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PRACTICE INSIGHTS

The use of environmental DNA for biodiversity monitoring in lentic and lotic ecosystems

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Abstract

1. Global biodiversity is facing an extinction crisis leading to increasing pressure on industries to monitor their potential environmental impact. Relatedly, there is demand for more efficient biodiversity monitoring methods, resulting in growing interest in the use of environmental DNA (eDNA). Many questions, however, regarding the reliability of this relatively novel method remain, particularly for non-specialist end-users of the technology.
2. Here, the use of commercially available (in the UK) eDNA assays for monitoring freshwater fish and invertebrate biodiversity was compared to conventional surveillance techniques. Samples were collected from different habitats, on varying spatial scales and using multiple sampling regimes to assess how eDNA results were affected.
3. For aquatic macroinvertebrates and fish, more taxa were detected by eDNA than conventional surveys conducted in parallel, and for fish, all taxa detected by conventional monitoring were confirmed by eDNA.
4. For aquatic macroinvertebrates, several species were only detected through conventional methods, and the number of families detected by eDNA was lower than for conventional monitoring at all sites.
5. eDNA results varied significantly between sampling locations within lentic sites and, for lotic sites, with the number of subsamples collected.
6. *Practical implication*. This study demonstrates the need for bespoke sampling protocols when collecting eDNA samples. It also improves understanding of using eDNA for detecting aquatic taxa that could inform species surveillance protocols. These are essential if eDNA is to be used by practitioners as a regulatory monitoring tool.

KEYWORDS
aquatic invertebrates, biological conservation, environmental DNA, freshwater fish, metabarcoding

1 | INTRODUCTION

Human activities are driving global biodiversity loss through habitat destruction, land use change, pollution and the movement of invasive non-native species (INNS) (Newbold et al., 2015; Steffen et al., 2007; Tilman et al., 2017; Vitousek et al., 1997). In recognition of this threat, there is increasing pressure for industries to minimise and monitor their environmental impact. Biodiversity assessments are critical for this, however, these activities are often resource-limited resulting in an ever-growing demand for more efficient surveillance methods. This has contributed to an increasing interest in the ecological applications of DNA which can be extracted from environment samples (Rees et al., 2014; Thomsen & Willerslev, 2015). Environmental DNA, or eDNA, originates from organic material (Thomsen & Willerslev, 2015) and, once isolated, can be analysed using polymerase chain reaction based methods or metabarcoding.

There are many potential benefits in using eDNA for ecological monitoring, including that eDNA is often considered to be more sensitive than conventional surveying methods, making it possible to detect species at lower population densities (Blackman et al., 2020; Fediajevaite et al., 2020; McDevitt et al., 2019; Rees et al., 2014). This is particularly important for monitoring recent introductions of INNS or protected species that are often rare and/or in decline. Furthermore, collecting eDNA samples is comparatively quick, species identification is not reliant on taxonomic expertise and a single sample can be tested for several taxonomic groups. It is also a non-invasive technique, meaning there is less risk of disturbing protected species or accidentally spreading INNS.

Despite the potential benefits, the application of eDNA is still relatively novel in ecology, and many uncertainties around the reliability of this method remain. Species detectability using eDNA is known to vary with habitat type (Bedwell & Goldberg, 2020; Rees et al., 2014), environmental conditions (Johnsen et al., 2020), season (Strand et al., 2019) and sampling regime (Bedwell & Goldberg, 2020; Rees et al., 2014) but the exact effect of these variables is not fully understood, particularly between species. Therefore, ideally, eDNA methods need to be bespoke for the target habitat/taxa but very few regulator-approved eDNA protocols are available. Furthermore, whilst numerous eDNA assays are commercially available the level to which these have been validated, in accordance with the 1–5 validation scale developed by Thalinger et al. (2021), is often unclear.

Here, the use of commercially available (in the UK) eDNA assays for freshwater fish and invertebrate biodiversity monitoring was compared to conventional species surveillance techniques. Samples were collected from different habitats, at different spatial scales and using multiple sampling regimes to investigate how these influenced the eDNA results. The primary aim of the study was to generate information for practitioners on the usefulness of off-the-shelf eDNA assays for monitoring frequently surveyed taxonomic groups in the absence of validated regulator approved protocols. With the additional aim to improve understanding of how the results of these assays differed with habitat type and sampling regime (including spatial distribution of samples) to help practitioners optimise eDNA survey designs.

