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Environmental DNA – An emerging tool in conservation for monitoring past and present biodiversity

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ABSTRACT

The continuous decline in Earth's biodiversity represents a major crisis and challenge for the 21st century, and there is international political agreement to slow down or halt this decline. The challenge is in large part impeded by the lack of knowledge on the state and distribution of biodiversity – especially since the majority of species on Earth are un-described by science. All conservation efforts to save biodiversity essentially depend on the monitoring of species and populations to obtain reliable distribution patterns and population size estimates. Such monitoring has traditionally relied on physical identification of species by visual surveys and counting of individuals. However, traditional monitoring techniques remain problematic due to difficulties associated with correct identification of cryptic species or juvenile life stages, a continuous decline in taxonomic expertise, non-standardized sampling, and the invasive nature of some survey techniques. Hence, there is urgent need for alternative and efficient techniques for large-scale biodiversity monitoring. Environmental DNA (eDNA) – defined here as: *genetic material obtained directly from environmental samples (soil, sediment, water, etc.) without any obvious signs of biological source material* – is an efficient, non-invasive and easy-to-standardize sampling approach. Coupled with sensitive, cost-efficient and ever-advancing DNA sequencing technology, it may be an appropriate candidate for the challenge of biodiversity monitoring. Environmental DNA has been obtained from ancient as well as modern samples and encompasses single species detection to analyses of ecosystems. The research on eDNA initiated in microbiology, recognizing that culture-based methods grossly misrepresent the microbial diversity in nature. Subsequently, as a method to assess the diversity of macro-organismal communities, eDNA was first analyzed in sediments, revealing DNA from extinct and extant animals and plants, but has since been obtained from various terrestrial and aquatic environmental samples. Results from eDNA approaches have provided valuable insights to the study of ancient environments and proven useful for monitoring contemporary biodiversity in terrestrial and aquatic ecosystems. In the future, we expect the eDNA-based approaches to move from single-marker analyses of species or communities to meta-genomic surveys of entire ecosystems to predict spatial and temporal biodiversity patterns. Such advances have applications for a range of biological, geological and environmental sciences. Here we review the achievements gained through analyses of eDNA from macro-organisms in a conservation context, and discuss its potential advantages and limitations for biodiversity monitoring.
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1. Introduction

The continuous decline in Earth's biodiversity remains one of the most critical challenges in the 21st century (Butchart et al., 2010). Worldwide, populations of wild flora and fauna are being depleted due to anthropogenic disturbances (Barnosky et al., 2011; Dirzo et al., 2014) and species extinctions rates exceed those of pre-human periods (Pimm et al., 1995; Barnosky et al., 2011), which greatly impacts human health and sustainability of our planet (Diaz et al., 2006). Although knowledge on biodiversity is incomplete or even un-described for numerous taxa and geographical regions (Vié et al., 2009), there is international political agreement to halt the current loss in biodiversity (UNEP, 2011). All such conservation efforts to save biodiversity essentially depend on biological monitoring for obtaining precise data on species distributions and population sizes on a relevant ecological and political time scale. Species monitoring has traditionally relied on physical identification of species by, for example, visual surveys and counting of individuals in the field using distinct morphological characters. However, in some cases these techniques fall short of actually performing efficient and standardized surveys, due to, for example, phenotypic plasticity and closely related species with very similar appearance in juvenile stages. Thus, there are examples of species databases flawed with errors (Daan, 2001). Additionally, traditional monitoring techniques have sometimes proven to be invasive on the species or ecosystem under study, such as marine surveys that has relied on highly destructive techniques (Baldwin et al., 1996; Jones, 1992), although see Robertson and Smith-Vaniz (2008). Furthermore, morphological identification is heavily dependent on taxonomic expertise, which is often lacking or in rapid decline (Hopkins and Freckleton, 2002; Wheeler et al., 2004). All such limitations of traditional biodiversity monitoring have created demand for alternative approaches.

Obtaining information of species, populations and communities by retrieving DNA from environmental samples (*environmental DNA – eDNA*) holds the potential of combating many of these challenges associated with biodiversity monitoring (Baird and Hajibabaei, 2012; Kelly et al., 2014b). The fact that DNA from higher organisms persists in the environment, where it can be sampled, extracted and analyzed, has been a major technological and scientific breakthrough within the last decade (Fig. 1). As species interact with the environment, they will continuously expel DNA to their surroundings. For higher organisms, this DNA may come from excreted cells or tissue such as urine (e.g. Valiere and Taberlet, 2000), faeces (e.g. Poinar et al., 1998), hairs and skin (e.g. Bunce et al., 2005; Lydolph et al., 2005), and obviously from dead individuals leaking genetic material. The macrobial eDNA may in some systems exists predominantly inside mitochondria or small cells (Turner et al., 2014), but owing to eventual membrane degradation, extracellular DNA will also be present in the environment (Nielsen et al., 2007). Once DNA is left in the environment, its preservation, and thus availability, varies with several orders of magnitude from weeks in temperate water (Dejean et al., 2011; Thomsen et al., 2012b) to hundreds of thousands of years in cold, dry permafrost (e.g. Willerslev et al., 2003). Accordingly, eDNA has been used to address applied and fundamental research questions within areas ranging from molecular biology, ecology, palaeontology and environmental sciences.

Within a single standardized sample, DNA from entire communities across taxonomic groups can potentially be analyzed simultaneously. The content of an eDNA sample is typically analyzed by amplification using *polymerase chain reaction* (PCR) and subsequent DNA sequencing. The amplification is done either by a single-species approach using specific primers or by multiple-species (multiple-taxon) approach using generic primers for a given focal group of organisms. Especially the fast advancing *next-generation sequencing* (NGS) technologies has made comprehensive biodiversity surveys possible for limited effort and costs (Shokralla et al., 2012). It has thus made the multiple-species eDNA approach especially powerful by *DNA metabarcoding* – mass DNA sequencing for the simultaneous molecular identification of multiple taxa in a complex sample (Taberlet et al., 2012a). Although similar in principle to classical *DNA barcoding* of simple DNA extracts (Hebert et al., 2003), the practical approach and target sequence is very different. While both rely on the fact that short standardized DNA regions – typically mitochondrial, chloroplast or ribosomal RNA (rRNA) genes – can be amplified by PCR, sequenced and subsequently used as *barcodes* to identify and discriminate taxons, DNA metabarcoding cannot efficiently utilize protein coding genes such as cytochrome oxidase I (COI), since interspecific genetic variation impedes the use of universal primers (Deagle et al., 2014). Also, proposed standardized DNA barcodes are usually >500 bp and seem to reach consensus on the mitochondrial COI for animals (Hebert et al., 2003), the plastid ribulose 1,5-bisphosphate carboxylase gene (rbcL) and the maturase K gene (matK) for plants (Hollingsworth et al., 2009) and the internal transcribed spacer (ITS) for fungi (Nilsson et al., 2009; Bellemain et al., 2010; Schoch et al., 2012). These target genes have basically been chosen due to their high resolution at the species level, but high copy number per cell of mitochondria, chloroplasts and rRNA genes also make them useful in eDNA studies, since they are more likely to be picked up than single-copy nuclear DNA. However, as

