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Perspective

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Utilizing aquatic environmental DNA to address global biodiversity targets

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Abstract

Achieving global biodiversity goals requires assessing, attributing and reversing the ongoing, unprecedented biodiversity decline in aquatic ecosystems, and relies on adequate data to inform policy and action. Analysis of environmental DNA (eDNA) has become established as a novel and powerful approach to assess the state and functioning of aquatic ecosystems, and although increasingly implemented by stakeholders its potential is not yet fully tapped. In this Perspective, we review the current state of aquatic eDNA research, focusing in particular on the policy relevance of eDNA and its utility in contributing towards the Kunming–Montreal Global Biodiversity Framework. We summarize key technological developments in eDNA science to measure organismal diversity, its potential for spatial and temporal upscaling to become a key reference for local to global biodiversity action, and the next steps needed to effectively implement eDNA for decision-making and reaching biodiversity targets. Using eDNA to support biodiversity assessment will particularly benefit the understanding of understudied ecosystems and allow the direct calculation of ecological indices and implementation of FAIR (findable, accessible, interoperable and reusable) and inclusive data curation. Important next steps for eDNA require proper method standardization and commonly agreed quality standards, populating reference databases, and overcoming methodological constraints in retrofitting novel eDNA-based approaches to existing biodiversity monitoring approaches.

Sections

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Key points

- Aquatic biodiversity is declining from local to global scales, yet in most regions, no or only minimal data on state and change of biodiversity are available.
- Representative, scalable and replicable monitoring of aquatic biodiversity is needed to achieve the Kunming–Montreal Global Biodiversity Framework targets.
- Environmental DNA (eDNA) analysis is a key technology to achieve a global measurement network of state and trends in biodiversity, and many of its technical aspects are ready to be implemented.
- eDNA analysis allows whole-community assessments, broad taxonomic coverage, high spatiotemporal resolution and calculation of environmental indices.
- Particularly for undersampled regions, large rivers, lakes and marine systems, eDNA metabarcoding might be an effective technology to rapidly gain biodiversity data.
- To make eDNA-based monitoring policy frameworks successful and trusted, inclusive development and uptake of international method standards are needed.

Introduction

The unprecedented decline of global biodiversity in terrestrial, freshwater and marine ecosystems[1–3], including the loss of functional and genetic diversity, is exceeding the planetary boundary of biosphere integrity[4] and threatening many ecosystem functions and services. Consequently, biodiversity loss has been identified as an urgent global challenge[5], and increasing political consensus exists that rapid measures to halt and reverse biodiversity loss are needed. The Kunming–Montreal Global Biodiversity Framework (GBF), adopted by the Parties of the Convention on Biological Diversity in December 2022 (ref. 6), sets out four overarching goals for 2050: (1) halt human-induced species extinction, (2) achieve sustainable use of biodiversity, (3) implement equitable sharing of benefits from biodiversity and (4) mobilize resources to close the biodiversity finance gap. The GBF also contains 23 targets to be reached by 2030, including conserving at least 30% of the world's land and sea areas, restoring degraded ecosystems, reducing pollution, ensuring sustainable use of biodiversity and performing adequate monitoring of biodiversity globally[6,7].

Freshwater and coastal marine systems are among the most affected by global change[8], and the main drivers of biodiversity decline in these ecosystems are well known[9]: habitat modification, pollution, invasive alien species, direct exploitation and climate change. Attributing the effects of these large-scale drivers to localized spatial and temporal scales is challenging, primarily because of the transport and mixing of water in aquatic systems. For example, diffuse and point-source inputs of chemicals in rivers have cumulative effects on aquatic biodiversity at a downstream catchment scale[10]. Furthermore, aquatic ecosystems cross countries, continents and are – via oceans and through the global water cycle – far more continuously integrated than terrestrial systems[11]. Consequently, effects of anthropogenic pressures have the potential to spread over large spatial extents and across political jurisdictions.

To implement actions that successfully address aquatic biodiversity loss, adequate assessments are necessary[7,12] and require monitoring of all aquatic biodiversity – including groundwater, surface water and marine systems. Such monitoring must be broadly applicable, comparable and scalable, and must be implementable for countries worldwide that have different baseline information on species diversity and abundance to effectively inform policymaking.

Environmental DNA (eDNA)[13] analysis is a key advance in biodiversity monitoring[14] that will help to achieve the GBF targets. eDNA is directly extracted from an environmental sample[15], such as water, soil or air (in contrast to DNA directly isolated from specimens) and analysed. Environmental samples might contain the DNA of all organisms present (including microorganisms, meiofauna and macrofauna), and include both intra- and extracellular DNA. eDNA in aquatic systems can also provide information on the diversity of organisms in adjacent terrestrial systems whose DNA is washed into the water bodies[16,17]. Environmental samples might also contain RNA (eRNA)[18,19], which is more difficult to handle, but can reflect more recent and metabolically active community signals than eDNA[20,21].

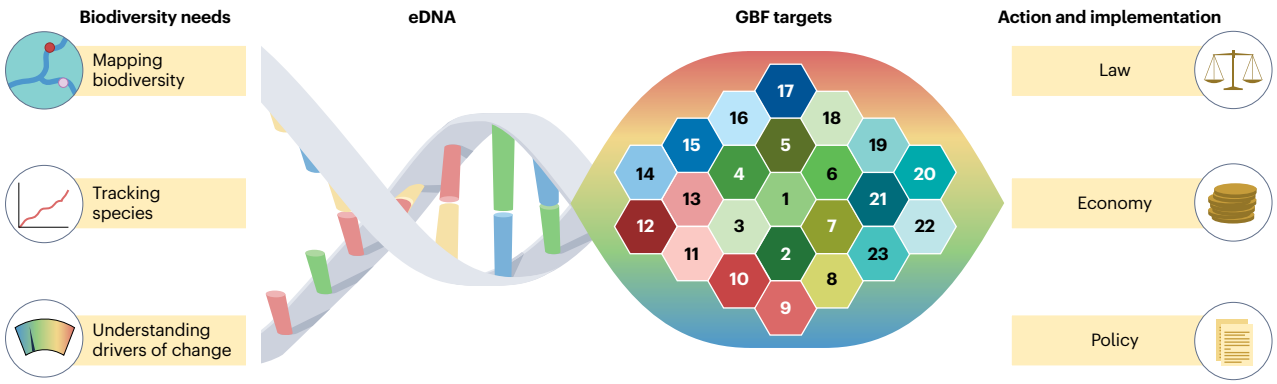
As eDNA can be sampled and analysed across all aquatic ecosystems to detect organisms in a universal manner, it can be applied to meet multiple GBF targets (Fig. 1). Methods based on eDNA are already established and being implemented for the early detection and mitigation of invasive alien species[22,23] (GBF target 6: reduce the introduction of invasive alien species by 50%) and the evaluation of the state of ecosystems under multiple stressors and in pre- and post-restoration states[24,25] (target 2: restore 30% of all degraded ecosystems). Collecting eDNA samples is becoming increasingly fast and cost-efficient[26], rendering this approach highly scalable across space and time. The simplicity of gathering water samples also grants eDNA analysis a high potential for automatization[27,28] and for its inclusion in citizen science projects[29–31]. Methods based on eDNA could, therefore, be implemented across the world, even in regions for which we currently lack adequate biodiversity data.

In this Perspective, we examine the current state of aquatic eDNA research, with a particular focus on the policy relevance of eDNA and its utility in contributing towards GBF targets (Fig. 1). We focus on eDNA only (not eRNA) because of its higher readiness level for biodiversity monitoring. First, we explain the technical aspects of eDNA-based biodiversity monitoring required for assessing baseline states and action–response effects. Second, we assess the use of eDNA at the level of communities and ecosystems, targeted species, and its ability to attribute environmental change to underlying drivers. Finally, we outline necessary steps, including standardization, data curation and data deposition, to make eDNA analysis globally accessible.

