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Environmental DNA-Based Methods in Biodiversity Monitoring of Protected Areas: Application Range, Limitations, and Needs

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Abstract: Novel methods for species detection based on collection of environmental DNA (eDNA) are not only important in biodiversity assessment in a scientific context, but are also increasingly being applied in conservation practice. The eDNA-based biodiversity detection methods have significant potential for regular use in biodiversity status assessments and conservation actions in protected areas (PAs) and other effective area-based conservation measures (OECMs) worldwide. Species detection based on DNA from environmental samples, such as water, sediment, soil, air, or organic material, has a broad application scope with precise, comprehensive, and rapid species identification. Here, we provide an overview of the application range of eDNA-based methods for biodiversity monitoring in PAs, evaluate environmental assessments in which this technology has already been implemented for nature conservation, and examine the challenges that can hamper further application in real world practice. Based on the outcomes of two projects, practical experience, and current scientific literature focusing on their application, we conclude that eDNA-based species detection methods provide promising novel approaches that have strong potential as supplement methods, or in some cases even as substitutes for the conventional monitoring methods used for PAs. This advancement is expected to affect decision-making in biodiversity conservation efforts in PAs and OECMs.

Keywords: eDNA; eDNA metabarcoding; biodiversity assessment; nature conservation; protected area management

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1. Overview of eDNA-Based Methods in the Context of Biodiversity Monitoring

As global ecosystems face increasing pressure from human development and accompanying climate change, biodiversity loss has become the key ecological challenge worldwide [1,2]. Especially during the last decades, a dramatic global decline in species richness and abundance due to degraded habitat quality and diversity has become indisputable in **terrestrial** [3–5] as well as **freshwater** [6–10] and **marine ecosystems** [11]. Protected areas (PAs) and areas with special management status are key for the conservation of local and regional biodiversity, as they harbor a higher species richness than most areas without such status. However, even PAs are not immune to negative trends in biodiversity. This is clearly illustrated by the dramatic decline in flying insect biomass, a loss of over 75%, even in PAs in Germany, over a time span of only 27 years [12]. This reflects not only the major decrease in species diversity and abundance, but also the effects on ecosystem services, such as pollination, pest control, and nutrient supply across the food chain. Habitat destruction and fragmentation, decreasing flower supply, land-use change including agricultural intensification or abandonment of traditional farming, excessive use of pesticides, increased occurrence of pathogens, introduction and spread of invasive

species, light pollution, increase in carbon dioxide, climate change, and the interactions of these factors have been recognized as main drivers for the ongoing biodiversity decline [13,14]. However, by applying sufficient measures and practices, such as effective, traceable, and accountable implementation of biodiversity policies, monitoring of biodiversity trends providing evidence-based indicators, and implementing sufficient measures in targeted areas, dramatic species decline can be mitigated [15,16]. Best practice can be promoted by applying recent advances in interdisciplinary biodiversity research.

The methodologies used must overcome several hurdles for effective implementation of different biodiversity monitoring programs. Monitoring on a large scale is costly and time consuming because it must occur at regular time intervals over a long period of time at the same observation sites e.g., [17]. Compounding financial and time constraints, a large number of organism groups would need to be monitored to obtain comprehensive biodiversity data for accurate ecological assessment allowing follow-up conservation action. Hence, biodiversity monitoring programs must typically target taxonomic indicator groups with high informative value [18], and in addition, must cope with a limited number of experts for several taxonomic groups [19]. Moreover, in several surveys, very short phenological timeslots for observation must be considered [20]. In inaccessible or dangerous terrain due to political conflicts, wild animals, or prevailing extreme weather conditions and high elevation, e.g., Bhutan or Himalaya [21], surveys are particularly challenging. On the other hand, biodiversity monitoring methods must meet high methodological standards. Methods must be reliable, reproducible, standardized, applicable to different taxonomic groups, usable across different geographic regions, consider different spatial scales, and be operator-independent, flexible, and applicable to different challenges over the course of a biodiversity monitoring program. Due to the multitude of benefits that a robust biodiversity monitoring program provides to human society, there is significant demand and interest in accommodating these challenges among different users, spanning the scientific community, industry, NGOs, and national, sub-national, and international clients [22]. For targeted implementation of biodiversity surveys, conventional and novel techniques are already available, which, however, have reached different degrees of maturity [17].