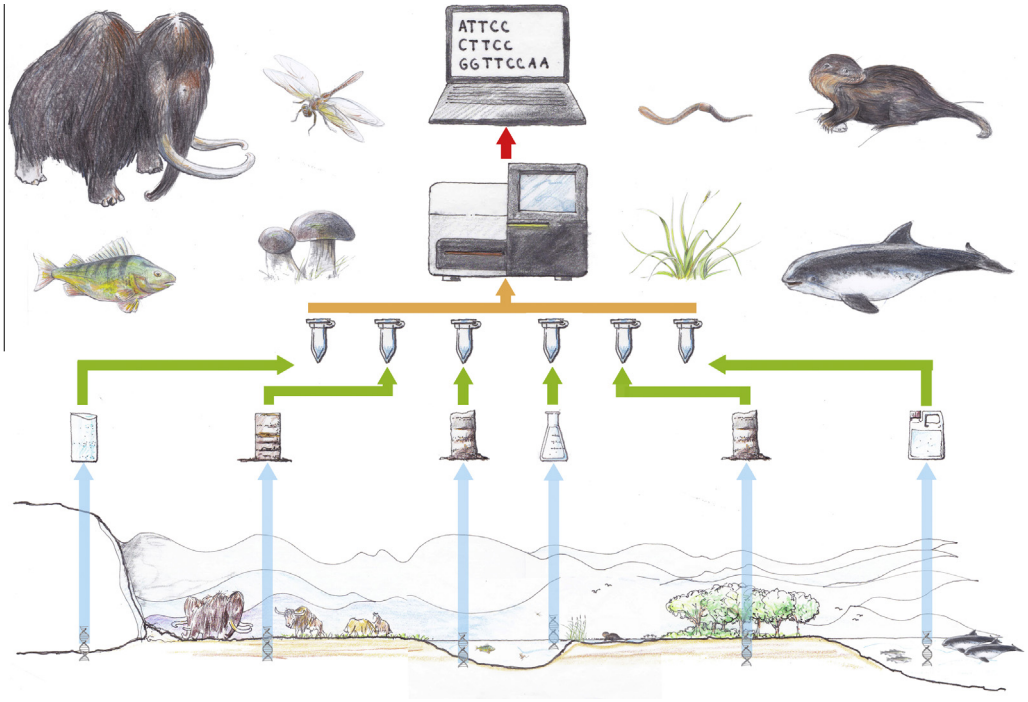

Fig. 1. The overall workflow for environmental DNA (eDNA) studies with examples of organisms that have been identified from environmental samples. Environments and their respective samplings from left to right: (i) glaciers; (ii) permafrost/tundra; (iii) aquatic sediments; (iv) lakes and streams; (v) terrestrial habitats; (vi) oceans. The first three are ancient environments while the latter three are modern. Color codes of arrows represent the different steps in the analyses: (blue) environmental sampling (ice cores, soil/sediment core samples, freshwater/seawater samples); (green) DNA extraction using procedures specific for the individual type of sample; (orange) PCR amplification of extracted DNA using generic or species-specific primers targeting biodiversity and subsequent sequencing of amplicons (here shown as Illumina MiSeq technology); (red) bioinformatic data-processing including error trimming, sequence sorting, and identification pipelines leading to various taxonomic level or MOTUs and the final interpretation and publication of results. Drawing by Lars Holm.

DNA from environmental samples, especially ancient samples, is often fragmented (e.g. Deagle et al., 2006; Willerslev and Cooper, 2005), analyses must rely on shorter DNA fragments than the traditional defined barcoding regions (e.g. Taberlet et al., 2007; Riaz et al., 2011; Epp et al., 2012). If DNA database coverage is low or taxons are unknown, target sequences are typically clustered into *Molecular Operational Taxonomic Units* (MOTUs). Yet, for less species-rich groups in low-biodiversity areas, a much shorter barcode sequence can prove informative at the species level, which permits eDNA metabarcoding with high taxonomic resolution (Bienert et al., 2012; Thomsen et al., 2012a).

Here we review and discuss the achievements of eDNA approaches in ancient and contemporary ecosystems for describing past biodiversity and for use in practical present-day conservation, respectively. We focus this paper on eDNA from *macro-organisms* (animals, plants and fungi) as they are the key target in conservation. We define eDNA as: *genetic material obtained directly from environmental samples* (*soil, sediment, water, etc.*) *without any obvious signs of biological source material*. In order to keep this focus, which is the topic of the current journal special issue, we exclude related studies of other complex samples such as faecal samples for assessment of biodiversity and diet content (e.g. Pompanon et al., 2012; Schnell et al., 2012; Valentini et al., 2009a) as well as analyses of arthropod bulk samples (e.g. Calvignac-Spencer et al., 2013; Hajibabaei et al., 2011; Ji et al., 2013), which apply very similar techniques and also have direct applications for conservation. Environmental DNA as defined here is thus an approach to obtain genetic material from the environment, on which a collection of molecular methodologies and analyses tools (such as DNA metabarcoding) subsequently can be applied to answer questions in e.g. palaeontology, ecology and conservation biology.

2. Achievements of eDNA

"Environmental DNA" arose with the idea of obtaining nucleic acids of microbes directly from environmental samples (Ogram et al., 1987; Olsen et al., 1986; Pace et al., 1986). This idea came from the recognition, that culture-based methods grossly misrepresent the composition of microbial populations as they occur in nature due to the fact that many microbes cannot be cultured, and eDNA thus represents a unique access to information on their genetic makeup (Brock, 1987). Later, eDNA from microorganisms has since been studied extensively in soil (e.g. Fierer and Jackson, 2006), permafrost (Johnson et al., 2007; Willerslev et al., 2004), freshwater (e.g. Fisher and Triplett, 1999) and seawater (e.g. Venter et al., 2004), which has given valuable insights to bacterial diversity (e.g. Zinger et al., 2011) and functional genomics (Tringe et al., 2005).

The discovery of diverse eDNA from macro-organisms established the approach as truly relevant in a conservation setting, and have been shown in several different environments both ancient and modern, terrestrial and aquatic (Fig. 1, Table 1). The nature of DNA from macro-organisms in environmental samples is different from targeting microbial organisms (prokaryotes and microbial eukaryotes), as the former is present only as parts of the organism (cellular remains or free DNA), whereas the latter may be detected by DNA deriving from whole, living organisms present in the samples.

2.1. Ancient environments

2.1.1. Terrestrial sediments

Environmental DNA as a method to assess the diversity of macro-organismal communities was first applied to sediments,

Table 1
Macro-organismal environmental DNA (eDNA) studies reviewed in this paper.

