Fishing for mammals: landscape-level monitoring of terrestrial and semi-aquatic communities using eDNA from riverine systems

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Abstract

1. Environmental DNA (eDNA) metabarcoding has revolutionised biomonitoring in both marine and freshwater ecosystems. However, for semi-aquatic and terrestrial animals, the application of this technique remains relatively untested.

2. We first assess the efficiency of eDNA metabarcoding in detecting semi-aquatic and terrestrial mammals in natural lotic ecosystems in the UK by comparing sequence data recovered from water and sediment samples to the mammalian communities expected from historical data. Secondly, using occupancy modelling we compared the detection efficiency of eDNA metabarcoding to multiple conventional non-invasive survey methods (latrine surveys and camera trapping).

3. eDNA metabarcoding detected a large proportion of the expected mammalian community within each area. Common species in the areas were detected at the majority of sites. Several key species of conservation concern in the UK were detected by eDNA sampling in areas where authenticated records do not currently exist, but potential false positives were also identified.

4. Water-based eDNA metabarcoding provided comparable results to conventional survey methods in per unit of survey effort for three species (water vole, field vole, and red deer) using occupancy models. The comparison between survey 'effort' to reach a detection probability of ≥0.95 revealed that 3-6 water replicates would be equivalent to 3-5 latrine surveys and 5-30 weeks of single camera deployment, depending on the species.

5. *Synthesis and applications.* eDNA metabarcoding can be used to generate an initial 'distribution map' of mammalian diversity at the landscape level. If conducted during times of peak abundance, carefully chosen sampling points along multiple river

courses provide a reliable snapshot of the species that are present in a catchment area. In order to fully capture solitary, rare and invasive species, we would currently recommend the use of eDNA metabarcoding alongside other non-invasive surveying methods (i.e. camera traps) to maximize monitoring efforts.

Keywords: biomonitoring, camera trapping, eDNA metabarcoding, latrine surveys, mammals, occupancy modelling, rivers

Introduction

Environmental DNA (eDNA) metabarcoding (the simultaneous identification of multiple taxa using DNA extracted from an environmental sample, e.g. water, soil, based on short amplicon sequences) has revolutionised the way we approach biodiversity monitoring in both marine and freshwater ecosystems (Valentini et al., 2016; Deiner et al. 2017). Successful applications include tracking biological invasions, detecting rare and endangered species and describing entire communities (Holman et al., 2019). Most eDNA metabarcoding applications on vertebrates to date have focused on monitoring fishes and amphibians (Hänfling et al., 2016; Valentini et al., 2016). What has become apparent from studies in lentic systems (ponds and lakes) is that semi-aquatic and terrestrial mammals can also be detected (Hänfling et al., 2016; Harper et al., 2019). As a result, there has been an increasing focus on the use of both vertebrate (Harper et al., 2019) and mammal-specific primer sets (Ushio et al., 2017; Leempoel et al., 2020; Sales et al., 2020a) for detecting mammalian communities using eDNA metabarcoding.

Mammals include some of the most imperiled taxa, with over one fifth of species considered to be threatened or declining (Visconti et al., 2011). Monitoring of mammalian biodiversity is therefore essential. Given that any optimal survey approach is likely to be species-specific, very few species can be detected at all times when they are present. This imperfect detection (even greater for elusive and rare species) can lead to biased estimates of occurrence and hinder species conservation (Mackenzie et al., 2002). For mammals, repeated surveys using several monitoring methods are usually applied. These include indirect observations such as latrines, faeces, hair, or tracks, or direct observations such as live-trapping or camera trapping surveys over short time intervals such that closure/invariance can be assumed and detectability

83 estimated (Nichols et al., 2008). Each of these methods has associated efficiency, cost
84 and required expertise trade-offs, which become more challenging as the spatial and
85 temporal scales increase.

86 eDNA sampling yields species-specific presence/absence data that are likely
87 to be most valuable for inferring species distributions using well established analytical
88 tools such as occupancy models (MacKenzie et al., 2002). These models resolve
89 concerns around imperfect detection of difficult to observe species. When coupled with
90 location-specific detection histories, these can be used to infer true occurrence states,
91 factors that influence occupancy rates, colonization-extinction probabilities, and
92 estimates of detection probability (MacKenzie et al., 2017). The use of eDNA sampling
93 to generate species-specific detection data has unsurprisingly increased in recent
94 years, and in many cases has outperformed or at least matched conventional survey
95 methods (Lugg et al., 2018; Tingley et al., 2019). Although comparisons between
96 eDNA analysis and conventional surveys for multi-species detection are numerous
97 (see Table S1 in Lugg et al., 2018), studies focusing on detection probability estimates
98 for multiple species identified by metabarcoding are rare (Abrams et al., 2019;
99 Valentini et al., 2016).

100 The aim of this study was to assess the efficiency of eDNA metabarcoding for
101 detecting semi-aquatic and terrestrial mammals in natural lotic systems in the UK. We
102 conducted eDNA sampling in rivers and streams in two areas (Assynt, Scotland and
103 Peak District National Park, England). Together these locations have the majority of
104 UK semi-aquatic and terrestrial mammalian species present (Table S1). Our
105 objectives were two-fold: first, we sought to establish whether eDNA metabarcoding is
106 a viable technique for monitoring semi-aquatic and terrestrial mammals by comparing
107 it to the mammalian communities expected from historical data, a group for which

eDNA sampling has rarely been evaluated in a natural setting. Secondly, we evaluate the detection efficiency of water- and sediment-based eDNA sampling in one of these areas (Assynt) for multiple species compared to multiple conventional non-invasive survey methods (latrine surveys and camera trapping).

