A field ecologist's guide to environmental DNA sampling in freshwater environments

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ABSTRACT

Environmental DNA, or eDNA—DNA shed from organisms and extracted from environmental samples—is an emerging survey technique that has the potential to transform biodiversity monitoring in freshwater ecosystems. We provide a brief overview of the primary methodological aspects of eDNA sampling that ecologists should consider before taking environmental samples in the field. We outline five key methodological considerations: (i) targeting single species vs multiple species; (ii) where and when to sample; (iii) how much water to collect; (iv) how many samples to take; and (v) recognising potential sources of false positives. The need to account for false negatives and false positives in eDNA surveys, and the power of species occupancy detection models in accounting for imperfect detection, is also discussed.

Key words: detection, eDNA, freshwater, monitoring, occupancy

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INTRODUCTION

Understanding spatial changes in biodiversity patterns is important for informing conservation efforts, but landscape-level data on species distributions can be logistically and financially difficult to collect. Environmental DNA (eDNA) sampling—the detection of extra-organismal DNA sourced from environmental samples without any obvious biological source material being present (Taberlet *et al.* 2012)—is an emerging survey technique that could, for many species, facilitate efficient and cost-effective collection of landscape-level data. DNA can be shed into the environment from various sources, including skin cells, mucous, faeces, or even individual hairs. Previous studies have shown samples of water, snow, soil, and air can be used to determine species presence-absence at a site (Dalén *et al.* 2007; Andersen *et al.* 2012; Lugg *et al.* 2017; Leontidou *et al.* 2018). In this brief review, we focus on the detection of eDNA presence-absence in freshwater ecosystems, which are globally imperilled and particularly amenable to eDNA sampling. Indeed, freshwater ecology is an area in which significant technical and practical progress has been made in the field of eDNA sampling (Cristescu and Hebert 2018), although similar progress is also starting to occur in marine environments (Foote *et al.* 2012; Thomsen *et al.* 2012; Kelly *et al.* 2014).

The first step in assessing the suitability of any emerging technology, such as eDNA sampling, is to compare the results it produces against traditional sampling methods (Lahoz-Monfort and Tingley 2018). As with other emerging technologies in ecology, such as drones (Hodgson *et al.* 2016), thermal cameras (Goodenough *et al.* 2017), and detector dogs (Cristescu *et al.* 2015), eDNA sampling in aquatic environments has been compared to traditional survey methods, such as trapping (Shaw *et al.* 2016), aural surveys (Valentini *et al.* 2016), and electrofishing (Evans *et al.* 2017). In many aquatic systems tested to date, eDNA sampling has been capable of detecting species or communities more effectively than traditional methods (see Lugg *et al.* 2017). Environmental DNA sampling lends itself to baseline data collection on species distributions, as well as routine monitoring programs that aim to track changes in species distributions over space and time. If more detailed data on population or individual health (e.g., reproductive output, juvenile recruitment, sex, genetic variation, abundance) are required, eDNA can highlight sampling locations for more traditional, time-intensive capture methods. However, eDNA methods also show some promise in this regard (providing population- and individual-based information), particularly as technologies improve (Sigsgaard *et al.* 2016; Bylemans *et al.* 2017).

Environmental DNA sampling has many elements that make it a promising survey method. It allows for landscape-level data to be collected cost-efficiently for many species without invasive sampling (e.g. capturing), or even sighting animals. Thus, eDNA sampling largely eliminates concerns around animal ethics. An additional benefit of eDNA sampling is that it reduces health and safety concerns for researchers and employers. For

example, eDNA sampling eliminates high-risk activities, such as entering waterways to check fyke nets for fish and platypuses (Serena 1994) or conducting backpack or boat electrofishing surveys for fish (Wilcox *et al.* 2016). This technique can potentially detect cryptic or rare species that evade capture with other methods, and detect species at life stages that were previously difficult to detect or distinguish between (Dejean *et al.* 2012).