The promise of eDNA

DNA is universal across all species, and traces of DNA are left in the environment by all organisms, for example, by shedding skin cells. Extraction of this DNA from an environmental sample, in theory, enables the detection of any organism, either by targeted screening for single species or assessment of whole communities[13,32] (Fig. 2). In both cases[15], the first step is sampling water in the focal environment and extracting the DNA present in these samples. For species-specific assays, a portion of DNA is amplified using primers specific to this taxon[33,34] and the number of copies present in the original sample is evaluated using a quantitative polymerase chain reaction (qPCR)[35] approach, such as real-time qPCR or digital PCR. When assessing whole communities via metabarcoding[14], a barcode gene

a eDNA can inform and support progress towards GBF targets



b Example contributions of eDNA to GBF targets

	GBF target	eDNA contributions
1	Plan and manage all areas to reduce biodiversity loss	Monitor and assess species richness to identify biodiversity hotspot and target species occurrences
2	Restore 30% of all degraded ecosystems	Evaluate pre- and post-restoration effectively
3	Conserve 30% of land, waters and seas	Effective single species gene flow assessment, effective population size estimates, eDNA-derived beta-diversity and community dynamics
6	Reduce the introduction of invasive alien species by 50% and minimize their impact	Species-specific assays for early detection to prevent invasive species spread
7	Reduce pollution to levels that are not harmful to biodiversity	Nuanced pollution indicators derived from eDNA data
14	Integrate biodiversity in decision-making at every level	Informed decision-making from effective biomonitoring with highly resolved spatial and temporal eDNA

Fig. 1 | Contribution of eDNA to the Kunming–Montreal Global Biodiversity Framework. a, The Kunming–Montreal Global Biodiversity Framework (GBF) identified 23 action-oriented global targets to be reached by 2030 (ref. 6). Many of these targets require information on the state, change and trends of biodiversity for scientific, political and economic decision-making. Using environmental DNA (eDNA) to provide information on biological diversity offers a potentially universal approach that can support GBF targets by providing baseline data and action–response information to guide decision-making. Establishing, tracking and assessing biodiversity hotspots, biodiversity trends and change is particularly important. **b**, Multiple targets can be directly assessed using existing eDNA technologies, which are sufficiently developed for implementation but not yet routinely used in most countries.

region is amplified using primers with a broader taxonomic scope. The amplified DNA is sequenced via high-throughput sequencing technologies[36], which yield millions to billions of sequences (termed 'reads'). These sequences are then bioinformatically processed and clustered into meaningful units (either as operational taxonomic units or as amplicon sequence variants) and compared with reference sequences in databases to assign them to specific organisms. These two approaches are well established and are currently the most used methods applied to eDNA samples[32,37]. New approaches are being developed to investigate the functional or genetic diversity of a group of organisms[38], to investigate trophic relationships[39] or to obtain information on the whole genome of the organisms present in the samples[40,41], but are not yet sufficiently developed for widespread biodiversity monitoring. Generally, sampling of eDNA is applicable to systems with minimal prior information and can also be integrated with data from other monitoring techniques such as remote sensing, camera or audio recordings[17,42].

Following pioneering work in the 1980s and 1990s to isolate microbial communities from environmental samples[43,44] and the development of DNA barcoding technology for species identification[45], the combination of these technologies in the late 2000s[46] led to eDNA becoming established as a research field in its own right[13]. Uptake of eDNA-based approaches has since vastly expanded in the field of biodiversity research owing to their non-invasiveness and increased efficiency for detecting species in a range of habitats at decreasing costs[47]. Technological advancements[36,48] have enabled progression from detecting single species – initially focused on protected or invasive alien taxa[46,49] – to comprehensive biodiversity assessments of many taxa with a metabarcoding approach. Consequently, the past 15 years have seen substantial growth in the number of datasets produced using this approach (Fig. 3), alongside the development of guidelines, large-scale projects and end-user applications[15,29,37,47,50–54].

The uptake of eDNA research has been particularly prevalent in aquatic ecosystems, including rivers, lakes and marine water bodies, because of the ease of sample collection. Gaps in biodiversity knowledge are often larger for aquatic ecosystems than for terrestrial ecosystems, necessitating the implementation of novel approaches tailored to aquatic ecosystems. Current eDNA research in aquatic environments has mostly focused on freshwater ecosystems (65% of studies compared with 25% on marine ecosystems and 10% on other/multiple systems) and more than half of the studies have focused on fish (52%)[51] – probably due to their high commercial and recreational values. Additionally, fish tend to shed greater amounts of DNA than species with sclerotized exoskeletons (such as crayfish) and are easier to detect owing to their high biomass and mobility[55].