Materials and Methods

Latrine surveys

Assynt, a heather-dominated upland landscape in the far northwest of the Scottish Highlands, UK (Fig. 1A), is the location of an ongoing 20-year metapopulation study of water voles (*Arvicola amphibius*) led by the University of Aberdeen (Fig. S1). Here, we mainly focus only on data collected in 2017. The metapopulation is characterized by 116 discrete linear riparian habitat patches (ranging from 90 m to nearly 2.5 km) distributed sparsely (4% of waterway network) throughout the 140 km^2 study area (Sutherland et al., 2014). Water voles use prominently placed latrines for territory marking (Fig. S2A). Using latrine surveys, a reliable method of detection (Sutherland et al., 2014), water vole occupancy status was determined by the detection of latrines that are used for territory marking (Sutherland et al., 2013). During the breeding season (July and August), latrine surveys were conducted twice at each site. In addition to water vole latrines, field vole (*Microtus agrestis*) pellets are also easily identifiable, and so field vole detections were also recorded along waterways as a formal part of the latrine survey protocol. Live-trapping was then carried out at patches deemed to be occupied by water voles according to latrine surveys to determine their abundances (this was used to determine which sites were sampled for eDNA; Fig. 1A).

Camera Trap Data

Camera traps were deployed at the beginning of July and thus overlapped temporally with the latrine survey in Assynt. Data were collected from cameras deployed at seven of these patches. Within each of these patches, cameras were deployed at the midpoint of the areas where active signs (latrines, grass clipping, burrows) were

137 detected, and if no signs were detected, at the midpoint of historical water vole activity
138 (J. Drake, C. Sutherland and X. Lambin, *pers. comm*.). These will also capture images
139 of any species present in the area that come within close proximity of the camera (Fig.
140 S3A-F).

141 Cameras were deployed approximately 1 m above ground on iron 'u-posts' to
142 avoid flooding, prevent knock-down by wind/wildlife, and optimize both depth of field
143 and image clarity. Cameras (Bushnell HD Trophy Cam, Overland Park, KA) were set
144 at normal detection sensitivity (to reduce false-triggers from grass/shadows), low night
145 time LED intensity (to prevent image white out in near depth of field), three shot burst
146 (to increase chance of capturing small, fast moving bodies), and 15 min intervals
147 between bursts (to increase temporal independence of captures and decrease
148 memory burden). The area each camera photographed was approximately 1-2 m^2.
149 Animals were identified on images and information was stored as metadata tags using
150 the R (R Core Team, 2018) package camtrapR following the procedures described in
151 Niedballa et al. (2018). Independence between detections was based on 60-minute
152 intervals between species-specific detections.

153

154 ***eDNA sampling***

155 A total of 18 potential water vole patches were selected for eDNA sampling in Assynt
156 from 25-27th October 2017. The time lag between the latrine/live-trapping and eDNA
157 surveys was because of two main reasons: (i) legitimate concerns around cross-site
158 DNA contamination during latrine/live-trapping where researchers moved on a daily
159 basis between sites as well as regularly handled and processed live animals (for
160 decontamination procedures see the Supplementary Material) and (ii) the selection of
161 eDNA sampling sites was based on the latrine surveys and abundance data provided

by live-trapping so could only occur after this was completed by August 6th. Water and sediment samples were collected from patches where water voles were determined to be absent (five sites; A1-A5); with 1-2 individuals present (three sites; A9, A16 and 18); 3-5 individuals (five sites; A6, A8, A11, A14 and A17); and 7-11 individuals (five sites; A7, A10, A12, A13 and A15; Fig. 1A). Each of these streams/rivers differed in their characteristics (in terms of width, depth and flow) and a representation of the sites is depicted in Fig. S4A-D. Three water (two litres each) and three sediment (~25mL) replicates were taken at each patch (further details of sample collection are provided in Appendix S1).

In addition to Assynt, eDNA sampling was also conducted on a smaller scale in the Peak District National Park, England (Fig. S5) to incorporate additional mammals that are not known to be present in Assynt (Table S1). Here, the occurrence of water vole was identified by the presence of latrines in two sites (P1 and P2) at the time of eDNA sampling (Fig. S2A), whilst no latrines were identified at one site (P3). At site P1, an otter (*Lutra lutra*) spraint was identified at the time of eDNA sampling (Fig. S2B). These three sites were sampled in March 2018 using the same methodology as in Assynt but were taken in close proximity (<50cm) to water vole latrines where present (Fig. S2A).

eDNA Laboratory Methods

DNA was extracted from the sediment samples using the DNeasy PowerMax Soil kit and from the water samples using the DNeasy PowerWater Kit (both QIAGEN Ltd.) following the manufacturer's instructions in a dedicated eDNA laboratory in the University of Salford. In order to avoid the risk of contamination during this step, DNA extraction was conducted in increasing order of expected abundance of water voles in

187 the eDNA samples (all field blanks were extracted first, followed by the sites with
188 supposedly zero water vole abundance, up to the highest densities last). Along with
189 field blanks (Assynt = 8, Peak District = 2), six lab extraction blanks were included
190 (one at the end of each daily block of extractions). A decontamination stage using a
191 Phileas 25 Airborne Disinfection Unit (Devea SAS) was undertaken before processing
192 samples from different locations. Additional information regarding decontamination
193 measures and negative controls can be found in the Supplementary Material.

