Received: 10 July 2023 | Revised: 11 December 2023 | Accepted: 30 January 2024

DOI: 10.1002/aqc.4111

RESEARCH ARTICLE

WILEY

Monitoring the threatened *Harttiella* (Siluriformes, Loricariidae) with environmental DNA: A comparison between metabarcoding and targeted digital PCR

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Funding information
Agence Nationale de la Recherche, Grant/Award Numbers: ANR-10-LABX-41, ANR-10-LABX-25-01; ANR, Grant/Award Number: ANR-17-CE02-007-01; DGTM; Office de l'Eau Guyane

Abstract

1. Environmental DNA has emerged as an efficient alternative to traditional sampling methods. A standard multispecies or a targeted single-species approach can be used for analysing environmental DNA samples. The costs, benefits, and drawbacks associated with these two approaches are quite different.
2. Here, a comparison between standard multispecies metabarcoding and targeted species assay using digital PCR (dPCR) for two threatened *Harttiella* species occurring in French Guiana (*Harttiella lucifer* and *H.* nsp. "Makwali") was performed.
3. Samples were collected in 11 sites of the upper Maroni River drainage basin and located in the Galbao mountain range, known to host the targeted species. The "Teleo" primer was used for the metabarcoding approach. To implement a new dPCR assay, specific primers and probes for the two targeted *Harttiella* species were developed.
4. This targeted dPCR assay detected *Harttiella* in seven sites. All of these sites were also positive for metabarcoding detection, and the habitat characteristics were favourable for the species.
5. This study demonstrated that both targeted (dPCR) and multispecies (metabarcoding) approaches can be used for the monitoring of the threatened *H. lucifer* and *H.* nsp. "Makwali" species.
6. From a conservation point of view, we recommend to use the metabarcoding approach to update the spatial distribution of the species, while the dPCR method can be used for a temporal follow-up of known *Harttiella* populations.

KEYWORDS
dPCR, environmental DNA, metabarcoding, neotropics, targeted species detection, threatened species

Jérôme Murienne and Sébastien Brosse should be considered as co-senior authors.

1 | INTRODUCTION

Freshwater habitats are crucial to life on Earth as they provide habitat for almost 10% of all known species while covering less than 1% of the Earth's surface area (Dudgeon, 2019). Nevertheless, freshwater ecosystems are hotspots for human activities that have led to overexploitation, water pollution, flow modification, destruction or degradation of habitat, and invasive species introductions (Dudgeon, 2019), leading to deep changes in biodiversity in more than 50% of the world's rivers (Su et al., 2021). Over the past 50 years, freshwater populations have declined by more than 80% which is more than any other species group (Albert et al., 2021). Moreover, not all freshwater ecosystems are impacted equally and tropical rivers and lakes are currently facing the highest biodiversity loss (WWF, 2022).

The Neotropical region hosts the richest freshwater fish fauna on Earth corresponding to one quarter of the global fish diversity (Lévêque et al., 2008). In addition, the presence of endemic species is remarkably high in neotropical freshwater ecosystems, with the Guiana Shield hosting 700 endemic species for almost 2200 species (Abell et al., 2008; Covain et al., 2012; Lemopoulos & Covain, 2019). Nevertheless, the Guiana Shield biodiversity is severely threatened by gold mining which induces deforestation, soil degradation, and river mercury contamination (Cantera et al., 2022; Coutant et al., 2023; Timsina et al., 2022). Considering this combination of high richness, high endemism, and increasing anthropogenic threats, particular attention should be paid to the management of the Guiana Shield freshwater ecosystems.