Despite these benefits, using eDNA sampling in biodiversity surveys is currently more complex than using most traditional survey methods. This is largely because eDNA sampling is a relatively new monitoring technique and standardised approaches have not been developed (see Goldberg *et al.* 2016). Thus, our objective in this paper is to provide a brief overview of the primary methodological aspects of eDNA sampling that ecologists who are interested in using eDNA should consider before taking environmental samples. Because our work is aimed at practitioners collaborating with a fully-equipped genetics laboratory, we do not consider other important, but more technical considerations, such as DNA extraction method, gene region, primer selection, and marker specificity (see Freeland 2017). Nor do we touch on quality control issues, such as field and laboratory negative controls, mock community positive controls, index switching (exclusive to metabarcoding and pooling samples), OTU thresholds, or read depth (Deiner *et al.* 2017). For excellent reviews incorporating such considerations see Bohmann *et al.* (2014), Cristescu and Hebert (2018), Deiner *et al.* (2017), and Rees *et al.* (2014).

Our review is structured around five key methodological considerations: (i) targeting single species vs multiple species or communities; (ii) where and when to sample; (iii) how much water; (iv) how many samples; and (v) mitigating false positive detections (Figure 1). We conclude with a discussion of the need to consider imperfect detection in eDNA surveys, and briefly highlight the power of species occupancy detection models (SODM) in this regard.

Targeting single species vs multiple species

Environmental DNA detection methods can be divided into two broad categories: single- and multi-species methods. Single-species methods focus on eDNA quantification from a single target species using probes that are specific to the species or population(s) of interest. Most single-species studies use real-time quantitative polymerase chain reaction (qPCR), although traditional PCR (Goldberg *et al.* 2011) and Droplet Digital PCR (ddPCR) (Doi *et al.* 2015) have also been used. Single-species detection methods have been used broadly to successfully detect rare (Laramie *et al.* 2015; Schmelzle and Kinziger 2016; Simpfendorfer *et al.* 2016) and invasive (Hunter *et al.* 2015; Hinlo *et al.* 2017; Klymus *et al.* 2017; Tingley *et al.* 2018) species in aquatic environments. Multi-species detection methods—also known as metabarcoding—take a broader, community-focused approach. High-throughput next generation sequencing (NGS) technologies for DNA sequencing enable all species from one or more target groups (e.g., fish, amphibians, or decapods) to be identified (Taberlet *et al.* 2012).

Whether to adopt a single- or multi-species eDNA approach depends primarily on the specific aims of the study. Clearly, a single-species approach is not appropriate when the scientific or management objective pertains to community-level patterns. But given the rich amount of data afforded by metabarcoding, why would one ever adopt a single-species approach, even if a study intends to focus solely on a single species? The answer to this question lies primarily in the relative complexity, sensitivity, and cost-efficiency of each eDNA approach.

Single-species approaches require species-specific primers/probes for a target DNA region, whereas metabarcoding requires primers designed to bind to conserved DNA regions across the target group. The ability to distinguish species within the target group then relies on a reference library of known sequences matched to species, with which the sequenced eDNA data can be compared. Publicly available sequence databases, such as GenBank (Clark *et al.* 2016), can be used to generate reference libraries at a broad level, but often native species are poorly represented in these databases. Therefore, a local reference library, consisting of genetic sequences of each species from the study area (as in Valentini *et al.* 2016), is recommended; this will also help account for any intraspecific variation within the gene region being sequenced. Once this library has been established, it can be used by future studies; however, its initial development can be expensive and time-consuming relative to the resources needed to design a species-specific primer. Different target gene regions will also provide different levels of species resolution, and this needs to be considered when undertaking metabarcoding surveys, as some gene regions may not differentiate closely related species (Bylemans *et al.* 2018). Primer bias can also result in some species not being detected in a sample when they may be present in the environment, as some species may amplify more readily than others (Elbrecht *et al.* 2017).

Metabarcoding requires bioinformatic pipelines to demultiplex samples—assigning sequences to samples—and assign genetic sequences (or haplotypes) to species (Coissac *et al.* 2012). Once again, these pipelines can be reused once developed, but the bioinformatic tools needed for metabarcoding are much more labour-intensive and require a different level of expertise compared to a single-species approach, which is much more routine in a molecular laboratory.

Few studies have directly compared the sensitivity of single- vs multi-species eDNA assays for individual species, although Harper *et al.* (2018) demonstrated that qPCR resulted in greater detectability for Great Crested Newts (*Triturus cristatus*) relative to metabarcoding.

