How will the 'molecular revolution' contribute to biological recording?

LORI LAWSON HANDLEY*

Evolutionary Biology Group, School of Biological, Biomedical and Environmental Sciences, University of Hull, Cottingham Road, Hull HU6 7RX, UK

Soaring throughput, plummeting costs, and increased sensitivity for assaying degraded or low-concentration DNA are driving a revolution in the way that we monitor biodiversity. Arguably the biggest 'game-changer' is environmental DNA (eDNA), which refers to free-floating DNA released by organisms into their environment. Rare or elusive species can be detected with greater sensitivity and accuracy using eDNA than by most conventional methods, and we have the capability to screen and describe whole communities, as well as perform targeted monitoring of single species. This paper discusses the basic approaches for molecular monitoring of biodiversity, provides case studies to demonstrate the effectiveness of the techniques, and considers any challenges and limitations that could impact molecular biological recording. It is argued that eDNA surveys offer exciting new opportunities to engage the public in biological recording and that molecular approaches will complement conventional surveys, enabling unprecedented insights into species distributions. Finally, with the number of eDNA studies increasing at a rapid pace, it is argued that there is a need to rapidly establish ways of managing molecular records. Integrating molecular records into existing biological records databases would enhance our understanding of species distributions and may be something that the Biological Records Centre should be considering to mark its landmark anniversary. © 2015 The Linnean Society of London, *Biological Journal of the Linnean Society*, 2015, **115**, 750–766.

ADDITIONAL KEYWORDS: biodiversity monitoring – citizen science – DNA barcoding – eDNA – environmental DNA – invasive species – metabarcoding – molecular food webs – trophic interactions.

Fifty years ago, the Biological Records Centre (BRC) was at the pioneering heart of a revolution in the way we record biodiversity (Pocock *et al*., 2015). Today, we are undergoing a revolution in the way we describe biodiversity. In recent years, we have progressed from being able to identify individual organisms from specific DNA sequences or 'barcodes' to analyzing hundreds of thousands of DNA barcodes from environmental samples, enabling us to describe whole communities. This high throughput approach, known as 'DNA metabarcoding', is particularly powerful for revealing cryptic biodiversity (Creer *et al*., 2010; Bik *et al*., 2012; Yu *et al*., 2012).

In addition to this increase in throughput, another revolution is ongoing in the field of environmental DNA or 'eDNA'. Organisms release DNA into their environments, for example through faeces, moulting, mucous secretion or releasing gametes. The sensitivity of DNA-based assays means that it is possible to detect tiny amounts of degraded eDNA present in the environment. This has great promise for biodiversity monitoring because it is non-invasive, and it has already been proven effective for monitoring rare and/or elusive species, particularly in freshwater environments. Indeed, eDNA monitoring in aquatic systems was identified as one of the 15 most important global conservation issues in a 2013 horizon scanning exercise (Sutherland *et al*., 2013) and may be a 'game changer' in biodiversity monitoring. The aim of the present review is to evaluate how these molecular approaches are likely to contribute to biological recording. The review briefly summarizes the main approaches; presents key case studies in eDNA

*E-mail l.lawson-handley@hull.ac.uk

monitoring of biodiversity, the detection of invasive alien species (IAS) and the detection of trophic interactions; demonstrates through an example how the public can be engaged in molecular biological recording; and discusses the burning issues relating specifically to eDNA analyses. Finally, some of the logistics of molecular biological recording are evaluated, such as whether it is cost-effective, and a debate is initiated on how the data should be managed.

THE APPROACH

WHY TAKE A MOLECULAR APPROACH FOR BIOLOGICAL RECORDING?

Between 10% and 50% of species from well-studied taxonomic groups (e.g. mammals, birds, amphibians, higher land plants) are threatened with extinction (Millennium Ecosystem Assessment, 2005) and current rates of species loss could be up to 1000 times higher than background rates (i.e. those before human influence; Millennium Ecosystem Assessment, 2005; Pimm *et al*., 2014). Despite good knowledge of certain taxonomic groups, it is considered that 15% more plant species and the great majority of animals are yet to be described (Pimm *et al*., 2014). We have a poorer understanding of the status of organisms in aquatic compared to terrestrial environments (Millennium Ecosystem Assessment, 2005), although freshwater ecosystems appear to be particularly vulnerable (McLellan *et al*., 2014). Indeed, populations of freshwater species declined by a mean of 76% between 1970 and 2010, which is almost double the rate of decline for populations of terrestrial species (McLellan *et al*., 2014).

Reliable cost-effective methods for large-scale screening of biodiversity are essential if we are to slow this rate of species loss. Traditional methods, based for example, on trapping, netting, and acoustic or observational surveys, can be costly, time consuming, and, sadly are often inefficient. Perhaps the greatest limitations of traditional surveys are (1) the difficulties associated with recording rare and/or elusive species and (2) dealing with taxonomically similar species, juvenile life stages or cryptic biodiversity. Methods based on DNA barcoding are promising with respect to complementing traditional approaches and helping overcome these limitations. This is becoming more feasible because of the rapidly decreasing costs associated with DNA sequencing and technological improvements for assaying degraded or low-concentration DNA. Another benefit of molecular methods is that they are easily auditable because samples can be split and analyzed in independent laboratories (Ji *et al*., 2013). However, DNA-based methods are not without their own limitations. It is

impossible, for example, to infer age structure of populations from DNA data, and there are other challenges, as discussed below.

DNA BARCODING OR METABARCODING?

The technology and application of DNA barcoding and metabarcoding in ecology has been reviewed extensively elsewhere (Valentini, Pompanon & Taberlet, 2009; Creer *et al*., 2010; Taberlet *et al*., 2012; Bohmann *et al*., 2014); therefore, just a brief introduction is provided here. In 'traditional' DNA barcoding, a short gene segment (500–800 bp) is amplified by the polymerase chain reaction (PCR) using widely conserved primers, and sequenced (using Sanger sequencing) from DNA extracted from a single individual. The DNA sequence or 'barcode' should be species-specific, allowing taxonomic identification by comparison with a public DNA data bank such as the International Barcode of Life (iBOL) Consortium's Barcode Library (Barcode of Life Data Systems; http://www.boldsystems.org). The most commonly used DNA barcodes are the mitochondrial cytochrome *c* oxidase subunit I gene (*COI*) for animals (Hebert, Ratnasingham & deWaard, 2003), the chloroplast ribulose biphosphate carboxylase gene (*rbcL*) gene for plants (Hollingsworth & Andra Clark, 2009), and the ribosomal internal transcribed spacer (*ITS*) for fungi (Schoch *et al*., 2012). Mitochondrial or chloroplast genes generally make attractive molecular markers because of their uniparental inheritance, high mutation rates, and the fact that they are found in multiple copies in a cell, coupled with the fact that it is relatively straightforward to design conserved PCR primers. However neither *COI*, nor *rbcL* are perfect. Some taxonomic groups, such as nematodes, are notoriously problematic to resolve using *COI*, and also exhibit extensive rearrangements in their mitochondrial genes (Powers, 2004). Therefore, the locus of choice for nematodes (and other meiofauna) is usually the 18S (small subunit or 'SSU') rRNA gene, which is a nuclear gene present in typically 50–100 copies (Floyd *et al*., 2002; Powers, 2004, although note that this region greatly underestimates diversity in some meiofaunal groups, Tang *et al*., 2012). The use of *COI* for barcoding a broad range of taxa has recently been criticized because the region does not have sufficiently conserved regions for primer design (Deagle *et al*., 2014). Some applications, such as the identification of museum specimens or environmental samples (see below), require shorter fragments for analyses than the standard barcodes because of DNA degradation problems. For this reason, a considerable amount of effort has gone into developing 'mini-barcodes' based on the minimum amount of sequence required for species identification. Mini-barcodes are

typically a 90–250-bp long portion of the primary barcoding genes (e.g. *COI*: *Hajibabaei et al*., 2006; Meusnier *et al*., 2008; *rbcL*: Little, 2014).

Arguably, the great disadvantage of DNA barcoding is that analyzing single organisms using traditional DNA sequencing methods is expensive and inefficient. Rather than focussing on single organisms, DNA metabarcoding characterizes species assemblages either from a homogenized 'soup' of whole organisms (e.g. as obtained from pitfall traps or other mass trapping methods; Yu *et al*., 2012) or from eDNA (see below). DNA from the whole community is PCR amplified using similar markers to those for standard barcoding/mini-barcoding, sequenced on a Next Generation Sequencing ('NGS') platform (e.g. an Illumina HiSeq or MiSeq sequencing system) and analyzed using bioinformatics pipelines. The throughput of next generation sequencers has increased a million fold ever since the turn of the century, whereas costs have plummeted (Glenn, 2011). Metabarcoding is revolutionizing our understanding of the diversity of microscopic eukaryotes (Bik *et al*., 2012) in environments that are traditionally difficult to study, such as soil (Porazinska *et al*., 2009), other sediments (Creer *et al*., 2010), and the deep sea (Fonseca *et al*., 2010). This approach is considered the leading technological advance for biodiversity measurement (Ji *et al*., 2013) and could lead to a shift in the focus of biodiversity monitoring away from reliance on indicator species, which are not always appropriate surrogates for the health of whole communities (Cushman *et al*., 2010).

A crucial question associated with metabarcoding studies is whether the method accurately reflects the true diversity both qualitatively and quantitatively. The validity of metabarcoding has been demonstrated by testing against artificially assembled samples of known composition (Hiiesalu *et al*., 2012; Yu *et al*., 2012) and several studies have demonstrated that metabarcoding generates reliable qualitative estimates of diversity (Fonseca *et al*., 2010; Hiiesalu *et al*., 2012; Yoccoz *et al*., 2012; Yu *et al*., 2012; Ji *et al*., 2013). For example, Yu *et al*. (2012) individually sequenced DNA barcodes from over 1300 insects collected in malaise traps at three sites in China. DNA from the 547 individuals representing different operational taxonomic units (OTUs) was then pooled into several mixtures, analogous to ecological communities with known composition, and mass sequenced by metabarcoding. A total of 598 OTUs were recovered during the bioinformatics steps from > 130 000 DNA sequences. The number of OTUs is greater than the number identified from individual sequencing perhaps because of the ability of metabarcoding to detect prey in predators' gut contents and parasite DNA in hosts (see later below). Encouragingly, there was high correlation between sequencing methods in their estimates of unweighted alpha and beta diversity (i.e. diversity within and between samples respectively, based on presence–absence data rather than abundance), demonstrating that metabarcoding accurately recovers these important indices.