One of the most promising approaches that can help overcome challenges of biodiversity monitoring and has the potential to facilitate field surveys and improve conservation measures in PAs is **species detection with DNA-based methods**. These methods enable species- and taxon-specific identification of organisms by aligning genetic sequences (i.e., barcodes) with reference sequences in a database (e.g., IBOL: https://ibol.org/, ABOL: https://abol.ac.at/; accessed on 5 April 2022) [23]. When more than a single species is targeted applying universal primers, the method is referred to as DNA metabarcoding [24]. Taberlet et al. 2012 [25] define DNA metabarcoding as a method for '*the automated identification of multiple species from a single bulk sample—containing entire organisms or from a single environmental sample containing degraded DNA*'. The method can be applied in at least three different target applications: identification of single organisms, characterizing the diversity of a bulk sample—an environmental sample containing organisms from different taxonomic groups [26] being studied, e.g., from a Malaise trap, or species identification from environmental DNA (eDNA). In contrast to organismal DNA, which is extracted directly from collected specimens, eDNA is regarded as a DNA target in the environment. DNA is emitted into the surrounding medium by organisms via skin, hair, gametes, urine, or feces [25,27,28]. Media that can be surveyed for species presence include water, soil, sediment, air, or organic materials, such as stomach content, feces, bird pellets, or even honey, which may contain a variety of plant pollen as well as DNA signatures of visiting pollinators [29]. The released DNA stays in the medium and may persist for periods varying from several days up to months [30] or even longer, as shown for lake sediments or arctic permafrost [31]. It enables the detection of species in a medium without the need for sighting, capturing, or acoustic detection.

Based on eDNA metabarcoding, a **spectrum of taxonomic groups and species** generally found in an environment can be identified from a single environmental sample. This

approach provides an overview of the species composition that is principally present in a surveyed habitat [24]. In addition to the detection of broader taxonomic units, eDNA-based methods allow targeted detection of **single species** [32]. Using species-specific primers, the presence or absence of a threatened species (e.g., amphibians in the Atlantic forest [33]), an indicator species (e.g., bioindicators of alpine freshwater environments [34]), an exotic species (e.g., Red-swamp crayfish, *Procambarus clarkii*, native in northern America and invasive in Europe [35]), or a parasite (e.g., the Rana virus *Batrachochytrium dendrobatidis* [36]) in a protected habitat can be verified. In PAs, species-specific eDNA-based methods have been applied for tracing large mammals, e.g., wild cats, lynx, wolves, or bears, based on hair samples [37] or excrement. Activities of protected species, e.g., migration of North American salmonids [38], are also assayed. In addition, eDNA metabarcoding analyses and species-specific assays are applicable not only in biodiversity monitoring but also in several other fields, such as ecology, e.g., in the analysis of stomach and gut contents [39]; paleobiology and palaeontology [40]; archaeoecology [26]; environmental impact assessment; environmental quality; citizen science (rapid test kits, e.g., 'frog in the water drop': https://www.uibk.ac.at/; accessed on 6 April 2022); agriculture and forestry; forensics; controls of food and traditional medical products as well as customs inspections on endangered and protected animals [41]; traceability of food; food safety; seed controls for specific ingredients or allergens; wood industry; and various industrial usages.

It is most likely that these multi-use methodologies will be increasingly applied to different monitoring projects and assessment of biodiversity, not only in scientific research, but also in practice. For example, within the framework of international guidelines and international reporting obligations, repeated biodiversity status analyses of PAs, such as national parks, UNESCO biosphere reserves, UNESCO world heritage sites, and European protected area networks (Natura 2000, Habitats Directive), must be conducted to evaluate the success of applied management activities. In general, any approach in the adaptive management of PAs requires accurate evidence of conservation outcomes [42,43]. Hence, eDNA-based assessments could be used for species detection and identification for this purpose, either as a supplement, or in some cases as a substitute, for conventional approaches as a part of regular monitoring campaigns [44].