2 | MATERIALS AND METHODS

2.1 | Site details

Lentic sites comprised of three reservoirs in Yorkshire, England (UK) owned by a regional water company (Table 1). Lotic investigations were carried out at three riverine locations also in Yorkshire (Table 2).

2.2 | Sample collection and processing

All surveys, eDNA and conventional, were conducted in September 2021. All samples for eDNA testing were collected using commercially available water filtering kits in accordance with the supplier's instructions (NatureMetrics). Where feasible, samples were collected without entering the water to avoid sediment disturbance. If this was not possible, surveyors entered the watercourse downstream of the sample collection point where access allowed. In adherence with biosecurity best practice all personal protective and survey equipment was cleaned and disinfected using Virkon™ Aquatic between sites. To minimise contamination risk, all consumables/equipment used for collecting eDNA samples were either sterile disposable or cleaned with a 10% bleach

TABLE 1 Lentic site locations.

Site	National Grid reference	Size (ha)
Baitings Reservoir	SE 00736 18794	23.9
Longwood Compensation Reservoir	SE 10061 17299	2.8
Green Withens Reservoir	SD 98856 16323	21.4

TABLE 2 Lotic site locations.

Site	National Grid reference	Watercourse	Catchment
Windeldon Clough	SE 15954 02221	Clough Beck	Don
Breary Banks	SE 16711 79986	River Burn	Ure
Booth Dean Clough	SE 03472 18795	River Ryburn	Calder

solution prior to re-use. Clean, disposable nitrile gloves were always worn for sample collection.

For sites used to investigate spatial variables, samples from different locations within a site were collected using separate, disinfected (by cleaning with a 10% bleach solution) containers. At each of these locations, samples were collected comprising of different numbers of subsamples but for logistical reasons, it was not possible to also use separate disinfected containers to collect individual subsamples. It was also not always feasible to collect samples consisting of one or three subsamples before those consisting of five. Therefore, it should be noted that there was some risk of residual DNA being transferred between subsamples.

Conventional aquatic macroinvertebrate samples were collected by 3-min kick sampling in accordance with standard Environment Agency England operating procedures (EA OPs). Macroinvertebrate samples were preserved in 70% industrial methylated spirit and transported to APEM Ltd bio-laboratories for identification to at least family-level. At all sites, fish population surveys were carried out by electro-fishing following standard EA OPs. Fish were identified to species level in-situ by morphological examination before being returned to the watercourse.

2.2.1 | Lentic sites

Assessment of different eDNA sampling regimes and spatial variation

At each reservoir, samples for eDNA analysis were collected from six locations around the reservoir perimeter (see Table S1; James et al., 2024). At each location, three water samples were collected and processed separately. One of the three samples consisted of 1 L of water collected from a single point within the sampling location and processed using an eDNA kit. The second and third samples consisted of 500 mL of water collected from three and five subsample points respectively (5 m apart where access allowed, range 1–5 m) at the sampling location. Water collected for each sample was mixed in separate sterile collection bags and 1 L of each of these samples was processed using a separate eDNA kit (it should be noted that at Green Withens there were four samples for which it was only possible to filter between 740 and 930 mL of water due to high suspended solids).

2.2.2 | Lotic sites

Comparing eDNA with conventional monitoring techniques

Samples for eDNA testing were collected in parallel to conventional aquatic macroinvertebrate and fish surveys at three sites: Windeldon Clough, Breary Banks and Booth Dean Clough (Table 2; James et al., 2024). For eDNA sampling, water samples were collected immediately prior to conventional surveying. For each eDNA sample, as much water as possible was passed through the eDNA filter. This resulted in total filtration volumes of 1070, 2050 and 1970 mL for Windeldon Clough, Breary Banks and Booth Dean Clough, respectively. Invertebrate eDNA samples were taken at the same site as the conventional survey, whereas fish eDNA samples were taken at the downstream point of the survey reach.