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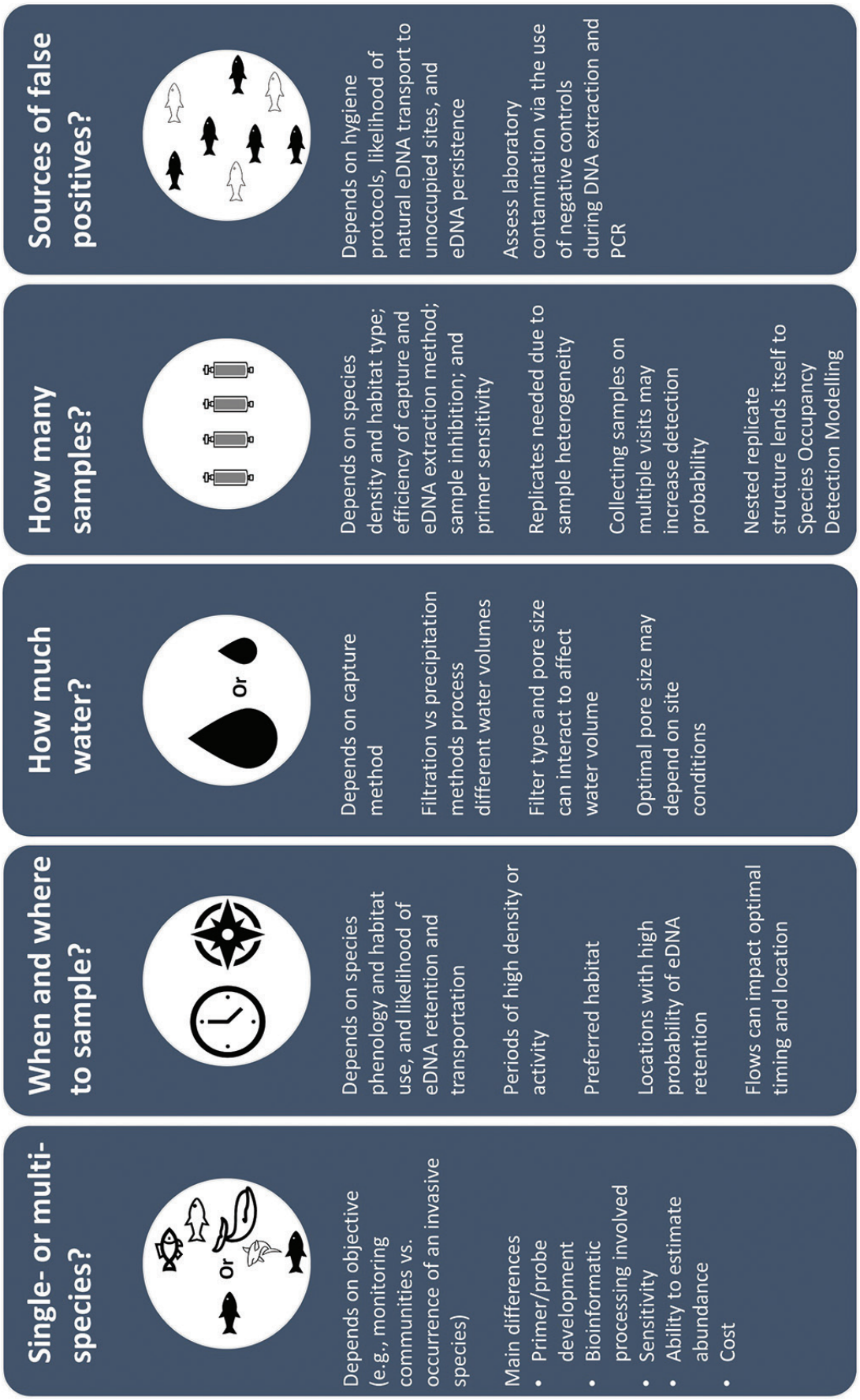

Figure 1. Key methodological considerations for eDNA sampling in the field, with notes on relevant methodological decisions.

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Nonetheless, this increased sensitivity needs to be evaluated against a study's aims and the nature of the study system. In some cases, accepting a slightly lower detection probability for a target species may be worthwhile if additional data on biotic interactions (e.g., presence of prey, competitors, predators, disease) would change scientific inferences or management decisions. In other cases, such as determining whether an endangered species is present at a site for an environmental impact assessment, a potentially less-sensitive metabarcoding assay may not be the optimal choice. There may be situations where combining approaches (metabarcoding and a single-species assay) is also appealing, particularly where sensitivity for the target species is paramount in the survey design, but there is also a need for the additional data on community composition. The advantage here is that sampling and DNA extraction have already been undertaken for one method; there is therefore a significant reduction in cost for the second method.