In the synopsis of our paper, we provide examples of current and potential future PA monitoring programs that involve assessment of eDNA. For this purpose, we review possible applications, highlight particular cases of the practical implementation of species and biodiversity monitoring in nature conservation areas, identify major challenges, and finally list future goals and needs for effective implementation of eDNA collection in PAs. Our analyses and considerations are based on a literature search (e.g., search engine: Scopus, Google Scholar), lessons learned from the two projects E.DNA (KWF/EFRE UiG 2019/20, KWF No 16048-31819-45776) and BioMONITec (Biodiversity Monitoring Technologies—Transfer of disruptive engineering technologies into conservation practice: COIN FFG 2021-2024, No 884138), as well as long-time experience in national and worldwide conventional nature conservation approaches and applied biomonitoring.

2. eDNA-Based Methodology—Advantages, Disadvantages and Requirements for Use in Protected Areas

eDNA-based methods are particularly advantageous, as they can enable simultaneous assessment of the entire species composition in a comparatively short time and with little effort, making them an ideal tool to support and complement **biodiversity monitoring** of a defined area [45]. The application of eDNA-based methods for species detection from environmental samples has significant potential in comprehensive surveys of various taxonomic groups, from single cell organisms to large mammals. Depending on the respective investigation, eDNA sample collection is generally fast, relatively cheap, and easy [46]. The costs of applying eDNA analyses for biodiversity monitoring are highly dependent on the respective costs per sample offered by the particular laboratory providers, as well as on the total number of samples, since the per-sample cost drastically decreases,

once a certain threshold value is met. In addition, the costs for DNA sequencing have been decreasing over recent years [47] as the methods have become widely available [48].

As a tool for **practical nature conservation**, there are several benefits of eDNA metabarcoding compared to conventional, morphology-based identification methods. The main advantages include the possibility of carrying out more comprehensive taxonomic surveys, the ability to cover spatially larger sampling areas, which is particularly crucial for PAs, the possibility of conducting non-invasive sampling of sensitive species in vulnerable ecosystems, and the ability to record traces of protected macroscopic organisms [49]. In addition to taxonomically comprehensive surveys, standardized approaches that enable comparisons of data between PAs should be available for biodiversity monitoring [50,51]. Regular biodiversity monitoring necessary for assessing and managing the status of protected goods—species and habitats—can be performed more economically using eDNA-based detection methods, which is also a key factor for PAs. Typically, taxon specific experts are not needed for sample collection. It can be conducted by non-geneticists, e.g., ecologists without knowledge of genetic analyses, geneticists, or even citizen scientists. This is of special importance for PAs, which often face a shortage of staff, especially of professional ecologists. Nevertheless, sampling training is mandatory for achieving desired outcomes. Thorough sampling is thus the prerequisite for accurate data analyses and species determination, which can then be outsourced. Finally, results of the eDNA metabarcoding analysis can be stored online and are accessible from any part of the world (e.g., IBOL, ABOL [52]). When successfully applied, eDNA-based species detection and identification may in some cases be even more reliable than expert taxonomic work, for example, in identifying larval stage insects, and may be much more effective when dealing with cryptic species e.g., deWaard et al., 2008 [53].

Aside from the listed advantages of eDNA detection methods, several major challenges must be considered when conducting species and community monitoring using eDNA [54]. The reliability of the assessment strongly depends on the sampled medium. Generally, eDNA detection is particularly successful when acquired from aquatic environments, but less reliable when collected from sediments and soil [55]. In this respect, the quality and quantity of the sample also rely on how much DNA is released from each species. Large amounts of DNA are, for instance, discharged from fish and amphibians [56,57]. In general, species can be better traced in particular habitats. The presence of frogs is preferentially and more easily confirmed in aquatic habitats compared to their terrestrial habitats. Species identification also depends on the densities of the organism group present in the investigated medium [49], also taking the spatial and temporal dynamics of eDNA into account [58,59]. Hence, in aquatic environments, assessment of species assembly proved to be more successful in small stagnant freshwater habitats, such as lakes or ponds, than in large running waterways, such as streams and rivers, because of the higher DNA concentrations in the stagnant water bodies [60]. However, challenges such as representative sampling, eDNA capture, and PCR inhibition still hamper complete species diversity detection in aquatic habitats. The success of species identification also varies among taxonomic groups due to the specificity of the primers used and differences in the completeness of the reference database, which in turn also depends on the level of taxonomic knowledge. Further challenges in applying eDNA-based methods include quantifying species abundance, relating species detections to the actual species assemblage of the habitat, and identifying species interaction. For habitat classification, there is also a need to assess the ecological status of key species [61]. In addition, the lack of experts is a major obstacle for data analysis and interpretation of eDNA metabarcoding results. A high risk of bias will result from the collection of samples by non-experts without adequate quality controls.