Source	References	Title	Sample habitat	Taxons studied	Geographical location	Aproximate ages (years BP)
Terrestrial sediments	Willerslev et al. (2003)	Diverse plant and animal genetic records from Holocene and Pleistocene sediments	Cave sediments, permafrost	Mammals, birds, plants	New Zealand; Siberia	Present – 400,000
	Hofreiter et al. (2003)	Molecular caving	Cave sediments	Mammals, birds	Arizona, USA	ca. 10,845
	Lydolph et al. (2005)	Beringian paleoecology inferred from permafrost-preserved fungal DNA	Permafrost	Fungi, plants, protists	Siberia	Present – 300,000–400,000
	Haile et al. (2007)	Ancient DNA chronology within sediment deposits: Are paleobiological reconstructions possible and is DNA leaching a factor?	Cave sediments	Birds, plants	New Zealand	Present – 3300
	Haile et al. (2009)	Ancient DNA reveals late survival of mammoth and horse in interior Alaska	Perennially frozen sediments	Mammals	Alaska, USA	ca. 7000–11,000
	Thomsen et al. (2009)	Non-destructive sampling of ancient insect DNA	Temperate dry sediments	Insects	New Zealand	3000
	Hebsgaard et al. (2009)	'The farm beneath the sand' – an archaeological case study on ancient 'dirt' DNA	Sand sediment	Mammals	South-west Greenland	AD 1030–1530
	Sønstebø et al. (2010)	Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate	Permafrost	Plants	Siberia	15,810–22,960 (uncal.)
	Epp et al. (2012)	New environmental metabarcodes for analyzing soil DNA: potential for studying past and present ecosystems	Soil, permafrost	Fungi, bryophytes, enchytraeids, beetle, birds	Norway; Siberia	Present, ca. 16,000–50,000
	Jørgensen et al. (2012)	A comparative study of ancient sedimentary DNA, pollen and macrofossils from permafrost sediments of northern Siberia reveals long-term vegetational stability	Permafrost	Plants	Siberia	12,500–46,000
	Jørgensen et al. (2012)	Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA meta-barcoding	Nunatak sediments	Plants	Southern Greenland	5528
	Willerslev et al. (2014)	Fifty thousand years of Arctic vegetation and megafaunal diet	Permafrost, soil	Plants, nematodes	Russia, Canada, Alaska, Svalbard	Present – 50,000
Aquatic sediments	Bhadury et al. (2006)	Molecular detection of marine nematodes from environmental samples: overcoming eukaryotic interference	Marine and estuarine sediments	Nematodes	England	Present
	Fonseca et al. (2010)	Second-generation environmental sequencing unmasks marine metazoan biodiversity	Marine beach sediments	Diverse metazoans	Scotland	Present?
	Pawlowski et al. (2011)	Eukaryotic richness in the abyss: insights from pyrotag sequencing	Marine sediments	Diverse eucaryotes	Weddell Sea; Arctic Ocean	Unknown?
	Chariton et al. (2010)	Ecological assessment of estuarine sediments by pyrosequencing eukaryotic ribosomal DNA	Estuarine sediments	Diverse eucaryotes	Sydney, Australia	Present
	Anderson-Carpenter et al. (2011)	Ancient DNA from lake sediments: bridging the gap between paleoecology and genetics	Lake sediment	Deciduous trees	Michigan; Wisconsin, USA	Up to ca. 3500
	Matisoo-Smith et al. (2008)	Recovery of DNA and pollen from NZ lake sediments	Lake sediment	Fish (probably gobiomorphus cotidianus)	New Zealand	AD 217–247
	Pedersen et al. (2013)	A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa	Lake sediment	Plants	South Greenland	Present – 10,500
	Parducci et al. (2012)	Glacial survival of boreal trees in Northern Scandinavia	Lake sediment	Trees (pine and spruce)	Norway	6500–22,000
	Parducci et al. (2013)	Molecular- and pollen-based vegetation analysis in lake sediments from central Scandinavia	Lake sediment	Plants	Norway; Sweden	900–10,400
	Giguet-Covex et al. (2014)	Long livestock farming history and human landscape shaping revealed by lake sediment DNA	Lake sediment	Domestic mammals, plants	France	Present – 5000
Ice	Willerslev et al. (1999)	Diversity of Holocene life forms in fossil glacier ice	Holocene ice cores	Plants, fungi, algae, protists	Northern Greenland	2000–4 000
	Willerslev et al. (2007)	Ancient biomolecules from deep ice cores reveal a forested Southern Greenland	Pleistocene ice cores	Plants, arthropods	Southern Greenland	Between ca. 450,000–800,000
Soil	O'Brien et al. (2005)	Fungal community analysis by large-scale sequencing of environmental samples	Forest soil	Fungi	North Carolina, USA	Present
	Buee et al. (2009)	454 Pyrosequencing analyses of forest soils reveal an	Forest soil	Fungi	France	Present

(*continued on next page*)

Table 1 (*continued*)

Source	References	Title	Sample habitat	Taxons studied	Geographical location	Aproximate ages (years BP)
		unexpectedly high fungal diversity				
	Yoccoz et al. (2012)	DNA from soil mirrors plant taxonomic and growth form diversity	Boreal, temperate and tropical soil	Plants	Norway; France; French Guiana	Present
	Taberlet et al. (2012)	Soil sampling and isolation of extracellular DNA from large amount of starting material suitable for metabarcoding studies	Grassland soil	Plants	France	Present
	Bienert et al. (2012)	Tracking earthworm communities from soil DNA	Soil	Earthworms	France	Present
	Andersen et al. (2012)	Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity	Soil from animal enclosuers	Mammals	Denmark	Present
Freshwater	Martellini et al. (2005)	Use of eukaryotic mitochondrial DNA to differentiate human, bovine, porcine and ovine sources in fecally contaminated surface water	Wastewater, rivers	Human, cow, pig, sheep	Quebec, Canada	Present
	Ficetola et al. (2008)	Species detection using environmental DNA from water samples	Ponds, artificial containers	Amphibians (american bull frog)	France	Present
	Jerde et al. (2011)	"Sight-unseen" detection of rare aquatic species using environmental DNA	Rivers	Fish	Illinois, USA	Present
	Goldberg et al. (2011)	Molecular detection of vertebrates in stream water: a demonstration using rocky mountain tailed frogs and IDAHO giant salamanders	Streams	Amphibians	Idaho, USA	Present
	Dejean et al. (2011)	Persistence of environmental DNA in freshwater ecosystems	Artificial ponds and containers	Amphibians, fish	France	Present
	Thomsen et al. (2012)	Monitoring endangered freshwater biodiversity using environmental DNA	Ponds, lakes, streams, artificial containers	Amphibians, fish, mammals, insects, crustaceans	Northern Europe	Present
	Takahara et al. (2012)	Estimation of fish biomass using environmental DNA	Lagoon, artificial ponds and containers	Fish	Japan	Present
	Minamoto et al. (2011)	Surveillance of fish species composition using environmental DNA	Rivers, dammed pool, artificial containers	Fish	Japan	Present
	Dejean et al. (2012)	Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*	Ponds	Amphibians (american bull frog)	France	Present
	Pilliod et al. (2013)	Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples	Streams	Amphibians	Idaho, USA	Present
	Wilcox et al. (2013)	Robust detection of rare species using environmental DNA: the importance of primer specificity	Streams	Fish (brook trout)	Montana, USA	Present
	Olson et al. (2013)	An eDNA approach to detect eastern hellbenders (*Cryptobranchus a. alleganiensis*) using samples of water	Rivers	Amphibians (eastern hellbender)	Indiana; Missouri, USA	Present
	Santas et al. (2013)	Noninvasive method for a statewide survey of Eastern Hellbenders *Cryptobranchus alleganiensis* using environmental DNA	Rivers, streams, creeks	Amphibians (eastern hellbender)	Ohio; Kentucky, USA	Present
	Goldberg et al. (2013)	Environmental DNA as a new method for early detection of New Zealand mudsnails (*Potamopyrgus antipodarum*)	River, artificial containers	Mollusk (new zealand mudsnail)	Idaho, USA	Present
	Takahara et al. (2013)	Using environmental DNA to estimate the distribution of an invasive fish species in ponds	Ponds	Fish (bluegill sunfish)	Japan	Present
	Mahon et al. (2013)	Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments	Rivers	Fish	Illinois, USA	Present
	Jerde et al. (2013)	Detection of Asian carp DNA as part of a Great Lakes basin-wide surveillance program	Lakes, rivers	Fish	Illinois, USA	Present
	Egan et al. (2013)	Rapid invasive species detection by combining environmental DNA with light transmission spectroscopy	Lakes	Mollusk (zebra mussel)	Michigan, USA	Present
	Piaggio et al. (2014)	Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA	Wetlands, artificial containers	Reptiles (burmese python)	Florida, USA	Present