Assessment of different eDNA sampling regimes and spatial variation

At all three lotic sites, two samples were collected at three different locations within each river (see Table S2; James et al., 2024). For one sample, 1 L of water was collected from a single point within the sampling location and processed using an eDNA kit. For the other sample, 500 mL of water was collected from three subsample points (5 m apart where access allowed, range 1–5 m) within the sampling location. For each sample, subsamples were mixed in a sterile collection bag and 1 L of the resulting mixture processed using an eDNA kit.

2.3 | Molecular analysis

All molecular analyses were performed by a commercial eDNA provider based in the UK (NatureMetrics) using internally modified assays (the details of which cannot be shared for intellectual property reasons).

All samples collected from lentic sites were analysed using a general invertebrate metabarcoding assay. Samples collected from lotic sites in tandem with conventional aquatic macroinvertebrate and fish surveys were analysed using invertebrate or fish metabarcoding assays respectively. All other samples collected from lotic sites were analysed using both invertebrate and fish metabarcoding assays.

2.4 | Statistical analysis

All analyses were carried out using R statistical software (version 3.6.0, R Core Team, 2019). A series of generalised linear mixed-effects models (GLMM) were run using the lme4 package (Bates et al., 2015) and assessed using residual plots as recommended by Thomas et al. (2015). The threshold for statistical significant used was $p \leq 0.05$.

2.4.1 | Lentic sites

Assessment of different eDNA sampling regimes and spatial variation

To assess whether the number of all invertebrate taxa (aquatic, terrestrial, microscopic and macroscopic) detected varied between samples that comprised of one, three or five subsamples, subsample number was included as a fixed effect with site number nested within reservoir as a random effect to account for replicate reservoirs and sites within. To determine whether the total number of invertebrate taxa detected varied between sites within a reservoir, site and reservoir were included as fixed effects, with subsample number included as a random effect.

2.4.2 | Lotic sites

Due to logistical limitations, not enough samples could be collected to enable robust statistical testing of the data gathered from the "*Comparison with conventional monitoring*" investigation. As such, interpretation of these data was based on descriptive statistics and graphical representations.

Assessment of different eDNA sampling regimes and spatial variation

To assess whether the total number of invertebrate taxa (aquatic, terrestrial, microscopic and macroscopic) or fish detected using metabarcoding varied between samples comprising of one or three subsamples, subsample number was included as a fixed effect with site number nested within river as a random effect to account for replicate sites and rivers. To determine whether the total number of invertebrate or fish taxa detected varied between sites across a river, site number and river were included as fixed effects and subsample number included as a random effect.

3 | RESULTS

3.1 | Lentic sites

3.1.1 | Assessment of different eDNA sampling regimes and spatial variation

The average number of invertebrate taxa (including aquatic and terrestrial macroscopic and microscopic organisms) detected by eDNA varied from 57.44 (±5.42 SE) at Baitings Reservoir to 36.06 (±5.45 SE) at Longwood Compensation and 34.22 (±3.02 SE) at Green Withens. Analysing the data for all reservoirs combined, the total number of invertebrate taxa detected did not vary with the number of subsamples per sampling site (GLMM, likelihood ratio test [LRT] = 2.27, $p = 0.32$), but did vary between the sampling sites across the reservoirs (GLMM, LRT = 14.94, $p = 0.01$; Figure 1).