Apart from the requirement to outsource wet lab and bioinformatics expertise for sample analysis, eDNA sampling can, in general, be conducted by non-experts, if several prerequisites are fulfilled to ensure successful implementation of eDNA sampling. First, sound ecological knowledge, species-specific expertise on the behavior and biology of

the sampled organisms, and experience with sampling in the field should be present. To be able to evaluate species lists obtained by eDNA metabarcoding, basic knowledge on laboratory practices e.g., Dully et al., 2021 [62], including DNA extraction, amplification, and sequencing is advantageous. On the other hand, understanding of workflows, basic bioinformatics experience, and knowledge on barcode alignment with reference databases are basic requirements for the expert entrusted with eDNA assessment. Thus, consultation or involvement of highly qualified experts is mandatory in eDNA-based biodiversity assessment. **Ecologists** are needed to identify and implement the sampling strategy, while **technicians** who are trained in the state-of-the-art laboratory work and in using bioinformatics pipelines, and **molecular biologists** who are experienced in interpreting the genetic results, should also be involved. In practice, the majority of eDNA samples collected by non-experts are processed and analysed by external technique providers. eDNA metabarcoding is still relatively cost-intensive, due to the required specialized equipment and expert handling in the context of regular monitoring. However, these methods may still be applied in PAs, as DNA-based methods are becoming increasingly standardized, and often the expertise of samplers is combined with that from companies specialized in performing molecular analyses (e.g., www.aimethods-lab.com; www.naturemetrics.co.uk; www.sinsoma.com; accessed on 7 March 2022).

3. Utilization of eDNA Metabarcoding in Biomonitoring in Protected Areas

Due to its advantages and application possibilities, these novel molecular methods are expected to have an immense implementation potential in future, including biodiversity monitoring practices in natural protection sites worldwide. As of April 2022, there are 251,947 terrestrial areas plus another 478 OECMs (other effective area-based conservation measures) under protection worldwide, covering nearly 17% of the global terrestrial area, including inland waters (https://www.protectedplanet.net/en; accessed on 20 April 2022). Another 17,910 marine PAs and OECMs cover 8% of the area of the world's oceans. Biodiversity includes ecosystems, biotopes and habitats, vegetation units, and ecological interactions, as well as almost all taxa and organismic categories. In the management of PAs, a shift towards evidence-based management and governance can be observed [63], which requires new monitoring capacities. For many categories, such as UNESCO sites or European protected area networks, **monitoring is mandatory**. That means biodiversity assessment must be carried out for evaluation of plant and animal diversity status, including habitat quality. In order to meet these requirements, ecological monitoring must occur regularly. Moreover, to enhance positive development of conservation targets, it is necessary to verify the effectiveness and success of the management measures applied [64]. Consequently, there is a high demand for applicable monitoring practices and related conventional as well as novel survey tools to facilitate these challenges and to achieve the desired conservation results [17].

However, as mentioned previously, PAs in particular have limited financial and staff capacities. Especially in the area of biodiversity assessment, they are largely dependent on external expertise. The number of existing experts is limited, and due to the peripheral location of many PAs, there are usually no experts available on-site. Consequently, eDNA-based methods open up completely new possibilities in this respect. Besides sampling, which can be performed by specially trained non-professional personnel, the required data expertise (taxonomic analysis) can be carried out by external experts at any time and from any location. Thus, for the first time, a basic prerequisite for systematic monitoring of conservation outcomes is being established.

To integrate eDNA sampling into standard conservation practice, a major focus is placed on the development of DNA-based methods applicable across ecosystems. In many cases, methods are established for the optimization of species-specific targets and for the investigation of species communities in different ecosystems [65]. However, there remains a large gap between testing and standard application in PAs, according to the published literature [66].

