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Challenges for assessing vertebrate diversity in turbid Saharan water-bodies using environmental DNA

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ABSTRACT

The Sahara desert is the largest warm desert in the world and a poorly explored area. Small water-bodies occur across the desert and are crucial habitats for vertebrate biodiversity. Environmental DNA (eDNA) is a powerful tool for species detection and is being increasingly used to conduct biodiversity assessments. However, there are a number of difficulties with sampling eDNA from such turbid water-bodies and it is often not feasible to rely on electrical tools in remote desert environments. We trialled a manually-powered filtering method in Mauritania, using pre-filtration to circumvent problems posed by turbid water in remote arid areas. From nine vertebrate species expected in the water-bodies, four were detected visually, two via metabarcoding and one via both methods. Difficulties filtering turbid water led to severe constraints, limiting the sampling protocol to only one sampling point per study site, which alone may largely explain why many of the expected vertebrate species were not detected. The amplification of human DNA using general vertebrate primers is also likely to have contributed to the low number of taxa identified. Here we highlight a number of challenges that need to be overcome to successfully conduct metabarcoding eDNA studies for vertebrates in desert environments in Africa.

Keywords: eDNA, spike, Mauritania, vertebrate, turbid, desert, biodiversity

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46 INTRODUCTION

47 Knowledge on species distribution is essential in many fields of biology, and the identification of
48 areas with high species richness is crucial to protect global and regional hotspots of biodiversity
49 (Pimm et al. 2014). Information about biodiversity distribution is still scarce in remote and
50 unexplored areas, such as deserts and arid regions (Brito et al. 2014). The Sahara desert is the
51 largest warm desert in the world and a poorly explored area due to the remoteness, size and long-
52 term political instability of the region (Brito et al. 2014). Small and fragile water-bodies
53 surrounded by sandy areas, such as mountain rock pools (gueltas) and floodplains, occur across
54 the Sahara desert acting as local biodiversity hotspots (Vale et al. 2015). For example,
55 Mauritanian mountain rock pools harbour a disproportionately high representation of desert
56 biodiversity (32% of the analysed vertebrates and 78% of the country's endemics; see Vale et al.
57 2015). Local human activities are also dependent on water-bodies, thus, the long-term
58 conservation of these fragile and unique ecosystems requires a better assessment of local
59 biodiversity levels and potential human-related conflicts.

60 Environmental DNA (eDNA) is a powerful and cost-effective tool for species detection and is
61 being increasingly used to conduct biodiversity assessments (Jerde et al. 2011; Thomsen et al.
62 2012). Most studies using eDNA "metabarcoding" for detecting vertebrates from water-bodies
63 concentrate eDNA prior to DNA extraction by filtering (e.g. Lopes et al. 2017; Robson et al.
64 2016). Filtering generally results in higher amounts of eDNA being extracted than precipitating
65 directly from water, since much higher volumes of water are processed when filtering (Adrian-
66 Kalchhauser and Burkhardt-Holm 2016; Eichmiller et al. 2016; Hinlo et al. 2017). However,
67 previous studies have so far focused on temperate and tropical environments, and the efficacy of
68 eDNA monitoring in water-bodies in extreme arid environments, such as desert water-bodies,

remains unknown. In particular, it was known from previous studies that the water in gueltas is often highly turbid (Verheye and Dumont 1984), which can lead to limitations in filtering any substantial volumes of water (Robson et al. 2016; Spens et al. 2017; Turner et al. 2014). One method to counter these limitations is pre-filtering of water samples through membranes with a larger pore size (see Majaneva et al. 2018). Another considerable difficulty with conducting research in remote desert environments is that it is often not feasible to rely on electrical tools. For the present study, we aimed to trial a manually-powered filtering method for collecting eDNA samples for metabarcoding, using pre-filtration to circumvent the problems posed by highly turbid water in remote arid areas. To validate the protocol we included a DNA spike: a known quantity of DNA from a pre-selected source (i.e. from species not present in the study area) added to environmental water samples prior to filtering.

MATERIALS AND METHODS

Field sampling

The study was conducted in Mauritania (latitude from N14.4 to N27.2 and longitude from W4.5 to W17.1) during an overland expedition in April 2017, coinciding with the dry season peak. This region has four mountain chains (Adrar Atar, Tagant, Assaba and Afollé) and is located in the transition between Sahara and Sahel ecoregions. Three permanent water-bodies were surveyed: Atar dam (N20.532 W13.044; c. 20,000 m²) and Hamdoûn rock pool (N20.323 W13.142; c. 5,000 m²) in the Adrar Atar mountains, and Matmâta river-bed (N17.887 W12.111; c. 10,000 m²) in the Tagant mountains (Figure 1). These water-bodies are characterised by high levels of water turbidity partially due to the faecal contamination by domestic animals and herdsmen exploitation (see Vale et al. 2015). They had previously been inspected during diurnal

and nocturnal surveys between 2007 and 2013 using several distinct methodologies (e.g. dip nets, live traps and camera traps) as well as indirect observations (e.g. faeces, footprints, tracks or burrows) to catalogue the vertebrates occurring there (Vale et al. 2015). During the current study, visual encounter surveys along the margins of the water-bodies were made by at least two persons to detect the presence of amphibians, fishes, mammals and reptiles.

Each water-body was then sampled at one sampling point. The original study design included multiple sampling points, but fieldwork was severely limited as a consequence of the difficulties encountered with filtering, namely that up to two hours were required to filter each water sample. At each sampling point 5 L water was collected in a container. The entire volume was first pre-filtered through a 1 μm polypropylene filter (Airwatertech, Belgium) housed in a Cintropur NW18 filtering capsule (Airwatertech, Belgium). This pore size was chosen based on our observations that pre-filtering water from Mauritanian gueltas through 20 μm , 10 μm and 5 μm pre-filters did not substantially reduce the suspended sediment in the filtrate, which resulted in rapid clogging during the final filtering step. Thus, a smaller pore size was necessary. A dual action hand-powered vacuum pump (Tribord, France) was used to draw the water through the filtration system. The filtrate from this first step was captured and 500 mL was transferred to a Nalgene 500 mL filter holder (ThermoFisher Scientific, USA) fitted with a 47 mm diameter 0.45 μm nitrocellulose Whatman filter (GE Healthcare, USA). At Atar and Hamdoûn, even after the pre-filtering step, it was necessary to use between two and four final filters to pass through the desired 500 mL water, due to filters clogging. Both the pre-filter and final filters were kept and stored separately in a 50 mL and a 2 mL tube respectively, which were topped up with 96 % ethanol. At each water-body, a negative control was also included, which consisted of filtering 2 L dH₂O through a sterile 0.45 μm filter. Disposable gloves were used for handling filters and

were changed between sampling points. All equipment was washed, soaked in 20 % bleach overnight and thoroughly rinsed between sampling sites.

