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Received: 6 October 2021 | Revised: 11 December 2021 | Accepted: 13 December 2021

DOI: 10.1002/edn3.274

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ORIGINAL ARTICLE

Assessing environmental DNA metabarcoding and camera trap surveys as complementary tools for biomonitoring of remote desert water bodies

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Abstract

Biodiversity assessments are indispensable tools for planning and monitoring conservation strategies. Camera traps (CT) are widely used to monitor wildlife and have proven their usefulness. Environmental DNA (eDNA)-based approaches are increasingly implemented for biomonitoring, combining sensitivity, high taxonomic coverage and resolution, non-invasiveness and easiness of sampling, but remain challenging for terrestrial fauna. However, in remote desert areas where scattered water bodies attract terrestrial species, which release their DNA into the water, this method presents a unique opportunity for their detection. In order to identify the most efficient method for a given study system, comparative studies are needed. Here, we compare CT and DNA metabarcoding of water samples collected from two desert ecosystems, the Trans-Altai Gobi in Mongolia and the Kalahari in Botswana. We recorded with CT the visiting patterns of wildlife and studied the correlation with the biodiversity captured with the eDNA approach. The aim of the present study was threefold: (a) to

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investigate how well waterborne eDNA captures signals of terrestrial fauna in remote desert environments, which have been so far neglected in terms of biomonitoring efforts; (b) to compare two distinct approaches for biomonitoring in such environments; and (c) to draw recommendations for future eDNA-based biomonitoring. We found significant correlations between the two methodologies and describe a detectability score based on variables extracted from CT data and the visiting patterns of wildlife. This supports the use of eDNA-based biomonitoring in these ecosystems and encourages further research to integrate the methodology in the planning and monitoring of conservation strategies.

KEYWORDS
biomonitoring, camera traps, deserts, DNA metabarcoding, eDNA, water bodies

1 | INTRODUCTION

Large-scale biodiversity loss has been documented in all types of ecosystems around the globe due to anthropogenic and climate change effects (Butchart et al., 2010; Díaz et al., 2019; Rosenzweig et al., 2008; WWF, 2020). Reliable biodiversity surveys are therefore needed to assess species conservation status over time and to plan and monitor management measures (Pimm et al., 2014), including the identification of biodiversity hotspots (Brooks et al., 2006; Myers et al., 2000). Desert ecosystems have been neglected in terms of scientific and monitoring efforts, resulting in knowledge gaps particularly for remote areas with difficult access, although they harbor diverse biological assemblages (Brito et al., 2014; Durant et al., 2012, 2014) and cover almost one fifth of the earth's land (Safriel et al., 2005). As climate change may impact environmental conditions in desert ecosystems disproportionately faster (Loarie et al., 2009), these unique systems should be placed at the center of attention.

Biomonitoring aims to provide detailed data on species' distribution, abundance, and diversity. Conventional, observer-based methods, such as visual censuses and systematic trapping, are time- and labor-intensive and mostly focus on a limited number of taxa (Thomsen & Willerslev, 2015). Technology-based methods gain importance in conservation research (Stephenson, 2020), whereof we compare two in this study. Non-invasive camera traps (CT) with infrared sensors are widely employed for conservation research and monitoring, in particular for larger terrestrial mammals (Caravaggi et al., 2017; Salvatori et al., 2021). They are becoming less effort-intensive thanks to AI-based tools to sort CT datasets. Nevertheless, there are also limiting factors for unbiased detectability of species, such as movement range (Burton et al., 2015; Caravaggi et al., 2020) or body mass. Small mammals are usually underrepresented in CT because their size is insufficient to trigger the camera sensor (Leempoel et al., 2020; but see Littlewood et al., 2021). They provide information on species' abundances, density, and richness while allowing for multispecies monitoring (Rovero & Zimmermann, 2016). Environmental DNA (eDNA) approaches offer valuable biodiversity assessment tools given the simultaneous analyses of complex DNA mixtures that enable to detect species' presences, estimate diversities, and relative abundances. Advances in DNA sequencing technologies facilitated an increase in eDNA studies over the last decade (Bohmann et al., 2014; Ruppert et al., 2019; Taberlet et al., 2018; Thomsen & Willerslev, 2015). In particular, DNA metabarcoding (i.e., PCR amplification of short but informative barcodes with universal primers and next-generation sequencing (NGS) of DNA mixtures (Taberlet et al., 2012)) allows the simultaneous assessment of whole communities. Most of these studies focus on aquatic organisms from freshwater ecosystems (Belle et al., 2019; Rees et al., 2014). Water samples are well suited to collecting eDNA due to high distribution capabilities of eDNA in water bodies (Rodgers & Mock, 2015; Valentini et al., 2016). Sampling being standardizable and relatively fast, the method requires only single visits to study sites (or repeated visits for temporal monitoring). Waterborne eDNA reflects temporally accurate biodiversity information due to the limited persistence of free eDNA in water for days or maximally weeks (Barnes & Turner, 2016). DNA degradation is the main cause impeding detection by eDNA-based techniques. Experimental studies have shown that the persistence of free aqueous eDNA (not bound to particles, i.e., sedimentary eDNA) depends on a number of factors, with, for example, higher temperatures, more solar radiation, and neutral or acidic *pH* leading to shorter detection periods (Pilliod et al., 2014; Strickler et al., 2015). However, its persistence depends on dynamic interactions of various biotic (e.g., rate of DNA shedding, microbial activity) and abiotic factors, hindering the drawing of general patterns. While most studies have been carried out in temperate areas, but see (Coutant et al., 2021; Ishige et al., 2017; Mena et al., 2021; Sales et al., 2020; Seeber et al., 2019), sampling eDNA from remote desert water bodies is particularly challenging because of DNA degradation, which is expected to be accelerated by extreme seasonal and daily temperature variations and high UV-B exposure found in this type of environment, technical difficulties caused by the filtration of typically turbid water samples (Egeter et al., 2018) and restrained accessibility. There are few studies to date using water

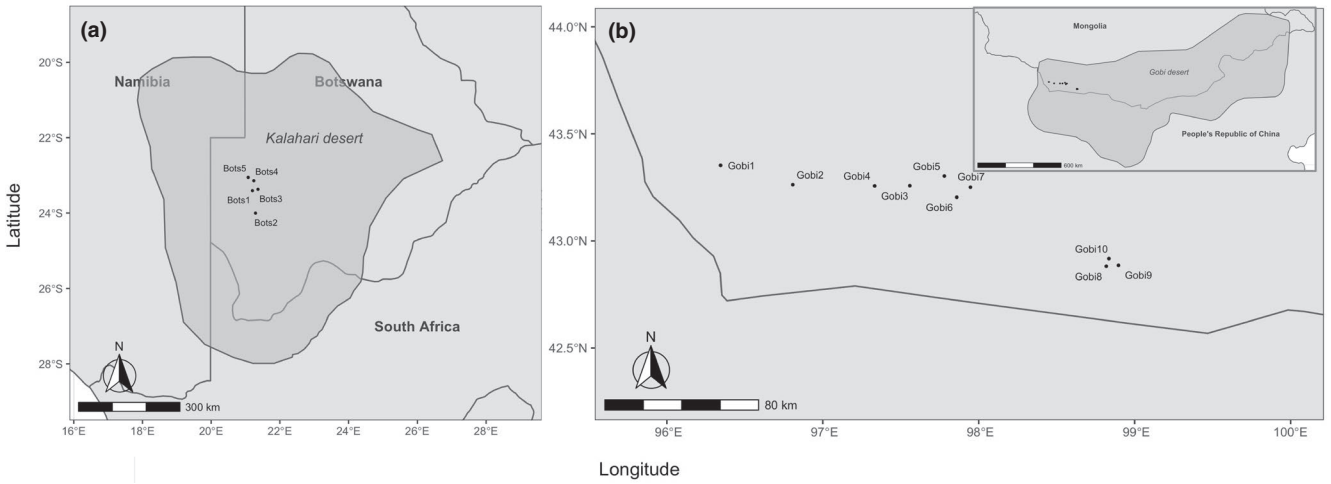

FIGURE 1 Sampling locations in (a) the Kalahari Desert and (b) the Trans-Altai Gobi Desert. Dark gray areas in each map indicate the extension of the Kalahari Desert and the Trans-Altai Gobi Desert, respectively