	Barnes et al. (2014)	Environmental conditions influence eDNA persistence in aquatic systems	Artificial containers	Fish (common carp)	Indiana, USA	Present
	Turner et al. (2014)	Particle size distribution and optimal capture of aqueous macrobial eDNA	Lakes, artificial containers	Fish (common carp)	Indiana, USA	Present
	Deiner and Altermatt (2014)	Transport distance of invertebrate environmental DNA in a natural river.	River	Crustacean, mussel	Switzerland	Present
	Pilliod et al. (2014)	Factors influencing detection of eDNA from a stream-dwelling amphibian	Streams	Amphibian (idaho giant salamander)	Idaho, USA	Present
	Tréguier et al. (2014)	Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds	Ponds	Crustacean (red swamp crayfish)	France	Present
	Jane et al. (2014)	Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams	Streams	Fish (brook trout)	Massachusetts, USA	Present
	Biggs et al. (2015)	Using eDNA to develop a national citizen science-based monitoring programme for the Great Crested Newt (Triturus cristatus)	Ponds	Amphibians (great crested newt)	England, Wales, Scotland	Present
	Deiner et al. (2015)	Choice of capture and extraction methods affect detection of freshwater biodiversity from environmental DNA	Lakes, rivers	Crustaceans, insects, mussels, general eucaryotes	Switzerland	Present
	Klymus et al. (2015)	Quantification of eDNA shedding rates from invasive bighead carp Hypophthalmichthys nobilis and silver carp Hypophthalmichthys molitrix	Artificial containers	Fish (carps)	Missouri, USA	Present
	McKee et al. (2015)	Evaluation of three simple treatments for the removal of quantitative PCR inhibition in environmental DNA samples	Wetlands	Sample spiked with target amphibian dna	Florida; Georgia; S. Carolina, USA	Present
	Sigsgaard et al. (2015)	Monitoring the near-extinct European weather loach in Denmark based on environmental DNA from water samples	Streams	Fish (weather loach)	Denmark	Present
	Strickler et al. (2015)	Quantifying effects of UV-B, temperature, and pH on eDNA degradation in aquatic microcosms	Artificial containers	Amphibians (american bull frog)	Idaho, USA	Present
	Takahara et al. (2015)	Effects of sample processing on the detection rate of environmental DNA from the Common Carp (Cyprinus carpio)	Ponds	Fish (common carp)	Japan	Present
	Turner et al. (2015)	Fish environmental DNA is more concentrated in aquatic sediments than surface water	Ponds, rivers	Fish (bigheaded carp)	Missouri; Indiana; Kansas, USA	Present
Seawater	Thomsen et al. (2012)	Detection of a diverse marine fish fauna using environmental DNA from seawater samples	Coastal water	Fish	Denmark	Present
	Foote et al. (2012)	Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals	Ocean, marine enclosure	Whales	Denmark	Present
	Kelly et al. (2014)	Using environmental DNA to census marine fishes in a large mesocosm	Sea-tank mesocosm	Fish	California, USA	Present

revealing DNA from extinct and extant mammals, birds and plants (Willerslev et al., 2003), followed by dry cave sediments, revealing DNA from past cave-dwelling mammals and birds (Hofreiter et al., 2003). Later, it was shown that also DNA from invertebrates such as insects (Epp et al., 2012; Thomsen et al., 2009) as well as fungi (Lydolph et al., 2005) is also preserved in ancient sediments. This so-called 'sedimentary ancient DNA' (*sed*aDNA or *dirt*DNA) has been shown to be of local origin, and organisms must be physically present at the site for their DNA to be deposited (Andersen et al., 2012; Haile et al., 2009; Lydolph et al., 2005; Yoccoz et al., 2012). DNA leaching through strata has not been observed in ancient frozen sediments (Hansen et al., 2006; Willerslev et al., 2007, 2004) or recently (<1000 years old) frozen sediments (Hebsgaard et al., 2009), but may occur between layers in non-frozen depositional settings (Andersen et al., 2012; Haile et al., 2007). Sedimentary eDNA has been speculated to derive from faeces, urine, epidermal cells and hair, which is based on the presence of DNA from coprophilic and keratinophilic fungi found in ancient sediments (Lydolph et al., 2005). The effectiveness of NGS techniques to reconstruct paleo-ecosystems from permafrost samples has been demonstrated to reveal rich diversity in past flora (Sønstebø et al., 2010) and study recent environmental change in Greenland (Jørgensen et al., 2012b). However, some results indicate that physical remains such as pollen and macrofossils are complementary rather than overlapping with *sed*aDNA, and in combination, reveal more detailed information on ancient plant communities than can be achieved by each individual approach (Jørgensen et al., 2012a; Parducci et al., 2013).

2.1.2. Aquatic sediments

Aquatic sediments (freshwater and marine) have also proven to be rich in eDNA. In fact, the extracellular DNA in marine sediments is considered by far the largest reservoir of DNA in the oceans (Dell'Anno and Danovaro, 2005), and especially anoxic conditions reduce nuclease degradation and favours long-term preservation of eDNA (Corinaldesi et al., 2011). For example, Bhadury et al. (2006) studied eDNA from nematodes, as an indicator of the meiofauna, in marine and estuarine sediments. Also, eDNA from estuarine sediments have been used to compare eukaryotic species assemblages to assess human impacts on these ecosystems (Chariton et al., 2010). Later and more comprehensive analyses of eDNA from marine benthic (Fonseca et al., 2010) and deep-sea sediments (Pawlowski et al., 2011) have deployed NGS, revealing differences in metazoan diversity at microgeographical scales and DNA from all major groups of eukaryotes and most marine metazoan groups, respectively.