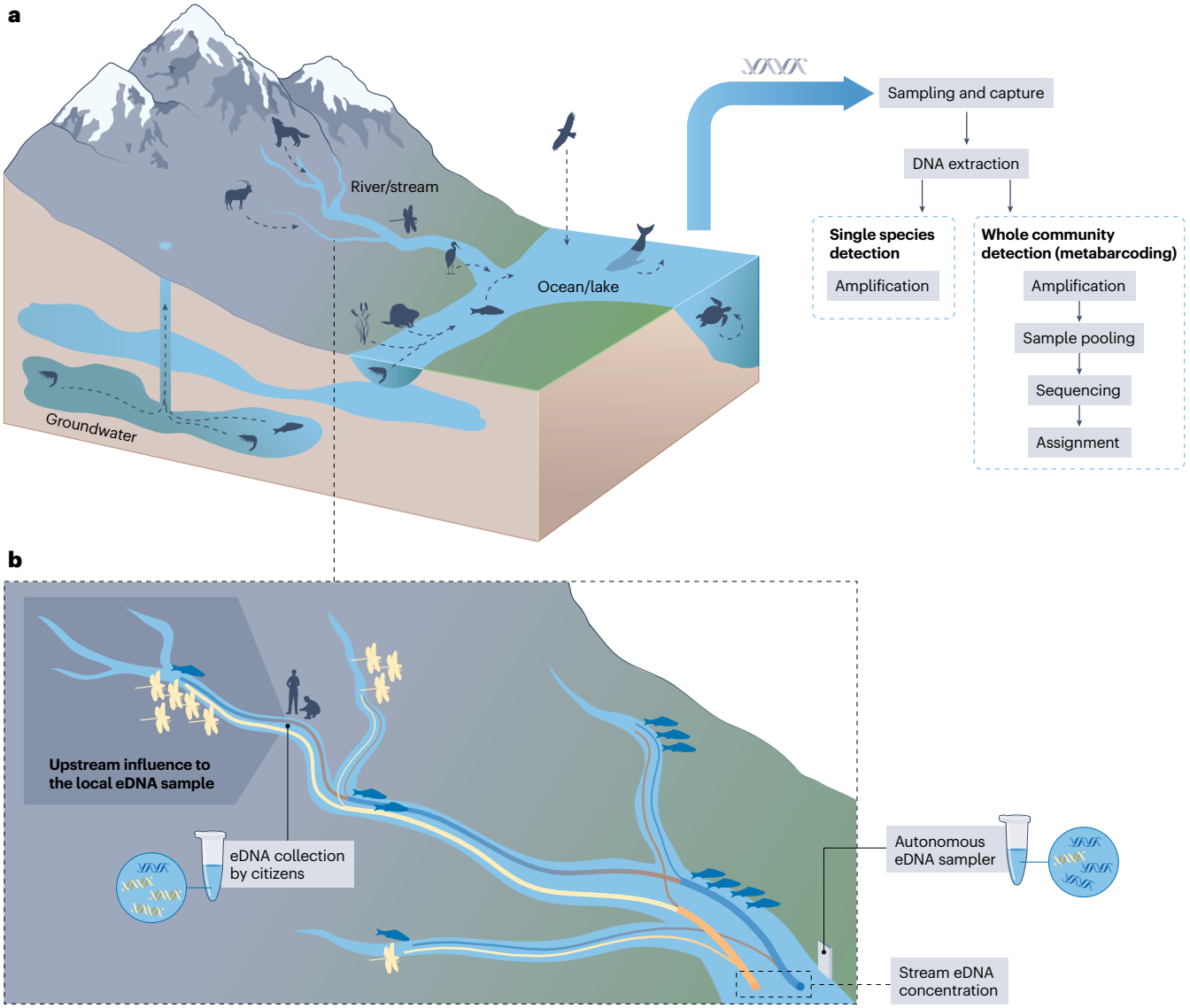


Fig. 2 | Use and application of eDNA sampled in lakes, rivers, groundwater and marine waters, and across different taxonomic groups. a, Aquatic environmental DNA (eDNA) is sampled from marine, freshwater and groundwater ecosystems, enabling spatial (for example, catchment wide) and temporal (for example, across seasons) integration. The basic principle of aquatic eDNA analysis include sampling and capturing the DNA (usually via filtration) followed by DNA extraction, amplification (either of single species or through metabarcoding), sequencing and taxonomic assignment by comparison to DNA reference databases. **b**, Environmental DNA collected in river networks enables gathering information on biodiversity from a wider spatial extent by integrating hydrological first principles and estimated production and decay of DNA. Collection of samples can be enhanced via automated methods (such as autonomous samplers) or via citizen science approaches, which enables accumulation of data at high spatiotemporal resolution.

The primary strength of using eDNA analysis for assessing biodiversity is the ability to infer species' presence without direct collection or observation[13], and that the same approach can be applied across the whole tree of life. The minimally invasive sampling involved is desirable from ethical, work-safety and systems perspectives, as massive upscaling can be achieved without unwanted side effects. However, indirect inference — detecting DNA and not the organisms themselves — is sometimes also seen as the greatest weakness of eDNA analysis. This approach limits inference and information on population structure (abundance or life stages) and requires cautious interpretation of the signal as positive detections might be linked to transport of DNA[56], sequencing errors[57] or database classification errors[58]. Therefore, eDNA workflows require rigorous methods and best practices at each stage to minimize the risk of false positives[59].

Using eDNA to support biodiversity policy

Successfully meeting the GBF targets requires the acquisition of three types of data (Fig. 1). First, biodiversity must be mapped across all scales and systems to identify priority areas for conservation and restoration

('map biodiversity'; for example, GBF targets 3, 6 and 14). Second, the spatial movements and temporal changes in abundance of species of interest (such as pathogens, invasive alien or endangered species) must be tracked ('track species'; for example, GBF targets 4 or 6). Finally, the responses of ecosystems to anthropogenic changes must be monitored and understood, including identifying pollution levels that do not harm biodiversity (as mentioned in GBF target 7) or tracking recovery success when restoring degraded ecosystems (see the 'Understand and attribute drivers of biodiversity change' section). The analysis of eDNA is a powerful approach to address all these aspects and to provide common information needed for implementing efficient regulation. In this section, the potential use of eDNA-based approaches for these three objectives is explored and examples of their usefulness for GBF targets presented.

Spatiotemporal mapping of aquatic biodiversity

Effective ecosystem assessment for policymaking, such as monitoring systems under pressure or identifying biodiversity hotspots, hinges upon efficient characterization of biodiversity, including adequate baseline data and mapping biodiversity trends over space and time[60]. Traditional sampling approaches such as gillnet fishing or trawling are still widely used, but are not sufficient to reach the ambitious targets of GBF and other regional initiatives (such as the European Union (EU) Water Framework Directive) because they are too labour

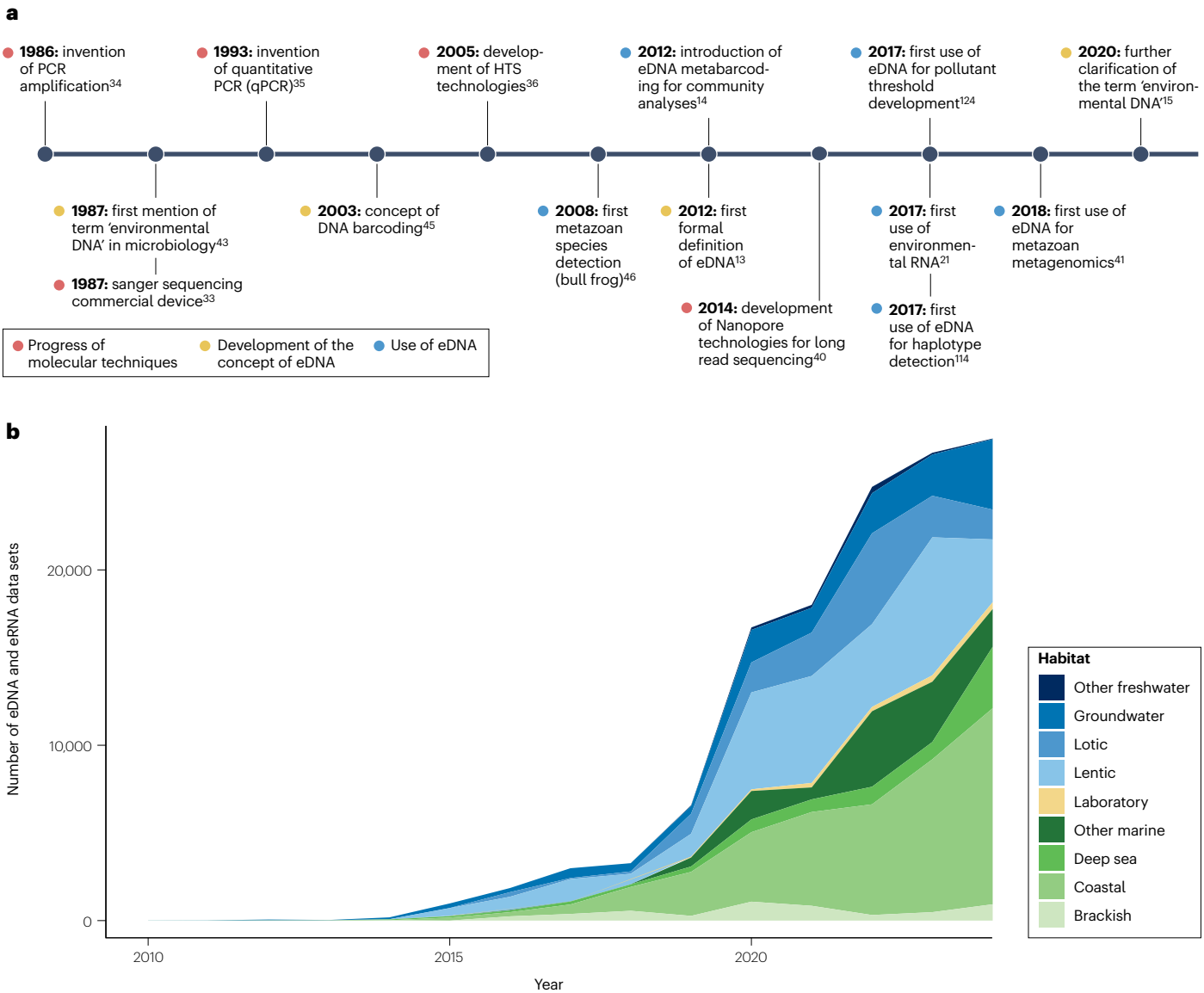


Fig. 3 | Development of eDNA-based technologies and availability of datasets. a, A schematic chronological timeline of important steps in the development of the concept of environmental DNA (eDNA) (yellow dots), progress of molecular techniques (red dots) and use of eDNA (blue dots). **b**, Temporal trends in aquatic data volume over time as the number of datasets published in the NCBI Sequence Read Archive on 20 September 2024. Here, a dataset is defined as a data package with a specific run number. Metadata on these datasets were retrieved using Entrez Programming Utilities and parsed using a list of keywords to include only eDNA and eRNA datasets from aquatic environments. HTS, high-throughput sequencing.