It could be argued that validating metabarcoding based on artificially constructed species assemblages does not guarantee the method will translate to real world situations. This is an important argument if biodiversity assessments based on metabarcoding are to be used to inform policy-making. To address this issue, Ji *et al*. (2013) compared metabarcoding data sets against three large-scale standard biodiversity data sets, comprising over 55 000 morphologically identified indicator specimens, from China, Malaysia and the UK that were collected for answering policy questions. Encouragingly, the data from metabarcoding and standard datasets were highly consistent, returned correlated diversity estimates, and produced the same conclusions for policy-making (Ji *et al*., 2013). Although these studies offer encouragement for the use of metabarcoding alongside standard methods for biodiversity assessment, one of the remaining major challenges is whether the methods are quantitative, as discussed further towards the end of this review.

eDNA

In 2003, a pioneering study obtained eDNA from Pleistocene animal and plant communities in the Siberian permafrost, as well as from more recent (600–3000-year-old) cave and coastal sediments in New Zealand (Willerslev *et al*., 2003). Subsequently, over 1000 papers have been published on eDNA, and the technology has been used to reconstruct both past and present flora and fauna from soil and other sediments (Sønstebø, Gielly & Brysting, 2010; Andersen *et al*., 2012; Jørgensen *et al*., 2012; Yoccoz *et al*., 2012; Pedersen *et al*., 2013), for non-invasive tracking of animals (e.g. from their faeces: Beja-Pereira *et al*., 2009; from footprints in snow: Dalén *et al*., 2007), for detecting genetically modified pollen in air (Folloni *et al*., 2012), and for detecting prey species in predator gut contents (as discussed further below). However, perhaps the greatest potential of eDNA technology is for monitoring current biodiversity in aquatic environments (Table 1) and we only began to realize this potential quite recently. The field took a leap forward in 2008 with the application of eDNA to detection of invasive American bullfrogs, *Rana catesbeiana*, in French wetlands (Ficetola *et al*., 2008). Subsequently, eDNA has been used to detect a number of rare, elusive or invasive species (Table 1), and the approach is shifting from targeting specific species to describing whole communities using

Table 1. Examples of targeted aquatic eDNA case studies by taxa

Taxon	Environment	Objective	Method	References
Amphibians				
Rocky Mountain tailed frogs (*Ascaphus montanus*)	Streams	Monitoring biodiversity	Standard and qPCR	Goldberg *et al.* (2011); Pilliod *et al.* (2013)
Idaho giant salamanders (*Dicamptodon aterrimus*)	Streams	Monitoring biodiversity	Standard and qPCR	Goldberg *et al.* (2011); Pilliod *et al.* (2013)
Spadefoot toad (*Pelobates fuscus*)	Ponds	Monitoring biodiversity	qPCR	Thomsen *et al.* (2012b)
American bullfrog, (*Rana catesbeiana*)	Ponds	Detection of IAS	Standard PCR	Ficetola, Bonin, & Miaud, 2008); Dejean *et al.* (2012)
Great crested newt (*Triturus cristatus*)	Ponds	Monitoring biodiversity	qPCR	Thomsen *et al.* (2012b); Biggs *et al.* (2014)
Fish				
Goldfish (*Carassius auratus*)	Bait shop tanks	Detection of IAS via bait trade pathway	Standard PCR	Nathan *et al.* (2014)
Prussian carp (*Carassius gibelio*)	River basin	Detection of IAS	Standard PCR	Keskin (2014a)
North African catfish (*Clarias gariepinus*)	River basin	Detection of IAS	Standard PCR	Keskin (2014a)
African jewelfish (*Hemichromis letourneuxi*)	Artifical ponds	Assess detection method in controlled lentic system*	qPCR	Moyer *et al.* (2014)
Silver carp (*Hypophthalmichthys molitrix*)	Chicago area waterway (large river and canal complex)	Detection of IAS	Standard PCR	Jerde *et al.* (2011); Mahon *et al.* (2013)
Bighead carp (*Hypophthalmichthys nobilis*)	Chicago area waterway (large river and canal complex)	Detection of IAS	Standard PCR	Jerde *et al.* (2011); Mahon *et al.* (2013)
Bluegill sunfish (*Lepomis macrochirus*)	Ponds	Detection of IAS	qPCR	Takahara *et al.* (2013)
European weather loach (*Misgurnus fossilis*)	Ponds and streams	Monitoring biodiversity	qPCR	Thomsen *et al.* (2012b)
Round goby (*Neogobius melanostomus*)	Bait shop tanks	Detection of IAS via bait trade pathway	Standard PCR	Nathan *et al.* (2014)
Nile tilapia (*Oreochromis niloticus*)	River basin	Detection of IAS	Standard PCR	Keskin (2014a)
Tubenose goby (*Proterorhinus semilunaris*)	Bait shop tanks	Detection of IAS via bait trade pathway	Standard PCR	Nathan *et al.* (2014)
Topmouth gudgeon (*Pseudoasbora parva*)	River basin	Detection of IAS	Standard PCR	Keskin (2014a)
Bull trout (*Salvelinus confluentus*)	Streams	Monitoring of species displaced by *S. fontinalis*, testing assay sensitivity and accuracy	qPCR	Wilcox *et al.* (2013)
Brook trout (*Salvelinus fontinalis*)	Headwater streams	Detection of IAS and testing assay sensitivity and accuracy; investigating DNA dynamics in streams	qPCR	Wilcox *et al.* (2013); Jane *et al.* (2015)

Table 1. *Continued*

Taxon	Environment	Objective	Method	References
Eurasian rudd (*Scardinius erythrophtalmus*)	Bait shop tanks	Detection of IAS via bait trade pathway	Standard PCR	Nathan *et al*. (2014
Reptiles				
Burmese python (*Python bivittatus*)	Large wetlands	Detection of IAS	Standard PCR	Piaggio *et al*. (2014)
Mammals				
Eurasian otter (*Lutra lutra*)	Streams and lakes	Monitoring biodiversity	qPCR	Thomsen *et al*. (2012b)
Harbour porpoise (*Phocena phocena*)	Seawater from a sea pen and open sea	Monitoring biodiversity	qPCR	Foote *et al*. (2012)
Crustaceans				
Water hog-louse (*Asellus aquaticus*)	Rivers and lakes	Detecting indicator species	Standard PCR	Mächler *et al*. (2014)
Northern River Crangonyctid (*Crangonyx pseudogracilis*)	Rivers and lakes	Detection of IAS	Standard PCR	Mächler *et al*. (2014)
Daphnia longispina	Rivers (downstream of lake where species present)	Investigating DNA dynamics in rivers	Standard PCR	Deiner & Altermatt, 2014
Chinese mitten crab, (*Eriocheir sinensis*)	Ships ballast	Detection of IAS	Microfluidic chip	Mahon *et al*. (2011)
River shrimp (*Gammarus pulex*)	River	Detecting indicator species	Standard PCR	Mächler *et al*. (2014)
Tadpole shrimp (*Lepidurus apus*)	Temporary pools	Monitoring biodiversity	qPCR	Thomsen *et al*. (2012b)
Red swamp crayfish (*Procambarus clarkii*)	Ponds	Detection of IAS	qPCR	Tréguier *et al*. (2014)
Molluscs				
Ancylus fluviatilis	Rivers and lakes	Detecting indicator species	Standard PCR	Mächler *et al*. (2014)
Zebra mussel (*Dreissena polymorpha*)	Lakes and rivers	Detection of IAS	Standard PCR	Lance & Carr (2012)
Quagga mussel, (*Dreissena rostriformis bugensis*)	Ships ballast; lakes and rivers	Detection of IAS	Microfluidic chip; standard PCR	Mahon *et al*. (2011); Lance & Carr 2012)
Golden mussel, (*Limnoperna fortuneii*)	Ships ballast	Detection of IAS	Microfluidic chip	Mahon *et al*. (2011)
Unio tumidus	Rivers (downstream of lake where species present)	Investigating DNA dynamics in rivers	Standard PCR	Deiner & Altermatt (2014)
Echinoderms				
Northern Pacific seastar (*Asterias amurensis*)	Mixed plankton or ballast	Detection of IAS	Standard PCR	Deagle *et al*. (2003)
Insects				
Scarce olive riverfly (*Baetis buceratus*)	Rivers	Monitoring biodiversity: vulnerable	Standard PCR	Mächler *et al*. (2014)
White-faced darter (*Leucorrhinia pectoralis*)	Ponds	Monitoring biodiversity	qPCR	Thomsen *et al*. (2012b)
Tinodes waeneri	Rivers and lakes	Detecting indicator species		Mächler *et al*. (2014)

Table 1. *Continued*

Taxon	Environment	Objective	Method	References
Fungal pathogens				
Crayfish plague (*Aphanomyces astaci*)	Large lakes	Pathogen detection	qPCR	Strand *et al*. (2014)
Batrachochytrium dendrobatidis (amphibian fungal pathogen)	Pond water and sediments	Pathogen detection	qPCR	Kirshtein *et al*. (2007); Hyman & Collins (2012)