samples to assess biodiversity in an arid or semi-arid environment, but see (Egeter et al., 2018; Seeber et al., 2019). Despite these challenges, eDNA methods may reveal valuable tools for general biodiversity assessments and the monitoring of iconic and threatened species in precious ecosystems with reduced accessibility.

Environmental DNA from terrestrial animals has been mostly assessed by analyzing scats (De Barba et al., 2014; Kartzinel et al., 2015; Swift et al., 2018), soil (Leempoel et al., 2020; Yoccoz et al., 2012; Zinger et al., 2019), stomach content samples (Kennedy et al., 2019; Masonick et al., 2019; Soininen et al., 2013), leeches blood meals (Abrams et al., 2019; Nguyen et al., 2021; Tilker et al., 2020; Weiskopf et al., 2018; Wilting et al., 2021), or carrion flies (Calvignac-Spencer et al., 2013; Gogarten et al., 2020; Rodgers et al., 2017; Schubert et al., 2015). Bulk tissue samples (mixtures of, e.g., insects or other macroinvertebrate specimens) are also increasingly used not only to assess invertebrate diversity but also as an indirect way to sample vertebrate DNA (Lynggaard et al., 2019). However, animals also leave DNA traces in water while drinking or bathing, which means this water can be sampled and analyzed to detect non-aquatic organisms. This has first been proven using PCR and Sanger sequencing in an experimental setting with coyote DNA (Rodgers & Mock, 2015). Further studies successfully analyzed eDNA of terrestrial animals shed in water bodies sampled across different natural environments, from salt-licks in a Bornean tropical forest (Ishige et al., 2017), water bodies (Seeber et al., 2019; Ushio et al., 2017, 2018) and ponds (Harper et al., 2019), stagnant and running water combined (Mena et al., 2021), to rivers and streams (Coutant et al., 2021; Sales et al., 2020; Sales et al., 2020). This approach is particularly relevant for desert ecosystems with extreme conditions, where waterholes are small and scattered, leading to a spatial concentration of terrestrial animals that must gather and use the few available water sources (Davis et al., 2017; Razgour et al., 2018; Vale et al., 2015). Albeit the close association of water resources and desert species, there are also numerous adaptations to reduce their dependence and some species, such as gazelles, do not always comply with this expectation, as documented in the Trans-Altai Gobi (Nasanbat et al., 2021).

While CT and eDNA are two key tools available for species monitoring, there is limited information available to help researchers choose the most appropriate method for their needs, to compare performance, and decide whether and when methods can be used together (Stephenson, 2020). In order to enable inter-method comparability and their complementary use, comparative studies are therefore needed. Here, we compare CT and eDNA, with a focus on vertebrate terrestrial taxa in two desert ecosystems. While eDNA approaches are still relatively recent, CT have been used far longer but are undergoing increased attraction for conservation monitoring (Rovero & Zimmermann, 2016). The complementary use of these two methods can be appropriate for many situations and in particular for environments that are not favorable for observer-based monitoring. Analyzing images of CT allows us to quantify relative densities of species per sampling site. Based on these data, we can identify variables that best describe visiting patterns and assess whether they are mirrored by eDNA sequence data. We expect, for example, to find DNA of those taxa that visit regularly, in great numbers and shortly before sampling. The aim of the present study was threefold: (a) to investigate how well waterborne eDNA captures signals of terrestrial fauna in remote desert environments, (b) to compare two approaches for biomonitoring in such environments, and (c) to draw recommendations for future eDNA-based biomonitoring.

2 | MATERIALS AND METHODS

2.1 | Sampling sites

Camera traps and water sampling were conducted at 10 different sites in the Gobi Desert in Mongolia and four different sites in the Central Kalahari Game Reserve, within the Kalahari Desert in

Botswana (Figure 1 and Table S1). The water bodies were natural in Mongolia and artificial in Botswana.

The Great Gobi A Strictly Protected Area (SPA) in Trans-Altai Gobi was created in 1975, covers 44,000 km^2 and hosts emblematic species such as the snow leopard (*Panthera uncia*), the brown bear (*Ursus arctos*), the Asian wild ass (*Equus hemionus*) and the Bactrian camel (*Camelus ferus*). For a detailed description of the environmental conditions of this ecosystem, see Nasanbat et al. (2021).

The Central Kalahari Game Reserve was created in 1961 and covers 52,800 km^2, where ecotourism is a fundamental source of income (Stone et al., 2017). It hosts a great diversity of emblematic African large mammals including the African elephant (*Loxodonta Africana*), the cheetah (*Acinonyx jubatus*), the lion (*Panthera leo*), the brown hyena (*Parahyaena brunnea*) and the South African oryx (*Oryx gazella*).

2.2 | Water sampling

At each sampling location (Figure 1), one water sample was taken following the methodology of Pont et al. (2018), with modifications. The water was filtered through a VigiDNA 0.45 μM cross-flow filtration capsule (SPYGEN), with disposable 200 ml sterile syringes for each filtration capsule. For the Mongolian samples, 10 L of water was filtered at each location. For the Kalahari samples, filtered volumes varied from 1 to 10 L (average 6.3 L) depending on water body size and water turbidity. To avoid eDNA degradation, water in the capsule was replaced by 80 ml of CL1 conservation buffer (SPYGEN) and stored at room temperature. Sampling in Mongolia took place in August 2018 and in Botswana in May 2019 (for all details on sampling locations and samples see Table S1).

2.3 | DNA extraction

Extractions were performed in a pre-PCR laboratory dedicated to low DNA-content analyses, using a protocol modified from Pont et al. (2018). The filtration capsules were shaken for one hour at 420 rpm and agitated manually for 2 min to ensure a maximum DNA yield from the filter. From each capsule, 45 ml was poured into three separate 50 ml Falcon tubes (15 ml each) and 33 ml of 96% ethanol and 1.5 ml of 3 M sodium acetate (*pH* 5.2) were added before overnight incubation at −20°C. After precipitation, tubes were centrifuged at 7000 *g* for 30 min at 6°C. Supernatants were discarded, and tubes were incubated at 56°C for 10 min to evaporate residual ethanol. 720 μl of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen) and 40 μl of proteinase K were added, and the mixture was transferred to 2 ml Eppendorf tubes for at least 2 h of incubation at 56°C. The DNA extraction was pursued at step 6 of the NucleoSpin Soil Kit protocol (Macherey-Nagel). The three sub-samples were pooled in the extraction column. Elution was done with 2 x 100 μl of SE buffer. Negative controls were included at all steps.