There was high turbidity in all water-bodies and at Hamdoûn it was only possible to filter 4 L through the pre-filter, before it clogged, even though these have a relatively high surface area of c. 260 cm², compared to the commonly used 47 mm diameter disc filters, which have a surface area of c. 17 cm².

In order to validate the filtering method, the entire procedure at each sampling point was repeated with the addition of a pre-prepared DNA positive control fragment. To prepare this "spike" of control DNA, the concentration of tissue-extracted DNA from five different amphibian species occurring in northern Maghreb, but known to be absent from Mauritania, was measured using the Qubit HS DNA Assay (ThermoFisher Scientific, USA). Separate 1.5 mL tubes were prepared, each containing desiccated DNA in the following amounts: *Discoglossus pictus* (50 ng), *Sclerophrys mauritanicus* (25 ng), *Discoglossus scovazzi* (1.25 ng) and *Pelophylax saharicus* (0.25 ng). At each site, before the pre-filtering step, this desiccated DNA was rehydrated in 1 mL dH₂O and added to the 5 L container, which was subsequently shaken thoroughly. This resulted in concentrations of DNA in the 5 L container of 10, 5, 0.25 and 0.05 ng/L for each species respectively. To assess whether the DNA in the spikes had become degraded between preparation in the laboratory and rehydration in the field, two spikes were rehydrated in the field and filtered through separate 0.45 µm filters, which were stored in the same manner as for other filters. At Matmâta, again due to the amount of time it took to filter water samples, only one 5 L water sample was sampled, which included the spike DNA.

DNA extraction

DNA extractions were carried out in a positive-pressure lab (CIBIO-InBIO, Vairão Campus, Portugal), following strict protocols including disposable lab wear, UV sterilization of all equipment before entering the lab, and cleaning lab surfaces with bleach between extraction batches. Only environmental and faecal samples are permitted to enter this lab. Other biological tissues, already-extracted DNA and PCR products are forbidden. Handling and cutting of all filters was done using flame-sterilized scissors and forceps, on disposable dissection trays. The larger pre-filters were quartered. Adapting the protocol used by Valentini et al. (2016), two of the quarters were placed in separate 50 mL tubes with 30 mL resuspension buffer each (50mM Tris, 10mM EDTA), and shaken thoroughly for 5 min at 20 Hz (Retsch 400 MM; Retsch, Germany). The buffer from both tubes was then combined and filtered through a 0.45 µm filter. From here, the extractions for all 0.45 µm filters was identical. Each filter was cut in half, placed in a 5 mL tube and excess ethanol was evaporated at room temperature. To each tube, 2 mL lysis buffer (0.1 M Tris-HCl, 0.1 M EDTA, 0.01 M NaCl, 1 % N-lauroyl sarcosine, pH 7.5–8; Maudet et al. 2004), was added and kept at 54 °C for 0.5 h. From here the EZNA Tissue DNA kit was used following the manufacturer's instructions, using 300 µL BL Buffer, 300 µL ethanol and eluting in 50 µL Elution Buffer.

PCR and sequencing

We used a primer designed to target all vertebrates – here referred to as 12SV5.1, as the original publication did not specifically name the pair (Riaz et al. 2011). The 12SV5.1 primer pair includes 12SV5.1_F ACTGGGATTAGATACCCC and 12SV5.1_R TAGAACAGGCTCCTCTAG that targets a c. 105 bp region of the mitochondrial 12S rRNA gene. PCR conditions were: 10 µL reactions containing 2 µL DNA, 5 µL Multiplex PCR Master Mix (Qiagen, USA) and 0.3 µM of each primer. The thermal cycling profile was an initial step of

95 °C for 10 min; then 35 cycles of 95 °C for 30 s, 51 °C for 30 s, and 72 °C for 30 s; with a final extension of 72 °C for 10 min. Amplification success was confirmed by electrophoresis in 2 % agarose gels stained with GelRed (Biotium, USA). To prepare samples for high-throughput sequencing, a second PCR was conducted using 10 µL reactions containing: 1 µM of each index-primer combination (Gansauge and Meyer 2013; Kircher et al. 2011), 5 µL 2X KAPA HiFi HotStart ReadyMix (Kapa Biosystems), and 2 µL of previous PCR product diluted 1:10 using 10mM Tris. The thermal cycling profile consisted of an initial step of 95°C for 3 min; 10 cycles of: 95°C for 30 s, 55°C for 30 s, and 72°C for 30 s; and a final extension of 72°C for 5 min. The resulting PCR product was cleaned using 1.2 X by volume AMPure XP beads (Beckman Coulter) following the manufacturer's instructions with the exception that 80% ethanol was used instead of 70%. Elution was done in 25 µL Tris 10mM. Library quality was assessed by measuring DNA concentration of each cleaned PCR product using Nanodrop 2000 (Thermo Fisher Scientific) and each PCR product was normalized to 15nM using 10mM Tris pH 8.5 0.1% Tween. The final pool was obtained by combining 5 µL from each normalised sample. The quality of the final pool was assessed by qPCR using KAPA Illumina Library Quantification (Illumina) following the manufacturer's instructions. Illumina paired-end sequencing was performed using a 300-cycle Illumina MiSeq Micro V2 Kit (Illumina) on an Illumina MiSeq sequencer housed at CIBIO-InBio (Vairão Campus, Portugal). The run was shared between projects, so that the pool for the present study represented 15% of the final loaded pool.