intensive, destructive and costly to scale up or implement – particularly in undersampled regions, such as tropical streams, open ocean or groundwater systems. By contrast, eDNA analysis allows greater spatial resolution and increased temporal and taxonomical scales compared with traditional approaches, and eDNA sampling is increasingly adopted by public agencies as part of their routine biomonitoring programmes[61–63]. eDNA-based approaches effectively complement traditional aquatic biodiversity monitoring as they overcome the challenges of morphology-based classification (such as juvenile life stages or cryptic species[64]) and enable access to undersampled and less-accessible systems such as groundwater ecosystems[65] or oceanic trenches[66].

Technological advances are currently driving this transition. The ever-increasing pace of technological developments in eDNA science has inevitably reduced the cost of processing water samples[67], enabling the collection of samples at high spatial and temporal resolution[68,69]. Although sample collection and eDNA capture can still be labour intensive, collecting water samples is becoming considerably faster owing to the development of autonomous robotic and passive samplers. Existing autonomous samplers[70–73] are capable of multisample capture and preservation. When coupled with a microfluidic block, they can even collect, filter, extract and perform multiple targeted qPCR assays of eDNA samples in situ[74,75]. These automated samplers are highly attractive for early detection of invasive alien species (addressing GBF target 6; see the 'Tracking endangered, harmful or invasive alien species' section), and can be operated for months at a time without intervention[76]; however, further technological improvements are possible and the cost of fully automated samplers is currently prohibitive for some applications. Low-cost and less technologically advanced alternative samplers are based on passive capture of eDNA on substrates, such as non-charged cellulose ester or even medical gauze secured in three-dimensional hollow printed housing[77,78]. Traditionally, monitoring biodiversity was often restricted to specialists, whereas these simple eDNA capture methods have the potential to include citizen scientists in eDNA-based biodiversity assessment[29–31], contributing to increased participation and improving integration of biodiversity into decision-making at every level (GBF targets 22 and 14, respectively).

Owing to transportation and mixing processes in water bodies, aquatic eDNA samples taken at a specific location reflect both the local community and organisms living in the surrounding environment. This feature can be leveraged to understand the spatial structure of biodiversity underpinning the observed data. In rivers, for example, depending on the discharge and other abiotic factors, eDNA can travel downstream from a few hundred metres to tens of kilometres[56,79,80], acting as a 'conveyor belt of biodiversity information'[16]. Thus, eDNA-based biodiversity measures not only correlate to upstream-averaged measures of land use and habitat types[17,81–83], but also provide space-filling projections of biodiversity by integrating signals from discrete sampling points into a continuous representation along a watercourse (Fig. 2). Similarly, in marine systems, eDNA is transported by currents and shaped by the stratification of the water column[84,85]. Coupling stream[86,87] and marine[88,89] eDNA data with hydrodynamic models is therefore essential to unravel and understand the spatial distribution of biodiversity. Specific models[90,91] enable spatial projection of pointwise eDNA samples beyond their collection point into space-filling taxon richness maps at high spatial resolution[92]. As such, they provide spatially integrated information on biodiversity.

The assessment of overall biodiversity is particularly relevant for GBF targets that focus on area-based or area-projected measures, such as target 1 ('plan and manage all areas to reduce biodiversity loss'), target 3 ('conserve 30% of land, water and seas') or target 14 ('integrate biodiversity into decision-making'). These targets are most effectively reached when overall biodiversity assessments of ponds, streams, groundwater and marine systems are comparable, implementable and accessible from local to global scales.

Tracking endangered, harmful or invasive alien species

Tracking or detecting species of particular interest or concern is often a key piece of information needed for policymaking and communication. In this context, eDNA analysis is a valuable tool for the detection and monitoring of individual species. Indeed, the first applications of eDNA were for the detection of either rare and endangered[49] or invasive alien species[46,93]. The detection and monitoring of individual species of interest is directly associated with several GBF targets, including target 4 ('halting extinction of [individual] endangered species') and target 6 ('reducing the introduction of invasive alien species'). Two examples of integrating eDNA information on targeted species into policy are the tracking of the endangered great crested newt in the UK using eDNA[94] and the almost globally implemented eDNA (and eRNA) tracking of pathogens (in particular SARS-CoV-2) in wastewater samples[95]. In both cases, real-time implementation and analysis of eDNA samples enables rapid dissemination to inform policy. eDNA-based methods are now implemented in many countries for routine monitoring of harmful algal blooms[96,97], invasive alien species, such as the quagga mussel[98] and European green crab[99], and of endangered species such as the above-mentioned great crested newt[94] or the harbour porpoise[49].

Importantly, however, an eDNA signal does not automatically indicate an organism's immediate temporal or spatial occurrence. The signal can be transported in space (for example, by water current or in the faeces of predators), reflect past occurrences (ancient DNA), be a transient signal of an organism passing by, or even enter the water via wastewater or terrestrial run-off[16,100–102]. An eDNA signal therefore contains different information compared with the direct observation of an organism, including potential false positives[103,104], and thus requires different interpretation. For example, detection of an alien species using eDNA analysis in a location where it has not previously been observed should not trigger a policy response for removal, but rather incentivize the deployment of other methods to confirm the species' presence. Similarly, eDNA-based techniques do not allow the collection of population demographics, such as age and size distribution or sex ratio, which can be required for endangered or invasive alien species monitoring[105]. Some of these limitations can be overcome by sampling eRNA rather than eDNA, as eRNA represents genes expressed in the sampled environment and degrades faster than eDNA[20]. As eRNA provides a more contemporary signal than eDNA, its analysis can potentially distinguish between live and dead material to reduce the number of false positives[18,21] or, in specific cases, even differentiate life stages (for example, in amphibians). However, eRNA analysis is still under evaluation and, with a few exceptions[19], is not yet ready for use in monitoring contexts to inform policymakers.

Approaches based on eDNA are potentially relevant for GBF target 4, which specifically links the halt of species extinctions to the prevention of genetic diversity loss. Addressing this target currently relies on indirect measures of genetic diversity based on abundance data for many species and subsequent estimation of effective population size. Determining abundance estimates from eDNA is challenging, but at least possible in some circumstances. Both targeted[106,107] and metabarcoding[108] approaches have demonstrated correlation between

copy number and/or read counts from eDNA and biomass or abundance, indicating that the method is at least semi-quantitative. However, this approach is strongly debated owing to the high potential for bias throughout eDNA workflows, which can create noise and error in the signal[109–113]. Possible solutions include adding internal standards of a known amount of synthetic DNA (so-called DNA spike-in) to compare read counts within species and across sites.