The extractions were tested for inhibitors with real-time quantitative PCR (qPCR) applying different dilutions in triplicates. qPCR reagents and conditions were the same as in PCR amplification (see below), with the addition of SybrGreen (Thermo Fisher Scientific). Based on the results, all samples were subsequently diluted 10-fold before PCR amplification.

2.4 | DNA metabarcoding

DNA extracts were amplified with two primer sets. The first primer pair targets a fragment of the mitochondrial 12S rRNA gene in vertebrates (Vert01 (Taberlet et al., 2018), corresponding to 12SV5F/R in (Riaz et al., 2011)), the second targets a fragment of the mitochondrial 16S rRNA gene of mammals (Mamm02 (Giguet-Covex et al., 2014; Taberlet et al., 2018)). Human-blocking primers were added to the PCR mixes to prevent amplification of human DNA contaminants (for details of all primers, see Table S2). The total PCR volume was 20 μl, including 2 μl of template DNA and 1 U AmpliTaq Gold 360 mix (Thermo Fisher Scientific), 0.16 mg/ml of bovine serum albumin (BSA, Roche Diagnostics), 2 μM of human-blocking primer, and 0.5 μM of each tagged forward and reverse primer (i.e., primers with eight variable nucleotides added to their 5' end, allowing further sample identification, see (Taberlet et al., 2018)). Each sample was amplified in 12 replicates per primer in three separate PCR plates. Thermocycling conditions were as follows: denaturation at 95°C for 10 min, followed by 40 cycles of 30 s at 95°C, 30 s at 49°C and 57°C for Vert01 and Mamm02, respectively, 1 min at 72°C, with a final elongation step of 7 min at 72°C. Each 96-well PCR plate contained 12 blanks, eight negative extraction controls, eight negative PCR controls, and eight positive controls (DNA assemblies of species not present in the studied regions). Blanks correspond to empty wells and allow to estimate the proportion of tag switches (i.e., false combination of tags, generating chimeric sequences) occurring during the sequencing process (Schnell et al., 2015). Successful amplification was confirmed on a 1.5% agarose gel, and PCR products were subsequently pooled per PCR plate. Pooled amplicons were purified using a MinElute PCR Purification Kit (Qiagen). Purified PCR products were quantified using a Qubit 2.0 Fluorometer (Life Technology Corporation).

Library preparation was performed using a TruSeq DNA PCR-Free Library Prep Kit (Illumina) with an adjusted beads ratio of 1.8 to remove small fragments. After adapter ligation, libraries were validated on a fragment analyzer (Advanced Analytical Technologies). Since larger and smaller fragments besides the target size remained after this step, additional post library bead purifications were performed. To remove large and small fragments from Mamm02 libraries, a ratio of 0.7 was used followed by a ratio of 1.1. For Vert01 libraries, a ratio of 1 was used to remove small fragments. Final libraries were quantified by qPCR, normalized to 1 nM and

pooled before 150 paired-end sequencing on an Illumina Miniseq Sequencing System with a Mid-Output Kit (Illumina).

2.5 | Bioinformatic data analyses

The bioinformatic processing of the raw sequence output was conducted using the *OBITools* package (Boyer et al., 2016). The subsequent steps were followed separately for each library. Forward and reverse reads were assembled with a minimum quality score of 40 and assigned to samples based on unique tag and primer combinations, allowing two mismatches with primer, and identical sequences were clustered. All sequences with less than five reads per library were discarded as well as those not corresponding to primer specific barcode lengths, that is, 56–132 bp for Vert01 and 53–84 bp for Mamm02 (Taberlet et al., 2018). This was followed by two different clustering methods. First, pairwise dissimilarities between reads were computed and lesser abundant sequences with single nucleotide dissimilarity were clustered into the most abundant ones. Second, using the *sumaclust* algorithm, we reduced remaining clusters based on a sequence similarity of 97% (Mercier et al., 2013). Sequences were then assigned to a taxon using a reference database in two steps. First, in silico PCRs were performed with the *ecoPCR* software (Ficetola et al., 2010) on the whole EMBL repository to build reference databases for both metabarcodes (4455 Mamm02 sequences; 16,292 Vert01 sequences, Appendix S1). Taxonomic assignments with these databases were performed using a 95% sequence similarity threshold. Unassigned sequences after this step were discarded from downstream analyses. Second, each taxonomic assignment was manually inspected, and each sequence queried using the BLAST algorithm of GenBank to account for potential mis-assignation, because we used a relatively low similarity threshold. This is more likely to occur for the Vert01 metabarcode, because the amplicons can be very similar between close species. For cases with multiple candidate species, the geographic range was taken into account to select the correct species.

Further data cleaning and statistical analyses were conducted in R (version 4.0.2). Sequences that were more abundant in extraction controls as well as in negative and positive PCR controls than in samples were considered contaminants and removed as well as known common contaminants that were not expected in the study areas (Furlan et al., 2020). To account for tag switching, we considered the leaking of a sequence to be directly linked to its abundance. We performed Wilcoxon signed-rank tests to assess the relationship between samples and blanks. Removal of tag-leaked sequences was done independently per library. Dysfunctional PCR replicates with too small read counts were also discarded. Absolute sequence read counts were transformed to relative read abundance (RRA). Sequences not present in at least two PCR replicates were discarded from downstream analyses. Finally, RRA values were grouped across replicates to obtain a mean value per sample (for a reference data cleaning workflow, see Axtner et al. (2019)). Environmental DNA was considered both as presence/absence and as RRA data for comparison with CT data.

One location in Kalahari (Bots1) had to be excluded from the analyses due to insufficient amplification, possibly due to a problem during sampling, storage, and/or the DNA extraction step.

2.6 | Camera trapping and image coding

Camera traps were set up on sampling sites (Figure 1) between 40 and 70 days before water sampling, in such a way as to cover a maximum of the water bodies and shorelines. In the Trans-Altai Gobi Desert, we used Reconyx HyperFire HC600 (Reconyx), with trigger time of 0.2 s, recovery speed of 0.9 s, sensitivity set to "medium," and detection range/field of view of 30 m/42°. We also used Scout guard 565F model camera, with a trigger time of 1.2 s, a recovery speed of 1 s, and a detection range/field of view of 10 m/52°. In the Kalahari Desert, we used Reconyx Professional HP2X HyperFire 2, with a trigger time of 0.2 s, a recovery speed of 1 s, and a detection range/field of view of 24 m/40°.

We retained images spanning up to 40 days before water sampling to be able to compare between locations. Images were manually examined and the number of individuals per taxon, the time and date of visit recorded in hourly intervals (to minimize the risk of counting several times the same individuals). We coded all individuals in the pictures, regardless of their interaction with the water body, assuming that their presence implied a need for water. Animals were identified to the lowest possible taxonomic rank (species or genus). For each taxon, we recorded *body mass* extracted from PanTHERIA (Jones et al., 2009; Pigot et al., 2020), the *total number of visits*, the number of *days of last visit before sampling*, and the *mean frequency of visits* as potential explanatory variables.