Sequence data

Reads produced on the Miseq were demultiplexed using BASESPACE

(basespace.illumina.com). The Obitoools package (v1.2.11; Boyer et al. 2016) was used for the

181 following: paired-end reads were aligned, alignments with a score < 50 were removed, reads
182 without both primer sequences were removed, reads within each sample were clustered only if
183 100% identical, primer sequences were trimmed, reads < 75 bp and > 120 bp were removed, as
184 were singletons. The resulting sequences were BLASTed against the NCBI Nucleotide database
185 using the MEGABLAST algorithm (Zhang et al. 2000). BLAST results were assigned to taxa
186 using MEGAN (Community Edition 6.10.8; Huson et al. 2016) with the default parameters apart
187 from the following: minScore=150.0, minPercentIdentity=97.0, topPercent=10.0,
188 minSupportPercent=0.0. One taxon, *Sus scrofa*, was observed in a number of samples, but with a
189 relatively low number of reads (maximum contribution to any sample was 0.9%). As one of the
190 other pools in the MiSeq run consisted of a high number of wild pig (*S. scrofa*) reads, these reads
191 were considered to be the result of tag jumping. Consequently, all taxa with a read count less
192 than 1% of the sample read count were removed. Eight other taxa (including hominids) were
193 excluded from the entire dataset based on relatively high read counts in either field, extraction or
194 PCR negatives, or in one case because the genus (*Podarcis*) is not known from Mauritania.

195 RESULTS

196 At the time of sampling, adults of the crowned frog (*Hoplobatrachus occipitalis*) and of the eel
197 catfish (*Clarias* sp.), were detected at all sites via visual surveys (Table 1). The west African
198 crocodile (*Crocodylus suchus*) and the Nile monitor (*Varanus niloticus*) were observed in the
199 water at Matmâta (Table 1). No other vertebrates were observed.

200 Cluster density for the run was 762,000/mm² and the Clusters Passing Filter was 88%. Prior to
201 bioinformatic filtering almost 350,000 reads were obtained across a total 40 PCR products (Table
202 S1). After filtering, before removing hominid-assigned reads and other potential contaminants,

203 over 264,099 reads remained (average = 7,181 reads / sample, excluding negative controls).
204 Following removal of human-assigned reads and other potential contaminants an average of 650
205 reads / sample was obtained. Hominid reads accounted for 184,675 of those filtered out, while
206 5,104 reads were attributed to taxa identified in negative controls (see Table S2). Using DNA
207 extracted from the final filters produced the majority of the reads and taxa detected (Table S3).
208 In only one case was a taxon identified at a site using DNA extracted from the pre-filter, but not
209 the corresponding final filter.

210 Table 1 includes a list of taxa that have been observed in these water-bodies in previous studies
211 (Vale et al. 2015), as well as taxa that were detected visually or via metabarcoding (regardless of
212 whether they were known from previous studies). Catfish DNA (assigned to *Clarias*) was
213 detected at all sampling sites and one barbel species (*Enteromius pobeguini*), known to occur in
214 the Atar region (Trape 2009), was identified at Hamdoûn (Table 1). The *Clarias*-assigned reads
215 are most likely *Clarias anguillaris*, which is known to inhabit water-bodies in the Adrar Atar
216 mountains and was identified during previous studies (Trape 2009; Verheye and Dumont 1984).
217 A number of reads from Hamdoûn were also assigned to *Enteromius eburneensis*, but this
218 species is not known from Mauritania (Vale et al. 2015). The *E. eburneensis*-assigned reads
219 could be variants of *E. pobeguini*-assigned reads, however, as *E. eburneensis*-assigned reads did
220 not match either species perfectly ($\leq 98\%$), it is also possible that these reads belong to a
221 separate species, such as *Enteromius macrops*. Neither *C. anguillaris* nor *E. macrops* have
222 representative 12S sequences in the NCBI Nucleotide database, so this remains unconfirmed.
223 Apart from one instance of *Canis lupus* and one instance of *Podarcis*, no other vertebrates were
224 identified, even though a number of other vertebrate taxa were expected to be present based on
225 previous surveys (Table 1). Reads assigned to *C. lupus* could be from domestic dog (*Canis lupus*

226 *familiaris*) or possibly African golden wolf (*Canis anthus*) as the target fragment is identical for
227 these species.

228 The two positive control spike DNA samples, rehydrated and filtered in the field, resulted in
229 recovery and detection of the three species with higher DNA amounts, while the species with the
230 lowest amount of DNA in the spike was not detected (Figure 2). DNA spiked in to the initial 5 L
231 water samples was only recovered from 0.45 µm filters, despite being first passed through the 1
232 µm pre-filters. The recovery of DNA spiked in to water samples varied across sites: two of the
233 spike species were detected at Hamdoûn, one at Atar and none at Matmâta.

234 DISCUSSION

235 Frogs were observed at the time of sampling in all water-bodies and reptiles in the water at
236 Matmâta, but only two of the expected genera, both fish, were detected via eDNA. There were a
237 number of limitations to this study that may have led to the apparent paucity of results, including
238 small sample size (as a result of lengthy filtering methods and expedition time constraints), the
239 use of a single primer, amplification of human DNA in high proportions, observed contamination
240 and a lack of complete reference databases.

241 Spens et al. (2017) recognized that further research is needed to identify optimal filtering
242 procedures for highly productive and/or turbid waters. An important consideration in the present
243 study is that the pre-filtering step required up to two hours of manual pumping in order to
244 produce the vacuum necessary to draw water through the filter as it became more clogged. This
245 is a prohibitive amount of time for most study scenarios, particularly those in remote areas,
246 where expeditions are time-constrained. This constraint limited our sampling protocol to one
247 sampling point per site. This alone may largely explain why many of the expected vertebrate

species were not detected, as multiple sampling points are required to maximize species detections (Goldberg et al. 2018). In any case, pre-filtering did not substantially aid the final filtering process as small particles remained in the filtrate and continued to cause clogging problems for the final filters. This is despite the fact that our pre-filtering step used 1 μm filters, which is a smaller pore size than for other reported pre-filtering steps (e.g. Djurhuus et al. 2018; Majaneva et al. 2018; Rees et al. 2014; Takahara et al. 2012). As we included eDNA captured from pre-filters in our analyses, and detected only a single vertebrate taxon from just one pre-filter sample, compared to detecting multiple vertebrate taxa from five (of the eight) final filter samples (Table S3), it is evident that eDNA was, at least, not being entirely retained by the pre-filter.