Matisoo-Smith et al. (2008) was, as far as we know, the first study to show that freshwater lake sediment, traditionally used for pollen records, also contain macro-organismal eDNA – in this case from fish. Later Anderson-Carpenter et al. (2011) successfully amplified plant DNA from up to 4600 years old freshwater lake sediment samples, and found that the results matched the taxonomic identity of the macrofossil in the respective samples. Since the DNA preservation is good under anoxic conditions, studies on eDNA from aquatic sediments, such as the above, holds potential for a better understanding of the ecological and evolutionary consequences of environmental change (Giguet-Covex et al., 2014; Parducci et al., 2012). Similar to results from terrestrial sediments, eDNA from lake sediments also complements analyses of pollen and macrofossils, validating the combination of traditional and molecular techniques to obtain a more comprehensive picture of biodiversity (Parducci et al., 2013; Pedersen et al., 2013).

2.1.3. Ice cores

Willerslev et al. (1999) showed for the first time that eukaryotic eDNA, representing a diverse assemblage of both plants, fungi, algae and protists, of both local and distant origin, could be obtained from Holocene ice cores in northern Greenland. Later, eDNA from much older, basal ice cores provided insights into a past ecosystem of plants and arthropods, indicative of a forested southern Greenland 450–800 thousand years ago – almost 2 million years younger than previously presumed (Willerslev et al., 2007).

2.2. Modern environments

2.2.1. Surface soil

While studies on soil eDNA began on ancient deposits (sediments), there have been several recent applications to modern ecosystems. Total soil DNA includes both intra- and extracellular DNA, the latter of which probably being the most significant part (Levy-Booth et al., 2007; Pietramellara et al., 2008). Metabarcoding of eDNA from surface soil has thus efficiently been used as a proxy for plant taxonomic diversity in several different terrestrial ecosystems (Taberlet et al., 2012c; Yoccoz et al., 2012). The applicability of soil eDNA has also been widely demonstrated for fungi (reviewed by Anderson and Cairney, 2004; Chase and Fay, 2009), for example by using DNA metabarcoding from various forest soils, revealing rich and diverse fungal communities (Buee et al., 2009; O'Brien et al., 2005). As for animal eDNA in modern soil samples, Bienert et al. (2012) demonstrated that earthworm communities – an important indicator group of ecosystem functioning and health – can be identified using next-generation sequencing, whereas Andersen et al. (2012) found that DNA from the soil surface reflects overall taxonomic richness and relative biomass of individual vertebrate species.

2.2.2. Freshwater

One of the first studies retrieving macro-organismal eDNA from freshwater, focused on DNA from human, cow, pig and sheep, as a method to detect the sources in faecally contaminated surface water (Martellini et al., 2005). Later, eDNA from American bullfrogs, an invasive amphibian species native to North America, was successfully retrieved from natural pond water samples in France (Ficetola et al., 2008). This study initiated a rapidly growing interest in aquatic ecosystems, which have generating numerous studies with direct applications to conservation (e.g. see this journal special issue). Many subsequent studies continued to focus on detection of invasive species. Dejean et al. (2012) demonstrated that eDNA can deliver improved detection of invasive species over traditional methods, using the original model species – American bullfrog. Jerde et al. (2011) studied two species of Asian carps in river systems, which demonstrated that the forefronts of the species were closer to invasion of upstream lake systems than initially seen by traditional surveillance methods. This approach were later expanded significantly (Jerde et al., 2013) and extended to other related fish species in a study that confirmed presence of eDNA also for low-abundance species in river systems (Mahon et al., 2013). Takahara et al. (2013) used eDNA to estimate presence of the invasive bluegill sunfish on Japanese mainlands and surrounding islands. Here eDNA also proved superior to visual detections. Recently, the eDNA approach has been expanded to invasive reptiles (Piaggio et al., 2014), snails (Goldberg et al., 2013) and crustaceans (Tréguier et al., 2014).

Another line of freshwater eDNA reseach concerns endangered species. Here, most studies have focused on detection of amphibians (Goldberg et al., 2011; Olson et al., 2013; Pilliod et al., 2013; Santas et al., 2013; Thomsen et al., 2012b) and even quantification (Pilliod et al., 2013; Thomsen et al., 2012b), which provides a potential new range of opportunities for estimating relative abundance. Quantification of fish biomass from fresh water samples has also been attempted (Takahara et al., 2012), as well as species composition and communities (Minamoto et al., 2011; Thomsen et al.,

2012b). Furthermore, it has been demonstrated that also endangered freshwater insects, crustaceans, fish and mammals can be monitored using eDNA, and that such an approach can account for entire lake faunas of amphibians and fish using high-throughput sequencing (Thomsen et al., 2012b).

Although Turner et al. (2014) recently found that aqueous macrobial eDNA likely exists predominantly inside mitochondria or small cells, the decay of eDNA in freshwater beyond the threshold of detectability has been demonstrated to happen at a scale of days or weeks (Dejean et al., 2011; Pilliod et al., 2014; Thomsen et al., 2012b). This contrasts situations in soil, where eDNA is proven to persist for decades (and perhaps centuries) after deposition (Andersen et al., 2012; Yoccoz et al., 2012). The rapid degradation time in freshwater ecosystems makes eDNA very useful in conservation, since a positive detection is likely to be associated with contemporary presence of species and populations while potentially misleading signals from past populations are not picked up. Nevertheless, long distance transport of eDNA from hundreds of meters to several kilometres have been reported in river systems, and should be taken into account for flowing waters (Deiner and Altermatt, 2014; Jane et al., 2014).

2.2.3. Seawater

It has been widely demonstrated that microbial (prokaryotic and eukaryotic) biodiversity can be studied by sequencing DNA from filtered seawater samples (Rusch et al., 2007; Sogin et al., 2006; Venter et al., 2004; Zinger et al., 2011). Recently however, Thomsen et al. (2012a) showed that it is possible to detect a rich marine fish fauna by metabarcoding of eDNA from seawater samples, and that such an approach can cover the fish diversity better than or equal to any of 9 methods conventionally used in marine fish surveys. The approach has been validated in a large sea-tank mesocosm, where also rank abundance of recovered eDNA correlated with abundance of corresponding species' biomass (Kelly et al., 2014a). Furthermore, Foote et al. (2012) showed that eDNA from whales can also be detected from seawater samples, and an experimental setup in sea-enclosures indicated the eDNA signal is evident only within short distances (metres) of animals. These are the first evidence that marine water samples contain detectable eDNA from macro-organisms. An experimental setup has shown that even small (100-bp) eDNA fragments in seawater degrades beyond detectability within days (Thomsen et al., 2012a). DNA degradation in seawater has previously been suggested to be substantially faster with an empirical turnover rate as low as 10 h (Dell'Anno and Corinaldesi, 2004). These studies indicate low probability of long-distance dispersal of eDNA in marine ecosystems. Although further studies are needed to validate the eDNA approach in contemporary marine environments, initial studies show promising perspectives for future monitoring and management of marine biodiversity and resources (Kelly et al., 2014b).