Contrary to terrestrial ecosystems, inventorying freshwater biodiversity is not straightforward and requires efficient non-invasive monitoring methods. Yet, most freshwater monitoring methods rely on destructive, time and labour-intensive methods that are often selective in species (Cilleros et al., 2019). During the past decade, environmental DNA (eDNA) has emerged as a sensible alternative to traditional sampling methods (Taberlet et al., 2012). This non-invasive approach uses DNA shed by organisms into the aquatic environment that can be extracted from water without first isolating any target individuals (Taberlet et al., 2012). eDNA samples can be analysed using single-species or multispecies approaches according to the primer specificity (i.e., specific vs. universal) (Valentini et al., 2016). Most fish eDNA studies used single-species approaches, mainly targeting exotic species but also endangered, common species or species of economic importance (Yao et al., 2022). The high detection sensitivity of single-species eDNA approaches makes them an efficient tool for monitoring endangered or elusive species (Baker et al., 2023; Brys et al., 2021; Everts et al., 2023; Lehman et al., 2020; Thomsen et al., 2012). Other studies used a multispecies approach to characterize species composition (Cilleros et al., 2019; West et al., 2020; Yamamoto et al., 2017), but it has been reported to be more time-consuming than a targeted approach, and it might also be less sensitive, despite a clear lack of knowledge on this last aspect (Harper et al., 2018; Lacoursière-Roussel et al., 2018). Differences in the cost, benefits, and drawbacks associated with the single-species and multispecies eDNA approaches were reported for lentic and marine systems (Gargan et al., 2022; Harper et al., 2018; Wood et al., 2019; Yu et al., 2022), but similar studies remain scarce for lotic systems and limited to a few temperate streams (Bylemans et al., 2019; Schenekar et al., 2020). For instance, McColl-Gausden et al. (2023) compared the single-species and multispecies detection methods and showed that single-species are generally more sensitive compared with metabarcoding. Nevertheless, those results are highly sensitive to detection thresholds and study design. The sensitivity of each eDNA approach being species- and habitat-specific, complementary studies comparing single-species and multispecies detection methods is needed to provide effective monitoring guidelines suitable for managers.

Here, a comparison between standard multispecies metabarcoding and targeted species assay using digital PCR (dPCR) was performed for two threatened *Harttiella* species (Siluriformes, Loricariidae) occurring in French Guiana streams, a territory located in the Guiana Shield and part of the northeastern Amazonian biome. Implications of our findings are discussed in light of the objectives of the French National Action Plan (2023–2032) targeting the *Harttiella* genus.

2 | MATERIALS AND METHODS

2.1 | *Harttiella* genus

The armoured catfish (Loricariidae) represents a highly diversified freshwater fish family including roughly 1000 species (Covain et al., 2012). Among them, the genus *Harttiella* contains rheophilic species, most of them being threatened. Six species (*Harttiella intermedia*, *Harttiella janmoli*, *Harttiella longicauda*, *Harttiella lucifer*, *Harttiella parva*, and *Harttiella pilosa*) are endemic to French Guiana and one (*Harttiella crassicauda*) has been only observed in Suriname (Covain et al., 2012). More recently, two potentially new species have been collected (*H.* sp. "aff. *lucifer*" and *H.* nsp. "Makwali"), which still await formal description. All *Harttiella* species except *H. longicauda* have a narrow geographical range and only occur in a single river basin. They are moreover confined to small streams that are often threatened by anthropic activities (e.g., mining activities and deforestation). Combined with the rarity of most of the *Harttiella* species, their low fecundity and dispersal capacities, and the threats to their habitats, the six formally described species occurring in French Guiana (*H. crassicauda* was never found in French Guiana) are considered as threatened by the International Union for Conservation of Nature (four species Critically Endangered, one Endangered, and one Vulnerable; Allard et al., 2017).