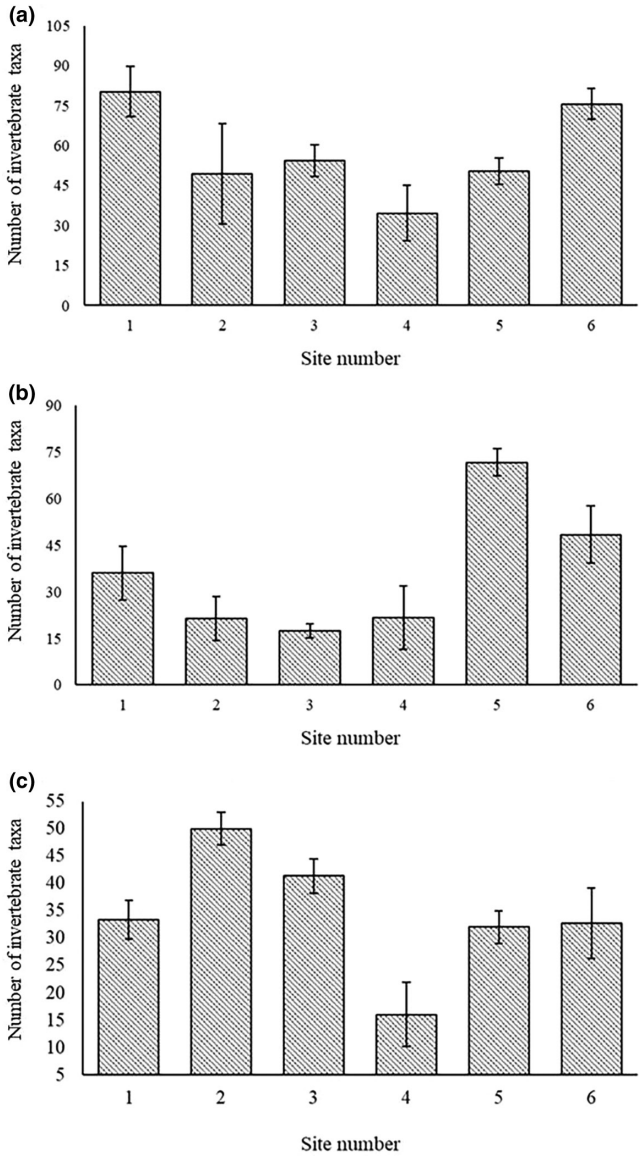

FIGURE 1 Number of invertebrate taxa detected using environmental DNA (mean ± SE) at each site within the reservoirs surveyed: (a) Baitings Reservoir, (b) Longwood Compensation Reservoir and (c) Green Withens Reservoir (Yorkshire, UK).

3.2 | Lotic sites

3.2.1 | Comparison with conventional monitoring

At all sites, a higher number of fish and aquatic macroinvertebrate taxa were detected using eDNA compared to conventional monitoring (Figure 2). For this comparison, non-aquatic and microscopic taxa detected by eDNA (which would not have been recorded by conventional methods) were excluded.

For each site, however, there were some aquatic macroinvertebrate taxa that were only detected by conventional monitoring and not through eDNA analysis (Figure 3). The percentage of the total taxa detected that were detected concurrently by both eDNA and conventional methods was relatively low, ranging from 16.8% to 22.75% (Figure 3). Furthermore, the number of aquatic macroinvertebrate families detected by conventional surveys was higher than for eDNA at all sites (Figure 4). For fish, all taxa found by conventional surveying were also detected through eDNA analysis.

3.2.2 | Assessment of different eDNA sampling regimes and spatial variation

The average number of invertebrate taxa (all species including macroscopic, microscopic, aquatic and terrestrial) detected within the lotic sites ranged from 59.00 (±7.61 SE) at Windeldon Clough to 73.00 at Breary Banks (±2.66 SE) and 60.00 at Booth Dean Clough (±5.02 SE). Analysing the data for all rivers combined, the total number of invertebrate taxa detected in samples comprising of three

subsamples was significantly more than for samples comprising of one subsample (GLMM, LRT = 3.75, $p = 0.05$, Figure 5). The total number of invertebrate taxa detected did not vary between the sampling sites across the rivers (GLMM, LRT = 0.79, $p = 0.67$).

The mean number of fish taxa detected within the lotic sites varied from 1.00 (±0.00 SE) at Windeldon Clough to 3.5 (±0.39 SE) at Breary Banks and 2.67 (±0.19 SE) at Booth Dean Clough. Analysing the data for all rivers combined, the total number of fish taxa detected in samples consisting of one subsample was significantly more than in samples consisting of three subsamples (GLMM, LRT = 6.15, $p = 0.01$, Figure 6). The total number of fish taxa detected did not vary between the sampling sites across the rivers (GLMM, LRT = 3.36, $p = 0.19$).