3. Ancient environments and extinctions

Ancient eDNA represents a challenge to obtain and verify given that it often has undergone significant degradation (Hansen et al., 2006; Willerslev and Cooper, 2005). Nevertheless, analyzing DNA in an evolutionary timeframe holds an important potential to understand changes in ecosystems and extinctions. This was recently evident by a study of Lorenzen et al. (2011), which analyzed the population history over the past 50,000 years using mtDNA from six big-bodied mammals (megafauna). Coupling this information with climate niche modelling and the archaeological records it became evident that each species reacted individualistically to climate changes and that it is basically impossible to predict which species would go extinct or survive over evolutionary time scales. Although the current study uses mtDNA from ancient remains and does not employ eDNA as such, it demonstrates that DNA can extend the time frame of population demography and help reveal the uncertainties associated with extinction predictions, providing relevant considerations for current species conservation attempts.

Many extinction models are based on predictions as to when the extinction happened (Nogués-Bravo et al., 2008). Again, such estimates rely on dating of macrofossils like teeth and bones. However, the chance of finding some of the youngest individuals in a dwindling population may be hard or impossible and scientists are very unlikely to come across "the last" macrofossil left behind. In this case eDNA from shed skin cells, urine or faces, which is likely to be present and distributed over a larger area, is a valid supplement to establish last appearance dates of species. This was attempted by Haile et al. (2009) in Stevens Village, Alaska, which is among the places where most bones of woolly mammoth have been dated. Dating of these bones have previously suggested an extinction date for woolly mammoth on mainland Alaska some 13,100 years BP. However, the eDNA results suggest survival of mammoth until some 10,500 years BP – ca. 3500 years after first human arrival into mainland Alaska and thus that man and beast co-existed several thousand years prior to extinction of the latter.

In a recent study by Willerslev et al. (2014) more than 200 permafrost samples from 21 sites across the Arctic covering the past 50,000 years were analyzed for plant chloroplast eDNA. Contrary to many pollen studies, the results reveal that the coldest and driest stages during the last ice age (the Last Glacial Maximum, LGM) around 20 thousand years ago was a major bottleneck in plant diversity and that the warmer periods before this bottleneck was richer and more diverse in plant composition than the Holocene period following the LGM, where many new taxa became dominant. As such the study suggests that not only climatic changes but also the recent vegetation history determines how ecosystems develop over time.

4. Pitfalls and challenges for eDNA

Despite obvious perspectives and useful applications using eDNA, several problematic issues associated with the approach needs to be considered. For a more thorough treatment of these issues see Coissac et al. (2012) and Pedersen et al. (in press).

4.1. Contamination

The most serious pitfall of eDNA is probably the risk of contamination and hence the possibility of false positive results. Contamination of samples can occur from taking the samples in the field to every step of analyses in the laboratory. If several localities are sampled after one another in the field, there is a risk of cross-contamination: target DNA carried unintentionally from one locality to another. Lab contamination is especially serious because of the frequent use of PCR in eDNA studies, generating billions of DNA copies, which can readily spread throughout the lab. The use of NGS technologies has further complicated the contamination issue, as they produce a very high throughput of DNA sequences likely to reveal tiny amounts of lab-source PCR products. Cross-contamination in the lab seems almost unavoidable, and it is essential to apply conservative cut-offs for minimum percentages of sequences obtained in a sample, and/or the amplification success in independent PCR reactions, before including a recovered taxon as authentic. A strict clean-lab protocol using decontamination procedures and physical separation of labs for pre- and post-PCR work will significantly limit the contamination

risks (Champlot et al., 2010; Willerslev and Cooper, 2005). Inclusion of DNA extraction blanks and PCR blanks, as well as field blanks, to monitor contamination is essential.

4.2. Inhibition

Humic acids or humic substances, co-extracted with DNA in environmental samples, strongly inhibit enzymes such as the *Taq* Polymerase used in PCR reactions to amplify DNA (Matheson et al., 2010). This obviously represents a bias in eDNA studies, which is probably most severe in soil samples, but also occurs in water samples contaminated with sediment particles (McKee et al., 2015; Sigsgaard et al., 2015). If not addressed sufficiently in each study, inhibition can lead to generation of false negative results. Both false positive and false negative results can have consequences for downstream conservation effort leading to over- or under-estimation of a species' occurrence, respectively.

4.3. Errors

Erroneous DNA sequences will, just as contamination, give rise to biased results. Errors can occur either before sampling in long-term preserved DNA (Hansen et al., 2006; Willerslev and Cooper, 2005), during PCR or during sequencing. PCR-generated errors include point mutations and formation of chimeric molecules (Acinas et al., 2005). However, most errors are probably generated during sequencing, and this has shown to overestimate actual microbial diversity by two orders of magnitude (Kunin et al., 2010). For animals, biodiversity signals are also obscured by PCR and/or sequencing errors (Sefc et al., 2007). Therefore, raw sequence data must be carefully filtered to limit false positives and to generate a reliable taxon list (Fig. 1). There is obviously a trade-off between conservative error trimming, and retaining as much information on diversity in the samples as possible (Coissac et al., 2012).

4.4. Reference DNA databases

Identification of DNA sequences derived from environmental samples depends crucially on reliable reference DNA-sequence databases. These are skewed in geographical and taxonomic overage (Kvist, 2013), but global initiatives addressing this need have been established, and databases are rapidly growing (www.ncbi.nlm.nih.gov/genbank, www.boldsystems.org). Accordingly, there are specific initiatives to provide DNA barcodes of all the world's fish (www.fishbol.org), mammal (www.mammaliabol.org) and bird species, (www.barcodingbirds.org) *etc*. It is clear, though, that the remaining gap in knowledge will for some time impair the usefulness of eDNA monitoring at lower taxonomic levels, where all species have not yet been sequenced. However, the highly frequent COI sequences in these databases are sub-optimal for eDNA metabarcoding (Deagle et al., 2014). We thus suggest, given the massive increase in DNA sequencing cost-efficiency, that future DNA reference databases focus on complete mitochondrial or even nuclear genomes for much wider applications than traditional DNA barcoding.

4.5. Single species detection vs. DNA metabarcoding

Broadly, two different approaches of species detection from eDNA have been deployed: single species detection by PCR or quantitative PCR (qPCR) and multi-species detection (eDNA metabarcoding) by NGS. The former is advantageous where the target is one or a few known species, for which species-specific primers and probes can be developed (Dejean et al., 2012; Takahara et al., 2013; Thomsen et al., 2012b). The approach has high specificity, sensitivity and quantification ability, but is hampered by the limit to detect only one target organism at a time. For more diverse systems, this approach quickly becomes cost-inefficient and even impossible due to lack of DNA extract for multiple reactions. Additionally, many issues remains for reliable quantification of biomass/individuals by eDNA using qPCR. These include Cycle threshold (Ct) cut-off values and percentage of positive replicates for defining true positives from background, as well as proper treatment of negative qPCR replicates (Ellison et al., 2006; Bustin et al., 2009). A standardized number of qPCR replicates is also important to be used consistently throughout a study. Finally, development of more sophisticated modelling for describing DNA production and degradation should be undertaken. The above issues remains to be properly addressed in qPCR based eDNA studies including those from our own group (Thomsen et al., 2012a, 2012b). Here, we did not define strict Ct cut-off values or include non-detect (no Ct) replicates as zero in the calculations of average DNA concentrations, which has been shown to increase accuracy and should be the practice (Ellison et al., 2006). Nevertheless, negative replicates are frequent in eDNA studies where DNA concentration is often low and the sample is a complex mixture of non-target DNA and environmental particles probably influencing oligo efficiency. Thus, template amount is a trade-off between avoiding inhibition and retaining a detectable concentration of target molecules. Fx. Biggs et al. (2015) finds eDNA detection in 1/12 replicates for some samples. Accordingly, some of the standard detection and quantification thresholds defined in the literature might have to be relaxed for eDNA studies. If high Ct values are included, sequencing of the product for verification is crucial (Thomsen et al., 2012a, 2012b).