Single-species eDNA assays using qPCR or ddPCR have revealed relationships between DNA copy number and species abundance within a sample, indicating that the method can be used to estimate density at a site (Klobucar *et al.* 2017; Tillotson *et al.* 2018). This provides additional information beyond just presence-absence and is therefore an additional consideration when designing an eDNA study. While, in theory, multi-species eDNA approaches can also estimate a measure of DNA copy number within a sample, this relationship is much more complex in metabarcoding due to the methodology employed, and therefore generally considered a relatively weak indicator of species abundance at a site (Fonseca 2018).

The final consideration as to whether to adopt a single- or multi-species approach—cost-efficiency—has not, to the best of our knowledge, been explicitly studied. Metabarcoding is considerably more expensive than single species PCR-based approaches when only considering a single target species, but it remains to be seen at what point (e.g. the number of species) metabarcoding is more cost-efficient. Clearly this will depend on the number of species present in a system and the availability of single-species eDNA assays (Shaw *et al.* 2017).

For the remainder of our review, we focus primarily on considerations for single-species eDNA studies, as this approach has been developed to a much greater extent than metabarcoding. However, many of our recommendations pertain to general sampling design and thus are also relevant for eDNA metabarcoding.

When and where to sample?

As with other survey methods, the timing and location of eDNA sampling needs to be carefully considered. The optimal timing of eDNA sampling will depend on a species' phenology, as well as the likelihood of DNA transportation and retention. When the primary objective is to maximise the probability of detecting a single species, sampling at a time of year when the target species is most active (e.g., the breeding season), or is in highest abundance (Buxton *et al.* 2017), may maximise the amount of DNA present in the system. This assumes, of course, that this information is known for the target species, and that DNA shedding rates are equivalent across different life stages of the target species.

In lotic (flowing) systems, the transportation and retention of eDNA is also important. Environmental DNA can be transported downstream to an unoccupied site or diluted to undetectable levels at the point of origin (Pont *et al.* 2018). Estimates of eDNA transportation differ greatly between studies. Balasingham et al. (2017) found residual eDNA at detectable levels at a maximum distance of 960m downstream, whereas Deiner and Altermatt (2014), in a faster flowing but similar-sized stream, detected a species' DNA 9.1 km downstream. The retention of eDNA in a system also needs to be considered, as eDNA can interact with environmental or biotic elements in the system that cause it to be retained, removed, or released (Shogren *et al.* 2017). Shogren *et al.* (2017) considered some of these complex issues by investigating the transport, retention and resuspension of eDNA empirically using controlled experimental streams. They suggest that the complexity of eDNA detection in lotic systems should be considered but that further progress is needed for predictive modelling of eDNA transport.

Where to take a sample within a site is also important. The optimal sampling location within a site will depend on a species' habitat preferences and the likelihood of DNA retention (Buxton *et al.* 2018). Targeting preferred habitat could increase the likelihood of detecting the target species but certain habitats will retain DNA for longer periods of time. For example, the DNA of a species that occupies vegetation around the stream edge may be retained at a site for a longer period compared to a species that prefers the water column in the middle of the stream, simply because of the water's physical movement.

Sampling from the water column compared to the sediment could yield different concentrations of eDNA and represent different timescales of species presence. eDNA in sediments is more concentrated than eDNA from the water column, due to eDNA settling through the water column (Turner *et al.* 2015). The timescales represented by sediment samples could be much longer compared to those of the water column (Turner *et al.* 2015) due to the exponential decay of eDNA; a higher starting concentration results in a longer decay time (Thomsen *et al.* 2012; Barnes *et al.* 2014).

Environmental conditions can also affect eDNA detectability via DNA degradation. Strickler *et al.* (2015), for example, concluded that habitats with less solar radiation and more alkaline water retained eDNA for longer periods. However, there can also be a trade-off;

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environments with higher levels of solar radiation that are warmer can increase DNA shedding rates from a target species, offsetting higher degradation rates (Robson *et al.* 2016). Other biotic factors, such as the presence of biofilm, have been shown to increase the degradation rate of eDNA in flowing systems (Shogren *et al.* 2018).