A number of studies have shown the benefits of using encapsulated filters, some with much greater surface areas than the more commonly used disc filters, and although this has not yet been explicitly tested for turbid water, these types of filtration units may provide an efficacious alternative (Civade et al. 2016; Eva et al. 2016; Lopes et al. 2017; Spens et al. 2017; Valentini et al. 2016; Vences et al. 2016). It may be that filtering, while efficacious in low-turbidity systems, is not easily applied in such turbid systems and other, low-volume methods, such as precipitation, bead extraction or centrifugation are the only solution (see Williams et al. 2017).

The primer pair chosen for the current study has been used in previous studies and the target fragment has been applied successfully in assigning taxonomy for vertebrates in these and other studies using closely-situated primer binding sites (Calvignac-Spencer et al. 2013; De Barba et al. 2014; Kelly et al. 2014; Kocher et al. 2017a; Kocher et al. 2017b; Port et al. 2016; Riaz et al. 2011; Rodgers et al. 2017; Shehzad et al. 2012a; Shehzad et al. 2015; Shehzad et al. 2012b; Xiong et al. 2016). Despite this, we did not detect some of the visually identified or expected

271 taxa. It is also somewhat surprising that livestock species, which are known to frequent these
272 sites, were not detected via metabarcoding. A partial explanation is that the primers have a
273 number of mismatches to some of the taxa being targeted at these sites, especially the reptiles
274 (Table 1). However, this does not explain why visually detected species with zero primer
275 mismatches were not amplified. Future studies should consider the use of more than one primer
276 pair for vertebrate species detection, as has been often recommended for invertebrate studies
277 (e.g. Alberdi et al. 2018; Corse et al. 2017).

278 The amplification of human DNA is also very likely to have contributed to the low number of
279 taxa being identified in the present study, as 68 % of the total reads obtained were assigned to
280 hominids. Amplification of human DNA is known to occur even using the most stringent
281 laboratory practices (Pääbo et al. 2004). Future studies should consider the use of human
282 blocking primers for this primer pair, similar to those used by Port et al. (2016).

283 Despite following strict protocols for extractions, a high number of hominid-assigned reads were
284 retrieved from all of our extraction controls, as well as some minor contamination from Felinae.
285 As these did not appear in our PCR negatives, this can be attributed to contamination during the
286 extraction step. This could be due to the use of shared laboratory reagents (namely the lysis
287 buffer) or incomplete flame sterilization of shared instruments. One PCR negative was
288 contaminated with Cercopithecinae, a group which is often studied in our institute. Finally, three
289 taxa (*Gallus gallus*, *Hemidactylus agrius* and *Phyllopezus pollicaris*) were identified from field
290 negatives. The source of this contamination remains unclear; it is possible the distilled water
291 used as a negative control became contaminated, or it may have occurred during downstream
292 extraction or PCR, in a non-systematic manner.

Of the four taxa detected via metabarcoding, only one could be confidently assigned at the species level due to the lack of available reference databases, which presents a major issue for eDNA studies in Africa (Coey et al. 2015). It is likely that there are more COI than 12S barcodes currently available for African species, but difficulties designing primers for COI for vertebrate metabarcoding studies have been highlighted by a number of studies, as they tend to co-amplify DNA from many other taxonomic groups (Deagle et al. 2014; Kondo et al. 2016). Until more African species are barcoded and added to publically available databases, the only method to overcome this issue is to build local reference DNA databases based using target taxa expected to be present in the study area. Of course, this requires a considerable amount of additional effort to obtain tissue samples from many different species, as well as *a priori* knowledge of species expected at field sites.

One of the problems with eDNA studies is the unquantified variability between samples in species detection probability. Using DNA spikes can help to account for inter-site variability, act as quality control for extraction and PCR procedures and compare different workflow methodologies at every step from field capture to sequencing platform. Although DNA spikes have been widely applied during DNA extraction, preservation and PCR steps, the number of studies that have used DNA spikes during field sampling are very limited (see Furlan and Gleeson 2017). Walker et al. (2017) used extracted DNA as spikes to assess degradation of salamander DNA in soil prior to DNA extraction. Pochon et al (2013) used entire larvae as spikes to assess the detection limits of their assays. Green and Field (2012) spiked *Escherichia coli* cells into environmental water samples prior to filtering to assess the variability of DNA recovery among samples. In other studies, water collected from tanks or ponds containing known vertebrate species has been injected into study systems, primarily to investigate survival of

316 eDNA in experimental systems (Barnes et al. 2014; Jerde et al. 2016; Shogren et al. 2016). It is
317 likely that the type of spike used will produce different recovery results. eDNA has a complex
318 physiochemical composition arising from the effects of biological, environmental and physical
319 conditions of a particular site. So it is difficult to discern what the ideal “eDNA spike” might
320 comprise, with options ranging from any combination of whole individuals, cells, mitochondria,
321 whole extracted DNA, sheared DNA of different lengths or synthesized DNA. Furlan and
322 Gleeson (2017) recommend using endogenous eDNA to identify potential false negative results
323 under certain scenarios, but note that endogenous eDNA concentrations will vary, precluding
324 comparison of DNA capture methods.

325 In the current study, the species with the lowest amount of DNA in the spikes was not detected
326 from the positive controls, indicating that there may have been some degradation of spike DNA
327 during the expedition or the methods of recovery/detection were not sensitive enough for DNA at
328 this low level. As DNA from the other three species was detected, this was not a substantial
329 problem for the study. The fact that DNA was recovered from spikes added to the 5 L water
330 samples at concentrations as low as 5 ng/L provided evidence, for Atar and Hamdoûn, that the
331 field filtering process and downstream analyses were indeed adequate. The reasons for the
332 observed variation across sites in the detection of spiked DNA are unclear. It may be that the
333 concentrations used are at the lower limits of detection in general for the methods used in this
334 study, or it may be that other site-specific factors influenced the results, such as incomplete DNA
335 extraction due to partial clogging of extraction spin columns. We used the EZNA Tissue DNA
336 extraction kit as it has achieved good results in our laboratory previously and has been used in a
337 number of other eDNA studies (e.g. Pérez et al. 2013), although we are not aware of any studies
338 comparing the efficacy of this kit with other methods. More sampling is required to examine

whether the individual water-body influences DNA recovery, but it is likely, as Liang and Keeley (2013) found that pH, ion concentration and suspended solid concentration all influenced DNA recovery from spiked samples under laboratory conditions. Green and Field (2012) also found that increased turbidity negatively affected amplification efficiency.

