies such as the Roche GS series, Illumina Genome Analyser series, and IonTorrent series that parallelise the sequencing process, producing thousands to billions of DNA sequences in single sequencing runs.

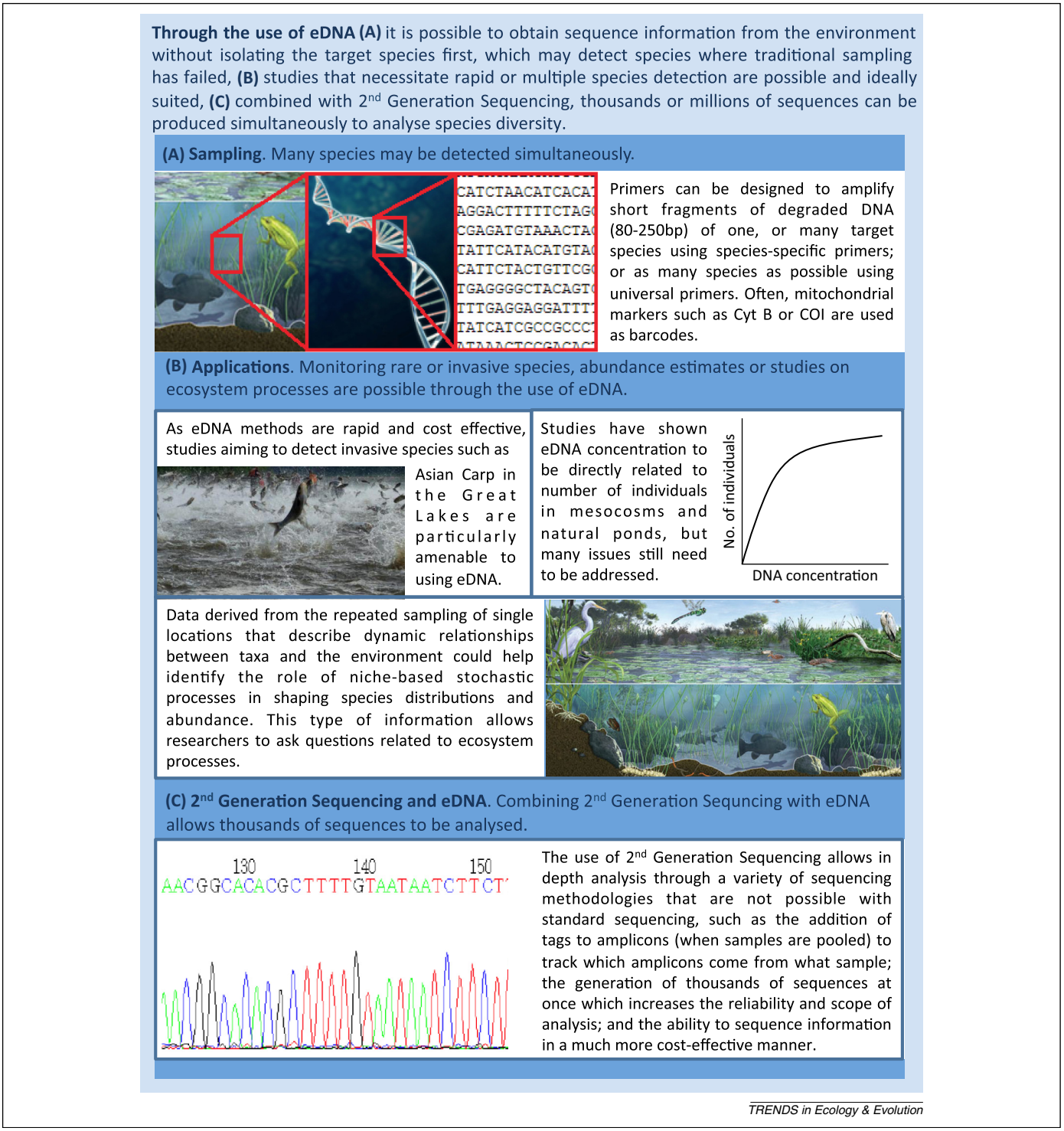

Figure 1. Summary of **(A)** the concept of environmental DNA (eDNA), **(B)** promising applications of eDNA, and **(C)** the advantages of combining eDNA with second-generation sequencing.

the subsequent genetic data analysis; however, the rapid emergence of commercial companies specialising in eDNA [e.g., SpyGen (http://www.spygen.fr/en/)] provides a way around this analytical bottleneck.