Species are ordered alphabetically by scientific name, within taxon group. *Note *H. letourneuxi* is an invasive alien species (IAS) in southern Florida. qPCR, quantitative polymerase chain reaction.
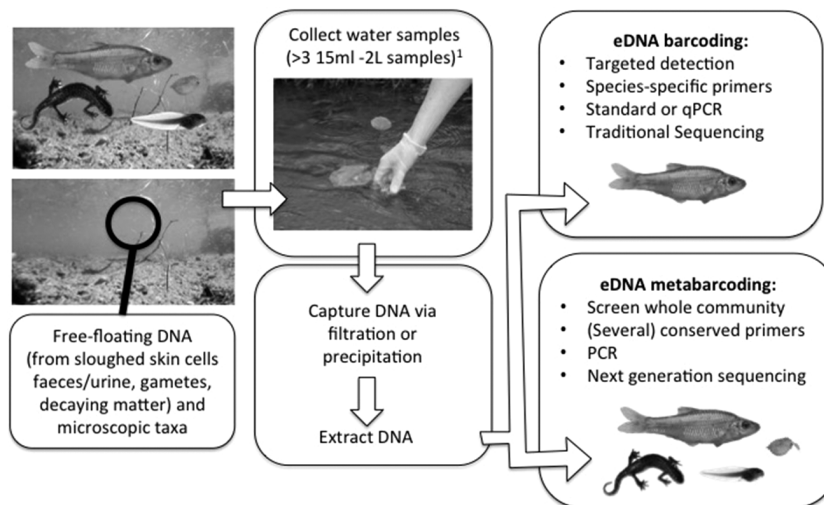

Figure 1. A basic overview of the steps taken in environmental DNA (eDNA) barcoding or metabarcoding studies. [1]Replicate samples should always be taken. General guidelines advise a minimum of three samples from a small pond, although this should be adapted to the specific environment under study. The number of samples required to obtain a high detection probability can be estimated using site occupancy modelling, as discussed in the text. Numerous protocols exist for DNA capture and extraction. Advice is provided in Rees *et al*. (2014). qPCR, quantitative polymerase chain reaction.

metabarcoding. Government agencies across the world are realizing the potential of eDNA to contribute towards biodiversity monitoring and early detection of invasive species, and are investing in the approach. However, we still have many challenges ahead before eDNA data can be used to inform policy and decision-making.

Perhaps the greatest advantage of this method is that it is non-invasive and very sensitive, with eDNA detection rates generally outperforming conventional survey methods (see below for more details). eDNA can be analyzed in several different ways depending on the environment under study and also on whether assays need to be targeted (i.e. species-specific) or describe whole communities (a basic overview of the approach is provided in Figure 1). Species-specific assays use standard or quantitative PCR (qPCR) with

primers that only amplify the target species. qPCR has the advantages of a much greater sensitivity, such that very low DNA concentrations can be detected and, by definition, the ability to quantify the number of copies of target DNA that are present. This can then be translated into estimates of relative abundance (further discussion is provided towards the end of this review).

Few metabarcoding studies have so far been carried out on eDNA, although this is set to rapidly change because there is huge appeal in describing whole communities from environmental samples. The first studies to blaze the trail for eDNA metabarcoding were carried out by the same group that recovered eDNA from the ancient animal and plant communities, described above. Andersen *et al*. (2012) investigated whether metabarcoding could accurately

recover vertebrate diversity from samples of soil collected from enclosures in safari parks, zoos, and farms. DNA sampled from the soil surface accurately reflected taxonomic richness and, interestingly, the relative biomass of the species present (Andersen *et al.*, 2012). Very few other studies have so far employed eDNA metabarcoding for biodiversity assessment (but see Thomsen *et al.*, 2012a; Kelly *et al.*, 2014), although the approach is being tested extensively at time of writing. The key question is whether this method can be used to generate sufficiently reliable estimates of species abundance as well as presence–absence (as discussed towards the end of this review). If it can, it will revolutionize the recording of biological diversity.

CASE STUDIES IN MOLECULAR BIOLOGICAL RECORDING

DETECTION OF INVASIVE ALIEN SPECIES

With new European Union regulation on invasive alien species introduced in January 2015, there is recognition of a pressing need for more effective early warning systems for invasive species (Schulz & Vedova, 2014; European Commision, 2015; Roy *et al.*, 2015). One of the great promises of eDNA is that it can be used to detect rare species that can easily go unnoticed. This makes it a particularly promising tool as an early warning system for detecting IAS (or indeed for pathogens) before they establish, as well as for monitoring establishment and spread, particularly in aquatic environments (Darling & Mahon, 2011).

As mentioned above, the first application of eDNA for detection of invasive species was on American bullfrogs, *R. catesbeiana* in France (Ficetola *et al.*, 2008). American bullfrogs are native to eastern North America but were introduced worldwide during the 20th Century. They are recognized as one of the world's 100 worst invasive species (DAISIE; http://www.europe-aliens.org; Dejean *et al.*, 2012) and have been linked to the decline of native amphibians via competition, predation, and spread of disease (Ficetola *et al.*, 2008). In France, there are three established populations and two are subject to control methods. An assay was developed based on standard PCR using species-specific primers (Ficetola *et al.*, 2008) and, later, the sensitivity of the method was compared with traditional methods based on auditory nocturnal and visual diurnal encounter rates carried out at the same time as eDNA sampling (Dejean *et al.*, 2012). American bullfrog eDNA was detected in 38 ponds out of 49 sampled compared to only seven sites for the traditional methods, suggesting that their distribution had been previously underestimated using traditional methods (Dejean *et al.*, 2012).

Encouragingly, positive eDNA results were obtained for all seven sites where bullfrogs were detected using traditional methods (Dejean *et al.*, 2012). One potential reason for the discrepancy between methods is the ability of eDNA to detect bullfrogs at very low densities and at any life stage. Subsequent to these pioneering bullfrog studies, species-specific assays have been developed and successfully deployed to detect other invasive species including fish, reptiles, crustaceans, molluscs, and echinoderms, as well as fungal pathogens (Table 1), and the number is rapidly increasing.

It is widely known that prevention is the most effective form of management for IAS (Leung *et al.*, 2002; Hulme, 2009). Perhaps one of the greatest opportunities for eDNA methods is in routine surveillance of invasion pathways aiming to detect IAS before they enter the environment. Invasive species can enter aquatic systems via a number of pathways (Hulme, 2009; Roy *et al.*, 2014). Ship ballast water is a particularly important source, and discharge standards are in place in some countries (e.g. the USA) to prevent IAS release (Frazier *et al.*, 2013). eDNA methods are likely to be particularly useful for identifying larval stages of marine invertebrates in ballast, where morphological identification is unreliable and impractical. Single-species PCR assays have been developed for detection of the northern Pacific seastar, *Asterias amurensis*, in mixed plankton or ballast water samples (Deagle *et al.*, 2003). However assays still typically need to be carried out in a laboratory, which is too time-consuming for effective ballast screening. There is an urgent need for on-board screening of ship's ballast before port entry to provide sufficient time to implement control measures (Mahon *et al.*, 2011). A leap in this technology came in 2011, when a portable microfluidic detection platform was developed for ballast water screening (Mahon *et al.*, 2011). The system combines DNA extraction and standard PCR with a carbon nanotube microfluidic detection chip to identify target species (Mahon *et al.*, 2011). Chip-based assays were successfully designed and tested for three ballast-transported IAS; quagga mussel, *Dreissena rostriformis bugensis*, Chinese mitten crab, *Eriocheir sinensis*, and golden mussel, *Limnoperna fortuneii* (Mahon *et al.*, 2011).

Targeted assays such as these are appropriate for active surveillance of priority species, although they have an obvious drawback: they miss nontarget IAS that may be present in the sample. Passive surveillance of IAS pathways using metabarcoding is therefore of great interest and may provide a substantial benefit for IAS management. The ability of metabarcoding to detect very rare species in complex samples such as mixed plankton or ballast has been

demonstrated by artificial spiking of indicator species with known biomass (Zhan *et al*., 2013). Metabarcoding assays for screening ballast are currently in development and undergoing field trials, although Mahon, Nathan & Jerde (2014) tested the utility of this approach for passive detection of IAS in the bait trade pathway of the North American Great Lakes Basin. Surprisingly, the study detected seven nonbait species in a total of six bait shops. Of greatest concern was the detection and subsequent confirmation of invasive white perch, *Morone americana*, in bait shops in three states across the Great Lakes region (Mahon *et al*., 2014). Because *M. americana* was not expected to be present in the sample, this perfectly illustrates the greater power of metabarcoding for detecting nontarget species.

MONITORING BIODIVERSITY FOR CONSERVATION

One of the great challenges in conservation is to effectively monitor rare and/or elusive species without causing disturbance. eDNA methods are particularly promising for the monitoring of rare or elusive species as a result of their sensitivity and non-invasive nature. Thomsen *et al*. (2012b) evaluated the use of eDNA for targeted monitoring of rare freshwater species from diverse taxonomic groups (amphibians: common spadefoot toad, *Pelobates fuscus*, great crested newt, *Triturus cristatus*; fish: European weather loach, *Misgurnus fossilis*; mammals: Eurasian otter, *Lutra lutra*; crustaceans: tadpole shrimp, *Lepidurus apus*; and insects: large white-faced darter, *Leucorrhinia pectoralis*). eDNA detection rates in natural freshwater ponds, where the species were known to be present, were 100% for *P. fuscus*, *M. fossilis*, and *L. apus*, and above 80% for *T. cristatus* and *L. pectoralis* (Thomsen *et al*., 2012b). Although detection was more difficult in larger freshwater systems, detection rates for the two species investigated (*L. lutra* and *M. fossilis*) were still comparable, and may provide a valuable complement, to traditional methods (Thomsen *et al*., 2012b). A small-scale metabarcoding study, using conserved vertebrate primers and carried out on the same pond water samples, detected all species of fish and amphibians previously recorded from the ponds, as well as several birds and a mammal (Thomsen *et al*., 2012b). This illustrates the potential for the monitoring of entire communities for biodiversity using metabarcoding, potentially shifting the focus away from a small number of indicator species.