How much water?

Filtration and precipitation methods have been used to capture eDNA and each method can process different volumes of water (Li *et al.* 2018). Precipitation involves adding sodium acetate and ethanol to water samples (as in Dejean *et al.* (2011) and Harper *et al.* (2018)). However, only a small subsample (typically 15 ml, but up to 30 ml (Eichmiller *et al.* 2016; Li *et al.* 2018)) can be used, which could impact the amount of eDNA that is recovered (Eichmiller *et al.* 2016). Filtration methods, in contrast, can process larger water volumes (Li *et al.* 2018). Below we focus on filtration, as it is the most commonly used method (Smart *et al.* 2015; Shaw *et al.* 2016; Hinlo *et al.* 2017; Lugg *et al.* 2017; Tingley *et al.* 2018). Filtration can involve on-site filtration by hand (Lugg *et al.* 2017), using a peristaltic pump (Goldberg *et al.* 2011), or the collection of site water in sterile water bottles, which are later filtered in the laboratory using a vacuum pump (Piaggio *et al.* 2014; Smart *et al.* 2015).

The eDNA capture method selected should optimise eDNA preservation until DNA extraction can take place. In the case of filtering methods, there are various types of filters available, each made of different materials, and of open or closed varieties. Closed filters, which consist of a filter that is enclosed in a casing, have been used more recently in eDNA studies (Lugg *et al.* 2017; Spens *et al.* 2017). The advantage of using a closed filtering system is increased DNA preservation (reduced degradation) and potentially lower contamination risk (Spens *et al.* 2017). More traditional, open filter units require the filter to be handled, as well as the use of a filter funnel and vacuum pump, thus increasing contamination risk. The most efficient filtering membrane used in closed filters is polyethersulfone (PES), whereas open filters contain mixed cellulose ester membranes, polycarbonate track-etched filters, or glass fibre filters (Spens *et al.* 2017). Membrane type can also influence filter effectiveness, regardless of pore size. For example, smaller volumes can be filtered with polyvinylidene difluoride (PVDF) Sterivex 0.45 μm filters than with PES Sterivex 0.22 μm filters, due to the hydrophobic nature and protein retention of the former. There are various preservation methods for filters (in the field or laboratory), including drying, freezing, ethanol, or the use of a buffer, such as Longmire's solution (Goldberg *et al.* 2016). The preservation method selected may depend on availability of field resources; for example, the availability of a freezer at field sites. PCR can now also be conducted in the field using portable qPCR machines, such as BioMeme® (Philadelphia, PA, USA), meaning the presence/absence of a target species can be confirmed within approximately one hour of sampling.

The pore size of the filter used to capture eDNA and the amount of water sampled can impact the amount and quality of DNA extracted from environmental samples. The source of DNA, for example extracellular or cellular DNA, could also dictate the most appropriate pore size (Taberlet *et al.* 2012). Some studies have found that smaller pore sizes can retain greater quantities and smaller particles of eDNA (Shaw *et al.* 2017). Turner *et al.* (2014) found that common carp (*Cyprinus carpio*) eDNA (an invasive species in Australia) ranged in size from > 180 μm to < 0.2 μm, and recommended using a 0.2 μm pore size to capture these smaller particles. Eichmiller *et al.* (2016) and Liang and Keeley (2013) both found that smaller pore sizes (such as those around 0.2-0.6 μm) enabled the extraction of more eDNA. However, Li *et al.* (2018) found that pore size did not affect eDNA yield or species detectability, although they only tested filter pore sizes between 0.45 μm and 1.2 μm.

Given the emerging consensus in the literature that smaller pore sizes do not hinder, but in some cases, improve eDNA quantification, why might one consider a filter with a larger pore size? There is an inherent trade-off between filter pore size and the volume of water that can be passed through the filter (Mächler *et al.* 2016; Minamoto *et al.* 2016). Smaller pores get clogged more easily, and thus limit the amount of water that can be filtered for a given sample (Li *et al.* 2018). Filtration of a larger water volume has, for some species, shown to increase detection rate (Mächler *et al.* 2016). Presumably, filtering larger volumes of water increases the chance of capturing a species' DNA from a site; filtering small amounts of water risks missing the species' DNA entirely. However, to the best of our knowledge, this has not been rigorously tested across a range of species and therefore more research is required to improve our understanding of how water volume interacts with filter pore size for detectability of different species.