194 A complete description of PCR conditions, library preparation and bioinformatic
195 analyses are provided in Appendix S1. Briefly, eDNA was amplified using the
196 MiMammal 12S primer set (MiMammal-U-F, 5′- GGGTTGGTAAATTTCGTGCCAGC-
197 3′; MiMammal-U-R, 5′- CATAGTGGGGTATCTAATCCCAGTTTG-3′; Ushio et al.,
198 2017) targeting a ˜170bp amplicon from a variable region of the 12S rRNA
199 mitochondrial gene. A total of 147 samples, including field collection blanks (10) and
200 laboratory negative controls (12, including six DNA extractions blanks and six PCR
201 negative controls), were sequenced in two multiplexed Illumina MiSeq runs. To
202 minimize bias in individual reactions, PCRs were replicated three times for each
203 sample and subsequently pooled. Illumina libraries were built using a NextFlex PCR-
204 free library preparation kit according to the manufacturer's protocols (Bioo Scientific)
205 and pooled in equimolar concentrations along with 1% PhiX (v3, Illumina). The libraries
206 were run at a final molarity of 9pM on an Illumina MiSeq platform using the 2 x 150bp
207 v2 chemistry.

208 Bioinformatic analysis were conducted using OBITools metabarcoding package
209 (Boyer et al., 2016) and the taxonomic assignment was conducted using ecotag
210 against a custom reference database (see Appendix 1). To exclude MOTUs/reads
211 putatively belonging to sequencing errors or contamination, the final dataset included

only MOTUs that could be identified to species level (>98%), and MOTUs containing less than 10 reads and with a similarity to a sequence in the reference database lower than 98% were discarded (Cilleros et al., 2019). The maximum number of reads detected in the controls for each MOTU in each sequencing run were removed from all samples (Table S7). For water voles, field voles and red deer (the most abundant wild mammals in terms of sequence reads in our dataset), this equated to a sequence frequency threshold of ≤0.17%, within the bounds of previous studies on removing sequences to account for contamination and tag jumping (Cilleros et al., 2019; Hänfling et al., 2016; Schnell, Bohmann, & Gilbert, 2015).

Occupancy/Detection Analysis in Assynt

The data collection from the different survey types described above (water-based eDNA, sediment-based eDNA, latrine and camera traps) produced the following site-specific detection/non-detection data:

(a) Latrine: two latrine surveys at 116 patches.
(b) w-eDNA: three water-based eDNA samples at 18 of the 116 patches surveyed.
(c) s-eDNA: three sediment-based eDNA samples at 18 of the 116 patches surveyed.
(d) Camera: six one-week occasions of camera trapping data at seven of the 18 patches surveyed by both Latrine and eDNA (w-eDNA + s-eDNA) surveys.

We chose to focus on three species that were detected by at least three of the four methods: water voles, field voles and red deer (*Cervus elaphus*). Water voles and field voles were recorded using all four survey methods and had detection histories for 14 surveying events ((Latrine × 2) + (w-eDNA × 3) + (s-eDNA × 3) + (Camera × 6)).

Red deer were not recorded during latrine surveys and had detection histories for 12 surveying events ((w-eDNA × 3) + (s-eDNA × 3) + (Camera × 6)). To demonstrate the relative efficacy of the four surveying methods, we restricted the analyses to the 18 sites where both latrine surveys were conducted and eDNA samples were taken, seven of which had associated camera trapping data. Although each surveying method differs in terms of effort and effective area surveyed, each are viable surveying methods that are readily applied in practice. A unit of survey effort here is defined as one latrine survey, one w-eDNA replicate, one s-eDNA replicate or one week of camera trapping. So, while the specific units of effort are not directly comparable, the relative detection efficacy per surveying method-specific unit of effort is of interest and will provide important context for designing future monitoring studies and understanding the relative merits of each surveying method. Analyzing the data using occupancy models allowing for method-specific detectability enables such a comparison in per unit effort efficacy between eDNA metabarcoding and multiple conventional survey methods.

A single season occupancy model (MacKenzie et al., 2002) was applied to the ensemble data where detection histories were constructed using each of the surveying events as sampling occasions (MacKenzie et al., 2017). The core assumption here is that the underlying occupancy state (i.e. occupied or empty) is constant over the sampling period, and therefore, every sampling occasion is a potentially imperfect observation of the true occupancy status. Because occasions represent method-specific surveying events, we used “surveying method” as an occasion-specific covariate on detection (Latrine, w-eDNA, s-eDNA and Camera). Our primary objective was to quantify and compare method-specific detectability, so we did not consider any

261 other competing models. For comparing the methods, we compute accumulation
262 curves as (MacKenzie & Royle, 2005):
263
264 $$p^*_{smk} = 1 - (1 - \hat{p}_{sm})^k$$
265
266 Where p^*_{smk} is the cumulative probability of detecting species *s*, when species *s* is
267 present, using method *m* after *k* surveying events based on the estimated surveying
268 method-specific detection probability for each species ($\hat{p}_{sm}$). We vary k from 1 to a
269 large number and find the value of *k* that results $p^*_{smk} \geq 0.95$. We conducted the same
270 analysis separately for water voles, field voles, and red deer. Analysis was conducted
271 in R (R Core Team, 2018) using the package unmarked (Fiske & Chandler, 2011).

Results

Mammal Detection via eDNA metabarcoding

The two sequencing runs generated 23,276,596 raw sequence reads and a total of 15,463,404 sequences remained following trimming, merging, and length filtering. After bioinformatic analysis, the final 'filtered' dataset contained 23 mammals (Tables S2 and S3). For mammals, ~12 million reads were retained after applying all quality filtering steps (see Appendix 1). Reads from humans, cattle (*Bos taurus*), pig (*Sus scrofa*), horse (*Equus ferus*), sheep (*Ovis aries*) and dog (*Canis lupus familiaris*), were not considered further as the focus of this study was on wild mammals (Table S4). *Felis* was included because of the potential of it being wildcat (*Felis silvestris*) or domestic cat (*F. catus*)/wildcat hybrids. A final dataset comprising ~5.9 million reads was used for the downstream analyses (Table S4).