For the management of PAs, proof of target achievement and thus of management effectiveness cannot be provided without solid evidence of the conservation status. For this purpose, eDNA-based methods have already proven to be applicable in the monitoring of PAs. For example, in the study results on airborne pollen patterns in Natura 2000 sites in the Italian Alps, eDNA metabarcoding was a '*powerful molecular tool to complement traditional biodiversity monitoring*' [67] as it enabled rapid detection of regional plant species. In this study, analyses of pollen DNA with metabarcoding allowed 68 taxa of 32 plant families to be determined, with finer taxonomic resolution than with the use of classical techniques, such as light microscopy. In addition, initial data on plant species composition were obtained. eDNA metabarcoding has also been applied in analyses of *soil* samples, as little has yet been ascertained regarding the composition of soil fauna in general using conventional approaches. For instance, in alpine environments in the Italian Gran Paradiso National Park, the edaphic soil fauna diversity and its composition related to environmental features, such as habitats, vegetation, soil, and topographic features, were surveyed with eDNA metabarcoding [68]. With the application of this method, 18 arthropod families could clearly be distinguished and identified. Key factors for forest soil community composition could be related to parameters such as vegetation and altitude of location, whereas soil pH and slope inclination had the most influential effect on species composition in the prairie soil, revealing the environmental needs of different alpine habitats. Moreover, novel molecular techniques enable comprehensive identification of soil microbial diversity. Fungi, for example, provide key functions in ecosystems in their role as decomposers or plant symbionts. Using classical determination approaches, however, it is challenging to observe and taxonomically identify fungal species. The study of Yan et al. [69] showed the response of soil fungi to ecological restoration in an active restoration site at Mt. Bold in Australia, indicating a shift of fungal communities towards a more natural species composition within only few years. This example shows how eDNA allows for accurate quantification of environmental changes, which makes it a useful monitoring tool in restoration campaigns. eDNA soil analyses can also be applied for confirmation of terrestrial distribution of animals. Two examples are the recording of the endangered sharp-tailed snake (*Contia tenuis*) on Salt Spring Island, British Columbia, Canada [70], and monitoring of the endangered parrot species kākāpō (*Strigops habroptilus*), in New Zealand [71]. However, species identification of mammals, birds, reptiles, and amphibians in their terrestrial habitats remains a major challenge, since the concentration of DNA traces on land is lower and the DNA residues are comparatively more difficult to detect than, for example, in a water medium, because the DNA is bonded to soil particles and immobile, requiring analysis of several soil samples to increase the confidence of species evidence. Moreover, several other environmental factors influence eDNA detection, including abiotic variables that may affect DNA degradation. Consequently, there are relatively few applications of eDNA-based methods in terrestrial environments. eDNA metabarcoding for status assessment was also performed in the Kruger National Park, South Africa, where bacteria communities, including pathogens in waterholes, are monitored to provide a baseline of bacterial diversity, which in future could serve as an indicator to identify ecosystem disturbance [72].

Application of eDNA analysis can be especially promising for monitoring in remote and dangerous terrain. For instance, cave salamanders (*Proteus anguinus*) are challenging to explore because their habitats are dangerous and difficult to access [73]. In PAs where large wild animals occur, for instance in South-African national parks, eDNA metabarcoding of animal traces, such as hair or feces, enables species identification without risking human or animal safety through direct interactions [30]. Even saliva on twigs, e.g., of giraffes, provides information about the presence and variety of browsing animals [49]. Aquatic eDNA samples from waterholes resulted in data about their visitors without requiring visual identification [74]. The assessment of species diversity based on eDNA metabarcoding of aquatic samples is just starting to be explored, and results are compared with conventional animal monitoring methods [60]; (T. Schenekar, pers. comm.).