In addition to indirect measures of genetic diversity based on abundance, eDNA analysis could enable direct measurement of intraspecific genetic diversity, even in hard-to-sample environments and for rare and elusive species. This approach is still in development and is not yet ready for direct implementation; however, it has shown promising results in particular cases. So far, investigations have focused on the detection of haplotypes in short sections of mitochondrial (mt)DNA used for metabarcoding, and demonstrated that eDNA captures the genetic diversity of sampled populations[38,114–117]. Such approaches can differentiate populations and identify regions with unique genetic variation based on the mtDNA marker genes[118]. However, these short mtDNA fragments generally have low power to resolve finer genetic structures within or between populations. Targeting longer fragments and nuclear DNA provides increased resolution but presents additional challenges related to the shorter persistence of longer DNA fragments in the environment, and that eDNA represents a mixture of DNA from different individuals. Current methods do not allow assigning genotypes to individuals, which restricts most genetic approaches to population-level estimates[119]. Likelihood-based DNA mixture models could be applied to address the problem of redundancy (multiple individuals sharing the same allele) as long as a relatively large panel of multi-allelic markers is available[120]. Technical challenges notwithstanding, microsatellite allele frequencies of round gobies (*Neogobius melanostomus*) or an Arctic diatom (*Thalassiosira hyalina*) estimated from aquatic eDNA have been shown to closely resemble allele frequencies from genotyped tissues[120,121].

A considerable challenge for estimating genetic diversity from eDNA data is the detection of errors generated during the amplification and sequencing steps. For species detection, many bioinformatic procedures remove genetic variability during regular data cleansing and cluster sequences at the species level. For population genetic-level analysis, keeping exact sequence variants while removing erroneous sequences is the primary goal and can be more challenging[119]. Resolving this challenge requires combined improvements in sequencing technologies and the computational steps to identify and remove errors.

Understand and attribute drivers of biodiversity change

For policymaking and management, attribution of biodiversity change is key: only when drivers of change (both positive and negative) are linked to their biodiversity effects can effective policy and management be achieved. Traditionally, attributing drivers to aquatic biodiversity change has relied on monitoring and morphological identification of a relatively small number of species (such as macroinvertebrates, benthic macrofauna, fish and diatoms) that are biological indicators of environmental pressures such as nutrient pollution[122]. However, these methods are often unsuitable for monitoring emerging drivers, such as pollution by nanoparticles or microplastics, and hinge on the (often incorrect) assumption that a few indicator groups represent overall ecosystem responses. eDNA-based methods have already led to marked improvements in our ability to document biodiversity[123] and identify pollutant thresholds[124] by integrating data from diverse taxonomic groups with only one sample. This approach, in comparison with traditional methods, will provide a more comprehensive – and at least more diversified – response of aquatic communities to anthropogenic changes, individually and in combination[24,25,125].

Current monitoring, whether with traditional or molecular tools, often focuses on the structure of communities (their richness and composition) rather than their functional properties[123]. However, the effect of biodiversity loss on ecosystem services cannot be fully understood from estimates of taxonomic diversity alone. To characterize ecosystem health in its entirety, structural and functional biodiversity must be measured as these parameters can respond differently to different stressors. This type of monitoring requires a shift from focusing on which species provides the function to understanding how the functional properties of the whole community respond to pressures[123,126]. For example, co-occurrence data generated from eDNA metabarcoding across the tree of life allow reconstructing multitrophic ecological networks, from which functional properties such as connectance and nestedness can be estimated[127]. Other emerging approaches for addressing ecosystem changes are based on the analysis of expressed genes via eRNA. The strength of these eRNA methods lies in monitoring the physiological state of populations or communities by detecting changes in the activity of single or multiple genes[18]. Shotgun sequencing of total RNA from environmental samples (metatranscriptomics) allows the identification of genes that are actively and collectively expressed under different conditions[128]. Changes in freshwater biofilm microbial community-level expression in response to pollutants have been demonstrated, even when changes in community composition were not detected[129]. Additionally, experiments have revealed changes in community-wide gene expression of aquatic organisms under heat stress through sampling experimental tank water, demonstrating that metatranscriptomics of extra-organismal eRNA can reveal gene expression response to environmental change[130]. These emerging measures of ecological condition, especially when using eRNA, are promising but not yet ready for widespread use for practitioners and to inform decision-making.

Advances in artificial intelligence and modelling are enabling new approaches for attributing biodiversity change to environmental and/or anthropogenic drivers. For example, eDNA coupled with machine-learning models is used to infer the ecological status of aquatic systems[131,132], and allows inclusion of a broader set of indicator taxa. Microorganisms, for example, differ considerably in their metabolism and sensitivity to different pollutant sources[133], but have been largely ignored in biomonitoring because they are difficult to culture and identify, and because knowledge of their ecological function is unavailable[134,135]. Validated approaches based on profiling communities using amplicon sequence variants and/or operational taxonomic units detect changes in the structure of communities of bacteria[133,136], freshwater invertebrates[25,137] and diatoms[138] against well-understood disturbance gradients, and have demonstrated strong improvements in the accuracy of ecological quality assessment[139,140]. Supervised machine learning combined with metabarcoding will complement and expand current morphology-based methods to produce the same ecological quality assessments at increased speed and cost efficiency, and without necessarily depending on taxonomic assignment using barcode reference databases[135] (Fig. 4). The ability of supervised machine learning to outperform traditional indicator value approaches, using training datasets with reference disturbance levels to estimate biotic indices, has been demonstrated[131,132,140–143].

Overall, eDNA analysis enables attributing changes in biodiversity to specific environmental factors[17,65,144], evaluating the impact of

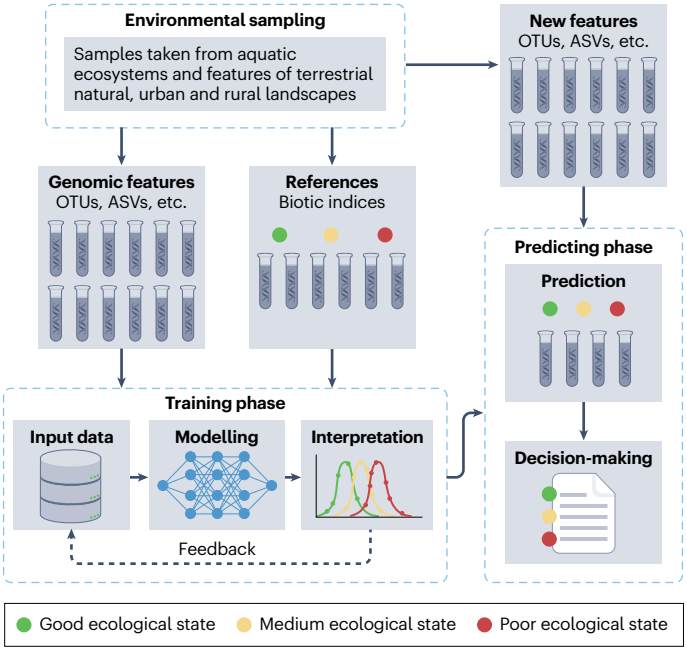


Fig. 4 | Machine learning enables taxonomy-free assessments of indices, such that simple, easily communicable predictions can be derived for decision-making. The principle of the machine-learning approach is to link genomic features of the environmental DNA (eDNA) samples (such as operational taxonomic units (OTUs), amplicon sequence variants (ASVs) or taxonomically assigned information) to environmental reference states. Specifically, during the training phase, the model learns relationships between genomic features and known environmental conditions, iteratively refining its predictive rules. In the predicting phase, the trained model applies these learned rules to analyse new eDNA samples, generating straightforward, actionable predictions. Through continuous feedback cycles, the model enhances its accuracy, improving its utility for environmental monitoring and management.

implementing protected areas[145,146], and calculating biodiversity and ecosystem state indices[47]. Aquatic eDNA analysis can also attribute change beyond aquatic ecosystems: imprints of terrestrial land use on aquatic eDNA are well established, showing that aquatic eDNA signals associate to the structure and diversity of terrestrial land use assessed by remote sensing at 400–1,000-m upstream distances[17]. This information can then be translated into effective practice for the prevention of biodiversity decline.