In Assynt, the wild species identified were the red deer (18/18 sites); water vole (15/18); field vole (13/18); wood mouse (*Apodemus sylvaticus* - 9/18); pygmy shrew (*Sorex minutus* - 4/18); wild/domestic cat (*Felis* spp. - 4/18); mountain hare (*Lepus timidus* - 4/18); rabbit (*Oryctolagus cuniculus* - 3/18); water shrew (*Neomys fodiens* - 3/18); common shrew (*Sorex araneus* - 2/18); edible dormouse (*Glis glis* - 2/18); grey squirrel (*Sciurus carolinensis* - 1/18); pine marten (*Martes martes* - 1/18); brown rat (*Rattus norvegicus* - 1/18); red fox (*Vulpes vulpes* - 1/18) and badger (*Meles meles* - 1/18; Fig. 1B). All of these species are distributed around/within Assynt (Table S1), with the exception of the edible dormouse and the grey squirrel. These are unequivocally absent from the region. The edible dormouse is only present in southern England and the grey squirrel is not distributed that far north in Scotland (Mathews et al., 2018).

296 Of the wild mammals in the Peak District, the water vole, field vole, wood mouse
297 and otter were found in two sites (P1 and P2). The red deer, pygmy shrew, common
298 shrew, water shrew, red squirrel (*Sciurus vulgaris*), grey squirrel, pine marten and
299 badger were each found at a single site (Fig. S5). Only rabbit was found in site P3. All
300 species identified are currently distributed within the Park (Table S1), except the red
301 squirrel and pine marten. The pine marten, which is critically endangered in England,
302 has only two reliable records that have been confirmed in the Park since 2000 and the
303 red squirrel has not been present for over 18 years (Alston et al. 2012).
304 Overall, water samples yielded better results than sediment samples regarding
305 species detection and read count for both areas sampled (Figs 1B and S5). In Assynt,
306 only the wild/domestic cat was exclusively detected in sediment samples (four sites),
307 whereas water samples recovered eDNA for ten additional species not found in the
308 sediment samples. The red deer, water vole, field vole, mountain hare and pygmy
309 shrew were also found in sediment samples in Assynt (Fig. 1B), and water vole and
310 wood mouse in the Peak District sediment samples (Fig. S5).
311
312 **Occupancy Analysis**
313 Of the 18 sites where both latrine and eDNA surveys were conducted, water voles
314 were detected at 13, and field voles were detected at 11. A total of seven wild
315 mammals were recorded at the seven sites with a camera trap from July 10th to
316 October 25th, 2017 (Fig. S3 and Table S5). There were several incidences where a
317 shrew could not be identified to species level using camera traps. For camera traps,
318 water voles were recorded at all sites, red deer at five out of seven, field voles and
319 weasels at three sites, water shrews and otters at two, and a red fox at a single site.

For the 18 sites in Assynt, estimated site occupancy (with 95% confidence intervals) from the combined surveying methods was 0.91 (0.63 – 0.98) for water voles and 0.88 (0.57 – 0.98) for field voles. Red deer were observed at every patch by at least one of the methods, and therefore occupancy was 1 (Table 1). For all three species, per sample detection probability was higher for eDNA taken from water than for eDNA taken from sediment (Table 1, Fig. 2). The surveying method specific efficacy pattern was similar for water voles and field voles (Table 1, Fig. 2): latrine surveys had the highest probability of detecting the species (0.77 and 0.52 respectively), followed by eDNA from water (0.57 and 0.40 respectively), then camera trapping (0.50 and 0.20 respectively), and finally eDNA from sediment (0.27 and 0.02 respectively). Detection probability was higher for water voles than field voles using all four methods (Table 1, Fig. 2). No effort was made to record red deer presence during latrine surveys. Like the water voles and field voles, red deer detection has higher using eDNA from water (0.67, CI: 0.53 – 0.78) compared to eDNA from sediment (0.10, CI: 0.04 – 0.21). Unlike the voles, which were more detectable by cameras than sediment eDNA, red deer detection on cameras was similar to sediment eDNA (0.10, CI: 0.04 – 0.24).

The patterns described above detail surveying event-specific detectability. We also computed the cumulative detection probability for each method and each species ($\hat{p}_{sm}$). The cumulative detection curves over 15 surveying events are shown in Fig. 2. The number of surveying events, *k*, required to achieve $p^*_{psm} \geq 0.95$ for water voles was 3 surveys, 4 samples, 10 samples, and 5 weeks, for latrines, water eDNA, sediment eDNA, and cameras respectively. The number of surveying events, *k*, required to achieve $p^*_{psm} \geq 0.95$ for field voles was 5 surveys, 6 samples, 141 samples, and 14 weeks, for latrines, water eDNA, sediment eDNA, and cameras respectively.

345 The number of surveying events, *k*, required to achieve $p^*_{psm} \geq 0.95$ for red deer was
346 3 samples, 30 samples, and 29 weeks, for water eDNA, sediment eDNA, and cameras
347 respectively (see also Fig. 2).

Discussion

Despite the increasing potential of eDNA metabarcoding as a biomonitoring tool (Deiner et al., 2017), its application has largely been focused on strictly aquatic or semi-aquatic animals, thus restricting management and conservation efforts of the wider ecosystem (Williams et al., 2018). Here, we demonstrate the ability of eDNA metabarcoding to provide a valuable 'terrestrial dividend' for mammals from freshwater lotic ecosystems, with a large proportion of the expected species from the wider landscape being detected in each of the two study locations. In particular, we have demonstrated that water-based eDNA sampling offers a promising and complementary tool to conventional survey methods for the detection of whole mammalian communities.

