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NEWS AND VIEWS

PERSPECTIVE

Making environmental DNA count

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The arc of reception for a new technology or method – like the reception of new information itself – can pass through predictable stages, with audiences' responses evolving from 'I don't believe it', through 'well, maybe' to 'yes, everyone knows that' to, finally, 'old news'. The idea that one can sample a volume of water, sequence DNA out of it, and report what species are living nearby has experienced roughly this series of responses among biologists, beginning with the microbial biologists who developed genetic techniques to reveal the unseen microbiome. 'Macrobial' biologists and ecologists – those accustomed to dealing with species they can see and count – have been slower to adopt such molecular survey techniques, in part because of the uncertain relationship between the number of recovered DNA sequences and the abundance of whole organisms in the sampled environment. In this issue of Molecular Ecology Resources, Evans *et al.* (2015) quantify this relationship for a suite of nine vertebrate species consisting of eight fish and one amphibian. Having detected all of the species present with a molecular toolbox of six primer sets, they consistently find DNA abundances are associated with species' biomasses. The strength and slope of this association vary for each species and each primer set – further evidence that there is no universal parameter linking recovered DNA to species abundance – but Evans and colleagues take a significant step towards being able to answer the next question audiences tend to ask: 'Yes, but how many are there?'

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The past three years has seen an explosion of interest in the use of environmental DNA (eDNA) in ecological and conservation applications. For researchers previously reliant on manual count data, eDNA – trace genetic material recovered from the habitat in which it was generated – has quickly become a potential new avenue through which to examine the world (Thomsen & Willerslev 2015). The idea is that multicellular organisms of all kinds shed cells containing diagnostic DNA into the environment; this DNA can then be recovered, sequenced and assigned to a taxon based upon its match to previously known sequences. Because the technique uses sequence data to assign taxonomy (i.e. DNA barcoding), and because of its reliance on high-throughput sequencing to process samples containing mixtures of many species, the technique is also known as metabarcoding.

A sizeable portion of microbial literature has focused on discovering new taxa and on describing the diversity of life that exists in habitats from deep-sea vents to patches of human skin (e.g. Grice *et al.* 2009). By contrast, 'macrobial' ecologists rarely aim at discovery *per se*; we generally know what species we are probably to find with eDNA. The goal has instead been to make biological surveys better, cheaper, easier or faster than alternative methods; that is, we are interested in making genetic tools useful for questions of larger scale basic ecology, conservation, environmental science and policy (Fig 1., illustrates some of these concepts).

The recent upsurge in interest is largely due to the shrinking gap between theory and practice of eDNA analysis for these purposes. In a 2012 special issue of Molecular Ecology, Lodge and colleagues (Lodge *et al.* 2012), Taberlet and colleagues (Taberlet *et al.* 2012), and others identified a set of hurdles that eDNA analysis would need to overcome before becoming a practical tool for field ecologists. We have since seen progress on several of these fronts – from primer design (Boyer *et al.* 2015) to the beginnings of PCR-free analysis (e.g. Zhou *et al.* 2013) – but Evans and coauthors take on one of the important remaining unknowns by quantifying relationship between species abundance and amplicon

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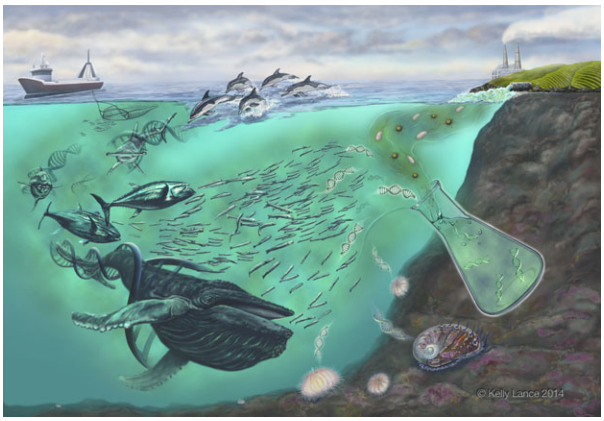

Fig. 1 Conceptual illustration of environmental DNA and some of its uses for science and 20 policy. Copyright Kelly Lance 2014.

abundance. This relationship is perhaps *the* key unknown for many field applications of eDNA, pointing the way to such uses as stock assessments, counts of endangered or invasive species, and other quantitative surveys for species and communities of interest.

Ecologists use two distinct methods of molecular detection to characterize environmental samples: quantitative PCR (qPCR), useful for quantitatively assessing changes in the concentration of particular target-species' DNA across samples, and high-throughput amplicon sequencing, in which a single region from all genomes present in a sample is amplified (using PCR) to create a multispecies community profile. The challenge of the latter method has been how to link a species' amplicon sequences to its field abundance in a quantitative way. Thus, researchers have had to choose between quantitatively surveying one or few species, and qualitatively surveying many species simultaneously.

Limited evidence has previously suggested that amplicon abundance increased with species' biomass (Kelly *et al.* 2014; Port *et al.* 2015). Evans and coauthors have substantially improved these early results, demonstrating remarkable consistency in the eDNA–biomass relationship across nine species and six primer sets (Evans *et al.* 2015; Figs. 3 and S2–S6); nearly every species-by-primer combination yields a positive and significant correlation. Another recent result reported an even stronger association (r^2=0.97) between eDNA sequence abundance and marine plankton species' biovolumes as estimated by visual (light microscopy) counts (de Vargas *et al.* 2015). These new studies draw the outer contours of what we might reasonably expect from abundance estimates via amplicon sequencing: somewhere between half (Evans *et al.* 2015) and nearly all (de Vargas *et al.* 2015) of eDNA variance among taxa might be attributed to variance in taxon abundance. Even such a wide confidence interval is remarkable given that each step in the analytical chain – from sampling to PCR to sequencing to bioinformatics – introduces some degree of bias into the estimate.

Several notes of caution are of course still warranted. First, because the eDNA–biomass abundance equation differs for each species (Evans *et al.* 2015), it is more appropriate to compare within-species trends, as Evans *et al.* do, rather than comparing community profiles wholesale. Even when measuring changes in abundance within a species, ecological context is bound to matter: detecting small changes in sardine abundance is easier when there is not a whale nearby, for example. The whale might swamp out any small change in sardine signal by dominating the finite number of reads in a high-throughput sequencing run. Similarly, the species richness of the sampled community will influence the absolute number of DNA reads recovered for a given taxon (as will the total number of reads per run, although a number of normalization techniques exist for this purpose, e.g. Love *et al.* 2014). The abiotic context probably also influences DNA transport distance and retention time, as illustrated by riverine and sediment sampling, respectively (Deiner & Altermatt 2014; Turner *et al.* 2015). And finally, primer mismatch percentage itself is not a reliable predictor of biomass–eDNA relationship, although biased PCR amplification – which itself can be driven by primer–template mismatches – can result in large differences between proportional biomass and proportional DNA sequences. The result is that, despite vast improvements in algorithms for PCR primer design in recent years, it is difficult to predict primer performance, and so empirical studies such as the one Evans and colleagues report in this issue are indispensible.

The future of eDNA and related molecular techniques seems bright, but the leap from potential to practicality hinges on ensuring that the data reflect real-world phenomena. Further developing quantitative biological assessments using DNA will make an already-powerful tool even more useful for applications in basic science as well as environmental policy.

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R.P.K. researched and wrote this piece in its entirety.