In the present study, spiked DNA was only recovered from 0.45 μm filters, despite the water being pre-filtered through a 1 μm filter beforehand. It may be that 0.45 μm is a more suitable pore size for eDNA recovery, particularly for extracellular DNA and this might be especially pertinent in environments with high concentrations of small-diameter suspended sediments (e.g. clay), as these are known to adsorb DNA, but are $< 1\mu\text{m}$. Alternatively, 1 μm filters have often been used to detect eDNA (Eichmiller et al. 2016; Turner et al. 2014), so it is also possible that the extraction technique used in this study for the pre-filters was not as efficient as for the final filters. Nitrocellulose filters have been used previously in many studies (reviewed by Rees et al. 2014) and resulted in the highest DNA yield in a comparison by Majaneva et al. (2018).

It is notable that species in the DNA spike were detected even at minute concentrations (at two of three sites), although indigenous amphibian eDNA was not. A possible explanation for this is that eDNA might be rapidly broken down in desert environments, as exposure to sunlight, UV and higher temperatures is known to expedite the DNA degradation process (Pilliod et al. 2014; Strickler et al. 2015). More research is needed to confirm this, but it may present another challenge for conducting metabarcoding studies in remote desert environments.

In conclusion, several constraints originated during the present study, which contributed to the very low number of taxa being identified via metabarcoding. First, high turbidity levels led to clogging of filters during field sampling, and this was not alleviated by pre-filtering. This

resulted in an increased amount of time being required per sample, which limited the study to one sampling point per site. Additionally, the use of a single general vertebrate primer set resulted in a high proportion of reads being assigned to hominids, which were likely the result of human contamination during DNA extractions. Finally, a lack of complete reference databases for Africa’s vertebrates hindered the taxonomic assignment of sequences at species level. To overcome these issues, we recommend the following: 1) alternative methods for capturing eDNA (such as high-surface area encapsulated filters) should be tested in challenging desert environments; 2) multiple primer sets should be used for vertebrate species detection; 3) human blocking primers should be incorporated during amplification; and 4) local reference DNA databases should be constructed. This study highlights the need for the development of better eDNA techniques catered for challenging and remote desert environments, which are required if eDNA studies are to be used for describing Africa’s understudied vertebrate biodiversity.

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REFERENCES

- Adrian-Kalchhauser, I., and Burkhardt-Holm, P. 2016. An eDNA assay to monitor a globally invasive fish species from flowing freshwater. *PloS One* **11**(1): e0147558. doi:10.1371/journal.pone.0147558.
- Alberdi, A., Aizpurua, O., Gilbert, M.T.P., and Bohmann, K. 2018. Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods Ecol. Evol.* **9**(1): 134-147. doi:10.1111/2041-210X.12849.
- Barnes, M.A., Turner, C.R., Jerde, C.L., Renshaw, M.A., Chadderton, W.L., and Lodge, D.M. 2014. Environmental conditions influence eDNA persistence in aquatic systems. *Environ. Sci. Technol.* **48**(3): 1819-1827. doi:10.1021/es404734p.
- Boyer, F., Mercier, C., Bonin, A., Le Bras, Y., Taberlet, P., and Coissac, E. 2016. obitools: a unix-inspired software package for DNA metabarcoding. *Mol. Ecol. Resour.* **16**(1): 176-182. doi:10.1111/1755-0998.12428.
- Brito, J.C., Godinho, R., Martínez-Freiría, F., Pleguezuelos, J.M., Rebelo, H., Santos, X., et al. 2014. Unravelling biodiversity, evolution and threats to conservation in the Sahara-Sahel. *Biol. Rev.* **89**(1): 215-231. doi:10.1111/brv.12049.
- Calvignac-Spencer, S., Merkel, K., Kutzner, N., Kühl, H., Boesch, C., Kappeler, P.M., et al. 2013. Carrion fly-derived DNA as a tool for comprehensive and cost-effective assessment of mammalian biodiversity. *Mol. Ecol.* **22**(4): 915-924.

- 400 Civade, R., Dejean, T., Valentini, A., Roset, N., Raymond, J.-C., Bonin, A., et al. 2016. Spatial
401 representativeness of environmental DNA metabarcoding signal for fish biodiversity
402 assessment in a natural freshwater system. *PloS One* **11**(6): e0157366.
403 doi:10.1371/journal.pone.0157366.
- 404 Coey, S., Fernández, C.A., Vogler, A.P., Yu, D., Atkinson, P., and Coates, A. Survey for African
405 freshwater fish using DNA barcoding. *In* 35th Annual Conference of the International
406 Association for Impact Assessment. Firenze Fiera Congress & Exhibition Center,
407 Florence, Italy, 20 - 23 April 2015 2015.
- 408 Corse, E., Megléc, E., Archambaud, G., Ardisson, M., Martin, J.-F., Tougaard, C., et al. 2017. A
409 from-benchtop-to-desktop workflow for validating HTS data and for taxonomic
410 identification in diet metabarcoding studies. *Mol. Ecol. Resour.* **17**(6): e146-e159.
411 doi:10.1111/1755-0998.12703.
- 412 De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., et al. 2014. DNA
413 metabarcoding multiplexing and validation of data accuracy for diet assessment:
414 application to omnivorous diet. *Mol. Ecol. Resour.* **14**(2): 306-323. doi:10.1111/1755-
415 0998.12188.
- 416 Deagle, B.E., Jarman, S.N., Coissac, E., Pompanon, F., and Taberlet, P. 2014. DNA
417 metabarcoding and the cytochrome c oxidase subunit I marker: not a perfect match. *Biol.*
418 *Lett.* **10**(9): 20140562. doi:10.1098/rsbl.2014.0562.