Based on these variables, we built an equation to evaluate the quantity of DNA of a given species in a given location, under the assumption that taxa frequenting a water body more often would be more likely to be detected and yield more reads. To this end, we used the maximum number of individuals recorded in a single picture for each taxon i at each station j, within each hour interval t (N_{it}). First, we calculated a CT based DNA detectability score with:

$$D_{ij} = m_i \sum_t \frac{N_{ijt}}{t} \quad (1)$$

where m_i is the body mass of species i and N_{ijt} is the maximum number of individuals recorded in a single picture of species i at station j and at time t [days] before water sampling.

This equation gives a value of the cumulative DNA detectability (D_{ij}) for each taxon in each location at a particular time, which we then summed up over the 40 days before water sampling to obtain a cumulative DNA detectability score through time. This approach assumes that the eDNA added by a taxon in the water remains constant until its next visit and reaches its maximum concentration at

the end of the recording period. We assume that the quantity of released DNA is proportional to species body mass m_i.

The second index (Dr_{ij}) is based on Equation (1) but considers the relative cumulative quantity of DNA in the water body at the end of the recorded period.

$$\mathrm{Dr}_{ij} = \frac{\sum D_{ij}}{\sum D_j} \quad (2)$$

The above-mentioned indexes do not account for the presence and constant turnover of eDNA of other taxa over the days preceding a visit, that is, the pool of eDNA in the water body. We recalculated the scores from Equation (2) to account only for the last 5 days before sampling ($Dr5_{ij}$), to reduce the pooling effect. Our three detectability formulas were tested using the Mamm02 dataset only. Overall, we excluded the Vert01 data because the amplification of mammals and birds using this primer yields mainly mammal and only a limited number of bird sequences (see Figure S2 for an overview on bird detection).

2.7 | Statistical analyses

We investigated the individual effect of all CT-derived variables (*days of last visit before sampling*, *total number of visits* separately, *body mass*, and *mean frequency of visit*) on the eDNA data, both qualitatively (presence/absence) and quantitatively (logit RRA).

The relative read abundances (RRA) were transformed to avoid zero values using Equation (3) with a sample size (S) of 12 samples per site (Smithson & Verkuilen, 2006):

$$\mathrm{RRA}' = \frac{(\mathrm{RRA} * (S-1) + 1/2)}{S} \quad (3)$$

A logit transformation was subsequently used to achieve normality:

$$\mathrm{logit}\left(\mathrm{RRA}'\right) = \log\frac{\mathrm{RRA}'}{1-\mathrm{RRA}'} \quad (4)$$

The non-linear correlations between some of the CT-derived variables suggested a more complex role of each variable to explain detectability (Figures S1 and S2). We aimed at disentangling from our hypothesis which variables were best explaining the observed eDNA detection. First, we used Kendall rank correlations to test separately the effect of each variable. Second, we used generalized linear mixed models (GLMM), with the *lme4* package (Bates et al., 2015), to investigate to what extent the variables (scaled and centered) influenced the likelihood of eDNA being detected. A logistic regression was performed on the presence/absence eDNA data and a normal regression for the RRA data (logit RRA) on the CT-derived variables. eDNA data (present/absent) were fitted to the explanatory variables extracted from CT data with a binomial distribution (Model 1, Supporting Information). Third, we used linear mixed-effect models (LMM), with the *lmer* package (Kuznetsova et al., 2017), to investigate the influence of the variables on the RRA data (Model 2, Supporting Information). For both model selections, we used Akaike information criterion (AIC) to select the model with the best fit, that is, the lowest AIC value, to reduce overfitting or underfitting the model (Burnham & Anderson, 2002). We chose the qualitative approach (Table 1, Model 1) to further investigate eDNA detection probability based on CT-derived variables because of its better explanatory power and ecological significance compared to the quantitative approach (Table 1, Model 2). We recalculated the predicted values of Model 1 for the three variables separately (*days of last visit before sampling*, *total number of visits*, and *body mass*) and combined through the cumulative detectability (D_{ij}, Equation 1).

TABLE 1 Results of the logistic regression for 0/1 eDNA data (categorical approach) and linear regression for the RRA data (quantitative approach)

	Presence/absence	Logit(RRA')
D_{ij} (Equation 1)	**$p < 0.001$**	**$p < 0.001$**
	AIC = 186.62	$R^2 = 0.136$
Dr_{ij} (Equation 2)	$p = 0.372$	$p = 0.879$
	AIC = 238.85	n. s.
$\mathrm{Dr5}_{ij}$	$p = 0.526$	$p = 0.157$
	AIC = 60.79	n. s.
Days of last visit before sampling	**$p < 0.001$**	$p = 0.065$
		n. s.
Total number of visits	**$p < 0.001$**	**$p < 0.001$**
		$R^2 = 0.29$
Mean frequency of visits	**$p < 0.001$**	**$p < 0.001$**
		$R^2 = 0.133$
Body mass	**$p < 0.01$**	**$p < 0.001$**
		$R^2 = 0.12$

Note: We used logit transformation on the RRA data and removed 0 and 1 values from the dataset to test for the linear regression, as shown in Equations 3 and 4. Significant *p*-values are shown in bold, n. s., stands for *not significant*. R^2 values show the Adjusted R^2.

3 | RESULTS

3.1 | DNA metabarcoding

After all quality filtering steps, we retained 1,254,585 reads of 93 different OTUs for the Vert01 assay that were assigned to 37 taxa. 747,628 reads of 51 different OTUs were assigned to 36 taxa for the Mamm02 assay (all species detected by eDNA can be found in Table S4, Supporting Information). We detected 18 taxa in the Trans-Altai Gobi and 21 in the Kalahari. Vert01 and Mamm02 primers are overlapping for some taxa, that is, these taxa can be amplified by both primer sets. Bird species detected with both

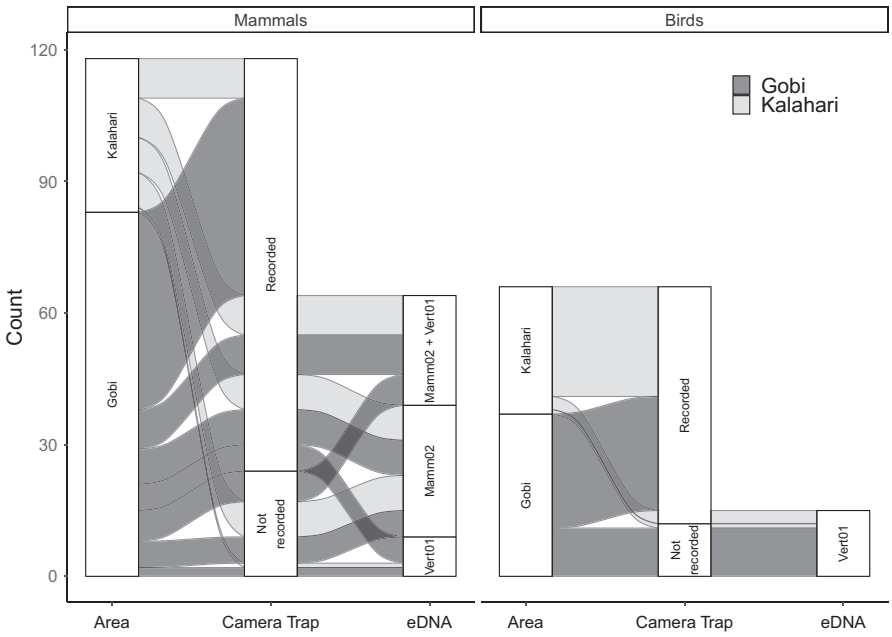

FIGURE 2 Comparison between areas of study, detection methods, and primers used for birds and mammals separately. The y-axis represents the number of different species in each particular location

eDNA and CT can be found in Figure S2. While most mammal taxa were detected by only one primer pair, nine were shared between assays in Kalahari and 16 in Gobi (Figure 2). Despite not being a prior goal of this project, we compared primer specificity and found that eight mammal taxa in Gobi and one in Kalahari were detected exclusively with the Vert01 primer set. The numerous presences of birds in the Vert01 dataset contributed to the variable detection score between primers, as we did not detect bird sequences in the Mamm02 results.