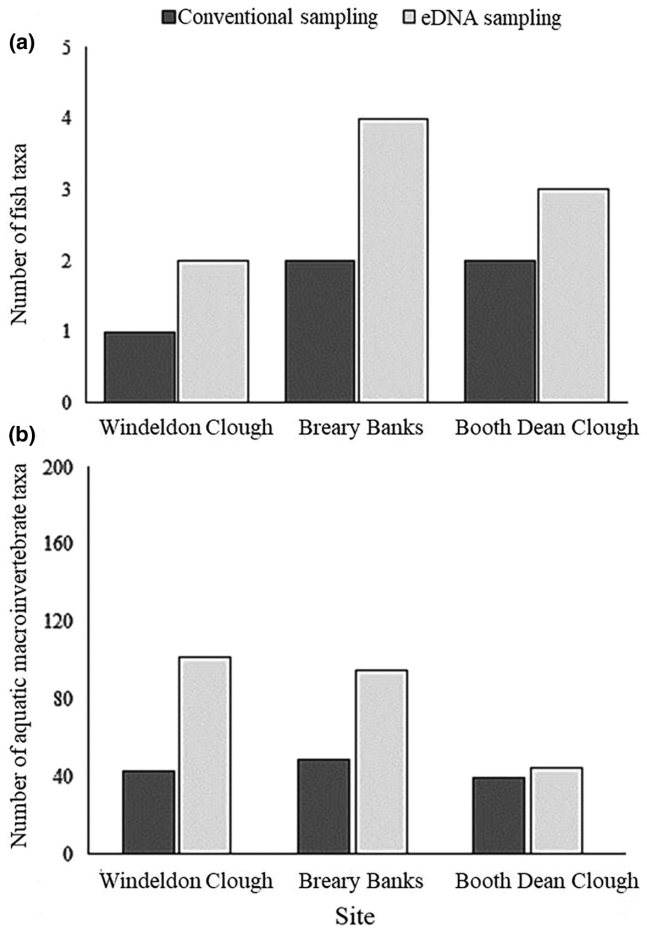

FIGURE 2 Number of (a) fish and (b) aquatic macroinvertebrate taxa detected in the three lotic sites surveyed using conventional and environmental DNA sampling techniques.

4 | DISCUSSION

Comparability between eDNA and conventional survey results varied between fish and aquatic macroinvertebrates. For fish, more species were detected using eDNA than electro-fishing conducted in parallel at each lotic site ($n = 3$). It should be considered though that detection of eDNA does not necessarily confirm species presence at the specific location the sample was taken, and these results could be influenced by the detection of DNA from an upstream population (Deiner & Altermatt, 2014), or DNA which has been introduced from outside of the system (Merkes et al., 2014). There was, however, good congruence between the fish species detected by both methods, with all the species located through electro-fishing being confirmed by eDNA and many of the additional species detected by eDNA being those known to be less frequently detected by electro-fishing (e.g. small species like *Gasterosteus aculeatus* or relatively rare species like *Lampetra* spp.).

For aquatic invertebrates, a greater number of taxa were detected by eDNA than kick-sampling conducted in tandem at each lotic site ($n = 3$), even after excluding non-aquatic and microscopic species. Unlike fish, however, there were several species detected by conventional surveying that were not detected by eDNA. Furthermore, a greater number of aquatic macroinvertebrate families were detected through kick-sampling than eDNA at all sites. This is likely due to differences in the detectability of different invertebrate families by eDNA, with eDNA analyses being more proficient at detecting more abundant, smaller, free-floating taxa and taxa that release more DNA into the environment (e.g. filter feeders as opposed to hard-bodied taxa like Coleoptera, Trichoptera and Hemiptera). These results suggest that the assay used can provide useful complementary species information but cannot currently be used to fully assess aquatic macroinvertebrate biodiversity.