Monitoring biodiversity in the marine environment is obviously more challenging as a result of the scales involved. Despite additional challenges to eDNA studies from marine water chemistry and the dilution effects of strong tides and currents, eDNA has already shown promise for monitoring marine mammals (Foote *et al*., 2012) and fish (Thomsen *et al*., 2012a). Foote *et al*., (2012) evaluated whether eDNA from harbour porpoises, *Phocena phocena*, could be detected in a four million litre sea pen holding four porpoises, and in natural field sites in the Baltic Sea. Porpoise eDNA was consistently detected in 15-mL samples from the sea pen, despite daily flushing of the pen by tidal water movements in the harbour basin. However, eDNA detection was less consistent in the natural sites, and was less successful than acoustic surveys. The study did, however, detect a nontarget species; the long-finned pilot whale, *Globicephala melas*, which is rare in the Baltic. One acknowledged limitation of the present study was the small volume of water collected in the natural sites (3 × 50-mL at each acoustic monitoring site), suggesting that much larger volumes of water might be required to adequately sample marine eDNA. More encouragingly, however, a metabarcoding study, carried out in the Sound of Elsinore, Denmark, recovered eDNA from 15 marine fish species from just three 0.5-L samples of seawater (Thomsen *et al*., 2012a). eDNA outperformed eight out of nine conventional survey methods in terms of number of species detected (Thomsen *et al*., 2012a). Common species and one rare vagrant (European pilchard, *Sardina pilchardus*) were detected, demonstrating the potential of eDNA for detecting rare species that are often missed by conventional methods. An extension of the technique for monitoring elusive species is discussed below.

So far, the majority of studies have focussed on monitoring of animals, particularly in aquatic environments. There is no doubt that more effort needs to be put into monitoring of plants in both aquatic and terrestrial environments. In one excellent study, eDNA was extracted from soil taken from meadow and heathland, and plant community diversity was inferred using metabarcoding (Yoccoz *et al*., 2012). eDNA-based diversity estimates were then compared with those from conventional above-ground surveys, and the estimates were highly consistent (Yoccoz *et al*., 2012). The same study also demonstrated that that species diversity can be recovered using eDNA even in more challenging tropical environments.

DETECTING RECORDS WITHIN RECORDS: MOLECULAR DETECTION OF TROPHIC INTERACTIONS

Although 'eDNA' in its strictest sense refers to free DNA in the environment, the term is often used more loosely to include prey DNA in the gut or faeces of predators, plant DNA in the guts of herbivores, parasite DNA in the host (or vice versa), or other interactions that leave behind traces of DNA. In an

imaginative use of the technology, haematophagus leeches were used as a screening tool for monitoring mammalian biodiversity (Schnell *et al.*, 2012). Remarkably, over 80% of leeches sampled in the Central Annamite region of Vietnam tested positive for mammalian DNA, and two of the species detected (Truong Son muntjac, *Muntiacus truongsonensis* and Annamite striped rabbit, *Nesolagus timminsi*) were only recently described and had not been confirmed in the study area despite extensive surveying, including over 2000 nights of camera trapping for *N. timminsi* (Schnell *et al.*, 2012). Surveying elusive species with leeches or other haematophagous species (e.g. mosquitoes and ticks) is inexpensive compared to camera trapping and other conventional methods, and could be promising for mammal biodiversity screening in certain situations. However, as discussed below, the field of 'molecular detection of trophic interactions' extends far beyond single species interactions and biodiversity screening.

Molecular tools have been used to study diet for over 10 years (Symondson, 2002), although interest in the field has soared recently thanks to improvements in technology for high-throughput assaying of degraded DNA, in combination with analyses of ecological networks from thousands of species' interactions (Roy & Lawson Handley, 2012; Clare, 2014; Symondson & Harwood, 2014). Indeed, this emerging field recently motivated a special issue of the journal *Molecular Ecology* (August 2014; Volume 23, Issue 15), and is already providing unprecedented insight into complex ecological interactions, which are almost impossible to study using conventional methods; for an excellent recent review, see Clare (2014). Molecular dietary analyses have already been performed on a range of animals, including mammals (Australian fur seals, *Arctocephalus pusillus*: *D*eagle, Kirkwood & Jarman, 2009; snow leopards, *Panthera uncia*: Shehzad et al., 2012; bison, *Bison bonasus*: Kowalczyk et al., 2011; Asian musk shrew, *Suncus murinus*: Brown *et al.*, 2014a), birds (little penguins, *Eudyptula minor*: Deagle et al., 2010; Cory's shearwaters, *Calonectris diomedea*: Alonso *et al.*, 2014), reptiles (slow worms, *Anguis fragilis*: Brown, Jarman & Symondson, 2012; smooth snake, *Coronella austriaca*: Brown, Ebenezer, & Symondson, 2014b), fish (e.g. predation of endangered Acigöl carp, *Aphanius transgrediens*, by invasive mosquitofish, *Gambusia affinis*, Keskin 2014b), terrestrial invertebrates (centipedes, *Lithobius* spp.: Eitzinger *et al.*, 2014; spiders, Araneae: Orbicularae: Chapman *et al.*, 2013; Welch *et al.*, 2014; ladybird beetles, *Harmonia axyridis*: Brown *et al.*, 2015), and marine invertebrates (rock lobsters, *Jasus edwardsii*; Redd *et al.*, 2014). By far the greatest effort so far has been invested in the molecular investigation of diet in insectivorous bats (Clare *et al.*, 2009, 2011, 2014a; Bohmann *et al.*, 2011; Razgour, Clare & Zeale, 2011; Zeale, Butlin & Barker, 2011; Alberdi *et al.*, 2012; Burgar *et al.*, 2014; Sedlock, Krüger & Clare, 2014; Clare, Symondson & Fenton, 2014b). Conventional methods for studying bat diet are fraught with challenges for obvious reasons: bats are nocturnal, highly generalist (even species considered specialists consume many closely-related prey species; Clare *et al.*, 2011), cryptic, feed on the wing, and are fast, agile flyers (Clare *et al.*, 2009, 2011, 2014a, b; Razgour *et al.*, 2011; Zeale *et al.*, 2011; Burgar *et al.*, 2014; Sedlock *et al.*, 2014). The challenges are exacerbated in bats from biodiversity hotspots because prey items are highly speciose and largely undescribed (Bohmann *et al.*, 2011; Burgar *et al.*, 2014). However, even when prey species are uncharacterized, DNA sequences can still be phylogenetically grouped into 'molecular operational taxonomic units' or 'MOTUs' (Floyd *et al.*, 2002) and used to compare diets within and between species. Key insights from these studies include: incredibly high diversity of prey even outside biodiversity hotspots (e.g. approximately 600 prey species in Canadian little brown bats, *Myotis lucifugus* (Clare *et al.*, 2014a), significant local variability in diet (*M. lucifugus*, Canada; Clare *et al.*, 2014a), strong seasonal, and annual variation in diet (big brown bats, *Eptesicus fuscus*, America; Clare *et al.*, 2014b), and resource partitioning (several species of Jamaican insectivorous bats; Emrich *et al.*, 2014) or niche differentiation (European pond bat, *Myotis dasycneme* and Daubenton's bat, *Myotis daubentonii*; Krüger *et al.*, 2014) in sympatric species.

INVOLVING THE PUBLIC IN MOLECULAR BIOLOGICAL RECORDING

One of the most important aspects of biological recording is engaging the public in nature, science and conservation (August *et al.*, 2015; Pocock *et al.*, 2015). An important question then is how can we do this with molecular recording? Does the technical nature of molecular work mean that it is off limits to amateur volunteers? A recent eDNA study on great crested newts (GCN), *Triturus cristatus*, led by the Freshwater Habitats Trust (FHT) and carried out in the UK, emphatically demonstrated that the answer to this question is 'no' (Biggs *et al.*, 2014; 2015). In the first part of this study, a targeted qPCR assay was developed and intensive surveys carried out on 35 ponds during the GCN breeding season, aiming to compare eDNA detection rates with traditional survey methods. Remarkably, detection rates were 99.3% for eDNA, which is substantially higher than for bottle trapping (76%), torch counting (75%) or egg

searching (44%), although they were similar to torch counting and bottle trapping combined (95%; Biggs *et al.*, 2014). Second, 80 volunteers were recruited to survey 239 ponds across England, where GCN had been recorded breeding in the previous year. An additional 30 sites were surveyed, first from ponds within the GCN range but with no prior record of newts, and, second, outside the GCN range, aiming to check for false positives. Volunteers were provided with written instructions and standardized sampling kits (including sterile gloves, a pipette, plastic bag, sampling ladle and six sterile 50-mL tubes containing absolute ethanol for sample preservation) and asked to collect 30-mL water samples at twenty locations around the pond margin, without entering the water. Volunteers then combined samples from a single pond in a plastic bag, homogenized the sample, transferred six 15-mL subsamples to individual 50-mL tubes containing ethanol, and posted the samples to FHT headquarters in Oxford, UK. Standard Habitat Suitability Index score sheets were also completed by volunteers at the same time as sample collection. GCN eDNA was detected in 91.2% of sites, indicating a small number of false negatives (8.8%) but, encouragingly, there was no evidence of false positives from the samples taken either from ponds within the range but without newts, or outside the GCN range. Twenty-six sites were also resurveyed by a professional to assess variability among surveyors. The same eDNA results were obtained in all but two of the volunteer and professional surveys, and a very low eDNA concentration was responsible for the discrepancy between surveys at these two sites (Biggs *et al.*, 2014).

These encouraging results demonstrate that large-scale collection of eDNA samples by volunteers is both feasible and effective and provides benefit to both the research and volunteer communities. The GCN study demonstrates the enormous contribution of volunteers to research because large-scale surveys over short time periods (e.g. coinciding with an organism's breeding season) would be far more logistically challenging with only a small research team. From the volunteer's perspective, monitoring of GCNs and other protected species is off limits to many potential recorders because specialist licenses are needed. With eDNA surveys, volunteers can become involved without the need for licenses. However a drawback is that collecting water samples is less rewarding than counting real organisms. As with all methods of biological recording, professional volunteer support and prompt feedback of results is essential to ensure that volunteers feel justifiably valued for their contributions. As discussed below, a combination of both conventional and eDNA methods is likely to prove the most powerful approach for biological recording, and engaging the public in both types of survey would be the ultimate outcome.