2.2 | Study sites and *Harttiella* habitat preference

Aquatic environmental samples were collected in January 2019 in 11 sites located on several streams flowing down from the Galbao mountains (French Guiana, Maroni drainage basin) (Figure 1). This location presents the advantage of harbouring several localities with

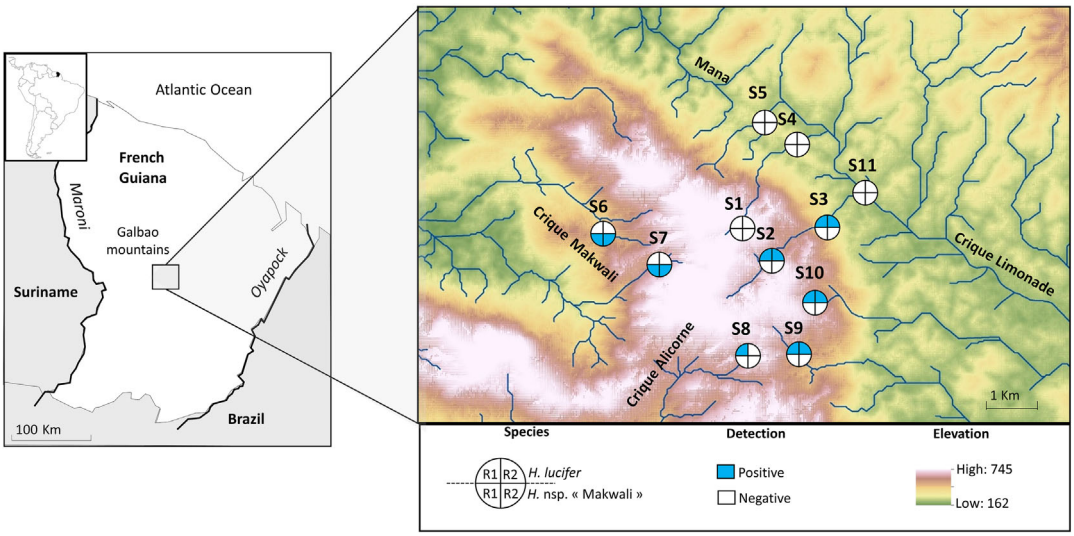

FIGURE 1 Localization of the sampling sites and digital PCR detection according to the targeted species. Sampling replicates are named "R1" and "R2."

favourable and unfavourable conditions for the presence of *Harttiella* species. *Harttiella* species are mainly found in altitude (120–800 m) and in the headwater streams (Covain et al., 2012). They are strictly dependent of small forest streams and inhabit fast-flowing water areas characterized by large-sized mineral substratum (boulders) and waterfalls (Brosse et al., 2013; Mol et al., 2007). Such habitats are also characterized by relatively low temperatures and high levels of O_2 saturation rate (Covain et al., 2012). Moreover, *H. lucifer* and *H.* nsp. "Makwali" are known to be present in some of the study sites but are never sympatric (Covain et al., 2012; Plan National d'Actions des Harttiella et des Anomaloglossus de Guyane). This provides a perfect opportunity to test in situ the specificity and the performance of the two detection methods.

Several site characteristics were recorded to assess *Harttiella* habitat preference: latitude, longitude, elevation (m), water depth (m), stream width (m), conductivity (µSiemens), temperature (°C), pH, turbidity (NTU), O_2 saturation (%), slope (%), and substratum particle size measured as a percentage of coverage of mud, silt, sand, gravel, pebbles, and boulders (Table S1). A principal component analysis was performed on these data using the FactoMineR package (Lê et al., 2008) to show the distribution of the 11 sites according to environmental variables accounting for the local habitat. All the metadata associated with the samples are listed in Supporting Information S1.

2.3 | Sampling and eDNA extraction

Twenty-two environmental samples were collected by filtering two replicates of 34 L of water at each site (Cantera et al., 2019). A peristaltic pump (Vampire sampler, Buerkle, Germany) and a single-use tubing were used to pump the water into a single-use filtration capsule (VigiDNA 0.45 µm; SPYGEN, le Bourget-du-Lac, France). The input part of the tubing was placed a few centimetres below the surface in zones with high water flow. Sampling was achieved in a turbulent area (rapid hydromorphologic unit) to ensure an optimal homogenization of the DNA throughout the water column. To avoid DNA contamination among sites, the operator always remained downstream from the filtration area and stayed on emerging rocks. At the end of the filtration, the filtration capsule was emptied of water, filled with 80 mL of CL1 conservation buffer (SPYGEN), and stored in the dark.