In terms of optimising survey design, eDNA results differed between sampling locations in lentic but not lotic sites. This is likely to be related to the lack of water flow in lentic systems meaning that eDNA

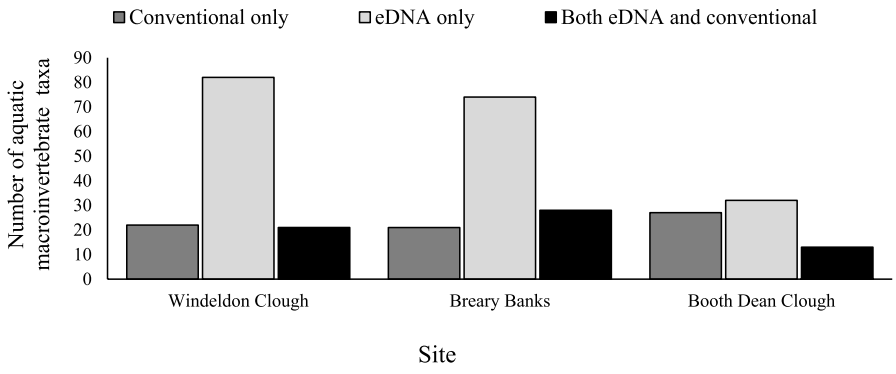

FIGURE 3 Number of aquatic macroinvertebrate taxa detected only through conventional surveys (dark grey), only through environmental DNA (eDNA) testing (light grey) and by both conventional and eDNA methods (black).

is not moved far, potentially <50 m from its point of origin (Bedwell & Goldberg, 2020; Dunker et al., 2016). While this may be helpful in trying to determine accurate distributions and micro-habitat uses, it can make detection more challenging than in lotic environments, particularly for low density or spatially confined populations. As such, eDNA surveys in lentic systems should aim to achieve as much spatial coverage as possible to maximise detection potential.

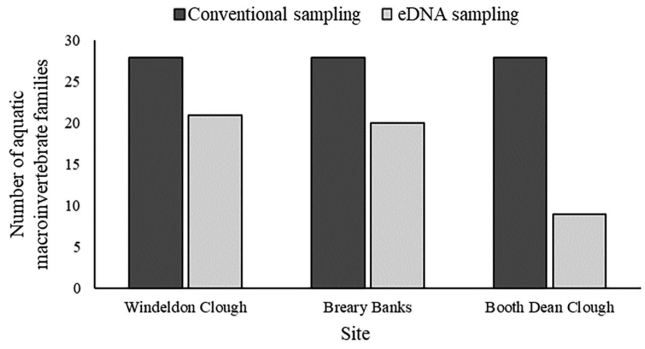

FIGURE 4 Number of aquatic macroinvertebrate families detected in the three lotic sites surveyed using conventional and environmental DNA (eDNA) sampling techniques.

In lentic systems, no significant difference was detected between samples comprising of different numbers of subsamples. The lack of sterilisation of some equipment (for logistical reasons) between collecting subsamples is a potential explanation for this. Disinfecting all equipment between subsampling locations may have provided finer scale information on the spatial distribution of eDNA within lentic systems. The main purpose of subsampling here, however, was to investigate whether collecting multiple subsamples across a transect would lead to increased detectability compared to point sampling. While we found no evidence of this, it is generally considered that samples from lentic sites should be collected to maximise spatial coverage. It should also be considered that while this result could be an artefact of sampling design it may also reflect a true similarity between samples comprising of different numbers of subsamples. The shoreline habitat appeared to be relatively homogenous within all lentic sites, so ecological communities were likely to have been similar between subsampling locations. Further work would help understand the effect of subsampling on eDNA results in lentic systems. From a practical perspective it should be noted though that fully disinfecting equipment between subsampling locations would require additional resources.