As the technologies have improved, the ability to detect trace quantities of eDNA and the breadth of environments more readily accessible to researchers have increased dramatically (Figure 1). Although the field of metagenomics (the study of many genomes) and metagenetics (the study of many genes) has until recently been considered applicable only to microorganisms, the idea of metagenetics in a macrobial sense is being applied to samples of eDNA in trace amounts left behind in the environment by organisms which are no longer present, as opposed to whole microorganisms that have been used in the latter fields. Such an approach facilitates community eDNA analysis [2] simultaneously from across the kingdoms of life, including, for example, plants, animals, fungi, and bacteria [3] (examples of which are shown in Table 1). In addition, eDNA offers researchers a glimpse of the DNA from elusive and endangered species,

Table 1. Examples of the wide range of eDNA applications

Sample	Application	Studies of importance	Refs
Applications with potential for conservation biology and policy-making decisions			
Blood meal	Species detection	DNA of rare mammals such as the elusive Truong Son muntjac (*Muntiacus truongsonensis*) identified in leeches collected in Vietnam	[58]
Faeces	Population genetics	Highly fragmented and isolated populations of giant panda (*Ailuropoda melanoleuca*) were analysed and landscape genetic patterns, divergence time, and population structure identified	[68]
Honey	Species detection	Plant and insect DNA identified in just 1 ml of honey	[69]
Seawater	Species detection	Harbour porpoise (*Phocoena phocoena*) and long-finned pilot whale (*Globicephala melas*) detected in the western Baltic	[30]
Snow	Species detection	Wolf (*Canis lupus*) DNA isolated from blood spots in the Italian Alps and Arctic fox (*Alopex lagopus*) DNA isolated from footprints	[70,71]
Soil	Species detection	Vertebrate mitochondrial DNA (mtDNA) identified in soil samples collected in a zoological garden and a safari park matched to the elephant and tiger inhabitants, respectively	[29]
Applications with potential for ecology (including palaeo- and macroecology)			
Cave sediments	Reconstructing past flora and fauna	Extinct biota identified from cave sediment in New Zealand, revealing two species of ratite moa and 29 species of plants from the prehuman era	[42]
Fresh water	Species detection and biomass estimation	Diversity of rare and threatened freshwater fish, amphibians, mammals, insects, and crustaceans was quantified in eDNA from small water samples collected in lakes, ponds, and streams	[28]
Ice cores	Reconstructing past flora and fauna	Plant and insect diversity from the past million years was catalogued from deep ice cores in Greenland	[72]
Nunatak sediments	Reconstructing past flora and fauna	Reconstruction of vegetation from the end of the Holocene Thermal Maximum [5528 $\pm$ 75 calibrated years before present (BP)] from bedrock protruding through ice sheets (nunatak sediments)	[43]
Permafrost	Reconstructing past flora and fauna, habitat conservation	Fungal, bryophyte, enchytraeid, beetle, and bird DNA identified in frozen sediment of late-Pleistocene age (circa 16 000–50 000 years BP)	[73, reviewed in 74]
Saliva/twigs	Species detection	DNA in saliva on browsed twigs identified browsing moose (*Alces alces*), red deer (*Cervus elaphus*), and roe deer (*Capreolus capreolus*), amplifying in some samples up to 24 weeks after the browsing event	[75]
Applications with potential for the understanding of ecosystems			
Air	Invasive-species detection	The presence of genetically modified organisms was detected from samples of air containing low levels of pollen	[76]
Fresh water	Wildlife-disease detection	Detecting the chytrid fungus *Batrachochytrium dendrobatidis*, which is likely to be a primary cause of amphibian population declines, in water samples	[77]
Fresh water	Invasive-species detection	The American Bullfrog (*Lithobates catesbeianus*) was successfully identified, showing that early detection of invasive species at low densities is possible and has implications for management	[44]

undetected invasive species, and species in habitats where they were previously unrecorded due to difficulty in locating such species or their active avoidance of conventional sampling methods. To date, in addition to proof of principle, eDNA studies have predominantly focused on species identification, as well as the detection of pathogenic, endangered, invasive, genetically modified, and game species and the reconstruction of diets and ancient communities (Table 1).