3.2 | Camera traps

We identified 38 taxa in Kalahari and 22 in Gobi with CT (Figure 3, Table S3, Table S4, Supporting Information). One camera from Kalahari could not be recovered, and this location was therefore excluded from all analyses (Bots2). Using the variables retrieved from the images, we assessed the correlations between them to better understand the visiting patterns of the recorded species. We observed a negative exponential correlation between *total number of visits* and *days of last visit before sampling* ($R^2 = 0.35$, p-value < 0.001, Figure S1A) and between *total number of visits* and *mean frequency of visits* ($R^2 = 0.31$, p-value < 0.001, Figure S1B).

3.3 | Comparison between eDNA and camera trap data

In total, 84 taxa were identified combining data from CT and eDNA, 59 in Kalahari and 31 in Gobi. Some species were present in both areas. Detailed overview on the performance of detection methods can be found in Figures 2 and 3, as well as primer differences within the successfully amplified species. Note that each occurrence in Figure 2 indicates a single species for each particular location.

3.4 | Detectability score and eDNA

Camera traps results were used as a reference to compare the detectability score of the eDNA approach in these environments. Using the score from Equation (1), we separated the cumulative curves by positive and negative eDNA results (Figure 4). D_{ij} increases drastically if there were visits to the water body the day before sampling or the same day. We found significant correlations with eDNA data for the raw CT variables and with the detectability score D_{ij} (Equation 1), both for the quantitative (RRA) or the presence/absence measure. Relative detectability approaches (Dr_{ij} and $Dr5_{ij}$) poorly explained the eDNA results compared to the absolute D_{ij} approach (Table 1).

3.5 | Modeling eDNA detection

The explanatory variables showed significant correlations with eDNA results when tested individually, except *days of last visit before sampling* for RRA (Table 1). We first used eDNA presence/absence data as our model response variable (Model 1). Variance in Model 1 was significantly explained by *days of last visit before sampling*, *total number of visits* but not *body mass (log transformed)*, which also had the lowest AIC score. In Model 2, the quantitative response variable (logit RRA) was not significant for any possible combination (Model 2, Supporting Information).

The lower fit of the RRA data led us to further explore the presence/absence approach (Model 1) regarding detectability by eDNA. Figure 5 illustrates the relationship between CT data and eDNA detection for the cumulative detectability score (D_{ij}) (Figure 5a) and for

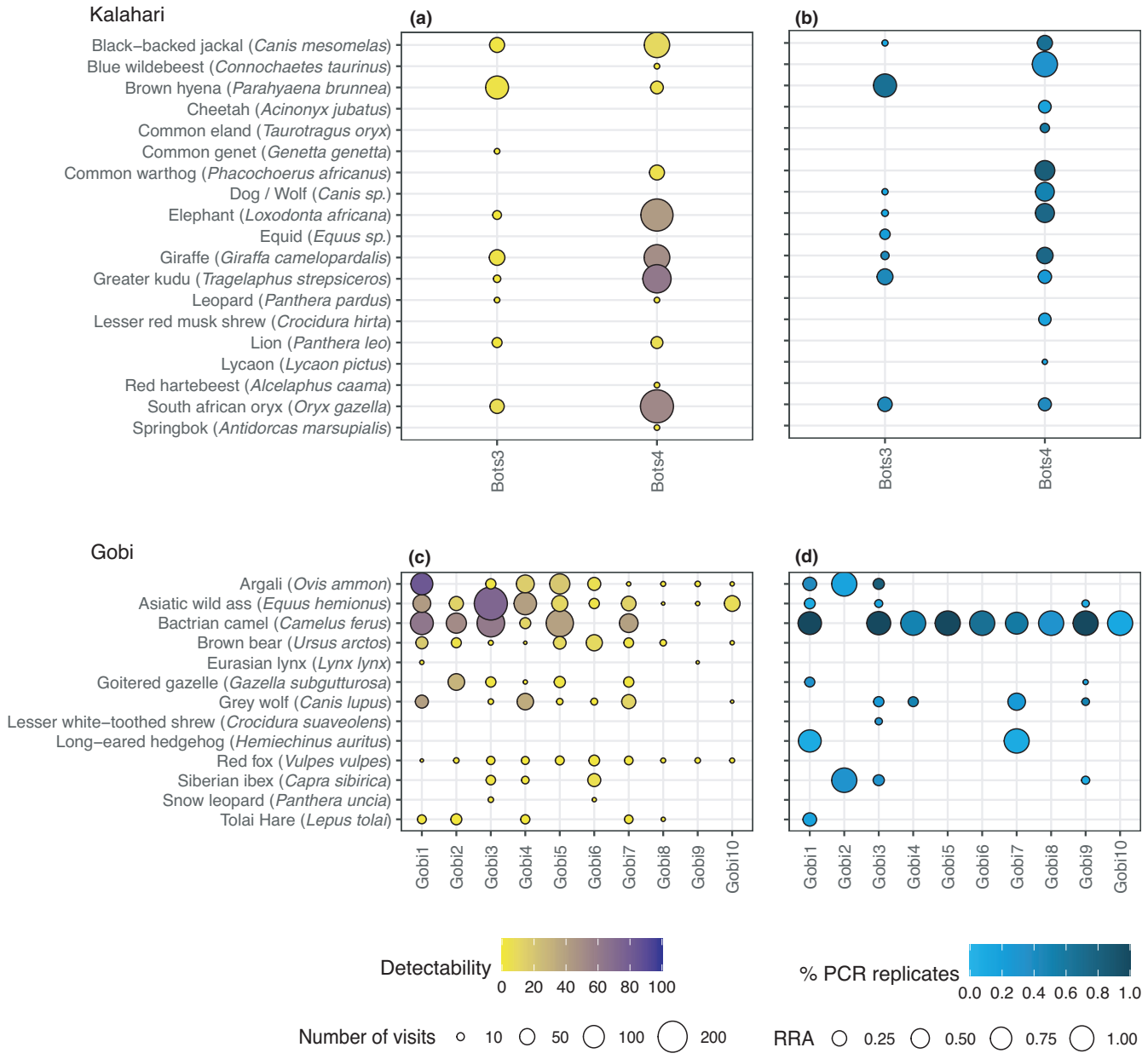

FIGURE 3 Bubble plot representing the detected mammalian species by each method. (a) Species recorded with camera traps in the Kalahari Desert. (b) Species detected with eDNA in the Kalahari Desert. (c) Species recorded with camera traps in the Trans-Altai Gobi Desert. (d) Species detected with eDNA in the Trans-Altai Gobi Desert

each of the three variables independently (Figure 5b-d), which were used to build the detectability score equation (Equation 1).