Detection of mammalian communities using eDNA metabarcoding

Of the species known to be common in both Assynt and the Peak District, eDNA metabarcoding readily detected the water vole, field vole and red deer at the majority of sites surveyed (Figs. 1B and S5). Pygmy, common and water shrews, wood mice and mountain hares were also detected by eDNA metabarcoding at multiple sites in Assynt (Fig. 1B). A higher eDNA detection rate is expected for aquatic and semi-aquatic mammals compared to terrestrial mammals in aquatic environments due to the spatial and temporal stochasticity of opportunities for terrestrial mammals to be in contact with the water (Ushio et al., 2017). The semi-aquatic water vole was generally detected by eDNA metabarcoding where we expected to find it and at relatively high read numbers (Figs. 1B, S1 and S5). This is in line with previous studies in lentic systems (Harper et al., 2019). However, the red deer was the only terrestrial species

372 detected by eDNA sampling at all sites in Assynt, and the terrestrial field vole at over
373 70% of surveyed sites.

374 In addition to lifestyle (semi-aquatic or terrestrial), the number of individuals of
375 each species (i.e. group-living) may be important for eDNA detection (Williams et al.,
376 2018). As a counter example to this, otters and weasels were notably absent in the
377 eDNA samples in Assynt despite being captured by camera traps (Fig. S3 and Table
378 S5). Otters were present in the water eDNA samples at two sites in the Peak District,
379 albeit at a lower number of reads in comparison to most of the other species detected
380 (Fig. S5; Table S2). This mirrors previous studies where eDNA analysis has performed
381 relatively poorly for otter detection in captivity and the wild (Harper et al., 2019;
382 Thomsen et al., 2012). Carnivores were generally detected on fewer occasions (e.g.
383 red foxes, badgers and pine martens; Figs. 1B and S5) or not at all (e.g. stoats and
384 American mink in addition to those discussed above) in comparison to smaller
385 mammals and red deer, and a similar pattern has been shown with North American
386 carnivores in a recent study using eDNA from soil samples (Leempoel et al., 2020).
387 For some of these species, species ecology/behavior such as a relatively large home
388 range and more solitary nature (e.g. red foxes) may go some way towards explaining
389 a lack of, or few, eDNA records. Furthermore, as demonstrated by Ushio et al. (2017)
390 poor efficiency for amplifying some mammal species might be associated to
391 suboptimal experimental conditions (e.g. inadequate primer design, primer bias, DNA
392 concentration, species masking and/or annealing temperatures).

393 Regarding the sampling medium for eDNA, we demonstrated that water is a
394 more effective method for detection of mammal eDNA than sediment (Table 1; Figs.
395 1B and S5). For one of our focal species, the water vole, 75% of sites which were
396 deemed unoccupied by latrine surveys and those with ≤2 individuals (8 sites) in

Assynt, returned a non-detection for sediment eDNA as opposed to 37.5% of sites for water (Figs. 1A, 1B and S1). Distinct temporal inferences are provided by eDNA recovered from water and sediment samples. DNA bound to sediments can remain detectable for a longer period (i.e. up to hundreds of years) and provide historical data, whereas, eDNA retrieved from water samples provide more contemporary data due to a faster degradation in the water column (Turner et al., 2015). It is worth investigating further if sediment eDNA could indicate the presence of a more 'established' population, where a certain threshold of individuals and long-term occupation (i.e. historical) is required for detection in sediment (Fig. S1; Turner et al., 2015; Leempoel et al., 2020).

Importantly, sparse or single eDNA records should be carefully verified. The edible dormouse and grey squirrel sequences identified within the Assynt samples (Fig. 1B) and red squirrel within the Peak District (Fig. S5) highlights the caveats associated with this technique. If management decisions had relied on eDNA evidence alone, false positives for these species could lead to unnecessary resources being allocated for management/eradication programmes as the edible dormouse and grey squirrel are classified as invasive species within Great Britain. These potentially arose due to sample carryover from a previous sequencing run on the same instrument (a known issue with Illumina sequencing platforms; Nelson et al., 2014) which included those species for the reference database construction. Controlling for false positives is certainly a huge challenge in eDNA metabarcoding and the need to standardize and optimize thresholds for doing so is an ongoing debate (Ficetola et al., 2015; Harper et al., 2019).

Even with these concerns around false positives highlighted, two records are potentially noteworthy in a conservation context for UK mammals because of the

422 relatively high read number associated with these records (Tables S2 and S3). The
423 first of these is the *Felis* records in sediment samples in multiple sites in Assynt (Fig.
424 1B). Even with 'pure' *F. silvestris* as reference sequences, it was not possible to
425 distinguish between the wild and domesticated species for this 12S fragment (data not
426 shown). Despite ongoing conservation efforts, there may now be no 'pure' Scottish
427 wildcats left in the wild in the UK but isolated populations (perhaps of hybrid origin)
428 may exist in this region (Sainsbury et al., 2019). Given that these eDNA detections
429 were all from sediment samples, it is possible that they may be historical rather than
430 contemporary (see above). The other significant eDNA record was the pine marten in
431 the Peak District. The pine marten (*Martes martes*) is known to occur in the Scottish
432 Highlands but had disappeared from most of the UK and recently has been recovering
433 from historical persecution, including a potential expansion of its range. Still, authentic
434 records from northern England are scarce or lacking altogether (Alston et al., 2012;
435 Sainsbury et al., 2019). However, a record of a recent roadkill exists from just outside
436 the Park's boundary (BBC News, 2018). The high number of reads recovered for the
437 Peak District sample (4293 reads versus 25 in the Assynt sample) adds credence to
438 this positive eDNA detection but further investigations are warranted into the potential
439 presence of this species in the area.
440
441 *Comparisons between surveying methods*
442 Comparisons of species detection by traditional survey approaches and eDNA
443 analysis are now numerous in the literature, and mainly focus on what is and what is
444 not detected within and across different methods (Hänfling et al., 2016; Leempoel et
445 al., 2020). Yet, there has been growing incorporation of occupancy modelling to
446 estimate the probability of detecting the focal species, in comparison to one other