- 419 Djurhuus, A., Pitz, K., Sawaya, N.A., Rojas-Márquez, J., Michaud, B., Montes, E., et al. 2018.
420 Evaluation of marine zooplankton community structure through environmental DNA
421 metabarcoding. *Limnol. Oceanogr. Methods* **16**(4): 209-221. doi:10.1002/lom3.10237.
- 422 Eichmiller, J.J., Miller, L.M., and Sorensen, P.W. 2016. Optimizing techniques to capture and
423 extract environmental DNA for detection and quantification of fish. *Mol. Ecol. Resour.*
424 **16**(1): 56-68. doi:10.1111/1755-0998.12421.
- 425 Eva, B., Harmony, P., Thomas, G., Francois, G., Alice, V., Claude, M., et al. 2016. Trails of
426 river monsters: Detecting critically endangered Mekong giant catfish *Pangasianodon*
427 *gigas* using environmental DNA. *Glob. Ecol. Conserv.* **7**: 148-156.
428 doi:10.1016/j.gecco.2016.06.007.
- 429 Furlan, E.M., and Gleeson, D. 2017. Improving reliability in environmental DNA detection
430 surveys through enhanced quality control. *Mar. Freshw. Res.* **68**(2): 388-395.
431 doi:10.1071/MF15349.
- 432 Gansauge, M.-T., and Meyer, M. 2013. Single-stranded DNA library preparation for the
433 sequencing of ancient or damaged DNA. *Nat. Protoc.* **8**(4): 737.
434 doi:10.1038/nprot.2013.038.
- 435 Goldberg, C.S., Strickler, K.M., and Fremier, A.K. 2018. Degradation and dispersion limit
436 environmental DNA detection of rare amphibians in wetlands: Increasing efficacy of
437 sampling designs. *Sci. Total Environ.* **633**: 695-703. doi:10.1016/j.scitotenv.2018.02.295.
- 438 Green, H.C., and Field, K.G. 2012. Sensitive detection of sample interference in environmental
439 qPCR. *Water Res.* **46**(10): 3251-3260. doi:10.1016/j.watres.2012.03.041.

- 440 Hinlo, R., Gleeson, D., Lintermans, M., and Furlan, E. 2017. Methods to maximise recovery of
441 environmental DNA from water samples. *PloS One* **12**(6): e0179251.
442 doi:10.1371/journal.pone.0179251.
- 443 Huson, D.H., Beier, S., Flade, I., Górska, A., El-Hadidi, M., Mitra, S., et al. 2016. MEGAN
444 community edition-interactive exploration and analysis of large-scale microbiome
445 sequencing data. *PLoS Comput. Biol.* **12**(6): e1004957.
446 doi:10.1371/journal.pcbi.1004957.
- 447 Jerde, C.L., Mahon, A.R., Chadderton, W.L., and Lodge, D.M. 2011. “Sight-unseen” detection
448 of rare aquatic species using environmental DNA. *Conserv. Lett.* **4**(2): 150-157.
449 doi:10.1111/j.1755-263X.2010.00158.x.
- 450 Jerde, C.L., Olds, B.P., Shogren, A.J., Andruszkiewicz, E.A., Mahon, A.R., Bolster, D., et al.
451 2016. Influence of stream bottom substrate on retention and transport of vertebrate
452 environmental DNA. *Environ. Sci. Technol.* **50**(16): 8770-8779.
453 doi:10.1021/acs.est.6b01761.
- 454 Kelly, R.P., Port, J.A., Yamahara, K.M., and Crowder, L.B. 2014. Using environmental DNA to
455 census marine fishes in a large mesocosm. *PloS One* **9**(1): e86175.
456 doi:10.1371/journal.pone.0086175.
- 457 Kircher, M., Sawyer, S., and Meyer, M. 2011. Double indexing overcomes inaccuracies in
458 multiplex sequencing on the Illumina platform. *Nucleic Acids Res.* **40**(1): e3.
459 doi:10.1093/nar/gkr771.

- 460 Kocher, A., de Thoisy, B., Catzeflis, F., Valière, S., Bañuls, A.L., and Muriénne, J. 2017a. iDNA
461 screening: Disease vectors as vertebrate samplers. *Mol. Ecol.* **26**(22): 6478-6486.
462 doi:10.1111/mec.14362.
- 463 Kocher, A., Thoisy, B., Catzeflis, F., Huguin, M., Valière, S., Zinger, L., et al. 2017b. Evaluation
464 of short mitochondrial metabarcodes for the identification of Amazonian mammals.
465 *Methods Ecol. Evol.* **8**(10): 1276-1283. doi:10.1111/2041-210X.12729.
- 466 Kondo, N.I., Nakagawa, M., Matsuzaki, S.-i., Ueno, R., Takamura, K., and Ito, H. 2016. DNA
467 barcoding, environmental DNA and an ongoing attempt of detecting biodiversity in Lake
468 Kasumigaura. *J. Integr. Field Sci.* **13**: 21-29.
- 469 Liang, Z., and Keeley, A. 2013. Filtration recovery of extracellular DNA from environmental
470 water samples. *Environ. Sci. Technol.* **47**(16): 9324-9331. doi:10.1021/es401342b.
- 471 Lopes, C.M., Sasso, T., Valentini, A., Dejean, T., Martins, M., Zamudio, K.R., et al. 2017.
472 eDNA metabarcoding: a promising method for anuran surveys in highly diverse tropical
473 forests. *Mol. Ecol. Resour.* **17**(5): 904-914. doi:10.1111/1755-0998.12643.
- 474 Majaneva, M., Diserud, O.H., Eagle, S.H., Boström, E., Hajibabaei, M., and Ekrem, T. 2018.
475 Environmental DNA filtration techniques affect recovered biodiversity. *Sci. Rep.* **8**(1):
476 4682. doi:10.1038/s41598-018-23052-8.
- 477 Maudet, C., Luikart, G., Dubray, D., Von Hardenberg, A., and Taberlet, P. 2004. Low
478 genotyping error rates in wild ungulate faeces sampled in winter. *Mol. Ecol. Resour.*
479 **4**(4): 772-775. doi:10.1111/j.1471-8286.2004.00787.x.