Examples of eDNA use for policy and beyond

eDNA-based biodiversity monitoring has the potential to scale from a local to a regional and eventually a global perspective, and enables data integration and the use of this information to downscale back to regional and local interventions[42]. eDNA approaches are already widely used across different scales, which helps to contribute to monitoring progress on GBF targets. These methods are particularly useful for spearheading activities in regions in which previous knowledge on biodiversity is limited; for example, eDNA-based approaches are being tested to study the biodiversity of deep marine habitats[147,148]. Similarly, subterranean habitats are one of the least studied environments and the use of molecular tools such as eDNA contributes to mapping their biodiversity[65,149]. Moreover, eDNA-based assessments are being developed and implemented in many of the world's largest rivers such as the Danube[150], Yangtze[144] or Rhine[151], which are not only home to enigmatic megafauna but are also severely threatened by anthropogenic activities.

Achieving target 1 of the GBF ('plan and manage all areas to reduce biodiversity loss') depends on understanding the initial state and change of overall biodiversity. In many cases, eDNA data are the very first baseline data, and linking these data to possible drivers of biodiversity change will ultimately guide restoration planning[152]. The ability of eDNA analysis to provide data on the state and change of individual species focuses on target 4 ('halt species extinction, protect genetic diversity, and manage human–wildlife conflicts'), which directly refers and links to the monitoring and managing of biodiversity, at the species or within-species level. Similarly, target 6 ('reduce the introduction of invasive alien species by 50% and minimise their impact') operates at the species level, aiming to decrease the impact of alien species in part through preventing their introduction in aquatic systems. To support this approach, eDNA analysis provides early detection and monitoring of alien species. Although initial attempts to detect invasive alien species (for example, the Asian carps *Hypophthalmichthys nobilis* and *Hypophthalmichthys molitrix*[93,153]) were partially challenged by detection failures (false absences), false presence indications and unknown lower thresholds of detectability, increasing standardization and the adoption of structured decision-making processes have resulted in a relatively broad application of eDNA methods to monitor Asian carp species in North America[23,154]. Similar efforts have been established for other aquatic alien species, such as invasive kelp (*Undaria pinnatifida*) and sea stars (*Asterias amurensis*)[155], tunicates (*Didemnum vexillum*)[156], dreissenid mussels (*Dreissena* spp.)[22] or freshwater aquatic plants (*Najas minor*)[157].

The contribution of eDNA analysis to GBF targets 1, 4 and 6 focuses on simply detecting the presence of species. However, eDNA analysis can provide insight into why and how biodiversity is affected; that is, mechanistic monitoring of aquatic systems and the effects of stressors. Classic assessments of environmental toxicology and environmental chemistry have focused on monitoring a highly diversified set of chemicals[10]. To reach target 7 ('reduce pollution to levels that are not harmful to biodiversity'), pollutant monitoring must be linked to an ecosystem's ecological health and resilience status. eDNA is effective in capturing how environmental stressors, particularly chemical pollution, have combined negative effects on the structure, stability and composition of aquatic communities across a wide range of taxa[158–160]. Target 10 ('enhance biodiversity and sustainability in agriculture, aquaculture, fisheries, and forestry') requires similar monitoring of effects on biodiversity, but in relation to the production and harvesting of natural resources. eDNA is already routinely used to monitor impacts of aquaculture (such as salmon farming) by examining marine benthic sediments[161] and has also been used to monitor the outflow of wastewater into natural streams, focusing on possible effects of different agricultural runoff[100].

Next steps for eDNA in the GBF context

eDNA-based methods have the potential to greatly improve our understanding of aquatic ecosystems and contribute to national and global biodiversity targets. For successful inclusion of eDNA analysis in monitoring programmes, careful action is needed as methods must be broadly applicable, comparable and scalable, and must be implementable for countries across the globe.

Two key barriers exist to the implementation of eDNA: first, the perceived limited comparability between results generated with eDNA-based approaches and those obtained with traditional methods[162–164]; and second, unequal access to technical infrastructure. eDNA metabarcoding technology is considered ready for implementation and allows – when needed – retrofitting of indices to current standards[47,135]. Discrepancies with traditional assessments occur because of amplification biases and incomplete reference databases, but also because eDNA can detect cryptic or elusive species[47,123,165] previously missed by traditional tools. Assessing biodiversity and achieving successful conservation outcomes will require effectively using eDNA alongside traditional methods, as biodiversity monitoring globally remains under-resourced. In high-income countries, existing monitoring programmes should be complemented with eDNA-based approaches, creating continuity for existing long-term monitoring and generally allowing comparison at the index level. Maintenance and overlap with other programmes will increase costs and require extra work over the short term, but secure existing data series in the long term. To encourage the rapid adoption of eDNA globally, in low- and middle-income countries, novel (and first) monitoring could use eDNA only.

This section discusses necessary steps towards a global application of eDNA to assess biodiversity within the context of the GBF. First, methods of standardization must be agreed and promoted (agree on minimal standards on sampling and processing, provide training opportunities and institutionalize quality control). Second, adequate reference databases, digital sequence data deposition and general access to these resources must be established. Finally, efforts must be made to increase inclusivity and remove technology barriers (collective benefit, authority to control, responsibility and ethics (CARE) principles and Nagoya Protocol).

Standardization

The development and implementation of international standards is an essential next step to make eDNA approaches broadly applicable. These next steps, including establishing standardized protocols and best practices for general implementation of eDNA, are particularly relevant for global initiatives, such as the GBF, that rely on common agreement beyond the borders and jurisdiction of individual countries.

eDNA has always been developed as a tool for practitioners, rather than for academic research only. Consequently, discussions on an adequate and replicable implementation paralleled the rapid advances in the field[153,162,166,167]. General protocols and guidelines were developed from the first species-based assessments, such as amphibians in the UK[29], to the monitoring of whole communities of invertebrates and fish, for example, in Switzerland[168], the EU[37,169], China[170] or the USA[93].

Despite these standardization efforts, eDNA-based biodiversity assessments still use various approaches throughout the world[171]. The selection of a certain filter material, DNA extraction methods, primers, sequencing technologies and post-processing steps can drastically affect the inferred taxa lists[172,173]. For multispecies detection approaches, such as eDNA metabarcoding, the effects of the choice of methods are most pronounced for rare species that are easily missed. Broader use of eDNA for monitoring thus requires standardization of practices[63,174], in particular a global agreement on general minimum standards for sampling and processing, reporting and data publishing. Here, specific recommendations for different freshwater ecosystems (for example, small stream versus large, stratified lake), environmental factors (turbidity, flow, pH and so on) and specific research questions and tasks (single species detection versus multispecies detection) must be accounted for[37,168,175]. This standardization is well underway – a first ISO standard is available in draft form after international voting[176], and several others are in development under the framework ISO/TC 147/SC 5 (Biological methods) – but must be continued. Given the ambitious 2030 GBF timeline, the implementation and standardization of the method in its current form might be more effective than deferring implementation considering the promise of upcoming new technologies.

Populating reference databases, FAIR curation of data and scalability

Comparability and scalability across taxa and regions are the biggest assets of eDNA analysis. These capabilities hinge, however, on adequate access to reference databases as most uses of eDNA rely on assigning specific sequences to taxonomic and genetic reference databases (although taxonomy-free approaches exist[139]). These reference databases are globally still highly unevenly distributed, and large geographic and taxonomic gaps are present[177–179]. For example, (sub) tropical systems and many invertebrate groups are far less represented than temperate systems and vertebrate groups. Populating, expanding and maintaining these reference databases is imperative.