There is now sufficient evidence that natural processes continuously deposit DNA into the environment in ways that make it possible to reconstruct ecological and evolutionary processes from easy-to-collect samples. Open questions include how accurate, unbiased, and detailed the eDNA record is and how best to extract and analyse the genetic information with the technologies currently available today – points of particular relevance because DNA degrades rapidly once exposed to oxygen, light, heat, DNases, or water [4]. Like the related study of ancient DNA (aDNA) (e.g., [5]), eDNA approaches require rigorous standards and controls, without which the information obtained might not only be noisy, but outright misleading.

A substantial eDNA literature now exists, which we draw on below to ask what will and could be achieved through the use of eDNA and how it will and could change what we understand about species and ecosystems. To do so, we discuss how eDNA approaches can be used to examine timely questions in ecology and evolution and consider how such insights might contribute to advances in these fields. The recent surge in eDNA studies, facilitated to a large extent by recent technological advances in affordable high-throughput sequencing, demands a critique of this emerging fields' scope of application as well as its limitations, to facilitate uptake of nascent opportunities while maintaining scientific rigour. We highlight particularly promising areas of eDNA research and evaluate priorities for additional work.

Describing ecosystem-level processes

Realistic inferences and predictions about the impact of environmental change on extant biota depend increasingly on our ability to transcend boundaries among traditional biological hierarchies in the wild, extending from individuals to species, populations, and communities. The implementation of so-called ecosystem-based approaches [6],

which take a more holistic view than single-species studies, is particularly amenable to eDNA, where trophic, energetic, and terrestrial–aquatic interactions can be detected and tracked. A recent demonstration of such functional links to biodiversity [7] was among the first to link functional traits and DNA metabarcoding studies. Using community traits from metagenomic aquatic samples, significant differences were detected between the community profiles derived from the commonly used 16S rRNA gene and from functional trait sets. Traits yielded informative ecological markers by discriminating between marine ecosystems (coastal versus open ocean) and oceans (Atlantic versus Indian versus Pacific). Another recent study [8] used eDNA for a community analysis in an ecotoxicology setting. This study examined the effect of elevated levels of triclosan, a common antibiotic and antifungal agent used in many consumer goods, on benthic invertebrate communities through microcosm experiments, and observed a pronounced loss of metazoan operational taxonomic units (OTUs) due to increased levels of triclosan.

Key ecosystems underpinning plant biological production and carbon and nutrient cycling can also be readily characterised using eDNA washed from root systems [9], generating insights into the dynamics of community structure and providing an ecological framework to investigate functional links among root-associated fungi, environmental variation and ecosystem diversity, and associated services. In this context, complementary multidisciplinary approaches, such as combining eDNA with aDNA and morphological analyses of micro- and macrofossils, show particular promise for elucidating the impact of changing climates on species and communities through time [3,10–13]. Macroecology, for example, is undergoing a small revolution as studies based on environmental samples transform our understanding of microorganismal abundance, range size, and species richness (e.g., [14–16]). Such insights provide a major impetus for understanding the distribution and drivers of diversity on our planet, from megafauna to viruses, particularly in regions that are difficult to study using more traditional methods (e.g., Antarctic lakes [17], deep-sea anoxic basins [18]).

One of the main advantages of eDNA approaches to understanding ecosystems is the relative ease with which eDNA samples can be collected, which enables researchers to analyse the dynamics of community diversity through time. Rather than looking at static snapshots that are limited by the difficulty of observation, researchers can now easily sample species in an area as often as geography permits, creating what could be imagined as a 'stop-motion eDNA video'. Moreover, data derived from repeated sampling of single locations could help identify the role of niche-based and stochastic processes in shaping species distributions and abundance [19].

Using eDNA to estimate relative abundance

A major opportunity provided by quantitative analysis of eDNA is to move beyond measures of the presence–absence of a species to its relative abundance in natural systems [20,21]. Such abundance estimates are, however, not straightforward. Although presence–absence measures can provide useful indicators of biological diversity, they are often insufficient to link biological diversity to ecosystem functioning [22]. Similarly, the ability to detect rare or endangered species with confidence is of clear conservation value, but mere presence does not necessarily indicate recruitment or persistence in a given habitat. Rapid measures of abundance or biomass across time and space would be more informative and, importantly, can reveal seasonal shifts in factors such as microhabitat use for feeding and/or reproduction or refuge use, as well as impacts of predation and competition. Approaches to date to estimate abundance using eDNA include [20], which used eDNA to detect Asian carp, and repeated sampling to generate an abundance index thereof (see also [23–25]); [26] showed that rank abundance of recovered fish eDNA sequences correlated with the abundance of the corresponding species' biomass in a large mesocosm; whereas [27] extended this and used occupancy models to correct for the fact that even eDNA has a less-than-perfect detection probability. An additional way to estimate abundance estimation is to base it on DNA concentrations.