CHALLENGES, LIMITATIONS AND IMPORTANT CONSIDERATIONS ASSOCIATED WITH MOLECULAR BIOLOGICAL RECORDING

QUALITY CONTROL AND VALIDATION

Validation is always an important step in biological recording. Observational records from the general public are typically validated by expert verification of photographs that are submitted with the record to online recording schemes. eDNA methods require very high-quality control standards and validation, particularly when the technology is applied for IAS detection and biodiversity monitoring for management purposes, because there can be serious financial implications of false positives (Darling & Mahon, 2011; Bohmann *et al.*, 2014; Rees *et al.*, 2014). It is therefore critical to adopt standardized quality control measures such as inclusion of sample and PCR blanks, and to carry out replication of sampling and PCR (for more advice on these issues, see Bohmann *et al.*, 2014; Ficetola *et al.*, 2014; Rees *et al.*, 2014). For legally sensitive work, it could be necessary to adopt similar procedures to those used for ancient DNA, which requires specialized containment facilities and rigorous sterile techniques to avoid contamination (Yu *et al.*, 2012). Of course, this will also require specialist training and increase costs.

DYNAMICS OF eDNA IN THE ENVIRONMENT

A particularly important question is whether the presence of eDNA equates to the presence of living organisms. To answer this, we need to understand the dynamics of eDNA, which determines its detectability, in different environments. Detectability of eDNA is governed by how much DNA is released into the environment (a product of the amount of DNA shed by individuals, density of animals, and their residence time in the environment; Pilliod *et al.*, 2014) and how long eDNA persists in the environment (a product of the rate of DNA degradation – as influenced by environmental chemistry, microbial composition, ultraviolet exposure, and temperature – and DNA transport – as influenced by water flow rates, currents and wave action etc.; Barnes *et al.*, 2014). Little hard data exists with respect to the DNA shedding rates of organisms (for an exception, see Pilliod *et al.*, 2013), although knowledge of DNA persistence in different environments is increasing. The consensus from several studies is that rate of DNA degradation in water varies widely between different environments, although DNA rarely persists for more than two weeks (Barnes *et al.*, 2014; Rees *et al.*, 2014). For example, in freshwater mesocosm experiments, the amphibian DNA concentration rapidly declined until

it could no longer be detected 2 weeks after removal of animals (Thomsen *et al*., 2012b). This suggests the rapid degradation of eDNA in freshwater, even in benign, controlled conditions (Dejean *et al*., 2011; Thomsen *et al*., 2012b). In an experiment aiming to investigate DNA degradation in seawater, Thomsen *et al*., (2012a) found that DNA fragments degrade beyond detectability within 0.9–6.7 days. This high rate of DNA degradation provides confidence that a positive result reflects real occurrence. The interpretation of results from soil and other sediments is more complicated because eDNA can persist over long time scales (Willerslev *et al*., 2003) and the data therefore reflect both current and previous biota. For example, an analysis of plant eDNA in temperate, modern agricultural sites demonstrated that crops cultivated 40–50 years ago could still be detected (Yoccoz *et al*., 2012). Interestingly, the same study also found a positive relationship between the number of DNA sequences in the soil and the years since crop abandonment (Yoccoz *et al*., 2012), indicating that eDNA is degraded at a steady rate. This information is helpful for modelling the dynamics of eDNA in sediments and for accurately interpreting results.

A second concern, which is often raised, is how applicable eDNA methods are for surveying in lotic environments or large water bodies, including the sea. eDNA surveys have already successfully been carried out on several stream or river-living species using eDNA (Table 1) and it is clear that eDNA methods are more challenging in fast-flowing rivers, large lakes and the marine environment than in ponds and small streams. However, this is also true for conventional surveying methods. As with conventional methods, the probability of detection will vary with species, organism density, stream size, flow rate, and season (Goldberg *et al*., 2011). A key issue with eDNA studies in flowing water, however, is how far DNA can be transported before it is degraded beyond levels of detection. Few studies have yet investigated the actual dynamics of eDNA in flowing water, and this is definitely an area that warrants further research. In one study, transport of eDNA from two lake-dwelling invertebrates, *Daphnia longispina* and *Unio tumidus*, was detected in a river up to approximately 12 and 9 km, respectively, downstream of the lake (Deiner & Altermatt, 2014). This indicates that eDNA can persist over relatively large distances in river systems, and that there may be species-specific transport distances for eDNA (Deiner & Altermatt, 2014).

The studies noted in the present review have demonstrated that eDNA surveys perform well, even in challenging environments, although the dynamics of eDNA in different environments are complex. It is clear from this that researchers need to carefully consider the ecology, behaviour, and, to an extent, the physiology, of their target organisms, as well as the flow dynamics and chemistry of their environment to fully understand the results of eDNA surveys.

ARE THE METHODS QUANTITATIVE?

Perhaps the most important question currently occupying the eDNA community, particularly those keen on metabarcoding approaches, is whether the methods can go beyond describing presence–absence and reliably be used for estimating abundance or biomass (Bohmann *et al*., 2014; Rees *et al*., 2014). Organism abundance and/or biomass is critical information for those who need to monitor and manage biodiversity or commercially exploited stocks. Obtaining quantitative estimates from eDNA is challenging because of the large number of factors that influence DNA dynamics in the environment, as discussed earlier above. Several approaches for estimating abundance have been explored including: measuring DNA concentration using qPCR (Takahara *et al*., 2012; Thomsen *et al*., 2012b; Pilliod *et al*., 2013) or digital droplet PCR (ddPCR; Nathan *et al*., 2014), counting the number of sequences per OTU in metabarcoding (Yu *et al*., 2012; Kelly *et al*., 2014), avoiding PCR (Zhou *et al*., 2013), and site occupancy modelling (Pilliod *et al*., 2013; Schmidt *et al*., 2013).

Encouragingly, methods based on qPCR have consistently demonstrated a positive (but nonlinear) relationship between animal density or biomass and eDNA concentration (Takahara *et al*., 2012; Thomsen *et al*., 2012b; Pilliod *et al*., 2013). The drawback of this method, however, is that qPCR is time consuming and costly because it focuses on one target species at a time. ddPCR is a relatively new method, which also focuses on target species but could potentially provide a much more cost-effective, although equally accurate, means of estimating eDNA concentration (Nathan *et al*., 2014). ddPCR essentially involves random partitioning of target DNA into several thousand individual droplets, which are then amplified by PCR. Random sampling means that droplets can contain target or background DNA or both. The fraction of positive droplets is then used to calculate the concentration of target DNA (Nathan *et al*., 2014).

In metabarcoding studies, in principle, the number of sequences per OTU could be taken as an estimator of species biomass. Unfortunately, it appears that this relationship is not a simple one. In the study by (Yu *et al*., 2012), the method employed accurately recovered unweighted alpha and beta diversity indices, although the number of sequence reads did not correlate with abundance. More recently, a metabarcoding experiment was carried out at Monterey Bay Aquarium in a large (4.5 million litre)

tank with known species composition, to evaluate whether species biomass could be accurately recovered (Kelly *et al.*, 2014). Although the rank abundances of eDNA and biomass were perfectly correlated, a complex, nonlinear relationship was found between the proportion of eDNA sequences recovered and the proportion of biomass in the tank (Kelly *et al.*, 2014). Taken together, these studies indicate that relating the number of sequences per OTU to biomass is complex, perhaps because there are several opportunities for bias during the sampling, extraction, PCR, and bioinformatics stages (Yu *et al.*, 2012). Further work is needed to investigate the efficacy of metabarcoding for recovering abundance/ biomass estimates in natural or semi-natural environments with known species composition. One possibility could be to use metabarcoding for describing species composition and qPCR or ddPCR for estimating abundance/biomass of species within the sample. However, a combined approach would obviously be more costly and time consuming. Another prospect, which is generating considerable excitement, is that of PCR-free metabarcoding. PCR can introduce taxonomic biases, as well as false negatives and positives, which can strongly influence abundance estimates (Zhou *et al.*, 2013). In the PCR-free approach, whole communities are sequenced after mitochondrial enrichment, and the individual barcodes are then retrieved during the bioinformatics steps (Zhou *et al.*, 2013). Although the method needs to be refined, one encouraging study demonstrated a correlation between the number of sequences and total biomass in bulk arthropod samples, suggesting this could provide a solution for estimating abundance (Zhou *et al.*, 2013).

Finally, statistical modelling offers another opportunity to improve quantitative estimates. Estimating the abundance of animal populations via conventional methods is notoriously difficult unless individuals can be individually identified. One route around this problem is to estimate site occupancy, namely the proportion of an area occupied by a species (MacKenzie *et al.*, 2002). Although this is obviously different from species abundance, the two measures are positively correlated (MacKenzie & Nichols, 2004), and so site occupancy can be considered as a proxy for abundance. Several recent studies have advocated the use of site occupancy modelling (SOM) for eDNA surveys (Pilliod *et al.*, 2013; Schmidt *et al.*, 2013; Ficetola *et al.*, 2014). SOMs account for imperfect detection (an inherent feature of eDNA studies) and can be used to obtain more reliable estimates of species prevalence, to estimate detection probability, and to determine the number of samples and site visits needed to obtain a high probability of detection (Kéry & Schmidt, 2008; Pilliod *et al.*, 2013; Schmidt

et al., 2013; Ficetola *et al.*, 2014), as well as to test for the effects of site characteristics, such as animal density, on detection probability (MacKenzie *et al.*, 2002; Kéry & Schmidt, 2008; Pilliod *et al.*, 2013; Schmidt *et al.*, 2013; Ficetola *et al.*, 2014). Incorporating site occupancy modelling as routine into eDNA surveys could therefore lead to major improvements in the method.

IS IT COST EFFECTIVE?

The costs associated with molecular analyses are plummeting but how do eDNA surveys compare with conventional methods? On the whole, they appear to be cheaper and less time consuming, with a higher catch per unit effort than traditional methods (Jerde *et al.*, 2011; Dejean *et al.*, 2012). For example, it was estimated that eDNA surveys for American bullfrogs were 2.5-fold cheaper and 2.5-fold less time consuming than traditional surveys (Dejean *et al.*, 2012). For great crested newts, the estimated savings are even greater, with eDNA surveys potentially costing six- to ten-fold less than torch counting and bottle trapping (Biggs *et al.*, 2014). Nevertheless, the cost of undertaking eDNA surveys is far from trivial, especially when a large number of replicates are required for validation purposes.