Advances in sequencing technologies enable the upscaling of reference database generation by several orders of magnitude while also drastically reducing costs. However, many – even formally described – species are still absent from public genomic repositories[180]. Novel nanopore sequencing technologies could allow the barcoding of thousands of specimens from a single amplicon pool at a sequencing cost of few US$ per specimen, offering the potential to catalogue all species within two decades[181]. The portability and applicability of sequencing technologies in field conditions is also rapidly improving; for example, portable sequencers have been used for DNA barcoding in the Ecuadorian Chocó Rainforest[182] and other remote locations. Populating databases is still a major bottleneck as implementation is lagging behind this progress in technologies, and also critically depends on taxonomists' knowledge and expertise. Scientists and end-users must be mobilized alike and commit to complementing the databases as the task is important, necessary and cannot be delegated. This challenge is particularly pronounced given the large number of undescribed or underrepresented taxa, discrepancies in taxonomic classifications and the need for rigorous validation of reference sequences. The complexity of maintaining and curating these databases has been widely discussed in the literature, touching on issues such as funding[177] and standardization efforts[58]. A comprehensive discussion of these aspects is, however, beyond the scope of this Perspective.

Alongside data generation, the raw data produced from environmental samples must be deposited in repositories (Table 1) whose long-term operability is guaranteed[183]. Priority should be given to open platforms specifically adapted to host genetic data, enabling better discoverability through advanced application programming interfaces (which enable the exchange of data between existing programmes) and research tools[184]. The International Nucleotide Sequence Database Collaboration, linking the Sequence Read Archive, the European Nucleotide Archive and the DDBJ Sequence Read Archive are currently among the most suitable options for depositing and indexing eDNA data. Ideally, eDNA repositories should facilitate the seamless exchange and integration of data across different platforms and disciplines. For example, biodiversity data portals such as the Ocean Biodiversity Information System (OBIS) and the Global Biodiversity Information Facility (GBIF)

Table 1 | Recommended environmental DNA data deposition strategy

Type of data	Format	Publication	Data repository
Sequencing data			
Demultiplexed raw data	Fastq	Link to repository	Genetic repository[a]
Sequencing information			
Platform used[b] Target marker[b] Primer sequences	Text/tabular	In text where relevant	Metadata (genetic repository)
Sample information			
MIxS MIMARKS-SU MIxS environmental package Controls: - type (positive or negative) - content for positive control	Tabular	Supplementary table	Metadata (genetic repository)
Reference databases			
Name Version Reference data	Text/fasta	Link to repository	Generalist repository if custom database Official repository if published database
Bioinformatics			
Tools/pipelines Versions	Text	In text where relevant	More complex and custom scripts should be deposited in a code repository
Biodiversity records			
DNA sequence or qPCR detection	Darwin Core	Link to repository	GBIF/OBIS
Community data matrix			
Taxonomic identification Sample identifier Read count/copy number	tabular	Supplementary table	Generalist repository (optional)

[a]GitHub and GitLab are not always accepted as valid repositories for data other than scripts because they do not provide DOI numbers. We thus suggest that only scripts and bioinformatics pipelines should be deposited there. [b]Part of the MIxS MIMARKS-SU checklist. For each type of data, we suggest a format for deposition, how to link or include the data in the original publication, and in which type of repository the different type of data should be deposited. Data deposition should comply with the FAIR principles. Genetic repositories include the Sequence Read Archive, DDBJ Sequence Read Archive and European Nucleotide Archive, generalist repositories include Zenodo, Figshare and Dryad, and code repositories include GitHub[a] and GitLab[a]. GBIF, Global Biodiversity Information Facility; OBIS, Ocean Biodiversity Information System.

can include biodiversity records derived from raw DNA data while providing a permanent link to the original repositories[185]. Publication of eDNA datasets in these global biodiversity databases is crucial to ensure that data can be used by policymakers with little to no specialist knowledge[186]. However, so far, few eDNA datasets have been made publicly available through these platforms. The key to successful adoption is the mandatory deposition of eDNA raw data along with relevant metadata provided in standardized data models, such as provided by the Genomics Standards Consortium (see the MIxS MIMARKS checklists[187]) or the Biodiversity Information Standards consortium TDWG (Darwin Core extended data model[188,189]). As part of ongoing international task forces, new metadata standards are currently being developed[190]. These data standards ensure that the most critical metadata are recorded and made available through a standardized vocabulary[191], which in turn can be aligned with other biodiversity community standards. Such metadata (Table 1) are crucial as they ensure that the data can be accurately interpreted and reused in various contexts, which is necessary to reach a broad range of stakeholders, including citizens.

eDNA data producers must focus on creating datasets that can be used and reused[186], which involves ensuring the longevity of data, adopting standard formats and metadata for ease of interpretation, and promoting open access to facilitate wide usage across different sectors. Thus, published eDNA data must adhere to the FAIR principles — findable, accessible, interoperable and reusable[192] — as this approach enhances the discoverability and usability of eDNA data across various scientific and non-scientific domains[186] (Table 1). Therefore, setting minimum guidelines for data reporting and analysis is recommended, and minimum information to be reported as part of any aquatic eDNA study has been proposed[190,193]. Data deposition must include the raw sequencing data and the essential metadata specific to the samples and their processing in the laboratory, but also the final community data matrix, easily interpretable by the stakeholders, as well as the bioinformatics tools and reference databases required to produce the results. These minimum guidelines will ensure a baseline of quality and comparability for eDNA research and application.

CARE and inclusivity

The successful, widespread implementation of eDNA hinges on removing barriers to its access and making the methods inclusive. Technological barriers and uneven distribution of knowledge and resources between high-income countries and low- and middle-income countries exist across biodiversity sciences. As eDNA analysis (similar to remote

sensing and other technology-driven approaches) depends on specialized laboratory and analysis techniques, overcoming discrepancies in lack of knowledge, training and resources is particularly relevant. If countries cannot afford eDNA-based monitoring or do not have access to the technologies to process and assess the samples, the existing inequality in biodiversity knowledge will further increase.

In addition to ensuring equitable access to eDNA technology, local and Indigenous knowledge on biodiversity must be both valued and integrated into technology-driven monitoring efforts. Although measuring biodiversity variables through proxies such as eDNA is technologically feasible, maintaining taxonomic and natural history knowledge, as well as connections to ecosystems and nature, is essential. Datafication of biodiversity and reliance on technological solutions run the risk of disconnecting people with nature[194], as sequencing information and eDNA approaches cannot replace observing and experiencing nature directly. Additionally, limits exist to the quality and applicability of data available from methods based on indirect collection of information, such as remote sensing or eDNA. Conversely, eDNA also has the potential to be a powerful tool that provides people with a new perspective on their surrounding environment and biodiversity, strengthening their interest in and commitment to nature[29–31]. Any eDNA-based approaches should, therefore, consider how to include the public and taxonomic experts such that the information on biodiversity from eDNA is accompanied by increased understanding and perception of biodiversity.