447 survey method, either for a single species (Lugg et al., 2018; Tingley et al., 2019) or
448 multiple species (Valentini, et al., 2016; Abrams et al., 2019). Simultaneous multi-
449 method comparisons for multiple species have been lacking and this study directly
450 addresses this for the first time.

451 The probability of detecting the water vole and field vole was higher for the
452 latrine surveys than eDNA sampling (both water and sediment) and camera traps
453 (Table 1; Fig. 2). However, when considering confidence intervals, there was
454 considerable overlap between latrine, water-based eDNA metabarcoding and camera
455 traps for both species, with only sediment-based eDNA metabarcoding yielding a low
456 probability of detection (Table 1). Detection probabilities for water-based eDNA
457 metabarcoding and camera traps were similar for water voles, with camera traps less
458 likely to detect the field vole than water-based eDNA. For the red deer (for which no
459 latrine survey was undertaken), water-based eDNA metabarcoding had a much higher
460 probability of detection than either sediment-based eDNA metabarcoding or camera
461 traps (which performed similarly; Table 1). Despite the increasing adoption of camera
462 traps in providing non-invasive detections for mammals (Hofmeester et al., 2019),
463 camera traps were outperformed by water-based eDNA metabarcoding for the three
464 focal species in this component of the study. Here, camera traps were deployed so as
465 to sample the habitat of the water vole (see Fig. S3), which may explain lower detection
466 for other terrestrial species in comparison to eDNA metabarcoding (see above).
467 Studies focusing on a single species often report that eDNA analysis outperforms the
468 conventional survey method in terms of detection probabilities (e.g. Lugg et al., 2018).
469 For metabarcoding, there is clearly a need to carefully consider the potential for cross
470 contamination between samples and how false positives (and negatives) could impact
471 detection probabilities using occupancy modelling with eDNA data (Brost et al., 2018;

Lahoz-Monfort et al., 2016). Among the recommendations made by Lahoz-Monfort et al. (2016) to account for these uncertainties, one was the simultaneous collection of data from more conventional surveying methods. Here, we have demonstrated general congruence between surveying methods for the water vole (Table S5; Fig. S1) and using certain species to apply a multiple detection methods model would be appropriate in further studies (Lahoz-Monfort et al., 2016). Alternatively, using repeated sampling and known negative controls in occupancy models that fully incorporate false-positive errors could be applied in the absence of other surveying data (Brost et al., 2018). Overall, multi-species metabarcoding studies may trade-off a slightly lower (but comparable) detection probability than other survey methods for individual species (Fig. 2) in favor of a better overall "snapshot" of occupancy of the whole mammalian community (Ushio et al., 2017).

The comparison between survey 'effort' for the four methods to reach a probability of detection of ≥0.95 is highly informative and provides a blueprint for future studies on mammal monitoring. Focusing on the water vole for example, three latrine surveys would be required. A total of four water-based and 10 sediment-based eDNA replicates or five weeks of camera trapping would be required to achieve the same result (Fig. 2). This increases for the field vole in the same habitat, with five latrine surveys and six water-based eDNA replicates. Sediment-based eDNA metabarcoding would be impractical for this species and camera trapping would take 14 weeks. What is important here is the spatial component and the amount of effort involved in the field. Taking 4-6 water-based eDNA replicates from around one location within a patch could provide the same probability of detecting these small mammals with three latrine surveys. In many river catchments, there may be 100s to 1000s of kilometers to survey that would represent suitable habitat, and only a fraction of that may be occupied by

any given species. This is particularly relevant in the context of recovery of water vole populations post-translocation or in situations where remnant populations are bouncing back after invasive American mink (*Neovison vison*) control has been instigated. On a local scale, finding signs of water voles through latrine surveys is not necessarily difficult, but monitoring the amount of potential habitat (especially lowland) for a species which has undergone such a massive decline nationally is a huge undertaking (Morgan et al., 2019).

The use of eDNA metabarcoding from freshwater systems to generate an initial, coarse and rapid 'distribution map' for vertebrate biodiversity (and at a relatively low cost) could transform biomonitoring at the landscape level. For group-living (i.e. deer) and small mammal species, carefully chosen sampling points (with at least five water-based replicates) along multiple river courses could provide a reliable indication of what species are present in the catchment area if conducted during times of peak abundance (i.e. Summer and Autumn). Then, on the basis of this, practitioners could choose to further investigate specific areas for confirmation of solitary, rare or invasive species (e.g. carnivores) with increased effort in terms of both the number of sampling sites and replicates taken. At present, we would recommend the use of eDNA metabarcoding alongside other non-invasive surveying methods (e.g. camera traps) when monitoring invasive species or species of conservation concern to maximize monitoring efforts (Abrams et al., 2019; Sales et al., 2020a).