- 480 Pääbo, S., Poinar, H., Serre, D., Jaenicke-Després, V., Hebler, J., Rohland, N., et al. 2004.
481 Genetic analyses from ancient DNA. *Annu. Rev. Genet.* **38**(1): 645-679.
482 doi:10.1146/annurev.genet.37.110801.143214.
- 483 Pérez, L.M., Fittipaldi, M., Adrados, B., Morató, J., and Codony, F. 2013. Error estimation in
484 environmental DNA targets quantification due to PCR efficiencies differences between
485 real samples and standards. *Folia Microbiol.* **58**(6): 657-662. doi:10.1007/s12223-013-
486 0255-5.
- 487 Pilliod, D.S., Goldberg, C.S., Arkle, R.S., and Waits, L.P. 2014. Factors influencing detection of
488 eDNA from a stream-dwelling amphibian. *Mol. Ecol. Resour.* **14**(1): 109-116.
489 doi:10.1111/1755-0998.12159.
- 490 Pimm, S.L., Jenkins, C.N., Abell, R., Brooks, T.M., Gittleman, J.L., Joppa, L.N., et al. 2014. The
491 biodiversity of species and their rates of extinction, distribution, and protection. *Science*
492 **344**(6187): 1246752. doi:10.1126/Science.1246752.
- 493 Pochon, X., Bott, N.J., Smith, K.F., and Wood, S.A. 2013. Evaluating detection limits of next-
494 generation sequencing for the surveillance and monitoring of international marine pests.
495 *PloS One* **8**(9): e73935. doi:10.1371/journal.pone.0073935.
- 496 Port, J.A., O'Donnell, J.L., Romero-Maraccini, O.C., Leary, P.R., Litvin, S.Y., Nickols, K.J., et
497 al. 2016. Assessing vertebrate biodiversity in a kelp forest ecosystem using
498 environmental DNA. *Mol. Ecol.* **25**(2): 527-541. doi:10.1111/mec.13481.

- 499 Rees, H.C., Maddison, B.C., Middleditch, D.J., Patmore, J.R.M., and Gough, K.C. 2014. The
500 detection of aquatic animal species using environmental DNA – a review of eDNA as a
501 survey tool in ecology. *J. Appl. Ecol.* **51**(5): 1450-1459. doi:10.1111/1365-2664.12306.
- 502 Riaz, T., Shehzad, W., Viari, A., Pompanon, F., Taberlet, P., and Coissac, E. 2011. ecoPrimers:
503 inference of new DNA barcode markers from whole genome sequence analysis. *Nucleic
504 Acids Res.* **39**(21): e145-e145. doi:10.1093/nar/gkr732.
- 505 Robson, H.L., Noble, T.H., Saunders, R.J., Robson, S.K., Burrows, D.W., and Jerry, D.R. 2016.
506 Fine-tuning for the tropics: application of eDNA technology for invasive fish detection in
507 tropical freshwater ecosystems. *Mol. Ecol. Resour.* **16**(4): 922-932. doi:10.1111/1755-
508 0998.12505.
- 509 Rodgers, T.W., Xu, C.C., Giacalone, J., Kapheim, K.M., Saltonstall, K., Vargas, M., et al. 2017.
510 Carrion fly-derived DNA metabarcoding is an effective tool for mammal surveys:
511 evidence from a known tropical mammal community. *Mol. Ecol. Resour.* **17**(6): e133–
512 e145. doi:10.1111/1755-0998.12701.
- 513 Shehzad, W., McCarthy, T.M., Pompanon, F., Purevjav, L., Coissac, E., Riaz, T., et al. 2012a.
514 Prey preference of snow leopard (*Panthera uncia*) in South Gobi, Mongolia. *PloS One*
515 **7**(2): e32104. doi:10.1371/journal.pone.0032104.
- 516 Shehzad, W., Nawaz, M.A., Pompanon, F., Coissac, E., Riaz, T., Shah, S.A., et al. 2015. Forest
517 without prey: livestock sustain a leopard *Panthera pardus* population in Pakistan. *Oryx*
518 **49**(2): 248-253. doi:10.1017/S0030605313001026.

- 519 Shehzad, W., Riaz, T., Nawaz, M.A., Miquel, C., Poillot, C., Shah, S.A., et al. 2012b. Carnivore
520 diet analysis based on next-generation sequencing: application to the leopard cat
521 (*Prionailurus bengalensis*) in Pakistan. Mol. Ecol. **21**(8): 1951-1965. doi:10.1111/j.1365-
522 294X.2011.05424.x.
- 523 Shogren, A.J., Tank, J.L., Andruszkiewicz, E.A., Olds, B., Jerde, C., and Bolster, D. 2016.
524 Modelling the transport of environmental DNA through a porous substrate using
525 continuous flow-through column experiments. J. Royal Soc. Interface **13**(119).
526 doi:10.1098/rsif.2016.0290.
- 527 Spens, J., Evans, A.R., Halfmaerten, D., Knudsen, S.W., Sengupta, M.E., Mak, S.S., et al. 2017.
528 Comparison of capture and storage methods for aqueous microbial eDNA using an
529 optimized extraction protocol: advantage of enclosed filter. Methods Ecol. Evol. **8**(5):
530 635-645. doi:10.1111/2041-210X.12683.
- 531 Strickler, K.M., Fremier, A.K., and Goldberg, C.S. 2015. Quantifying effects of UV-B,
532 temperature, and pH on eDNA degradation in aquatic microcosms. Biol. Conserv. **183**:
533 85-92. doi:10.1016/j.biocon.2014.11.038.
- 534 Takahara, T., Minamoto, T., Yamanaka, H., Doi, H., and Kawabata, Z.i. 2012. Estimation of fish
535 biomass using environmental DNA. PloS one **7**(4): e35868.
536 doi:10.1371/journal.pone.0035868.
- 537 Thomsen, P.F., Kielgast, J.O.S., Iversen, L.L., Wiuf, C., Rasmussen, M., Gilbert, M.T.P., et al.
538 2012. Monitoring endangered freshwater biodiversity using environmental DNA. Mol.
539 Ecol. **21**(11): 2565-2573. doi:10.1111/j.1365-294X.2011.05418.x.