WILL IT REPLACE TRADITIONAL METHODS?

A key take home message from the present review is that eDNA sampling should never be undertaken naively. It is just as important to understand the ecology of the study organism and its environment as it is with conventional surveying. eDNA surveys should be designed on a case-by-case basis with the target organisms and environment in mind. eDNA methods cannot determine the age class of organisms, which is an important component of biodiversity monitoring and therefore there are clear advantages in combining both methods. The most effective approach will be to use them in combination.

CONCLUSION: THE NEXT PHASE IN THE LIFE OF THE BRC?

To conclude, hopefully, the present review has demonstrated that the molecular revolution will contribute to biological recording, complementing traditional surveys and offering new and exciting opportunities for engaging the public in citizen science. A final important consideration is how we should manage the data generated from molecular surveys. Should molecular data be integrated into current biological records databases or maintained separately? Although the International Barcode of Life Data

Systems portal (http://www.boldsystems.org/) has over 3.5 million georeferenced DNA sequences, its purpose is quite distinct from biological records databases and it was never intended as a tool for mapping species distributions. The Atlas of Living Australia (ALA; http://www.ala.org.au/), which is the Australian node of the Global Biodiversity Information Facility (GBIF; http://www.gbif.org/), is an incredible resource that incorporates biological records, mapping functions, taxonomic information, and species descriptions (even including vocalizations of certain animals). The ALA portal also links with the GenBank search engine for retrieving DNA sequences from individual species. However, this is still quite different from treating the molecular data as a biological record. To my knowledge, this has yet to be attempted. Integrating molecular records into existing national biological records databases would increase our understanding of species distributions and facilitate direct comparisons of the methods. This will not be a trivial enterprise but is perhaps something that the Biological Records Centre (and their equivalent organizations outside the UK) should be pioneering to take biological recording into its next phase.

ACKNOWLEDGEMENTS

REFERENCES

Alberdi A, Garin I, Aizpurua O, Aihartza J. 2012. The foraging ecology of the mountain long-eared bat *Plecotus macrobullaris* revealed with DNA mini-barcodes. *PloS ONE* **7:** e35692.

Alonso H, Granadeiro JP, Waap S, Xavier J, Symondson WOC, Ramos JA, Catry P. 2014. An holistic ecological analysis of the diet of Cory's shearwaters using prey morphological characters and DNA barcoding. *Molecular Ecology* **23:** 3719–3733.

Andersen K, Bird KL, Rasmussen M, Haile J. 2012. Meta-barcoding of 'dirt' DNA from soil reflects vertebrate biodiversity. *Molecular Ecology* **21:** 1966–1979.

August T, Harvey M, Lightfoot P, Kilbey D, Papadopoulos T, Jepson P. 2015. Emerging technologies for biological recording. *Biological Journal of the Linnean Society* **115:** 731–749.

Barnes MA, Turner CR, Jerde CL, Renshaw MA, Chadderton WL, Lodge DM. 2014. Environmental conditions influence eDNA persistence in aquatic systems. *Environmental Science & Technology* **48:** 1819–1827.

Beja-Pereira A, Oliveira R, Alves PC, Schwartz MK, Luikart G. 2009. Advancing ecological understandings through technological transformations in noninvasive genetics. *Molecular Ecology Resources* **9:** 1279–1301.

Biggs J, Ewald N, Valentini A, Gaboriaud C, Dejean T, Griffiths RA, Foster J, Wilkinson JW, Arnell A, Brotherton P, Williams P, Dunn F. 2015. Using eDNA to develop a national citizen science-based monitoring programme for the great crested newt (*Triturus cristatus*). *Biological Conservation* **183:** 19–28.

Biggs J, Ewald N, Valentini A, Gaboriaud C, Griffiths RA, Foster J, Wilkinson J, Arnett A, Williams FAD. 2014. *Analytical and methodological development for improved surveillance of the great crested newt.* Defra Project WC1067. Freshwater Habitats Trust: Oxford.

Bik HM, Porazinska DL, Creer S, Caporaso JG, Knight R, Thomas WK. 2012. Sequencing our way towards understanding global eukaryotic biodiversity. *Trends in Ecology & Evolution* **27:** 233–243.

Bohmann K, Evans A, Gilbert MTP, Carvalho GR, Creer S, Knapp M, Yu DW, de Bruyn M. 2014. Environmental DNA for wildlife biology and biodiversity monitoring. *Trends in Ecology & Evolution* **29:** 358–367.

Bohmann K, Monadjem A, Lehmkuhl Noer C, Rasmussen M, Zeale MRK, Clare E, Jones G, Willerslev E, Gilbert MTP. 2011. Molecular diet analysis of two african free-tailed bats (Molossidae) using high throughput sequencing. *PloS ONE* **6:** e21441.

Brown DS, Burger R, Cole N, Vencatasamy D, Clare EL, Montazam A, Symondson WOC. 2014a. Dietary competition between the alien Asian musk shrew (*Suncus murinus*) and a re-introduced population of Telfair's skink (*Leiolopisma telfairii*). *Molecular Ecology* **23:** 3695–3705.

Brown DS, Ebenezer KL, Symondson WOC. 2014b. Molecular analysis of the diets of snakes: changes in prey exploitation during development of the rare smooth snake *Coronella austriaca*. *Molecular Ecology* **23:** 3734–3743.

Brown DS, Jarman SN, Symondson WOC. 2012. Pyrosequencing of prey DNA in reptile faeces: analysis of earthworm consumption by slow worms. *Molecular Ecology Resources* **12:** 259–266.

Brown PMJ, Ingels B, Wheatley A, Rhule EL, de Clercq P, van Leeuwen T, Thomas A. 2015. Intraguild predation by *Harmonia axyridis* (Coleoptera: Coccinellidae) on native insects in Europe: molecular detection from field samples. *Entomological Science* **18:** 130–133.

Burgar JM, Murray DC, Craig MD, Haile J, Houston J, Stokes V, Bunce M. 2014. Who's for dinner? High-throughput sequencing reveals bat dietary differentiation in a biodiversity hotspot where prey taxonomy is largely undescribed. *Molecular Ecology* **23:** 3605–3617.

Chapman EG, Schmidt JM, Welch KD, Harwood JD. 2013. Molecular evidence for dietary selectivity and pest suppression potential in an epigeal spider community in winter wheat. *Biological Control: Theory and Applications in Pest Management* **65:** 72–86.

Clare EL. 2014. Molecular detection of trophic interactions: emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications* **7:** 1144–1157.

Clare EL, Barber BR, Sweeney BW, Hebert PDN, Fenton MB. 2011. Eating local: influences of habitat on the diet of little brown bats (*Myotis lucifugus*). *Molecular Ecology* **20:** 1772–1780.

Clare EL, Fraser EE, Braid HE, Fenton MB, Hebert PDN. 2009. Species on the menu of a generalist predator, the eastern red bat (*Lasiurus borealis*): using a molecular approach to detect arthropod prey. *Molecular Ecology* **18:** 2532–2542.

Clare EL, Symondson WOC, Broders H, Fabianek F, Fraser EE, MacKenzie A, Boughen A, Hamilton R, Willis CKR, Martinez-Nuñez F, Menzies AK, Norquay KJ, Brigham M, Poissant J, Rintoul J, Barclay RM, Reimer JP. 2014a. The diet of *Myotis lucifugus* across Canada: assessing foraging quality and diet variability. *Molecular Ecology* **23:** 3618–3632.

Clare EL, Symondson WOC, Fenton MB. 2014b. An inordinate fondness for beetles? Variation in seasonal dietary preferences of night-roosting big brown bats (*Eptesicus fuscus*). *Molecular Ecology* **23:** 3633–3647.

Creer S, Fonseca VG, Porazinska DL, Giblin-Davis RM, Sung W, Power DM, Packer M, Carvalho GR, Blaxter ML, Lambshead PJD, Thomas WK. 2010. Ultrasequencing of the meiofaunal biosphere: practice, pitfalls and promises. *Molecular Ecology* **19:** Suppl 1: 4–20.

Cushman SA, McKelvey KS, Noon BR, McGarigal K. 2010. Use of abundance of one species as a surrogate for abundance of others. *Conservation Biology* **24:** 830–840.

Dalén L, Götherström A, Meijer T, Shapiro B. 2007. Recovery of DNA from footprints in the snow. *Canadian Field-Naturalist* **121:** 321–324.

Darling JA, Mahon AR. 2011. From molecules to management: adopting DNA-based methods for monitoring biological invasions in aquatic environments. *Environmental Research* **111:** 978–988.

Deagle BE, Bax N, Hewitt CL, Patil JG. 2003. Development and evaluation of a PCR-based test for detection of *Asterias* (Echinodermata: Asteroidea) larvae in Australian plankton samples from ballast water. *Marine and Freshwater Research* **54:** 709–719.

Deagle BE, Chiaradia A, McInnes J, Jarman SN. 2010. Pyrosequencing faecal DNA to determine diet of little penguins: is what goes in what comes out? *Conservation Genetics* **11:** 2039–2048.

Deagle BE, Jarman SN, Coissac E, Pompanon F, Taberlet P. 2014. DNA metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biology Letters* **10:** pii: 20140562.

Deagle BE, Kirkwood R, Jarman SN. 2009. Analysis of Australian fur seal diet by pyrosequencing prey DNA in faeces. *Molecular Ecology* **18:** 2022–2038.

Deiner K, Altermatt F. 2014. Transport distance of invertebrate environmental DNA in a natural river. *PloS ONE* **9:** e88786.

Dejean T, Valentini A, Duparc A, Pellier-Cuit S, Pompanon F, Taberlet P, Miaud C. 2011. Persistence of environmental DNA in freshwater ecosystems. *PloS ONE* **6:** e23398.

Dejean T, Valentini A, Miquel C, Taberlet P, Bellemain E, Miaud C. 2012. Improved detection of an alien invasive species through environmental DNA barcoding: the example of the American bullfrog *Lithobates catesbeianus*. *Journal of Applied Ecology* **49:** 953–959.

Eitzinger B, Unger EM, Traugott M, Scheu S. 2014. Effects of prey quality and predator body size on prey DNA detection success in a centipede predator. *Molecular Ecology* **23:** 3767–3776.