The opportunity to estimate abundance based on concentrations of eDNA relies in part on the assumption that the release of eDNA from faeces, secretions, or tissues is correlated with the abundance or standing biomass of the respective individuals. Although such correlations have been demonstrated in a few studies (e.g., [28,29]), there are three core challenges that must be overcome before informative relative abundance data can be generated. First, robust information on the persistence of eDNA in the wild from a broad range of climates and habitats is necessary. It is well established that eDNA decay rates vary considerably under different environmental conditions [30–32], which will result in biased estimates of abundance. Second, our understanding of how environmental factors, including digestive systems for faecal matter-based studies, affect eDNA concentrations needs to be improved [33–36]. Finally, the assumption needs to be tested that eDNA sequence copy numbers accurately reflect the original composition of DNA in an environmental sample [37] and are not altered somewhere along the analytical pipeline (Box 1).

Water sampling illustrates the complexity of interpreting eDNA-based studies. Detection probability is likely to be dependent on the interplay between the density of target species, the amount of DNA released via excretion, and variation in rates of dilution and diffusion depending on the environment, temperature, microbial communities, and the rate of DNA degradation, to name but a few of the variables. In the studies performed to date (e.g., [25,28,32]), waterborne eDNA appears to yield near-real-time, local, and reliable-but-noisy estimates of species frequencies, although DNA concentration may fall to sub-detectable levels once organisms are removed from the environment over relatively short time spans (around 2 weeks in Northern European artificial ponds [28]). By contrast, in soil or lake sediments, detectable traces of plant and animal eDNA persist for centuries or millennia (e.g., [33,38–41]) or even tens to hundreds of millennia when frozen (e.g., [10,41–43]). Comprehensive replicated sampling surveys are required to evaluate eDNA abundance and dynamics across a range of species and study sites.

Box 1. Improving eDNA data recovery in the laboratory

Recent years have seen rapid improvements in sequencing technologies and we are only beginning to see the associated opportunities for eDNA research. However, continued improvements to current eDNA protocols are conceivable for all aspects of laboratory work.

Sequencing library preparation

Future eDNA studies are likely to take an increasingly metagenomic approach. Instead of PCR enriching a relatively small number of markers before sequencing, the eDNA extract will be sequenced in its entirety. If, however, PCR is avoided completely, libraries have to be prepared directly from potentially highly degraded eDNA. Most existing library preparation protocols are optimised for high-quality DNA and are inefficient for highly degraded DNA [78–80]. To overcome this limitation, eDNA methods can benefit from developments in the field of aDNA, which routinely produces potentially relevant protocols in this regard (e.g., [79]).

Target enrichment

Until the sequence output of second-generation sequencing platforms becomes sufficient to avoid informative marker targeting, enrichment methods are needed. Although PCR represents the basic option, hybridisation-based sequence capture might offer an alternative [81]. With an ability to target short molecules, under relatively permissive levels of mismatch [82] such methods might bypass major disadvantages of PCR enrichment.

Blocking of undesired molecules

A further approach to increase the percentage of informative markers is to prevent non-target molecules from being enriched and sequenced by sequestering them with blocking oligonucleotides (e.g., [83]). The approach has so far mostly been used to exclude a relatively small set of contaminating molecules from being sequenced. However, as the amount of eDNA sequence data increases, it is conceivable that 'blocking libraries' for common environmental contaminants will be created. For example, blocking GC-rich molecules can reduce the amount of bacterial DNA sequenced in a library.

Direct shotgun sequencing

The power of Illumina-based direct shotgun sequencing of bulk insect samples was recently demonstrated [84], with subsequent informatics recovery of informative markers from the output. By avoiding the biases introduced by all target-enrichment strategies, as sequencing costs drop and outputs increase, we might for the first time obtain directly quantifiable data representing the unbiased components of an eDNA extract. With the arrival of third-generation single-molecule sequencers (e.g., Pacific Biosciences [85], Oxford Nanopore GridION™ and MinION™ [86]) that remove the need for amplification during library building, these benefits will increase yet further.