4 | DISCUSSION

Studying fauna in terrestrial environments using eDNA is generally more challenging than in aquatic habitats because the presence and concentration of eDNA is less homogeneous across the area of sampling (Leempoel et al., 2020; Lyet et al., 2021). To study terrestrial and semiaquatic environments using eDNA, sampling water can therefore be more advantageous over other sample types (Harper et al., 2019; Rodgers & Mock, 2015). Biomonitoring in arid or semi-arid ecosystems represents an additional challenge given the harsh environmental conditions and often vast spatial areas. However, the rare and spatially dispersed water bodies attract organisms and operate as DNA pools that record and temporarily preserve the information of visiting animals, thus representing unique opportunities for sampling. In this study, we successfully show the detection of terrestrial fauna using desert water. We analyzed the correlation between CT image data and eDNA sequence data and showed the interplay between the visiting patterns of species and the probability to detect them using eDNA. We detected terrestrial organisms in all our water samples with both primer sets, which illustrates the

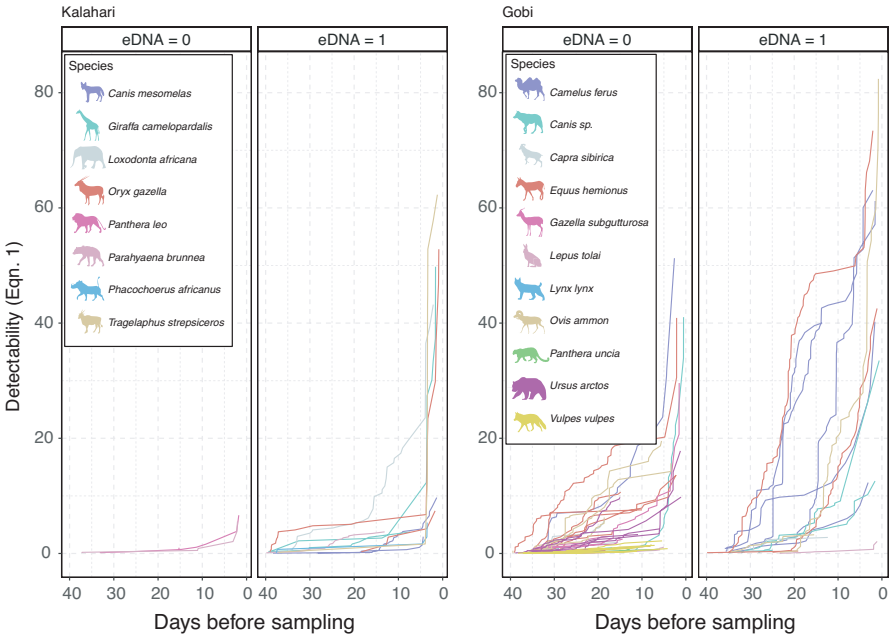

FIGURE 4 Detectability curves for each of the sampled areas calculated using Equation (1). Lines in the "0" box indicate that no eDNA could be recovered. Lines in the "1" box yield eDNA. This figure does not show species which were recorded only once, but these were included in Figure 3. Each species is represented by a silhouette

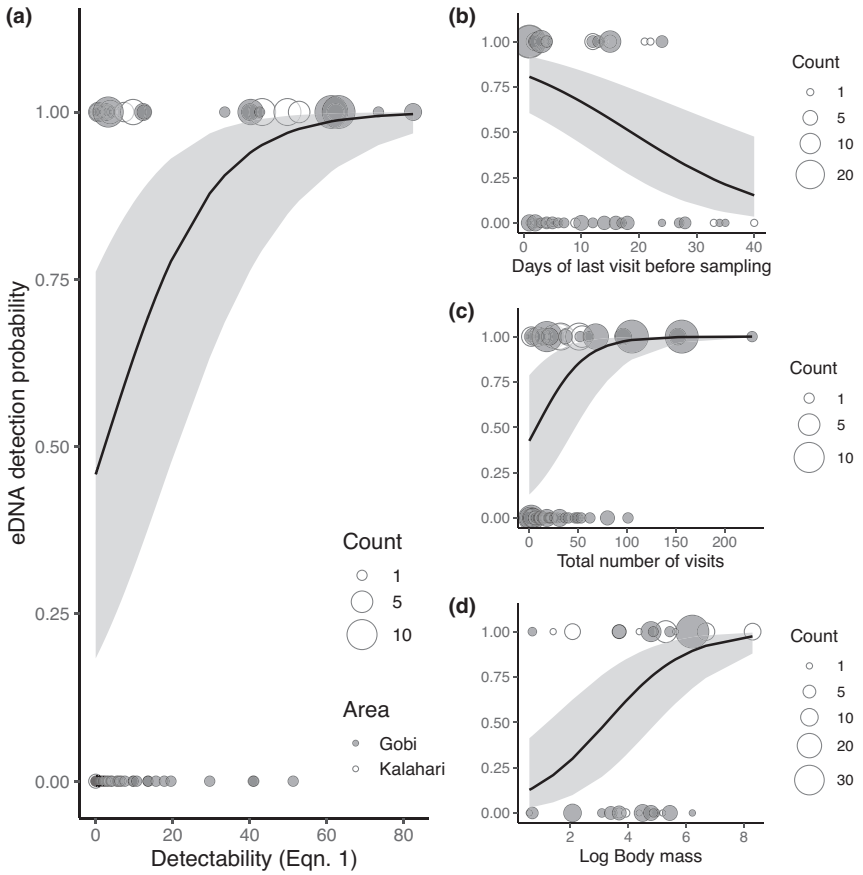

FIGURE 5 (a) Modeled prediction of eDNA detection for cumulative detectability (Equation (1). (b–d) Modeled prediction for each variable involved in Equation (1) separately. All with *p*-value below 0.001. Size of the dots indicates count of occurrences, and gray scale indicates the area. The black line indicates the model's predicted values with its confidence interval in gray

capacity of our assays to detect terrestrial fauna using waterborne eDNA from desert ecosystems. While CT is widely used for biomonitoring, only few studies compare CT and eDNA data for terrestrial animals (Leempoel et al., 2020; Lyet et al., 2021; Mena et al., 2021; Sales et al., 2020; Sales et al., 2020) and fewer explored the potential of waterborne eDNA for arid and semi-arid ecosystems. Seeber et al. (2019) used samples of water bodies in two African ecosystems to study the presence of mammal species, comparing a hybridization capture approach to conventional PCR. Furthermore, Egeter et al. (2018) sampled water in three Saharan water bodies for a mainly methodological DNA metabarcoding study focusing on the water filtration process. We observed clogging of the filters during filtration

in the Kalahari due to the turbidity of the water resulting in variable volumes (Table S1), although we used the filter pore size as recommended by Egeter et al. (2018). Alternatively, Abrams et al. (2019) and Weiskopf et al. (2018) investigated the suitability of leech-derived eDNA as a survey tool for vertebrate species by comparing it to CT detections. Taking the analyses one step further, Tilker et al. (2020) combined the data obtained with these approaches to identify species responses to environmental factors.

DNA metabarcoding relies on "universal" primers that are designed for restrained taxa or groups. Variable numbers of mismatches between primers and templates are the presumed main cause for the preferential amplification of certain taxa and the under- or non-representation of others (Piñol et al., 2015; Piñol et al., 2019). The multiplexing of primers and barcodes is an attempt to minimize these effects (Alberdi et al., 2018; Galan et al., 2018; Jusino et al., 2019; Krehenwinkel et al., 2017). However, in our study, while we chose the Vert01 primers to target birds and reptiles in addition to mammals, mammal sequences were also the most amplified with Vert01 primer, duplicating eDNA detections. The redundancy of the information raises the question of the utility of primer multiplexing in this context (Figure 2).