Emrich MA, Clare EL, Symondson WOC, Koenig SE, Fenton MB. 2014. Resource partitioning by insectivorous bats in Jamaica. *Molecular Ecology* **23:** 3648–3656.

European Commission. 2015. Invasive alien species. Available at: http://ec.europa.eu/environment/nature/invasivealien/index_en.htm

Ficetola GF, Bonin A, Miaud C. 2008. Population genetics reveals origin and number of founders in a biological invasion. *Molecular Ecology* **17:** 773–782.

Ficetola GF, Miaud C, Pompanon F, Taberlet P. 2008. Species detection using environmental DNA from water samples. *Biology Letters* **4:** 423–425.

Ficetola GF, Pansu J, Bonin A, Coissac E, Giguet-Covex C, De Barba M, Gielly L, Lopes CM, Boyer F, Pompanon F, Rayé G, Taberlet P. 2014. Replication levels, false presences and the estimation of the presence/absence from eDNA metabarcoding data. *Molecular Ecology Resources* DOI: 10.1111/1755-0998.12338.

Floyd R, Abebe E, Papert A, Blaxter M. 2002. Molecular barcodes for soil nematode identification. *Molecular Ecology* **11:** 839–850.

Folloni S, Kagkli DM, Rajcevic B, Guimarães NCC, Van Droogenbroeck B, Valicente FH, Van den Eede G, Van den Bulcke M. 2012. Detection of airborne genetically modified maize pollen by real-time PCR. *Molecular Ecology Resources* **12:** 810–821.

Fonseca VG, Carvalho GR, Sung W, Johnson HF, Power DM, Neill SP, Packer M, Blaxter ML, Lambshead PJD, Thomas WK, Creer S. 2010. Second-generation environmental sequencing unmasks marine metazoan biodiversity. *Nature Communications* **1:** 98.

Foote AD, Thomsen PF, Sveegaard S, Wahlberg M, Kielgast J, Kyhn LA, Salling AB, Galatius A, Orlando L, Gilbert MTP. 2012. Investigating the potential use of environmental DNA (eDNA) for genetic monitoring of marine mammals. *PloS ONE* **7:** e41781.

Frazier M, Miller AW, Lee H, Reusser DA. 2013. Counting at low concentrations: the statistical challenges of verifying ballast water discharge standards. *Ecological Applications* **23:** 339–351.

Glenn TC. 2011. Field guide to next-generation DNA sequencers. *Molecular Ecology Resources* **11:** 759–769.

Goldberg CS, Pilliod DS, Arkle RS, Waits LP. 2011. Molecular detection of vertebrates in stream water: a dem-

onstration using Rocky Mountain tailed frogs and Idaho giant salamanders. *PloS ONE* **6:** e22746.

Hajibabaei M, Smith MA, Janzen DH, Rodriguez JJ, Whitfield JB, Hebert PDN. 2006. A minimalist barcode can identify a specimen whose DNA is degraded. *Molecular Ecology Notes* **6:** 959–964.

Hebert PDN, Ratnasingham S, deWaard JR. 2003. Barcoding animal life: cytochrome c oxidase subunit 1 divergences among closely related species. *Proceedings of the Royal Society of London Series B, Biological Sciences* **270:** Suppl 1: S96–S99.

Hiiesalu I, Oepik M, Metsis M, Lilje L. 2012. Plant species richness belowground: higher richness and new patterns revealed by next-generation sequencing. *Molecular Ecology* **21:** 2004–2016.

Hollingsworth ML, Andra Clark A. 2009. Selecting barcoding loci for plants: evaluation of seven candidate loci with species-level sampling in three divergent groups of land plants. *Molecular Ecology* **9:** 439–457.

Hulme PE. 2009. Trade, transport and trouble: managing invasive species pathways in an era of globalization. *Journal of Applied Ecology* **46:** 10–18.

Hyman OJ, Collins JP. 2012. Evaluation of a filtration-based method for detecting *Batrachochytrium dendrobatidis* in natural bodies of water. *Diseases of Aquatic Organisms* **97:** 185–195.

Jane SF, Wilcox TM, McKelvey KS, Young MK, Schwartz MK, Lowe WH, Letcher BH, Whiteley AR. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Molecular Ecology Resources* **15:** 216–227.

Jerde CL, Mahon AR, Chadderton WL, Lodge DM. 2011. 'Sight-unseen' detection of rare aquatic species using environmental DNA. *Conservation Letters* **4:** 150–157.

Ji Y, Ashton L, Pedley SM, Edwards DP, Tang Y, Nakamura A, Kitching R, Dolman PM, Woodcock P, Edwards FA, Larsen TH, Hsu WW, Benedick S, Hamer KC, Wilcove DS, Bruce C, Wang X, Levi T, Lott M, Emerson BC, Yu DW. 2013. Reliable, verifiable and efficient monitoring of biodiversity via metabarcoding. *Ecology Letters* **16:** 1245–1257.

Jørgensen T, Kjaer KH, Haile J, Rasmussen M, Boessenkool S, Andersen K, Coissac E, Taberlet P, Brochmann C, Orlando L, Gilbert MT, Willerslev E. 2012. Islands in the ice: detecting past vegetation on Greenlandic nunataks using historical records and sedimentary ancient DNA meta-barcoding. *Molecular Ecology* **21:** 1980–1988.

Kelly RP, Port JA, Yamahara KM, Crowder LB. 2014. Using environmental DNA to census marine fishes in a large mesocosm. *PloS ONE* **9:** e86175.

Kéry M, Schmidt BR. 2008. Imperfect detection and its consequences for monitoring for conservation. *Community Ecology* **9:** 207–216.

Keskin E. 2014a. Detection of invasive freshwater fish species using environmental DNA survey. *Biochemical Systematics and Ecology* **56:** 68–74.

Keskin E. 2014b. Molecular evidence for the predation of critically endangered endemic *Aphanius transgrediens* from the stomach contents of world wide invasive *Gambusia affinis*. *Mitochondrial DNA* Aug 1: [Epub ahead of print]: 1–6.

Kowalczyk R, Taberlet P, Coissac E, Valentini A, Miquel C, Kamiński T, Wójcik JM. 2011. Influence of management practices on large herbivore diet – case of European bison in Białowieża Primeval Forest (Poland). *Forest Ecology and Management* **261:** 821–828.

Krüger F, Clare EL, Greif S, Siemers BM, Symondson WOC, Sommer RS. 2014. An integrative approach to detect subtle trophic niche differentiation in the sympatric trawling bat species *Myotis dasycneme* and *Myotis daubentonii*. *Molecular Ecology* **23:** 3657–3671.

Lance RF, Carr MR. 2012. Detecting eDNA of invasive dreissenid mussels: report on capital investment project. ANSRP Technical Notes Collection. ERDC/TN ANSRP-12-2. Vicksburg, MS: US Army Engineer Research and Development Center. Available at: http://el.erdc.usace.army.mil/publications.cfm?Topic=technote&Code=ansrp

Leung B, Lodge DM, Finnoff D, Shogren JF, Lewis MA, Lamberti G. 2002. An ounce of prevention or a pound of cure: bioeconomic risk analysis of invasive species. *Proceedings of the Royal Society of London Series B, Biological Sciences* **269:** 2407–2413.

Little DP. 2014. A DNA mini-barcode for land plants. *Molecular Ecology Resources* **14:** 437–446.

Mächler E, Deiner K, Steinmann P, Altermatt F. 2014. Utility of environmental DNA for monitoring rare and indicator macroinvertebrate species. *Freshwater Science* **33:** 1174–1183.

MacKenzie DI, Nichols JD. 2004. Occupancy as a surrogate for abundance estimation. *Animal Biodiversity and Conservation* **27:** 461–467.

MacKenzie DI, Nichols JD, Lachman GB, Droege S, Andrew Royle J, Langtimm CA. 2002. Estimating site occupancy rates when detection probabilities are less than one. *Ecology* **83:** 2248–2255.

Mahon AR, Barnes MA, Senapati S, Feder JL, Darling JA, Chang HC, Lodge DM. 2011. Molecular detection of invasive species in heterogeneous mixtures using a microfluidic carbon nanotube platform. *PloS ONE* **6:** e17280.

Mahon AR, Jerde CL, Galaska M, Bergner JL, Chadderton WL, Lodge DM, Hunter ME, Nico LG. 2013. Validation of eDNA surveillance sensitivity for detection of Asian carps in controlled and field experiments. *PloS ONE* **8:** e58316.

Mahon AR, Nathan LR, Jerde CL. 2014. Meta-genomic surveillance of invasive species in the bait trade. *Conservation Genetics Resources* **6:** 563–567.

McLellan R, Iyengar L, Jeffries B, Oerlemans N, eds. 2014. *Living Planet Report 2014: species and spaces, people and places*. Gland: WWF.

Meusnier I, Singer GAC, Landry JF, Hickey DA, Hebert PDN, Hajibabaei M. 2008. A universal DNA mini-barcode for biodiversity analysis. *BMC Genomics* **9:** 214.

Millennium Ecosystem Assessment. 2005. *Ecosystems and human well-being: biodiversity synthesis*. Washington, DC: World Resources Institute.

Moyer GR, Díaz-Ferguson E, Hill JE, Shea C. 2014. Assessing environmental DNA detection in controlled lentic systems. *PloS ONE* **9:** e103767.

Nathan LM, Simmons M, Wegleitner BJ, Jerde CL, Mahon AR. 2014. Quantifying environmental DNA signals for aquatic invasive species across multiple detection platforms. *Environmental Science & Technology* **48:** 12800–12806.

Pedersen MW, Ginolhac A, Orlando L, Olsen J, Andersen K, Holm J, Funder S, Willerslev E, Kjær KH. 2013. A comparative study of ancient environmental DNA to pollen and macrofossils from lake sediments reveals taxonomic overlap and additional plant taxa. *Quaternary Science Reviews* **75:** 161–168.