Methods of eDNA collection have been most successfully applied in PAs in **freshwater ecosystems**, such as ponds, lakes, rivers, and streams, for example, in [75]. For the most accurate and comprehensive assessment possible, eDNA collection in freshwater ecosystems should also be combined with conventional ecological surveys on the ground ('**ground truthing**' [76]). Such combined datasets enable a comprehensive overview about the quality and biodiversity status of the ecosystem under evaluation, also within the framework of environmental impact assessments. Currently, aquatic samples are taken primarily from freshwater systems where invertebrates, fish, and amphibians are the focus of ecological assessment, as reported in [77]. For example, the produced list of aquatic insects present in a sampled medium can provide an introductory overview on the ecological status of the waterbody and may also be used to identify single indicator species or groups. A useful application area is the survey of macrozoobenthos in flowing waters for the assessment of water quality and ecological status. A prominent example of this is the Himalayan state of Bhutan. The massive expansion of hydropower as a renewable energy source has had a significant impact on the country's remarkable river systems. These need to be systematically monitored [78] in order to mitigate ecological damage. This inspection cannot be guaranteed by applying conventional methods and capacities only. If needed, findings on species abundance, community composition, and ecological role can furthermore be investigated in more detail by conventional approaches. Besides freshwater ecosystems, eDNA metabarcoding has already proved to be particularly useful in saltwater ecosystems in connection with marine PAs [79,80]. Gold et al. 2021 referred to molecular methods as '*a promising alternative for marine ecosystem monitoring*' [69] as there exist large data gaps regarding species identification and species communities in marine habitats. In addition, on-site work in marine habitats is particularly challenging, dangerous, costly, and time-consuming, and hence, any facilitation in this regard is welcomed within the framework of the performed monitoring of the marine fauna. In the study of Gold et al., fish communities were investigated using eDNA metabarcoding in comparison to underwater visual census surveys. Out of 25 visually observed species, 19 could be confirmed with eDNA metabarcoding, providing optimism but also addressing further efforts for future applications in marine environments. However, the strengths and limitations of the different approaches still need to be assessed in more detail and for specific monitoring goals in the future.

In PAs, eDNA surveys can serve as a selection tool in biodiversity assessment for particular indicator species and can support efforts to further engage citizens in nature protection. In this regard, eDNA approaches can be very suitable in regional initiatives that attempt to generate data on the presence or absence of species of different taxonomic groups in PAs, and can contribute to regional barcode reference databases. One example of such an initiative is the Austrian Citizen Science campaign called BioBlitz, in which species are collected in the run of the Days of Biodiversity to generate DNA barcodes of species living in the investigated PAs. Citizen scientists contribute their findings, which are verified by taxonomic experts [81]. The information may, in such cases, act as the starting point for further monitoring programs in PAs.

As demonstrated above with examples of applications in PAs, eDNA-based species detection methods have already proven to be a promising novel approach that is expected to have strong potential as a supplement, or, in some cases, even as a replacement, for conventional monitoring methods in conservation. It is anticipated that conventional methods could be eclipsed, especially for complex monitoring, such as soil fauna investigation.

4. Challenges and Limitations of eDNA-Based Methods in Protected Area Monitoring

Despite a promising outlook for the application of eDNA-based methods in conservation, several challenges remain to be confronted. Currently, different protocols exist for the survey of the same medium and taxonomic groups that do not always produce comparable data sets [50,82,83]. Hence, the first step would be to develop standardized methods for eDNA field sample collection and analysis. Some methodological approaches, e.g., for

water ecosystems and soil, already exist in a standardized form [82]. However, specific protocols for different media, types of samples and target groups are still missing. As DNA has proven to be present in sediments and soil and is stable for periods of several days or even months [31], the uncertainty of the actual physical presence of the detected species is high [49]. In aquatic environments, the DNA may be displaced over several km and often cannot be assigned to a specific location [84]. Thus, the selection of sampling locations also influences sampled eDNA quality and requires expert knowledge of species-specific occurrence, ecology, and behavior. Another major challenge is that taxon-specific primers must be identified in advance of laboratory analyses [85]. In addition, DNA inhibitors might prevent amplification of the target genomic region by the associated primer [86]. Furthermore, the quality of results is limited by the quality of existing reference databases. Knowledge about soil bacteria species communities, for example, is currently still scarce. For assessment of biodiversity in such cases, however, (molecular) operational taxonomic units ((M)OTUs) can be used [55]. In some cases, sampling of eDNA is not the optimal solution; for freshwater insects, bulk sampling is suggested, as insects do not shed much DNA into their environment [87]. Moreover, for a precise, correct, and complete species list of an investigated habitat, complete taxonomic databases are required. Thus, regional databases should ideally already be established for correct species assignment in a monitoring campaign; however, they should be collected from the same standardized source. The systematic use of eDNA metabarcoding for monitoring in PAs requires decision-makers to be aware of the importance and possibilities of this methodology. Appropriate capacity should be built and trained. Therefore, it is likely to be several years before eDNA-based methods can become established as a standard tool in nature conservation.