In practice, the choice of pore size will depend on the conditions of the study area. For example, in more turbid environments, such as farm dams or heavily disturbed sites, larger pore sizes may be required, as they will clog less often, enabling a larger volume of water to be processed. In clearer, faster-flowing streams, it may be preferential to use a smaller pore size to ensure smaller eDNA particles are captured. Collecting a larger quantity of water is also likely to become more important as water body size increases, although collecting additional water samples can help alleviate this issue.

How many samples?

The optimal number of samples to take at each site depends on the detectability of the target species. A species' detectability using eDNA depends on various ecological factors, including abundance (more individuals = more eDNA), life-history (fully aquatic or not), and

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habitat (fast-flowing river vs lentic pond). Detection probability also depends on the efficiency of the capture and DNA extraction methods, any sample interference (inhibition), and primer sensitivity (see Goldberg *et al.* 2016). The number of samples taken should reflect this detectability, as accurately estimating site occupancy of a species is generally the main objective of any biodiversity survey. Detectability can be estimated with a pilot study at field sites where a species is simultaneously observed, or via mesocosm or laboratory trials. In the absence of resources for such studies, detectability can be estimated using taxonomically- or ecologically-similar species, although this approach has not, to the best of our knowledge, been validated for eDNA sampling.

The reason that replicate samples are recommended is that there is likely to be heterogeneity between samples (Schmidt *et al.* 2013; Furlan *et al.* 2016; Shogren *et al.* 2017), due to the nature of random sampling, and the potential for uneven distribution of eDNA molecules in the environment (Hunter *et al.* 2015). This heterogeneity is introduced by the water sampling process; for any given water sample, there is an associated 'availability' probability (Schmidt *et al.* 2013). That is, there is a probability that the water sample in question actually captures a species' DNA from the site when present. Note that this replication is distinct from replication at the qPCR level (technical replication), in which multiple qPCR assays are run on each water sample (see ***Accounting for imperfect detection: SODM for eDNA data***).

Greater numbers of water samples may be required at sites that present difficult filtration conditions (e.g. high turbidity or PCR inhibition). Under such conditions, increasing the number of samples collected could increase the probability of capturing the eDNA of the target species in the sampled water. Mächler *et al.* (2016) detected a positive relationship between the volume of water sampled and detection rate for one macroinvertebrate species but found no relationship for two others. They speculated that this relationship could exist for the other two species, but that the smallest volume of water tested did not reach the lower limit of detection for those species, and thus no relationship was observed. A review undertaken by Willoughby *et al.* (2016) found that water volume did not impact species detectability, but the number of replicate samples taken at a site did, as also suggested by Furlan *et al.* (2016), Schmidt *et al.* (2013) and Shogren *et al.* (2017). The effect of water volume on species detectability is likely to be species-specific.

A final consideration is whether to collect replicate samples on a single site visit (which minimises travel costs) or whether to stagger sample collection over multiple visits to reduce stochastic variability in eDNA detections. Although focused on a single species, the results of Smart *et al.* (2016) suggest that the latter approach - collecting water samples on repeat site visits - is the more cost-efficient approach.

Recognising potential sources of false positives

As with many survey methods (e.g., aural detections, point-count surveys), there is potential for one or more species to be detected at an unoccupied site (i.e., false positive detections) with eDNA sampling. False positives can enter the detection process via several pathways in the field, including sample contamination, eDNA transport, and eDNA persistence (Darling and Mahon 2011; Evans *et al.* 2017).

Contamination can be minimised by ensuring protocols are in place to avoid contaminating samples and sites with a target species' DNA. Such protocols should, at a minimum, involve the sterilisation of field equipment, such as boots, and the use of single-use gloves and sampling equipment (e.g. syringes). Decontamination of field equipment for re-use (e.g. boots, buckets, trays, sampling poles, bottles) can be undertaken using a 10% commercial bleach solution (Smart *et al.* 2015). Entering the water should be avoided completely, if possible, and may necessitate extra sampling equipment (e.g. sampling poles). False positive detections at the site level can also arise via the movement of eDNA by a non-human source; for example, a predator could move an animal's body from one site to another, or leave traces of another species via its faeces.