The potential to use eDNA sequencing as a high-throughput means of obtaining measures of abundance across large scales and many taxa simultaneously offers the promise of detecting cooperative and competitive relationships through robust tests of co-occurrence. Within the next 3–5 years, a coordinated global network of eDNA surveillance and monitoring activities can be envisioned as proof of principle is established across a range of environments and their resident taxa, moving eDNA from an emerging field to one at the forefront of biodiversity science. The applicability of such data would provide a potential framework for global ecosystem network prediction and enable the development of ecosystem-wide dynamic models [22]. Such analyses will, for example, allow exploration of long-standing issues relating to the nature and dynamics of shifts in community assembly (e.g., [3,10,41–43]).

eDNA in applied conservation biology

One of the most attractive facets of eDNA is its potential as a rapid and cost-effective tool for applied conservation biology, including early detection of invasive species and monitoring of otherwise difficult-to-detect species. The use of eDNA as an early-warning system for the detection of invasive species [20,44–46] and pathogens [47] at low density, at any life stage or season, and through *ad hoc* sampling of substrates as diverse as ship ballast water, aquaculture transits, or habitats at high risk can alert regulatory authorities before the establishment of alien species. Indeed, the method has already demonstrated particular promise. The US Fish and Wildlife Service, for example, have implemented an eDNA-based approach to monitor invasive Asian carp in the Midwest, USA (Figure 2A), providing a labour- and cost-effective alternative to traditional large-scale sampling methods such as electrofishing and/or manual netting [20]. Uptake of eDNA methodologies into biomonitoring of invasive species for fisheries appears to be increasing, with events such as the American Fisheries Society symposium in September 2013 entitled 'Environmental DNA (eDNA) Analysis – a New Genetic Tool for Monitoring, Managing, and Conserving Fishery Resources and Aquatic Habitat', which covered the topics of Asian carp in the Great Lakes, the invasive New Zealand mud snail, and the invasive African jewel fish (https://afs.confex.com/afs/2013/webprogram/Session2539.html).

Despite the promise of using eDNA as an early-warning system, eliminating false positives remains a major challenge (see Box 2 for an extended discussion). The mere presence of eDNA does not necessarily indicate the presence of the relevant organism, due to the potential for eDNA dispersal (in particular for air- or waterborne eDNA) or contamination. Where there is the potential for high connectivity, such as in aquatic systems, this challenge may be tempered if the study design incorporates risk assessment of target eDNA emanating from sources such as sewage and wastewater, bilge water discharge, excrement from predatory fish or waterfowl, dead fish carried on barges and boats from elsewhere, or even carry-over from PCR and sequencing chemistries. For example, [48] shows that invertebrate eDNA can travel up to, and potentially further than, 12 km along river systems. In short, robust control of false positives to assess and control for contamination are critical in eDNA analyses, as is the case for aDNA studies (e.g., [5]).

An extension to the use of eDNA in conservation biology is its use in species monitoring through diet analyses (e.g., [49,50]). Traditionally, diet analyses were performed either by directly observing what an animal ate or by collecting its faeces and examining prey fragments under a microscope. These results were then used in ecological studies of, for example, predator ecology, interspecific competition, or niche partitioning. For some animals, however, these approaches are unfeasible, as is the case with insectivorous bats, which prey aerially in the dark and masticate or void the larger prey fragments. eDNA has provided an alternative or complementary approach and metabarcoding, in which second-generation sequencing is performed on amplicons originating from faecal or other bodily extracts amplified with tagged universal primers [51] (Figure 2C), has made it more efficient and cost-effective to obtain diet information on a large scale (e.g., [34,52–55]; reviewed in [56,57]).