On the other hand, the DNA metabarcoding approach is powerful and cost-efficient (Thomsen et al., 2012a, 2012b; Yoccoz et al., 2012) and the technology for mass DNA sequencing continues to improve (Schadt et al., 2010; Shokralla et al., 2012). The main drawback, when using generic primers for metabarcoding, is primer affinity bias leading to certain sequences (species) amplifying less efficiently than others (Deagle et al., 2014). This will in turn limit the results to species with best primer affinity or to species, which is already known to be locally present so specific-primers can be designed (Riaz et al., 2011; Wilcox et al., 2013). However, such limitations will continuously become less crucial due to optimization and publication of generic primers for metabarcoding studies.

High-throughput sequencing has also added another level of complexity to the analyses of results, and the massive amount of sequences needs to be filtered in a conservative way to remove low abundant sequence reads that might originate from errors or chimeric sequences. Such *denoising* is crucial to discriminate authentic molecular diversity from errors and hence to give accurate estimation of the biodiversity represented in the samples and a number of programs are available for sequence data e.g. Amplicon Noise (Quince et al., 2011). A range of tools has been developed to aid processing of NGS data, such as OBITools (www.grenoble.prabi.fr/trac/OBITools) and QIIME (Caporaso et al., 2010). The translation of DNA sequence diversity obtained from metabarcoding of e.g. an environmental sample into actual "species" richness and diversity is not straightforward. First, retrieved sequences are clustered in MOTUs representing supposedly discrete taxons, however this delimitation is sometimes based on arbitrary criteria (Coissac et al., 2012), and is unlikely to be standardized across taxa. When taxonomic coverage of the DNA database is low or non-existent, sequences will remain clustered into MOTUs. The task of retrieving actual species names (important units of conservation) from eDNA metabarcoding results are more challenging due to: (i) limited knowledge of inter- and intra-specific sequence diversity of the targeted DNA-fragment and considerable gaps in DNA sequence databases (Kvist, 2013), (ii) limited genetic variation

of target genes of some taxa (Waugh, 2007) and (iii) the limitation of eDNA to short DNA sequences with poor taxonomic resolution. Nevertheless, similar constraints are also associated with species identification using morphological characters. Progress are being made to standardize and improve the clustering and taxonomic identification of sequences from metabarcoding studies (Jones et al., 2011; Puillandre et al., 2012; Zhang et al., 2013). However, for obtaining sheer estimates of diversity and richness based on sequence diversity, DNA metabarcoding can essentially be independent of DNA databases and clustering sequences into MOTUs can provide estimates comparable between different localities or at different conditions within a locality.

See further discussion on bioinformatic methods and challenges in Coissac et al. (2012).

4.6. Interpretation of results

Another important issue in eDNA studies concerns the critical interpretation of final results. Here, important caveats associated with eDNA detection compared to traditional surveys includes the failure to distinguish living vs. dead organisms, particular life stages (eggs, juveniles, adults) and hybrid species. The latter is linked to the dominant use of mitochondrial markers. Despite advantages of being useful as DNA barcodes and plentiful in excreted cells, mtDNA can only detect the maternal lineage of a hybrid species. Finally, eDNA, like any other monitoring approach, will only detect a proportion of the total sites occupied by a given species. Using site occupancy models, Schmidt et al. (2013) demonstrated the importance of using rigorous analyses of presence/absence data to obtain more reliable estimates on species occupancy based on eDNA. Occupancy models can also be used to calculate the number of eDNA samples needed to reach a cumulative detection chance of fx 95% (Schmidt et al., 2013).

4.7. What is the temporal and spatial scale?

For eDNA to be applicable in monitoring and conservation of contemporary biodiversity, it is crucial that results reflect the present state of an ecosystem. While studies of eDNA from water samples have demonstrated rapid degradation times suggesting that results are fairly consistent in space and time (Dejean et al., 2011; Thomsen et al., 2012a, 2012b), eDNA in soil seems to be able to persist for decades or centuries (Andersen et al., 2012; Yoccoz et al., 2012). This obviously questions the use of eDNA in soil from terrestrial ecosystems in a conservation context and necessitate further studies. However, the potential release of "*ancient*" eDNA from bottom sediments to the water column (Turner et al. 2015) might also complicate the use of aquatic eDNA as strict contemporary biodiversity surveys. An extreme and highly intriguing example of the borderline between "ancient" and "contemporary" eDNA was recently demonstrated (Overballe-Petersen et al., 2013). Here the authors show experimentally that bacteria can take up ancient and damaged mammoth DNA down to just 20 bp by natural transformation. As a consequence, the diverse pool of DNA in the environment could potentially have shaped the evolution of bacteria by incorporation of this DNA into their genomes through the history of life on Earth. Furthermore, such transformed eDNA fragments could even be re-deposited in the environment following cell-death, which could make eDNA fragments continuously available thousands or millions of years after they were first shed from its original source.

Likewise, the spatial scale of eDNA monitoring is essential to consider for any inferences on the proximity of target organisms compared to their DNA traces left behind. Transport of target eDNA from another locality by e.g. predators or man-made structures represents a potential, yet often insignificant possibility (Jerde et al., 2013). Transport of eDNA within an ecosystems remains an issue especially in flowing waters (Deiner and Altermatt, 2014; Jane et al., 2014; Pilliod et al., 2013) and marine environments (Thomsen et al., 2012a), where long-distance transport is possible.

4.8. Skepticism

The fate of eDNA-based monitoring of biodiversity relies heavily on the validity of results from the scientists and other organizations applying the approach. Skepticism about the general approach from conservation planners will appear, if false results from scientists or companies are unintentionally published. Here, there is a lesson to be learned from ancient DNA research, where enthusiasm in the initial research phase generated spectacular results that were later disproved. These include claims of DNA sequences surviving for millions of years in dinosaur bones (Woodward et al., 1994) or amber inclusions (Cano et al., 1992). However, it is the nature of any new research field to make mistakes, which for ancient DNA meant the adoption of rigorous lab procedures to avoid contamination and high standards for authentication of results. From this history a plea for strict general protocols and authentication of results in conservation-related eDNA studies emerges. Similarly, the implementation of eDNA in conservation management remains a challenge and is subject of debate (Darling and Mahon, 2011).