The presence of trace DNA of humans and domesticated animals in eDNA studies represents a common issue, the possible sources of such contaminations being numerous (Furlan et al., 2020). We removed the obvious cases (pig, cow); however, we kept two taxa in the Kalahari dataset which are ambiguous and unconfirmed by CT. The detection of *Equus* sp. DNA in one Kalahari sample could be explained by the presence of horse, zebra, or donkey or the transport of such DNA, for example, by a predator. In addition, in the Kalahari samples, we amplified DNA assigned to *Canis* sp. (dog/wolf). The sequences differ from the ones found in the Gobi samples, therefore excluding a cross-contamination, but its source remains unclear.

Previous CT vs. eDNA studies found that smaller animals are less likely to trigger CT and risk being overlooked in this type of biomonitoring studies (Leempoel et al., 2020; Lyet et al., 2021; Sales et al., 2020). Smaller species probably release less DNA into the water than larger species and are hence less likely to be detected due to the presence of other species' DNA. Mena et al. (2021) conducted a comparative study of different traditional survey methods (pitfalls, grids, mist nets, and CT) and aquatic eDNA for the detection of terrestrial mammals in tropical forests. The overall results point out the benefits of eDNA surveys, in terms of detection scores, labor-effort and costs, but depend very much on the species and sampling area. It must be noted that in the present study, we analyzed 40 days of CT data and compared it to the results of only one water sampling event for each locality (Figures 2 and 3). This has to be kept in mind when evaluating the performance of the eDNA assays, since a 40 days period is well beyond the persistence of free eDNA in water according to literature (Barnes & Turner, 2016). In the light of these shortcomings, we consider it encouraging to have detected relying on eDNA more than half of the mammals recorded with CT (Figure 2). We also acknowledge the limitations of taking a single sample per water body, as it may be underrepresenting the wildlife diversity. Additional samples from the same water body would likely increase detected species numbers, and we advocate that future studies aim to determine the optimal number of samples per location.

In this study, DNA was already present in the water prior to the placement of CT and the a priori DNA composition is unknown. We used the RRA approach as a proxy for species abundances, but it could be biased by the variable *body mass* of a species or by its behavior (e.g., drinking, bathing, and defecating), which affects the release of DNA (Harper et al., 2019). We did not find different detection scores between methods for nocturnal/diurnal species, but this is due to the capacity of CT to detect nocturnal species, contrasting to line transects (Coutant et al., 2021). For Gobi, the coverage of species is biased by the overwhelming presence of Bactrian camels (*Camelus ferus*). These animals are big and recurrent in the area, as confirmed by CT. To overcome this limitation, we suggest adding blocking primers for dominant species, if known beforehand. The usage of blocking primers is recommended for this type of study (De Barba et al., 2014; Vestheim & Jarman, 2008). Egeter et al. (2018) used Vert01 primers without human-blocking primers and obtained 68% of total reads assigned to hominids. In our study, we had fewer human sequences (9.36% of sequences for Mamm02 and 37.5% for Vert01), indicating a good performance of the blocking primer. A higher concentration of blocking primer than the one we used would increase the risk of co-blocking targeted taxa (Shehzad et al., 2012; Taberlet et al., 2018). In fact, the high abundance of camel and human DNA could explain the low detection rates of other less recurrent species, mainly carnivore species, despite the frequent CT recordings. For example, the locally rare and emblematic brown bear (*Ursus arctos*), whose residual population in the Gobi Desert numbers a few dozen individuals, was detected only once with the Vert01 primer pair (and therefore is not present in Figure 3d). This low detection of carnivore eDNA was also observed for Botswana and could indicate that further factors, such as drinking technique or contact with water (bathing), are involved besides the species body mass, see for example (Lyet et al., 2021). We therefore encourage future metabarcoding studies to investigate the factors associated with successful eDNA detection of carnivores.

The negative correlation between the variables *total number of visits* and *days of last visit before sampling* (Figure S1A) was expected because species visiting a water body many times are also likely to have visited it recently, and it could only be biased by migratory or nomad species that visit a water body in great numbers but low frequency. We excluded this potential confounding effect because taxa with high *total number of visits* were also the ones with the lowest *mean frequency between visits* (Figure S1B). Hence, we were able to use these variables as predictors to calculate and model eDNA detection probabilities.

We tested several equations to combine the explanatory variables retrieved from CT data into a comprehensive index to account for the expected detectability of species in each location, both using a categorical (0/1) and quantitative (RRA) approach (Table 1). When visualizing the increasing detectability score D_{ij} (Equation 1) by eDNA detection (Figure 4), there was a sudden increase for some

species at the end of the monitored period. Such increase is due to the visits occurring not long before water sampling, which have a major impact on the overall score through time. Dr_{ij} and $Dr5_{ij}$ could not be visualized across time because only single values per species and location can be obtained. We found better correlation between RRA and $Dr5_{ij}$ compared to Dr_{ij} (Table 1), probably due to lower DNA degradation, but none were significant, which highlights the complexity of defining detectability scores. However, these scores are a simplification of reality, as they do not account among other factors for DNA decay (Barnes & Turner, 2016). Using RRA as a proxy for species relative abundance must, however, be taken with caution because of the biases that DNA extraction, amplification, and sequencing imply (for an overview of the biasing factors, see (Fonseca, 2018). Furthermore, the categorical approach homogenizes the coverage of each species and, in practice, increases the weight of low RRA species in our test (Deagle et al., 2019). This is of particular interest when using RRA data, as it provides a more realistic proxy in terms of abundances. Overall, the better correlation of RRA for $Dr5_{ij}$ suggests the RRA approach to be a better proxy for species detection when water samples are taken frequently. The categorical approach is recommended when the sampling is done only once (as in the present study) or sparsely. In addition, these scores only make sense when comparing CT and eDNA data. Still, the characteristics of the sampled area need to be accounted for when drawing detectability scores. For instance, Lyet et al. (2021) sampled river water to detect mammal species and they defined their detectability score based on the camera trap detection rate and the pluviometry of the day. Nonetheless, our results are promising, and optimizing the accuracy of these scores will improve cross-validation of both methodologies, both for comparative studies and when using eDNA as a complementary tool to CT. However, the complexity of interacting ecological factors complicates building a simple equation to reliably infer eDNA detection probability. In this line, a purpose-built experiment should be carried out to tackle this matter, with a limited number of species in a controlled environment.

Another goal of this study was to draw guidelines for future studies aiming to use eDNA as a biomonitoring tool in desert environments. We built models based both on eDNA 0/1 data (Model 1) and RRA data (Model 2). All variables used in Model 1 except *body mass* were significant, suggesting that the categorical transformation of our eDNA data is more advisable when the goal is species detection rather than its relative abundance. We used this model as a reference to calculate detection probabilities for each variable and area independently, in order to disentangle the effect of each and visualize them (Figure 5). Interestingly, the positive eDNA detections based on *Final cumulative detectability* are clearly divided into two groups (Figure 5a). This suggests that our detectability score fails to properly reflect the true detectability of some species, which could be explained by the poor correlation observed for *days of last visit before sampling* (sparse distribution of non-detection occurrences, Figure 5b) and *body mass* (Figure 5d). In this line, these last two variables should be studied more in depth to properly understand their impact on eDNA detectability. The good fit of *total number of visits* (Figure 5c) is, as mentioned before, influenced by *days of last visit before sampling* (Figure S1B).