- 540 Trape, S. 2009. Impact of climate change on the relict tropical fish fauna of Central Sahara:
541 threat for the survival of Adrar mountains fishes, Mauritania. Plos one **4**(2): e4400.
542 doi:10.1371/journal.pone.0004400.
- 543 Turner, C.R., Barnes, M.A., Xu, C.C., Jones, S.E., Jerde, C.L., and Lodge, D.M. 2014. Particle
544 size distribution and optimal capture of aqueous microbial eDNA. Methods Ecol. Evol.
545 **5**(7): 676-684. doi:10.1111/2041-210X.12206.
- 546 Vale, C.G., Pimm, S.L., and Brito, J.C. 2015. Overlooked mountain rock pools in deserts are
547 critical local hotspots of biodiversity. PloS One **10**(2): e0118367.
548 doi:10.1371/journal.pone.0118367.
- 549 Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P.F., et al. 2016.
550 Next-generation monitoring of aquatic biodiversity using environmental DNA
551 metabarcoding. Mol. Ecol. **25**(4): 929-942. doi:10.1111/mec.13428.
- 552 Vences, M., Lyra, M.L., Perl, R.G.B., Bletz, M.C., Stanković, D., Lopes, C.M., et al. 2016.
553 Freshwater vertebrate metabarcoding on Illumina platforms using double-indexed
554 primers of the mitochondrial 16S rRNA gene. Conserv. Genet. Resour. **8**(3): 323-327.
555 doi:10.1007/s12686-016-0550-y.
- 556 Verheye, H.M., and Dumont, H.J. 1984. The calanoid copepods of the Nile system.
557 Hydrobiologia **110**(1): 191-212. doi:10.1007/Bf00025791.
- 558 Walker, D.M., Leys, J.E., Dunham, K.E., Oliver, J.C., Schiller, E.E., Stephenson, K.S., et al.
559 2017. Methodological considerations for detection of terrestrial small-body salamander

- 560 eDNA and implications for biodiversity conservation. *Mol. Ecol. Resour.* **17**(6): 1223-
561 1230. doi:10.1111/1755-0998.12667.
- 562 Williams, K.E., Huyvaert, K.P., and Piaggio, A.J. 2017. Clearing muddied waters: Capture of
563 environmental DNA from turbid waters. *PloS One* **12**(7): e0179282.
564 doi:10.1371/journal.pone.0179282.
- 565 Xiong, M., Shao, X., Long, Y., Bu, H., Zhang, D., Wang, D., et al. 2016. Molecular analysis of
566 vertebrates and plants in scats of leopard cats (*Prionailurus bengalensis*) in southwest
567 China. *J. Mammal.* **97**(4): 1054-1064. doi:10.1093/jmammal/gyw061.
- 568 Zhang, Z., Schwartz, S., Wagner, L., and Miller, W. 2000. A greedy algorithm for aligning DNA
569 sequences. *J. Comput. Biol.* **7**(1-2): 203—214. doi:10.1089/10665270050081478.

TABLES

Table 1. Taxa expected and/or detected, either visually or via metabarcoding (read count indicated). Also shown are the number of base mismatches for each species for the primer pair used in this study.

		Actinopterygii			Amphibia		Reptilia			Aves		Mammalia	
	Site	<i>Clarias anguillaris</i> ^a	<i>Enteromius pobeguini</i>	<i>Enteromius eburneensis</i> ^b	<i>Hoplobatrachus occipitalis</i>	<i>Sclerophrys xeros</i>	<i>Crocodylus suchus</i>	<i>Varanus niloticus</i>	<i>Ardea cinerea</i>	<i>Bubulcus ibis</i>	<i>Vanellus spinosus</i>	<i>Canis lupus</i> ^c	<i>Canis anthus</i>
Expected	Matmâta	✓			✓	✓	✓	✓	✓	✓	✓		✓
	Atar	✓			✓	✓							✓
	Hamdoûn	✓	✓		✓	✓							✓
Visual observation	Matmâta	✓			✓		✓	✓					
	Atar	✓			✓								
	Hamdoûn	✓			✓								
eDNA detection	Matmâta	3691										777	
	Atar	148											
	Hamdoûn	1549	683	2676									
Forward primer mismatch		0*	0	0*	0	0	2*	2	0	0	0*	0	0
Reverse primer mismatch		0*	0	0*	0	1	2*	1	0	0	0*	0	0

^a Reads assigned to *Clarias*, likely *C. anguillaris*

^b Reads assigned to *E. eburneensis*, likely either *E. pobeguini* or *E. macrops*

^c Reads assigned to *C. lupus*, likely either *C. anthus* or *C. lupus familiaris*

* Primer binding site sequence unavailable for this species. Indicated are the number of mismatches to other species within the same genus.

FIGURE CAPTIONS

Figure 1. Locations and photographic representations of sampling sites: Atar dam (photo by G. Velo-Antón), Hamdoûn rock pool (photo by G. Velo-Antón), and Matmâta river bed (photo by JC Brito). Base map: Map data ©2018 GeoBasis-DE/BKG (©2009), Google, Inst. Geogr. Nacional, ORION-ME. Altitude adapted from USGS (2006).

Figure 2. Number of reads obtained following recovery of spiked DNA, which for positive controls (PC1 and PC2) was rehydrated in 1 ml dH₂O before filtering and for field samples was rehydrated in 1 ml dH₂O before adding to 5 L water samples.

Draft

Figure 1. Locations and photographic representations of sampling sites: Atar dam (photo by G. Velo-Antón), Hamdoûn rock pool (photo by G. Velo-Antón), and Matmâta river bed (photo by JC Brito). Base map: Map data ©2018 GeoBasis-DE/BKG (©2009), Google, Inst. Geogr. Nacional, ORION-ME. Altitude adapted from USGS (2006).

214x217mm (300 x 300 DPI)

Figure 2. Number of reads obtained following recovery of spiked DNA, which for positive controls (PC1 and PC2) was rehydrated in 1 ml dH₂O before filtering and for field samples was rehydrated in 1 ml dH₂O before adding to 5 L water samples.

273x143mm (300 x 300 DPI)