It is clear that eDNA metabarcoding is a promising tool for monitoring semi-aquatic and terrestrial mammals in both lotic (this study) and lentic systems (Harper et al., 2019; Ushio et al., 2017). We detected a large proportion of the expected mammalian community (Table S1). Water-based eDNA metabarcoding is comparable or out-performs other non-invasive survey methods for several species (Fig. 2).

However, there remain challenges for the application of this technique over larger spatial and temporal scales. Technical issues of metabarcoding in laboratory and bioinformatic contexts have been dealt with elsewhere (Harper et al., 2019) but understanding the distribution of eDNA transport in the landscape and its entry into natural lotic systems is at an early stage (and incorporating such variables in occupancy modelling approaches). This clearly requires more detailed and systematic eDNA sampling than undertaken here, particularly in an interconnected river/stream network with organisms moving between aquatic and terrestrial environments. Leempoel et al. (2020) recently demonstrated the feasibility for detecting terrestrial mammal eDNA in soil samples but this study has shown that sampling a few key areas in freshwater ecosystems (e.g. larger rivers and lakes) within a catchment area could potentially provide data on a large proportion (if not all) of the mammalian species within it, even when some species are present at low densities (Deiner et al., 2017). In this regard, future studies might also investigate the value of citizen science, where trained volunteers can contribute to data collection at key sites, thus scaling up the reach of research whilst raising public awareness and the significance of mammalian conservation concerns (Parsons et al., 2018).

Data availability statement

Data available via the Dryad Digital Repository https://doi.org/10.5061/dryad.d51c59zzf (Sales et al., 2020b).

Authors' contributions

ADM, XL, CS, OSW, IC, SM, NGS, SSB, EO, BH and LLH conceived the study. Monitoring and live-trapping of water voles was part of XL, CS, EB and JD's ongoing work in Assynt. JD analysed the camera trap data. DAD advised on primer set/data validation and provided information and data on mammals in the Peak District. ADM, NGS, SSB and MBM carried out the eDNA sampling. MBM, NGS, SSB, CB and ADM performed the laboratory work. NGS, OSW, LRH, MBM, CB and ADM carried out the bioinformatic analyses. NGS, ADM, IC and MBM analysed the eDNA data. CS and JD conducted the occupancy modelling. ADM, NGS, CS, JD, MBM and LRH wrote the paper, with all authors contributing to editing and discussions.

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690

691 **Tables**

692

693 Table 1. Estimated site occupancies and detection probabilities, with associated 95%
694 confidence intervals in brackets, obtained for water-based eDNA (w-eDNA), sediment-
695 based eDNA (s-eDNA) and conventional survey methods (Latrine and Camera) in
696 Assynt, Scotland.

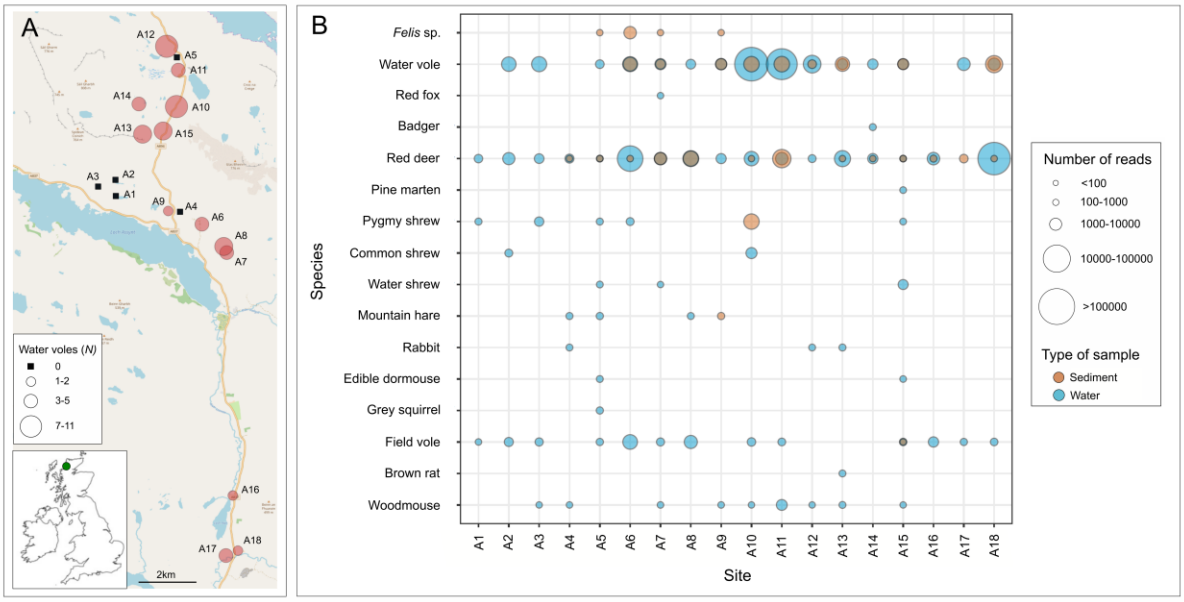
		Detection probability			
Species	Occupancy	*Latrine*	*w-eDNA*	*s-eDNA*	*Camera*
Water vole	0.91 (0.63 – 0.98)	0.77 (0.59 – 0.89)	0.57 (0.43 – 0.71)	0.27 (0.16 – 0.41)	0.50 (0.35 – 0 .65)
Field vole	0.89 (0.57 – 0.98)	0.52 (0.34 – 0.69)	0.40 (0.26 – 0.55)	0.02 (0.00 – 0.14)	0.20 (0.10 – 0.37)
Red deer	1.00 (1.00 – 1.00)	--	0.67 (0.53 – 0.78)	0.10 (0.04 – 0.21)	0.10 (0.09 – 0.24)

697

FIGURES

Figure 1. Panel A shows the environmental DNA (eDNA) sampling sites in Assynt, Scotland; the size of sites corresponds to abundance categories based on summer live trapping. Panel B is a bubble graph representing presence-absence and categorical values of the number of reads retained (after bioinformatic filtering) for eDNA (water in blue and sediment in orange) from each wild mammal identified in each site in Assynt (A1-A18).