Natural transport of eDNA downstream is also a potential source of false positives in lotic systems. Methods to reduce the chance of false positive detections in lotic systems include ensuring adequate distance between sampling sites, sampling over a short time period, or ensuring that sampling sites are not connected by waterways (Lugg *et al.* 2017). However, the exact location of an individual may be irrelevant when conducting management over large spatial scales (e.g., at the scale of entire catchments). Similarly, sampling along a waterway in multiple locations can provide insight into this movement via estimation of eDNA concentrations of target species from samples (samples further away from the location of the target species will likely display lower eDNA concentrations).

False positive detections can also arise in the field via prolonged eDNA persistence after the extirpation of a species at a site. Detecting a species in conditions that rapidly degrade eDNA (e.g., high UV, low alkalinity) suggests that an organism was present not long before sampling. In conditions that favour eDNA persistence, eDNA could be from the time of sampling, or it could simply be persisting in the environment, making the timescale of inference more uncertain. This source of false positives is likely less of a concern than the potential sources outlined above, given that many studies have found that eDNA degrades rapidly (days to weeks) in freshwater environments (Dejean *et al.* 2011; Piaggio *et al.* 2014). A species' DNA in the environment could be sourced from a live or dead organism, potentially resulting in a false positive result if the assumption is that the site is occupied by live organisms (Darling and Mahon 2011; Evans *et al.* 2017).

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False positive detections can also occur in the laboratory as a result of sample contamination, or as a result of more technical aspects of eDNA analysis, such as primer specificity (Wilcox *et al.* 2013) or bioinformatic processing (Deiner *et al.* 2017). There are steps that can be undertaken to determine laboratory contamination via the use of negative controls (during DNA extraction and PCR) and/or mock communities for metabarcoding.

Accounting for imperfect detection: SODM for eDNA data

Given the above considerations, detection is likely to be imperfect with eDNA data. Accounting for imperfect detection, using any survey technique, is vital to ensure accurate estimates of site occupancy. Fortunately, a rich statistical literature on species occupancy detection models (SODM) has been developed to account for imperfect detection in wildlife survey data, and these models are a natural framework in which to analyse eDNA data (Dorazio and Erickson 2018; Strickland and Roberts 2019). SODM estimate site occupancy whilst accounting for imperfect detection, and thus are ideal for eDNA data, in which replicate samples are collected at a set of sites, and there is potential for both false positive and false negative detections (Schmidt *et al.* 2013; Lahoz-Monfort *et al.* 2016; Guillera-Arroita *et al.* 2017; Lugg *et al.* 2017).

Hierarchical SODM, which account for nested detection processes, are particularly attractive for the analysis of eDNA data. This is because multiple PCRs are often nested within multiple water samples taken at each site. For example, Hunter *et al.* (2015), Lugg *et al.* (2017) and Schmidt *et al.* (2013) use a three-level SODM considering (i) the latent presence/absence of a species, given the occupancy probability; (ii) the probability of the eDNA being contained (or 'available') within the water sample, given (i); and (iii) the probability of detecting the species' eDNA using PCR, given (ii). Using such hierarchical models enables imperfect detection to be incorporated at the different levels of the eDNA sampling process. Guillera-Arroita *et al.* (2017) have shown how these models can be extended to account for false positive detections.

SODM can also be applied to multi-species metabarcoding. For example, Valentini *et al.* (2016) used SODM to estimate detection probabilities for multiple amphibian species.

Conclusion

Environmental DNA sampling has been used in a wide variety of ecological applications to date, and important methodological considerations are beginning to emerge from this diverse literature. Here we have highlighted the need to carefully consider these methodological aspects before implementing an eDNA-based monitoring program. When applied appropriately and complemented by analytical methods that account for imperfect detection, eDNA sampling can be an effective survey method for documenting the distributions of native and non-native species in freshwater systems. Its high sensitivity and use as a species- or community-level survey tool means that it has the capacity to complement, or in some situations replace, more traditional sampling methods. However, as with any emerging technology, it is important that we evaluate the strengths and limitations of eDNA sampling relative to traditional methods, and carefully consider the scientific or management objective at hand.

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