Modeling with RRA data (logit transformed) was more ambitious because we also had to cope with the issue of PCR-introduced biases, which were minimized when transforming our eDNA data to a categorical approach. The best model fit was obtained with *total number of visits* and *days of last visit before sampling* (Model 2), but none of these variables were significant. The RRA per taxa is assumed to be correlated to the released DNA, that is, the initial biomass of taxa in a sample (Deagle et al., 2019). It was difficult to correlate RRA data to visiting patterns to the water body. In fact, the degradation of DNA in the water combined with the continuous turnover of new DNA creates a complex multivariate dynamic system of DNA concentration and quality in the water body which is captured only once at the moment of sampling. In our study, the 40 days range of CT monitoring exceeds the duration free eDNA remains detectable in water. This could explain the lack of significance for the explanatory variables of Model 2. We tested this same model only with data from the last 5 days before sampling ($Dr5_{ij}$), and we obtained a better fit but remaining non-significant. Nevertheless, the proposed DNA detectability scores calculated from CT data successfully represented the detection of species through eDNA, surpassing 75% of positive detection for D_{ij} scores above 25 (Figure 5a). Furthermore, species that visited the water bodies more than 25 days before sampling were never detected (Figure 5b), which indicates the maximum day-span between sampling events. However, these numbers apply only to our particular study system. DNA degradation and its detectability through DNA metabarcoding are very sensitive to environmental conditions of the sampled area, and future studies should target the effects of additional biophysical (such as *pH*, temperature, UV radiation, water body size, and depth) or biological variables (such as bacterial activity). Increasing the resolution and ecotype range of this kind of study will contribute to defining the probability of species detection through eDNA and contribute to improving sampling strategy for future research.

The direct comparison of detection success is strongly biased by the different survey lengths of this study and we found, therefore uncontested, CT to detect more taxa than the eDNA approach. Both methods have undoubtedly pros and cons, and it is crucial to weigh those and adapt the sampling strategy to the respective study system. Our results on detectability suggest that CT is preferential over eDNA for monitoring species when eDNA sampling cannot be made at regular intervals or to cover long monitoring periods. It ultimately also depends on the targeted taxa, being preferential when you study medium- to large-sized organisms which can be easily differentiated morphologically (Mena et al., 2021). Species abundances and densities can also be deduced from image data more easily. However, to rely on cameras implies the risk of losing data (over 40 days, 1/14 cameras were lost) and demands repeated visits which may be complicated in remote areas (at least installation and recovery of cameras), where eDNA sampling offers valuable study opportunities (McInnes et al., 2021). Also, CT does not offer the same options as eDNA in terms of possible population genetic studies (Bohmann

et al., 2018; Nguyen et al., 2021; Sigsgaard et al., 2016, 2020; Tilker et al., 2020; Wilting et al., 2021) and the extension to other phyla (as, e.g., invertebrates, plants, and bacteria). eDNA sampling is appealing due to its sensitivity, standardization, and non-invasiveness, as well as the independence from taxonomic experts for taxa identification. eDNA techniques may enable the detection of elusive species and the taxonomic differentiation of morphologically similar as well as of cryptic species (Thomsen & Willerslev, 2015). In terms of quantifying the abundance of species, the eDNA approach has to cope with the dynamics of DNA in the water body released by the visiting individuals. They create a continuous turnover of available DNA that can easily bias the inference from amplified DNA to species relative abundances. Furthermore, genetic data at this scale are per se unable to provide certain population dynamic parameters (sex, age, and absolute numbers of individuals). As we have seen in our data, regular sampling in short intervals is necessary to provide a complete picture in terms of species richness. Alternatively, eDNA surveys can be used, for example, on a broad geographic scale to get a first glimpse of the biodiversity of the area that can be locally refined with CT (Sales et al., 2020). In fact, the combination of the two methods is increasingly used in biomonitoring studies (Sales et al., 2020; Sales et al., 2020), which is advantageous due to their complementary strengths. This could be particularly beneficial when the study system includes both large and small taxa of interest. The water samples are going to be dominated by the DNA of large taxa, which are easily recorded by CT, but they complicate the amplification of small taxa. Dividing methodological efforts, for instance by including blocking primers of the large dominant species, will facilitate the detection of smaller species, which are more likely to be missed by CT.

Our results show that water bodies concentrate information about large remote regions that are difficult to access and monitor using conventional, observer-based methods. They function as eDNA reservoirs containing information about valuable ecosystems. In light of the manifold risks facing wildlife—particularly species in remote and threatened ecosystems—further cross-method tests are needed, to validate their parallel application and support their integration into conservation monitoring strategies.

ACKNOWLEDGMENTS

We thank the Ministry of Environment, Natural Resources Conservation and Tourism (Botswana) for the issuing of a research permit (ENT 8/36/4 XXX II [10]), and the Bavarian Association for Bird Protection (LBV, Germany) for fieldwork and logistic support in Gobi. We sincerely acknowledge the contributions made by Kealeboga Matshameko, Norman Keoagile, and Mogomotsi Mokgadi in water collection and field work proceedings. We thank Julie Guenat and Jérôme Wassef for their contribution in the laboratory and image coding, respectively. We thank Jérémy Gremion and Ara Monadjem for bird species identifications. We also thank Jonas Bylemans for the constructive discussions during data analyses and Peter James Stephenson for comments on an earlier version of the manuscript.

FUNDING INFORMATION

EM-C was supported by a fellowship in Life Sciences (Faculty of Biology and Medicine, University of Lausanne). We thank the Botswana International University of Science and Technology (BIUST) for providing funding and infrastructure support to MB and CN. This project was supported by a Swiss National Science Foundation grant to PC (nr. 31003A_179378) and to LF (nr. 310030_192512).

CONFLICT OF INTERESTS

PT is co-inventor of a patent related to the Vert01 primers for vertebrate identification using degraded template DNA. This patent only restricts commercial applications and has no impact on the use of this locus by academic researchers. All other authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in the paper.

AUTHOR CONTRIBUTIONS

LF, PC, and OG designed the study and supervised all analyses. BN, SR, OG, PC, MB, and CN conducted fieldwork. CA provided logistic support. CS carried out laboratory work. BN, EM-C, SR, FC, and PC carried out species identifications and image coding based on camera trap data. EM-C conducted bioinformatics and data analyses and prepared the figures. EM-C and JS wrote the paper, with input from all other authors.

DATA AVAILABILITY STATEMENT

The DNA metabarcoding data generated for this study is available on DRYAD (https://doi.org/10.5061/dryad.wpzgmsbp2).

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How to cite this article: Mas-Carrió, E., Schneider, J., Nasanbat, B., Ravchig, S., Buxton, M., Nyamukondiwa, C., Stoffel, C., Augugliaro, C., Ceacero, F., Taberlet, P., Glaizot, O., Christe, P., & Fumagalli, L. (2021). Assessing environmental DNA metabarcoding and camera trap surveys as complementary tools for biomonitoring of remote desert water bodies. *Environmental DNA*, 00, 1–16. https://doi.org/10.1002/edn3.274