Figure 2. Figures on the left show estimated detection probabilities of each survey method for each of three focal species; the vertical lines are 95% confidence intervals. Figures on the right show the method- and species-specific cumulative detection probability with increasing number of sampling events; the horizontal dashed line shows a probability of 0.95 for reference.


A
A12
A5
A11
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A10
A13
A15
A3
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A1
A9
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A6
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A7
A16
A17
A18
2km
Water voles (N)
0
1-2
3-5
7-11
B
Felis sp.
Water vole
Red fox
Badger
Red deer
Pine marten
Pygmy shrew
Common shrew
Water shrew
Mountain hare
Rabbit
Edible dormouse
Grey squirrel
Field vole
Brown rat
Woodmouse
Species
A1
A2
A3
A4
A5
A6
A7
A8
A9
A10
A11
A12
A13
A14
A15
A16
A17
A18
Site
Number of reads
<100
100-1000
1000-10000
10000-100000
>100000
Type of sample
Sediment
Water

Figure 1.

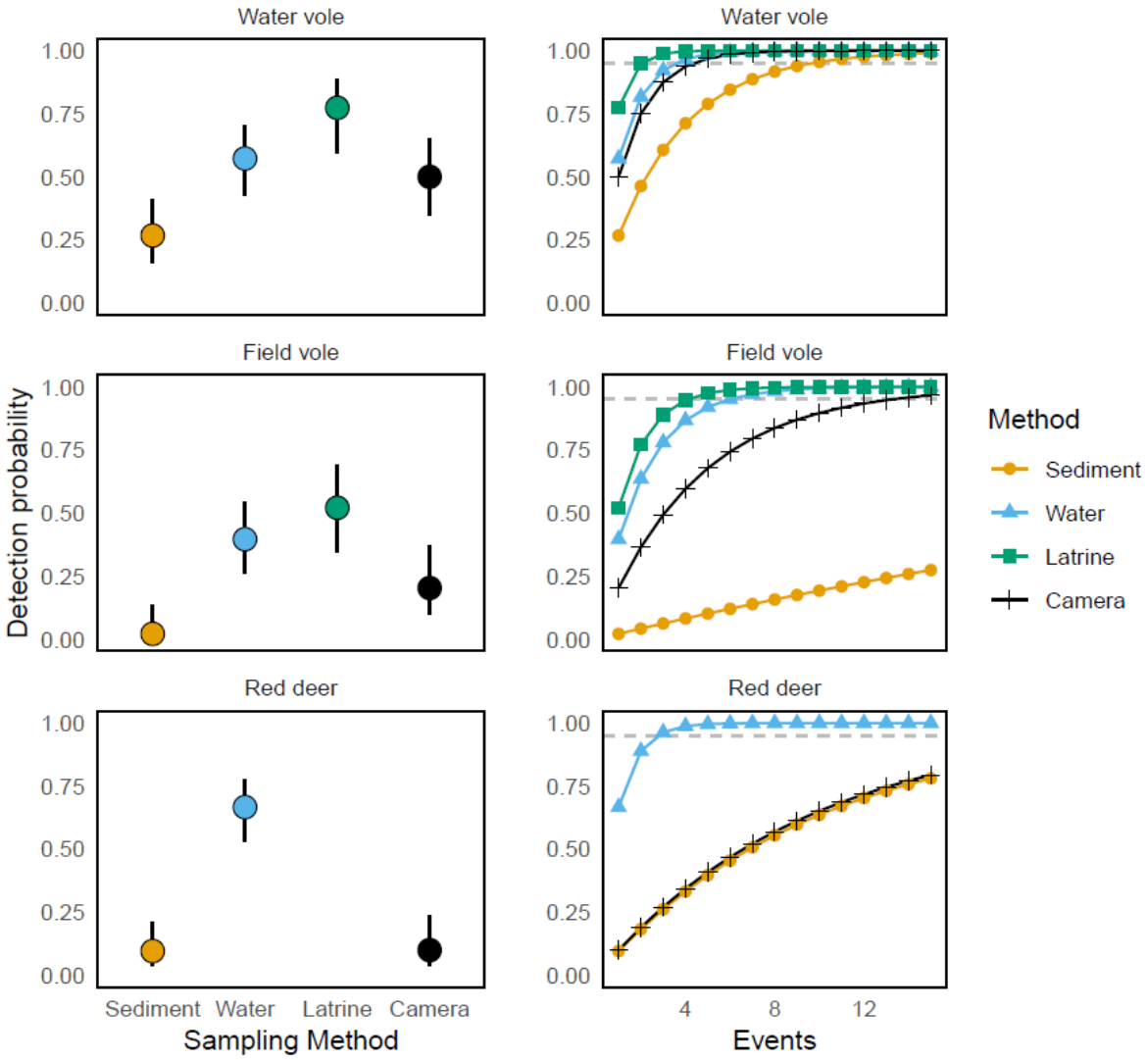
Water vole
Water vole
Field vole
Field vole
Red deer
Red deer
Detection probability
1.00
0.75
0.50
0.25
0.00
Sediment
Water
Latrine
Camera
Sampling Method
4
8
12
Events
Method
Sediment
Water
Latrine
Camera

Figure 2.