4.9. Future directions?

Moving from fundamental research to an applied tool in conservation, several aspects need to be more thoroughly investigated before eDNA approaches can be fully matured and integrated into conservation. In this regard we suggest that future research on both terrestrial and aquatic ecosystems should focus on:

(i) The temporal and spatial distribution of eDNA in different habitats, which gives information as to what part of the biodiversity is monitored in space and time.
(ii) More precise links between eDNA concentration and species abundance whether this is measured as total biomass or density of individuals.
(iii) The exact sources of eDNA, whether this comes from sloughed epithelial cells, intestinal cells, faeces and urine, *etc.*, which might vary with life stages of the target organism and could therefore greatly influence abundance estimates. Klymus et al. (2015) shows that an important source of aquatic eDNA might be faeces as eDNA shedding rates increase with feeding.
(iv) Physio-chemical factors influencing eDNA availability and degradation such as temperature, pH and salinity. Barnes et al. (2014) showed that a high degree of biological activity, measured as chlorophyll concentration, biological oxygen demand (BOD) and total eDNA, decreased the degradation rate of eDNA, perhaps due to shielding of eDNA from UV radiation by algae. Furthermore, Strickler et al. (2015) explored the effect of various abiotic factors (temperature, UV-radiation and pH) on eDNA degradation, which indicated that high DNA degradation rates were associated with conditions favourable for microbial growth.

Finally, we find it intriguing to explore the possibility of obtaining much longer fragments than the usually targeted 100–200 bp, target nuclear genes and test the eDNA metabarcoding approach more thoroughly in truly high-diverse ecosystems such as the tropics. Such advances would lead the way for important conservation measures like species-level identification and population genetics using environmental samples.

5. Perspectives of eDNA

Obtaining accurate data on species distributions and extinctions is by no means trivial. Establishing when a species is extinct will inevitably be an assumption based on the last remains of a species. Bones, hair, tissue and other visible fossil remains left behind will only represent a fraction of a given species' history on Earth. DNA left in the environment represents the invisible traces from past species still left for scientists to analyze. eDNA is likely to be much more ubiquitous in the environment than the macrofossils not yet destroyed beyond recognition due to millennia of weather and mechanical degradation, simply because of the sheer amount of cells in an organism or its remains left in the environment through time. As such, eDNA is easier to sample in a stratified and comparable manner across ancient ecosystems in order to establishing more accurate last appearance dates of extinct species. Likewise, information on contemporary species distributions, including those thought to be recently extinct can be difficult to obtain. Here, eDNA can be used to efficiently monitor invasive, cryptic, endangered or presumably extinct species of conservation concern. Analyses of ancient environments using eDNA does not speak directly to present-day conservation issues, but understanding how species become extinct in the past provides important information of the unpredictability often associated with population fluctuations and the role of humans in these processes (Haile et al., 2009; Lorenzen et al., 2011) and it demonstrates how eDNA can be an important (or the only) source to provide this information (Willerslev et al., 2014). Acknowledging this history provides a more robust platform for applying eDNA in conservation.

Keeping in mind the limitations and methodology challenges mentioned in Section 4, eDNA approaches does offer some great advantages over traditional methods in biodiversity monitoring. Here we highlight the following:

(i) *Standardization*: Even though there are still methodological optimization to be done (Deiner et al., 2015; Takahara et al., 2015), obtaining an environmental sample can be carried out in a very standardized manner across localities in a given type of habitat. This is more difficult using traditional methods where, in general, results depend on the taxonomic knowledge and experience by personnel carrying out the surveys.

(ii) *Non-invasiveness*: eDNA is a truly non-invasive method that inflicts no damage on the species or habitats under study.

(iii) *Sensitivity*: In situations where species of conservation importance have cryptic life styles or require the study of juvenile life stages that are difficult to identify from closely related species, even high-quality taxonomic expertise are often inadequate. Here eDNA methods can prove superior to traditional methods in detection of species (e.g. Dejean et al., 2012; Biggs et al., 2015).

(iv) *Cost-effectiveness*: It would be wrong to state generally that eDNA is more cost-efficient than traditional methods as this depends on the target species (Herder et al., 2014). Nevertheless, several studies report shorter handling time and lower cost using eDNA compared to traditional monitoring techniques (Jerde et al., 2011; Biggs et al., 2015; Sigsgaard et al., 2015). As the price of sequencing per base-pair continues to decline exponentially, eDNA will in many cases undoubtedly become superior to some traditional methods, especially when using a metabarcoding approach.

(v) *Independence of weather conditions*: For several species (e.g. amphibians), traditional surveys are difficult outside particular seasons or certain weather conditions where vocal activity of adults are peaking. However, eDNA may remain in their habitat also outside of these high-activity periods (e.g. from juveniles), extending the time for monitoring. For other species (e.g. fish), harsh weather conditions can impede the usage of traditional fishing equipment but not eDNA sampling.

The advance in DNA sequencing technologies has significantly expanded the possibilities of using eDNA and is expected to continue improving in the future. Although the first studies using eDNA relied on cloning and subsequent Sanger sequencing of PCR products and many still do, there is no doubt that the new emerging sequencing technologies will have profound impact on eDNA studies (Shokralla et al., 2012), and become a fully integrated part of ecologists' toolbox (Baird and Hajibabaei, 2012; Taberlet et al., 2012b; Valentini et al., 2009b). Furthermore, new generations of powerful technologies such as novel real-time sequencing techniques e.g. PacBio RS by Pacific Bioscience® or Nanopore-based sequencing by Oxford Nanopore Technologies®, carbon nanotube chips (Mahon et al., 2011) and real-time laser transmission spectroscopy (Egan et al., 2013; Li et al., 2011), are awaiting full trial of their promising potential in eDNA approaches. It is thus expected that the use of eDNA in conservation and biological monitoring will move from single-marker analyses of species or communities to meta-genomic surveys of entire ecosystems for predicting spatial and temporary biodiversity patterns (Davies et al., 2012; Kelly et al., 2014b).

We wish to emphasize that eDNA approaches will complement rather than replace traditional monitoring. This is evident from the literature on sediments, where macrofossils, pollen and classical surveys on the flora complements sedaDNA (Parducci et al., 2013; Pedersen et al., 2013), and inferior detection probabilities of eDNA for some freshwater taxa (Thomsen et al., 2012b). Furthermore, the final process of eDNA analyses (the one that can hardly be standardized) is the interpretation of the results, and here the necessity of well-trained taxonomists and ecologists to meaningfully interpret results and recommend subsequent actions cannot be stressed enough.

Ultimately, we are looking to the most comprehensive way of utilizing eDNA for the benefit of our planet and all of its inhabitants. Environmental DNA will merely be a means to monitor biodiversity and provide fast and efficient insights on the distribution of species, estimation of abundance and ultimately perhaps population sizes, which all provides the basis of taking appropriate conservation actions. As such, it will never directly combat the biodiversity crisis, which remains a more complicated issue requiring especially political will, commitment and action.

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