In order to use eDNA metabarcoding for biodiversity monitoring programs across PAs, suitable assessment and research questions must be formulated in advance. It is imperative that the applied eDNA-based method fits into the framework describing the goal of the biodiversity monitoring approach in the PA, and that suitable indicators and related questions are defined in advance (Dalton et al. submitted). The first step is to determine whether the monitoring target is already known, or whether it still needs to be identified. In this context, key questions include [27]: Are the desired taxa well-represented in the environmental sample? What type of material should be collected? Are specific sampling protocols available? Which genetic markers and primer sets should be used? Does a comprehensive reference database of DNA barcodes for the surveyed species group exist? Depending on the research assessment and monitoring task in a PA, DNA-based techniques are generally not applicable for every monitoring objective, and in several cases, conventional approaches will ensure more detailed and reliable assessments. Hence, the success of the applied DNA methods depends on the monitoring goal within the PA.

For biodiversity assessment in PAs, ecological information on the species assembly derived from eDNA metabarcoding is restricted and faces many limitations. **Species abundance** is hardly estimable [77]. Studies suggest, abundance should be assessed only when sufficient reference data are available; however, the data should still be interpreted cautiously in this case [88,89]. Consequently, in most cases only presence/absence data are generated. Information about life stage, demographic structure, reproductive success, and fitness of a species is generally lacking. This information is, however, needed to implement suitable management actions in PAs, requiring 'classical' ecological surveys for comprehensive assessment of the status of the indicator group. **Hybrids** can rarely be distinguished, because in most cases maternal mitochondrial DNA is used for eDNA-based approaches [90]. If hybrids need to be determined, specific primers must be developed and applied. Several animal species transfer very little DNA derived from their prey. This can yield false results of the actual occurrence of species in a medium (e.g., predators [91]). Depending on the DNA concentration and applied methodology, e.g., filter extraction method or sample preservation, species detection probability differs. Hence, harmonized optimally performing sampling protocols must be developed or optimized for use in the field, so that they can be applied globally across the widest possible range of PAs [92],

simplifying their application by various experts as well as non-experts. In addition, based on the analyzed biodiversity data, better reporting standards would be needed to compare the ecological status of a similar environmental medium and follow-up protection measures in different PAs [93].

In general, eDNA metabarcoding is expected to be cheaper than applied conventional methods. However, if no protocol exists, establishing a novel metabarcoding methodology could be expensive. Beyond that, cost efficiency largely depends on the targeted taxonomic group, the respective applied method, and the number of samples to be processed. PAs often suffer from very limited budgets for biodiversity monitoring and conservation measures. A study by James et al. 1999 [94], based on a World Conservation Monitoring Center survey in 1996 across 600 PAs of 180 countries, with altogether 3.7 million km^2 under protection, investigated the global mean budget and personnel devoted to PAs worldwide, revealing that in the US PAs, the amount spent was US $893 per km^2. The mean amount in developed countries at that time was reported to be $2058 per km^2, while the mean in developing countries was capped at $157 per km^2. However, biomonitoring in developing countries in tropical PAs presents a particular challenge, as these complex ecosystems harbor an exceptionally large diversity of species, the majority of which are still unknown.

Beyond the application of eDNA-based methods in PAs, this novel method will also contribute to practical decision-making applications, such as environmental impact assessments, which could accelerate and compliment environmental legal procedures. In this application area, eDNA metabarcoding shows limitations, for instance, along linear structures, such as railways, or in construction planning, in which soil sample eDNA analyses would only represent a small fraction of the evaluated area, hence providing only point-based information. Thus, expert consultation is needed to survey target areas and assess the occurrence of priority plant and animal species. Despite these challenges, there are strong initiatives in some European countries to use this technique also in environmental impact assessment. In the North Adriatic Sea of Italy, genetic techniques are used in biomonitoring to survey marine diversity around three offshore gas platforms [95]. Finland, as another example, is preparing a plan to regularly implement it in environmental monitoring [96], while Canada has a guideline on the use of eDNA analysis to manage invasive and at-risk aquatic species [51].