Piaggio AJ, Engeman RM, Hopken MW, Humphrey JS, Keacher KL, Bruce WE, Avery ML. 2014. Detecting an elusive invasive species: a diagnostic PCR to detect Burmese python in Florida waters and an assessment of persistence of environmental DNA. *Molecular Ecology Resources* **14:** 374–380.

Pilliod DS, Goldberg CS, Arkle RS, Waits LP. 2013. Estimating occupancy and abundance of stream amphibians using environmental DNA from filtered water samples. *Canadian Journal of Fisheries and Aquatic Sciences. Journal canadien des sciences halieutiques et aquatiques* **70:** 1123–1130.

Pilliod DS, Goldberg CS, Arkle RS, Waits LP. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Molecular Ecology Resources* **14:** 109–116.

Pimm SL, Jenkins CN, Abell R, Brooks TM, Gittleman JL, Joppa LN, Raven PH, Roberts CM, Sexton JO. 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science* **344:** 1246752.

Pocock MJO, Roy HE, Preston CD, Roy DB. 2015. The Biological Records Centre: a pioneer of citizen science. *Biological Journal of the Linnean Society* **115:** 475–493.

Porazinska DL, Giblin-Davis RM, Faller L, Farmerie W, Kanzaki N, Morris K, Powers TO, Tucker AE, Sung W, Thomas WK. 2009. Evaluating high-throughput sequencing as a method for metagenomic analysis of nematode diversity. *Molecular Ecology Resources* **9:** 1439–1450.

Powers T. 2004. Nematode molecular diagnostics: from bands to barcodes. *Annual Review of Phytopathology* **42:** 367–383.

Razgour O, Clare EL, Zeale M. 2011. High-throughput sequencing offers insight into mechanisms of resource partitioning in cryptic bat species. *Ecology and Evolution* **1:** 556–570.

Redd KS, Ling SD, Frusher SD, Jarman S, Johnson CR. 2014. Using molecular prey detection to quantify rock lobster predation on barrens-forming sea urchins. *Molecular Ecology* **23:** 3849–3869.

Rees HC, Maddison BC, Middleditch DJ, Patmore JRM, Gough KC. 2014. REVIEW: the detection of aquatic animal species using environmental DNA – a review of eDNA as a survey tool in ecology. *Journal of Applied Ecology* **51:** 1450–1459.

Roy HE, Lawson Handley LJ. 2012. Networking: a community approach to invaders and their parasites. *Functional Ecology* **26:** 1238–1248.

Roy HE, Peyton J, Aldridge DC, Bantock T, Blackburn TM, Britton R, Clark P, Cook E, Dehnen-Schmutz K, Dines T, Dobson M, Edwards F, Harrower C, Harvey MC, Minchin D, Noble DG, Parrott D, Pocock MJ, Preston CD, Roy S, Salisbury A, Schönrogge K, Sewell J, Shaw RH, Stebbing P, Stewart AJ, Walker KJ. 2014. Horizon scanning for invasive alien species with the potential to threaten biodiversity in Great Britain. *Global Change Biology* **20:** 3859–3871.

Roy HE, Rorke SL, Beckmann B, Booy O, Botham MS, Brown PMJ, Harrower C, Noble D, Sewell J, Walker K. 2015. The contribution of volunteer recorders to our understanding of biological invasions. *Biological Journal of the Linnean Society* **115:** 678–689.

Schmidt BR, Kéry M, Ursenbacher S, Hyman OJ, Collins JP. 2013. Site occupancy models in the analysis of environmental DNA presence/absence surveys: a case study of an emerging amphibian pathogen. *Methods in Ecology and Evolution* **4:** 646–653.

Schnell IB, Thomsen PF, Wilkinson N, Rasmussen M, Jensen LRD, Willerslev E, Bertelsen MF, Gilbert MTP. 2012. Screening mammal biodiversity using DNA from leeches. *Current Biology* **22:** R262–R263.

Schoch CL, Seifert KA, Huhndorf S, Robert V, Spouge JL, Levesque CA, Chen W, Fungal Barcoding Consortium, Fungal Barcoding Consortium Author List. 2012. Nuclear ribosomal internal transcribed spacer (ITS) region as a universal DNA barcode marker for fungi. *Proceedings of the National Academy of Sciences of the United States of America* **109:** 6241–6246.

Schulz M, Vedova BD. 2014. Regulation (EU) No 1143/2014 of the European parliament and of the council of 22 October 2014 on the prevention and management of the introduction and spread of invasive alien species. *Official Journal of the European Union* **L317:** 35–55.

Sedlock JL, Krüger F, Clare EL. 2014. Island bat diets: does it matter more who you are or where you live? *Molecular Ecology* **23:** 3684–3694.

Shehzad W, McCarthy TM, Pompanon F, Purevjav L, Coissac E, Riaz T, Taberlet P. 2012. Prey preference of snow leopard *Panthera uncia* in South Gobi, Mongolia. *PloS ONE* **7:** e32104.

Sønstebø JH, Gielly L, Brysting AK. 2010. Using next-generation sequencing for molecular reconstruction of past Arctic vegetation and climate. *Molecular Ecology* **10:** 1009–1018.

Strand DA, Jussila J, Johnsen SI, Viljamaa-Dirks S, Edsman L, Wiik-Nielsen J, Viljugrein H, Engdahl F, Vrålstad T. 2014. Detection of crayfish plague spores in large freshwater systems. *Journal of Applied Ecology* **51:** 544–553.

Sutherland WJ, Bardsley S, Clout M, Depledge MH, Dicks LV, Fellman L, Fleishman E, Gibbons DW, Keim B, Lickorish F, Margerison C, Monk KA, Norris K, Peck LS, Prior SV, Scharlemann JP, Spalding MD, Watkinson AR. 2013. A horizon scan of global conservation issues for 2013. *Trends in Ecology & Evolution* **28:** 16–22.

Symondson WOC. 2002. Molecular identification of prey in predator diets. *Molecular Ecology* **11:** 627–641.

Symondson WOC, Harwood JD. 2014. Special issue on molecular detection of trophic interactions: unpicking the tangled bank. Introduction. *Molecular Ecology* **23:** 3601–3604.

Taberlet P, Coissac E, Pompanon F, Brochmann C, Willerslev E. 2012. Towards next-generation biodiversity assessment using DNA metabarcoding. *Molecular Ecology* **21:** 2045–2050.

Takahara T, Minamoto T, Doi H. 2013. Using environmental DNA to estimate the distribution of an invasive fish species in ponds. *PloS ONE* **8:** e56584.

Takahara T, Minamoto T, Yamanaka H, Doi H, Kawabata Z. 2012. Estimation of fish biomass using environmental DNA. *PloS ONE* **7:** e35868.

Tang CQ, Leasi F, Obertegger U, Kieneke A, Barraclough TG, Fontaneto D. 2012. The widely used small subunit 18S rDNA molecule greatly underestimates true diversity in biodiversity surveys of the meiofauna. *Proceedings of the National Academy of Sciences of the United States of America* **109:** 16208–16212.

Thomsen PF, Kielgast J, Iversen LL, Møller PR, Rasmussen M, Willerslev E. 2012a. Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PloS ONE* **7:** e41732.

Thomsen PF, Kielgast J, Iversen LL, Wiuf C, Rasmussen M, Gilbert MTP, Orlando L, Willerslev E. 2012b. Monitoring endangered freshwater biodiversity using environmental DNA. *Molecular Ecology* **21:** 2565–2573.

Tréguier A, Paillisson JM, Dejean T, Valentini A, Schlaepfer MA, Roussel JM. 2014. Environmental DNA surveillance for invertebrate species: advantages and technical limitations to detect invasive crayfish *Procambarus clarkii* in freshwater ponds. *Journal of Applied Ecology* **51:** 871–879.

Valentini A, Pompanon F, Taberlet P. 2009. DNA barcoding for ecologists. *Trends in Ecology & Evolution* **24:** 110–117.

Welch KD, Schofield MR, Chapman EG, Harwood JD. 2014. Comparing rates of springtail predation by web-building spiders using Bayesian inference. *Molecular Ecology* **23:** 3814–3825.

Wilcox TM, McKelvey KS, Young MK, Jane SF, Lowe WH, Whiteley AR, Schwartz MK. 2013. Robust detection of rare species using environmental DNA: the importance of primer specificity. *PloS ONE* **8:** e59520.

Willerslev E, Hansen AJ, Binladen J, Brand TB, Gilbert MTP, Shapiro B, Bunce M, Wiuf C, Gilichinsky DA, Cooper A. 2003. Diverse plant and animal genetic records from Holocene and Pleistocene sediments. *Science* **300:** 791–795.

Yoccoz NG, Bråthen KA, Gielly L, Haile J, Edwards ME, Goslar T, Von Stedingk H, Brysting AK, Coissac E, Pompanon F, Sønstebø JH, Miquel C, Valentini A, De Bello F, Chave J, Thuiller W, Wincker P, Cruaud C, Gavory F, Rasmussen M, Gilbert MT, Orlando L, Brochmann C, Willerslev E, Taberlet P. 2012. DNA from soil mirrors plant taxonomic and growth form diversity. *Molecular Ecology* **21:** 3647–3655.

Yu DW, Ji Y, Emerson BC, Wang X, Ye C, Yang C, Ding Z. 2012. Biodiversity soup: metabarcoding of arthropods for rapid biodiversity assessment and biomonitoring. *Methods in Ecology and Evolution* **3:** 613–623.

Zeale M, Butlin RK, Barker G. 2011. Taxon-specific PCR for DNA barcoding arthropod prey in bat faeces. *Molecular Ecology* **11:** 236–244.

Zhan A, Hulák M, Sylvester F, Huang X, Adebayo AA, Abbott CL, Adamowicz SJ, Heath DD, Cristescu ME, MacIsaac HJ. 2013. High sensitivity of 454 pyrosequencing for detection of rare species in aquatic communities. *Methods in Ecology and Evolution* **4:** 558–565.

Zhou X, Li Y, Liu S, Yang Q, Su X, Zhou L, Tang M, Fu R, Li J, Huang Q. 2013. Ultra-deep sequencing enables high-fidelity recovery of biodiversity for bulk arthropod samples without PCR amplification. *GigaScience* **2:** 4.