Addressing the sociocultural dimensions of eDNA, particularly when data originate from territories of Indigenous communities, necessitates the adoption of the CARE principles[195]. These principles ensure that eDNA research is conducted in a manner that respects the rights and traditions of local communities, promoting an ethical approach and facilitating the local populations acceptance of biodiversity research. Incorporating the CARE principles aligns eDNA practices with broader societal values and legal frameworks, such as the Nagoya Protocol[196] and GBF target 13 ('fair and equitable sharing of genetic resources'). In some situations, however, open-access goals such as the population and maintenance of reference databases can bypass traditional mechanisms for benefit sharing, potentially disadvantaging the countries or communities where the genetic material originated. Public deposition without consultation can undermine the authority of these communities over resources tied to their cultural identity and governance systems. Moreover, eDNA can also complicate traditional frameworks, especially when DNA is transported across national boundaries by natural processes such as ocean currents, river flows and air[197]. All stakeholders — nations, Indigenous groups and local communities — must be included in discussions about the implementation of CARE principles, data sharing and benefit sharing to ensure that the benefits of eDNA research are distributed fairly and with due regard to the sovereignty and cultural significance of the data sources[194,198].

Biodiversity credits and the marketing of biodiversity

One of the four overarching goals of the GBF is to mobilize the business and finance sectors to close the gap in funding biodiversity. In particular, target 19 ('Mobilize $200 billion per year for biodiversity') encourages solutions to incentivize international companies and finance to engage in the monitoring of biodiversity and contribute monetarily to its protection. Several initiatives are being promoted to market biodiversity and to incentivize the development of activities and technologies that are not harmful for the environment or that mitigate deleterious actions, such as green bonds or biodiversity credits (quantifiable units of biodiversity improvement that should provide an incentive for funding conservation projects)[199,200]. Although such market-based solutions to environmental challenges are gaining substantial attention at the global scale, deep uncertainties exist regarding the process and their benefits[199]. Without proper transparency and accountability, these schemes might be ineffective and lead to greenwashing and inequitable outcomes, as has occurred for carbon credits[201].

A challenge to the broad use of biodiversity credits is the difficulty in quantify biodiversity improvements accurately and in a standardized manner. Sampling and identification methods vary substantially, are not all easily implementable across the globe, and the multidimensionality of biodiversity cannot be captured in one single number. eDNA metabarcoding has been suggested as a useful method to help the development of marketing metrics for biodiversity, as its simplicity and scalability could allow the collection of comparable data across the globe. However, this approach also requires global standardization of methods and a clear reference to its limits. Moreover, eDNA is particularly well suited for aquatic environments but is less proven for terrestrial ecosystems, which might limit the comparability of this data[201,202].

Glossary

Amplicon sequence variant
Inferred unique sequence(s) derived from high-throughput sequencing after removal of erroneous sequences.

Biological indicator
Taxonomic group, such as fish, macroinvertebrates or diatoms, specifically used to assess environmental conditions in relation to legislative frameworks.

DNA barcoding
Identification of a specimen using a short DNA fragment called a (genetic) marker.

High-throughput sequencing
Approaches used to sequence millions of DNA sequences in a rapid and cost-effective manner (also known as next-generation sequencing).

Marker (or genetic marker)
A DNA sequence of a gene or part of a gene with a known location in the genome used to identify specific species.

Metabarcoding
Identification of the multiple organisms represented in a sample by sequencing a common DNA marker using high-throughput sequencing.

Operational taxonomic unit
An operational definition of clustered sequences based on their sequence similarity (for example, >97% similarity) to reflect approximated taxonomic units.

Polymerase chain reaction (PCR)
The process used to multiply target DNA sequences in a sample to facilitate their identification.

Primer
A short, single-stranded DNA sequence (~18–25 bp) used to target a region of the gene to be amplified during the polymerase chain reaction.

Quantitative PCR (qPCR)
Dye-based or probe-based PCR method that allows the quantification of target DNA at each PCR amplification cycle.

Read (amplicon read)
Individual sequence of base pairs (here, amplified through PCR) that corresponds to a single DNA fragment.

Species-specific assay
An approach in which a single species is targeted, typically using standard, quantitative or digital PCR (as opposed to metabarcoding).

Summary and future directions

Addressing biodiversity decline and implementing global measures to assess, manage and restore biodiversity are essential and at the core of the GBF framework. The huge progress in molecular-based biodiversity monitoring, particularly the use of eDNA, has created the first feasible and coherent approach to monitor aquatic biodiversity. To successfully implement eDNA biomonitoring as a reliable and widely trusted basis for policy and decision-making, a new common agreement on its use is needed, including common protocols, data standards and standardization for quality assurance. Further, implementation of eDNA approaches hinges on increased FAIRness and open accessibility of biodiversity data globally, which also presents an opportunity to enhance collaboration, improve conservation efforts and advance research. Rapid completion of reference database, curation and integration of FAIR principles are needed. Although progress towards these goals, in particular where and by whom data are stored, is becoming more systematically addressed in academic research, agreement has not yet been reached and implemented regarding data produced by public stakeholders and private companies. Sample repositories (such as the Global Genome Biodiversity Network) and data centres established for eDNA data should not only be accessible and implemented for high-income countries (for example, the International Nucleotide Sequence Database Collaboration), but also for low- and middle-income countries. As these repositories are funded by public money and provide collective benefits, all data collected through governmental programmes should be included – even when data collection and analysis is executed by private companies.

Coherent, integrated and complete data storage and access will allow establishing new universally valid metrics for describing biodiversity and elevate the quality and comparability of biodiversity data. In the framework of the GBF, regional to global progress on reaching targets could be tracked by new eDNA-based indicators on the state, change and pressures of biodiversity. Particularly in aquatic systems, these metrics can be calculated at high spatial and temporal resolutions: transport of eDNA in the water allows predictions of biodiversity across catchments and water bodies, whereas matching eDNA sampling to existing monitoring stations of water quality allows synergy with existing infrastructure. A pragmatic approach should be chosen when designing new metrics versus retrofitting to existing monitoring programmes: that is, complementing rather than replacing existing monitoring programmes, yet building new monitoring timelines where no data are available. Swiftly establishing a global network of coherent aquatic biodiversity monitoring using eDNA is realistic and required to achieve GBF targets.

A possible way forwards is institutionalizing eDNA monitoring activities through regional centres (examples of such attempts include the Defra DNA Center of Excellence in the UK, the Joint Research Center in the EU and the National eDNA Reference Centre in Australia). These centres coordinate activities that include funding, supporting and driving research that is relevant to policy implementation, reaching out to local monitoring personnel, receiving samples from citizens, performing quality checks through internal positive control, and generating reference material and certification standards. Such regional eDNA excellence centres can facilitate capacity building and act as coordinating and executing building blocks, linking both local and global biodiversity monitoring centres (similar to GEO BON). The Canadian Centre of Biodiversity Genomics at the University of Guelph and other federal initiatives are examples of such centres. Global join up of these initiatives and centres is crucial to ensure international standards and comparability of datasets across countries. Finally, to future proof the data acquired and ensure long-term comparability, adequate biobanking of samples is a necessary next step, which should, at a minimum, include storing metadata, raw sequencing output, and the extracted DNA samples in quality and quantity sufficient to be used by generations to come[203]. Together, these measures will elevate the accessibility and extent of aquatic biodiversity data to levels needed for achieving GBF targets and securing global biodiversity.

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Author contributions

Overall project lead and coordination by F.A. Overall conceptualization by F.A., M.C. and R.C.B. Discussion of content by all authors. Lead writing of article by F.A., M.C. and R.C.B. Writing and lead for specific sections by F.A., M.C., L.C., F.K., L.L.-H., F.L., X.Z., Y.Z. and R.C.B. Conceptualization of figures by F.A., M.C., R.C.B., L.C. and Y.Z. Reviewing and editing of manuscript before submission by all authors.

Competing interests

X.Z. directs a translation project at Nanjing University that develops apparatus for routine eDNA biomonitoring. The remaining authors declare no competing interests.

Additional information

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