5. Future Perspective

eDNA-based methods represent a promising technology in biodiversity monitoring, and are currently expanding into different fields of applied practice. Use of species-specific assays and eDNA metabarcoding in practical nature conservation is expected to fundamentally change assessment opportunities, services, and workflows, and will provide new answers on research, assessment, and management questions.

Potential areas of application of eDNA collection and analysis in the management of PAs include the following:

- **Implementation of effective long-term monitoring** of changes in species composition, especially in the air (e.g., pollen), water (e.g., zoobenthos, diatoms [97]), and soil (microbes, fungi). These investigations may go beyond taxon-specific monitoring and may cover entire species communities.
- **Early detection of biological threats in vulnerable ecosystems**, such as invasive species (e.g., pathogens [67,98]) or farmland and forest (e.g., spotted lanternfly (*Lycorma delicatula*) in northeastern USA; [99]). Robust analytical protocols may contribute to the implementation of an **early warning system**.
- **Systematic detection of rare or cryptic species** that may be of crucial importance for conservation and thus for management of the sites [80].
- Possibilities for **systematic recording of ephemeral natural phenomena and phenological changes** that can be of outstanding importance in the management of a site (e.g., research on shifts in phenology of bryophytes in relation to meteorological factors over time, https://www.lunduniversity.lu.se; accessed on 11 April 2022).

- **Detecting unexpected or unintended trends** in biodiversity in the context of PA management [100].

In order to successfully implement novel eDNA-based species detection methods into PA monitoring programs and increased efficiency of biodiversity monitoring, the following steps should be taken:

(1) Improvement of **reference data libraries**. Several countries with networks of PAs and national parks still have to build up such libraries, and urgently need to sequence more species before they can even consider applying this method (e.g., West Africa: [101]). (2) Acquisition of comprehensive scientific ecological knowledge to support monitoring planning and application of novel genetic methods to different environments. (3) Standardization of lab and field protocols. (4) Harmonization of several guidelines, which should ideally result in a common worldwide-applicable guideline as an initial guidance [102], [Dalton et al. submitted]. (5) Suitable method selection. The most useful, straightforward and cost-efficient methods should be identified and offered to managers and implemented through local, national, and global standards. (6) Development of common workflows as field data collection, data analysis, taxonomic determination, and data management become increasingly decoupled. (7) National training services. eDNA metabarcoding, especially in repeat applications (biodiversity monitoring), places high demands on data management and data handling. Data science and handling of big data require new capacities at the responsible agencies. In this regard, services should be offered nationally to support staff training. (8) Assessment of the method's suitability in each context. In each case, a critical examination must ascertain whether eDNA metabarcoding is able to support ecological field research and assessment at all, and whether the method is able to provide the desired information about the investigated environment or habitat.

In order to further fuel such implementation, several steps would have to be tackled. A significant gap exists between park management practitioners, academic labwork, and data analysis and interpretation, and this must be bridged in future. For a successful 'real world' application and implementation of DNA-based techniques in biodiversity monitoring, mutual understanding from all working perspectives must be worked on. A basic knowledge would have to be acquired by all parties on all steps in the workflow. Furthermore, these workflows must be simplified, and additional administrative and coordination services must be provided in the PAs to ensure a fluent handling process.

To conclude, eDNA analyses are a promising and applicable tool for a variety of monitoring-associated research and management questions in PAs. Different eDNA-based methods have their advantages and limits, so they should be implemented together by a broader group of experts, including molecular biologists, ecologists, and bioinformaticians. The methods have the potential to systematically support biodiversity monitoring and assessment in PA management cycles worldwide. However, the systematic use of eDNA also places high demands on the management of the PA; systematic workflows ranging from data collection to evaluation (big data) and archiving must be developed, tested, and standardized. Ideally, the workflows can be organized based on a labor-sharing approach in collaborations with experts with an ecological background, as not all steps need to be carried out by the PA management body alone. It is expected that the new technologies will be introduced gradually over the next few years, and will bring about a major change in the key processes of PA management.

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