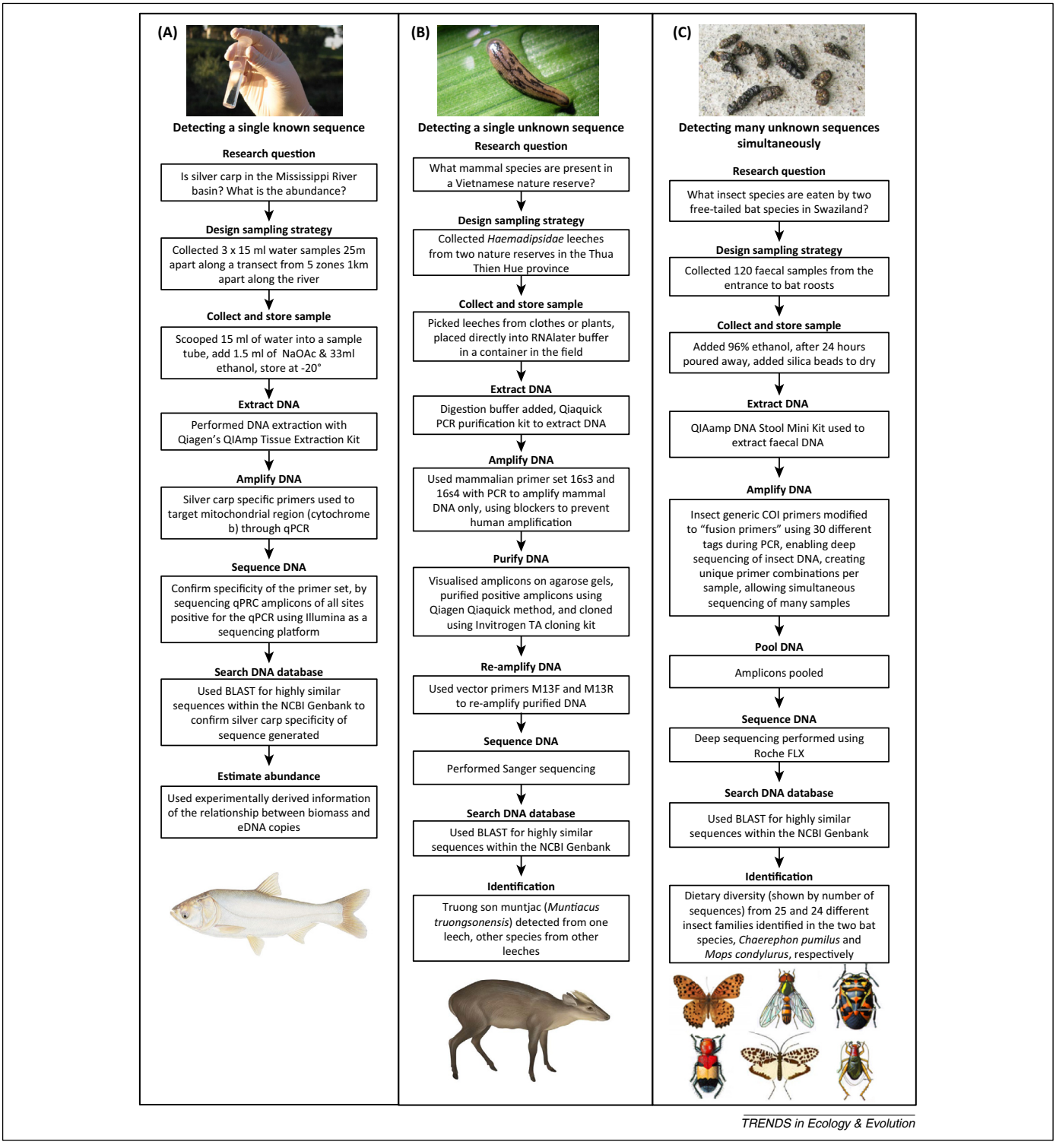

Figure 2. Exemplary environmental DNA (eDNA) case studies illustrating three research questions and the experimental procedures followed. eDNA studies can be designed in various ways to address the research question. **(A)** Detection and abundance estimation of invasive Asian carp in a water sample [20,87]. **(B)** Detection of mammal species in leech blood meals [58]. **(C)** Detection of insect prey in bat faeces [34]. Each example follows a general framework (in bold) and a specific procedure (in boxes).

Because predators or blood-sucking insects feed on biodiversity, collecting either faecal material or the insect itself for molecular diet analysis can identify rare or cryptic species that traditional monitoring methods such as camera traps might miss. Recent studies on this include stomach-content analyses of parasitic invertebrates such as leeches [58] (Figure 2B), carrion flies [59], mosquitoes [60], and ticks [61] to reveal their vertebrate hosts. In one case, Vietnamese terrestrial leeches of the genus *Haemadipsa* revealed the presence of an endemic rabbit species that had not been detected despite monitoring the site for several thousand nights with camera traps [58]. Leeches are currently being used to search for the highly endangered saola antelope in Vietnam and Laos [Saola

Box 2. Sources of uncertainty from eDNA and how they can be overcome

Source 1. False positives (type I error: eDNA detected where target species is not present) resulting from false detection of eDNA from other sources, such as tributaries into a major river, ballast water discharge, sewage and waste water, excrement from animals that prey on the target species, dead target species carried on boats, or unsterilised equipment (see [20,32,88]).