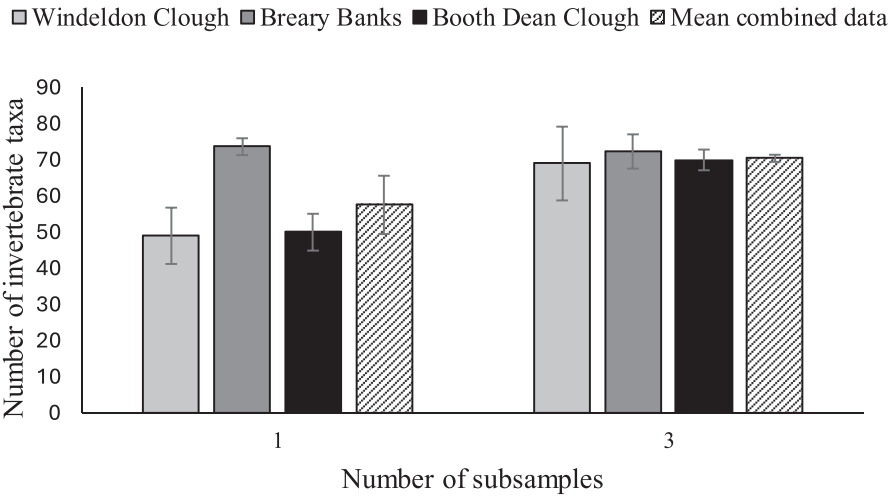

FIGURE 5 Number of invertebrate taxa detected in environmental DNA samples consisting of one or three subsamples taken from each lotic site and sites combined (mean ± SE).

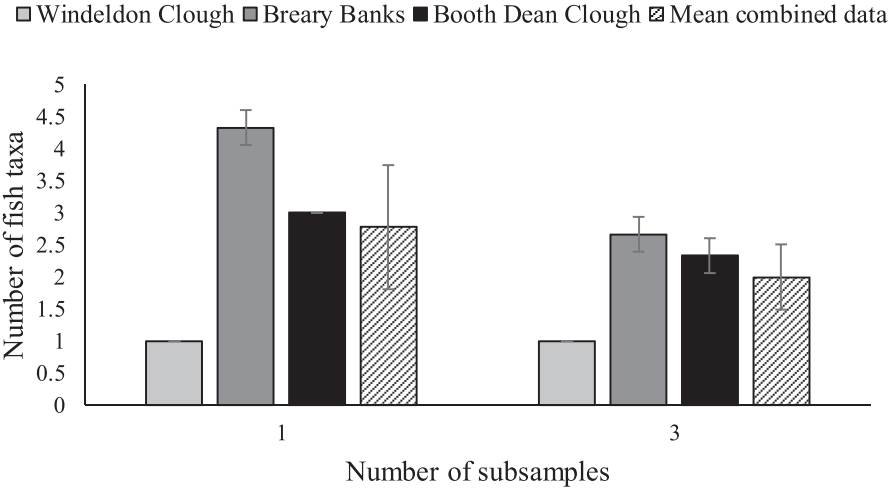

FIGURE 6 Number of fish taxa detected in environmental DNA samples consisting of one or three subsamples taken from each lotic site and sites combined (mean ± SE).

In lotic systems, the effect of sampling regime (i.e. the number of subsamples collected) was taxa specific. For invertebrates, more taxa were detected in samples consisting of three compared to one subsample, whereas for fish the reverse trend was observed. The reasons for these trends are unclear but likely related to numerous factors including population complexity and abundance, propensity to release DNA into the environment and species mobility. Further work investigating the effect of sampling regime on eDNA results (in lentic and lotic systems) is required but overall, this emphasises the need for taxa specific eDNA sampling protocols to be generated to maximise the outcomes of such surveys.

Overall, this study provides information which, in the absence of regulator-approved protocols, can be used by practitioners to inform decisions surrounding implementation of the eDNA assays investigated. The generation and sharing of such information is imperative for understanding, and potentially improving, eDNA assay validation in accordance with the widely adopted scale developed by Thalinger et al. (2021).

AUTHOR CONTRIBUTIONS

Jo James designed the study, collected eDNA samples, analysed and interpreted the data and wrote the manuscript. Emily M. Moore wrote and reviewed the manuscript. Rachel Naden conceptualised the research, designed the study and collected eDNA samples. Ben Aston conceptualised the research and reviewed the manuscript. Stephanie J. Bradbeer reviewed the manuscript and collected eDNA samples. Paul D. Stebbing designed the study and reviewed the manuscript.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflict of interest.

PEER REVIEW

The peer review history for this article is available at https://www.webofscience.com/api/gateway/wos/peer-review/10.1002/2688-8319.12361.

DATA AVAILABILITY STATEMENT

Data generated from this study are available in Dryad: https://doi.org/10.5061/dryad.1c59zw44f (James et al., 2024).

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

Table S1. eDNA subsample collection locations at each lentic site included in the sample regime investigation.

Table S2. eDNA subsample collection locations at each lotic site included in the sample regime investigation.

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