Solution 1. To ensure false positives do not occur via contamination between samples when using the same equipment, equipment must be sterilised thoroughly or, preferably, not reused [20]. Quality control to avoid false positives should be implemented in the sampling strategy; for example, blank samples can be taken into the field to ensure contamination does not occur in the transport phase [20] and samples can be taken from adjacent areas where target species are known not to occur [20]. Sampling design should incorporate a risk assessment of target and non-target eDNA.

Source 2. False positives resulting from PCR primers and probes that do not have a high enough level of specificity, allowing the amplification of 'lookalike' non-target DNA [32,45,88].

Solution 2. In silico testing of species-specific DNA-based probes and primers, such as comparing sequences with the Basic Local Alignment Search Tool (BLAST), or using ecoPCR software, as well as *in vitro* testing of probes and primers against target tissue-derived DNA [32,88]; genetic distances should also be reported [20].

Source 3. False negatives (type II error: eDNA not detected where target species is present) resulting from insufficient sensitivity or failure of methods to perform as expected [88].

Solution 3. Rigorous testing of primers against target species' DNA must be undertaken to ensure successful amplification, as well as optimising protocols to be confident of species detection before sample collection begins.

Source 4. The inability of eDNA to distinguish between live or dead organisms [88], including digested or faecal remains of target organisms derived from their predators (e.g., birds preying on fish).

Solution 4. Repeated temporal sampling of the same area will alleviate this problem to some extent. Because dead bodies, predators' faecal matter, or other introduced sources of DNA decompose and degrade over time, a species that is permanently present in an environment will still be detected after the introduced contaminants have degraded beyond the point of DNA amplification. The study's risk assessment should include any visually observed dead organisms.

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Advantages of eDNA as an assessment tool

Although advances in technology can themselves propel new conceptual insights, uptake will depend crucially on the cost-effectiveness of any new tools and the ease and efficacy of the approach. It is worth noting that, as with the introduction of DNA barcoding *sensu stricto* [62], which aimed to complement the Linnaean system of taxonomy, eDNA will most likely exert a pervasive impact through its integration with existing approaches rather than necessarily replacing them. A study from 2012 [30] demonstrates the advantage of this combined approach. By evaluating the use of eDNA in detecting marine mammals, it was shown that conventional static acoustic monitoring devices that recognise echolocation were more effective in detecting the harbor porpoise (*Phocoena phocoena*) in natural environments; however, eDNA detected the rare long-finned pilot whale (*Globicephala melas*), demonstrating how eDNA and conventional sampling can work together.

Recent work on eDNA from water samples (e.g., endangered hellbender salamanders [*Cryptobranchus a. alleganiensis*] [63]) demonstrates the benefits of eDNA analysis, which not only is less labour intensive but, importantly, is noninvasive, thereby minimising disruption to already fragile microhabitats and reducing disease transfer and stress to target species. Filtering of water samples in this case enabled the reliable detection of target eDNA even where specimens occurred at low frequencies (as also shown in [28,30,31]). In the case of the hellbender salamander, the greatest saving was in person-hours; whereas, typically, large teams are required for traditional sampling by rock lifting, a single researcher can collect and filter water. Another example in this context examined direct comparisons between eDNA and traditional estimates based on auditory and visual inspection of the invasive American bullfrog *Rana catesbeiana* [44]. Findings revealed a higher efficiency of the former in both sensitivity and sampling effort.

Various cost-effective and simple protocols can be employed to enhance effectiveness. With a diverse array of sampling (e.g., water/soil volume), concentrating (e.g., precipitation versus filters), DNA extraction (e.g., kits and protocols), primer optimisation, and PCR protocols (e.g., efficacy of quantitative PCR [qPCR] [64]) available, it is of high priority to compare their efficacy and application under a range of biological and abiotic conditions [65]. Protocols and sampling kits can be developed to enable citizen-science approaches, such as that proposed by the Freshwater Habitats Trust and partners (Spygen, ARC and University of Kent) in the UK. In 2013 this group undertook an extensive trial of the eDNA approach to test for the presence and abundance of the endangered great crested newt (*Triturus cristatus*) in British freshwaters. Results were promising [93] and suggest that community engagement with eDNA sampling is feasible; however, they, along with the stakeholders, methodological developers, resource managers, and policy makers, must be made aware of the current levels of uncertainty associated with eDNA (discussed in Box 2). This is critical when eDNA methodology is being used to inform management or development decisions, such as those faced by local planning authorities responsible for enforcing environmental regulations with regard to planning developments and endangered species.

The future of eDNA in ecology and wildlife monitoring

It is enticing to imagine the possibilities that eDNA could open up, if advances in molecular ecology, bioinformatics, and sequencing technologies continue to accelerate. The main advantages of eDNA are rooted in its autonomous nature; with a reduced need for human taxonomists, ecologists, or biologists, sampling can access inhospitable environments, target elusive species, and provide a vast reduction in labour costs. In the future, it may be possible to implement mechanical sampling of eDNA, similar to that of oil spill-sampling buoys or military sonobuoys. When combined with the technology to transmit live data such as that used by the US National Weather Service (http://earth.nullschool.net/), technology currently being developed by Oxford Nanopore Technologies to sample

Box 3. Outstanding questions

- Can we catalogue the variables that will affect eDNA half-life and can we set standards to determine whether the samples are degraded past the point of use (e.g., [32,89])?
- How do we best preserve samples for later analyses of eDNA (e.g., [90])?
- What are the dispersive properties of eDNA in various environments (e.g., [33,91]) – how readily is eDNA transported between horizons and environments (e.g., [92])?
- How can we more rapidly and cost-effectively analyse field samples? One method still in the testing phase is a mobile DNA sampler that sends results to the laboratory directly from the field (http://www.environmentalhealthnews.org/ehs/news/2013/beach-tests).
- As with the field of metagenomics, how can we more powerfully and reliably define and assign taxonomies to eDNA sequences?
- How quantitative is eDNA data – can conversion factors be meaningfully implemented to account for sampling, biomass, and amplification biases?

and analyse DNA using a handheld MinION™ device, and the current ongoing project to map the Earth's surface in 3D (http://www.bbc.co.uk/news/science-environment-16578176), it is not beyond the realm of possibility to imagine a situation where eDNA videos could be recorded in real time from automated sampling stations. Such stations could remotely relay sequence information of interest, with additional data overlaid, – including, for example, water depth, hydrological or other environmental movements, temperature, and pH – that could help identify how long eDNA had been in the environment and where it was likely to have originated from. On a smaller scale, this approach could be applied to human samplers targeting environments of interest, sampling eDNA, and remote uploading information via smartphone, creating a network of live biodiversity assessment, or the implementation of 'eDNA traps' similar to camera traps. On a larger scale, this approach could be applied to the sampling of inaccessible habitats, such as the Arctic or the deep sea, by remote samplers.

A more realisable goal in the short term is the potential for the use of eDNA in population genetics, with, for example, applications for conservation genetics and phylogeography. To date, to the best of our knowledge, such an approach has not yet been attempted. If eDNA stores sufficient population-specific information within molecular markers (e.g., mitochondrial haplotypes), it is possible that eDNA could be used directly for population genetic studies. With repeated sampling across temporal and geographical scales, this information could feed in to questions related to biogeography or palaeoecology.

The next step for eDNA

eDNA has proven its worth in detecting not only common species, but also endangered, undetected invasive, or elusive native species. As with most technological advances, limitations remain, as do many challenges that need to be overcome to move beyond mere species detection (Box 3). The potential implementation of eDNA approaches across disciplines indicates that it will be critical not only to sample, extract, and sequence eDNA in an efficient and cost-effective manner, but also to handle and analyse efficiently and reliably the typically massive data sets generated by second-generation sequencing platforms. Future eDNA studies should aim to refine and improve the processing, analysing, and organisation of what has been referred to as a 'tidal wave' of sequence information [66]. Although detailed bioinformatic considerations are beyond the scope of this review, they are crucial to consider when conducting an eDNA study. Although eDNA methods applicable to a broad range of environments and their resident taxa are currently being tried and tested, work remains to be done to ensure their reliability and repeatability (Box 1), particularly with regard to false positives and negatives (Box 2). The current evidence outlined above indicates that such effort is warranted, with exemplary eDNA studies including multiple approaches to address such uncertainties (Box 2). eDNA is on the brink of making significant contributions to our understanding of invasive species, community and ecosystem processes underpinning biodiversity and functional diversity, and wildlife and conservation biology.

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