During eDNA sampling, one operator was looking for *Harttiella* specimens using a hand net downstream from the water filtration site. The upstream area was then investigated using the same capture method once filtration was completed.

For DNA extraction, each filtration capsule was agitated for 15 min on an S50 shaker (cat Ingenieurbüro™) at 800 rpm and then transferred into a 50 mL tube before being centrifuged for 15 min at 15,000×*g*. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of the tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50 mL tube and stored for at least one night at −20°C. Tubes were centrifuged at 15,000 *g* for 15 min at 6°C, and the supernatants were discarded. After this step, 720 µL of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen) was added. The tubes were then vortexed, and the supernatants were transferred to 2 mL tubes containing 20 µL of Proteinase K. The tubes were finally incubated at 56°C for 2 h. Afterwards, DNA extraction was performed using the NucleoSpin Soil kit (MACHEREY-NAGEL GmbH & Co., Düren Germany) starting from step six and following the manufacturer's instructions. The elution was performed by adding 100 µL of SE buffer twice. Four negative extraction controls were also performed. They were amplified and sequenced in the same way and in parallel to the field samples to monitor possible laboratory contaminants. After the DNA extraction, the samples were tested for

inhibition by quantitative PCR (qPCR) following the protocol in Biggs et al. (2015). Briefly, qPCR was performed in duplicate for each sample. If at least one of the replicates showed a different cycle threshold than expected (at least two cycle thresholds), the sample was considered inhibited and diluted fivefold before the amplification.

2.4 | Detection by multispecies metabarcoding

The "Teleo" primer pair (Forward 5′-ACACCGCCCGTCACTCT-3′, Reverse 5′-CTTCCGGTACACTTACCATG-3′) (Valentini et al., 2016) was used as it has been shown to provide a species-level discrimination of the local fish fauna (Cantera et al., 2019; Cantera et al., 2022; Cilleros et al., 2019; Coutant et al., 2020; Coutant et al., 2023). DNA amplifications were performed in a final volume of 25 µL including 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 0.2 µM of primers, 10 mM of Tris–HCl, 50 mM of KCl, 2.5 mM of $MgCl_2$, 0.2 mM of each dNTP, and 3 µL of DNA template. Human blocking primers with a final concentration of 4 µM and 0.2 µg/µL of bovine serum albumin (Roche Diagnostic, Basel, Switzerland) were added to the mixture (De Barba et al., 2014). We performed 12 PCR replicates per field sample. The forward and reverse primer tags were identical within each PCR replicate. The PCR mixture was denatured at 95°C for 10 min, followed by 50 cycles of 30 s at 95°C, 30 s at 55°C, and 1 min at 72°C, and a final elongation step at 72°C for 7 min. This step was done in a room dedicated to amplified DNA with negative air pressure and physical separation from the DNA extraction rooms (with positive air pressure). The purified PCR products were pooled in equal volumes to achieve an expected sequencing depth of 500,000 reads per sample before library preparation.

Four libraries were sequenced using an Illumina HiSeq 2500 (2x125 bp) (Illumina, San Diego, CA, USA) and the HiSeq SBS Kit v4 (Illumina, San Diego, CA, USA). Sequencing was performed following the manufacturer's instructions at Fasteris facilities (Geneva, Switzerland). To monitor possible contaminants, 14 negative extraction controls and four negative PCR controls (ultrapure water, 12 replicates) were amplified per primer pair and sequenced in parallel to the sample.

The sequence reads were analysed using the functions of the OBITools package following the protocol described in Valentini et al. (2016). Briefly, forward and reverse reads were assembled using the `illuminapairedend` program. Subsequently, the `ngsfilter` program was used to assign the sequences to each sample. A separate data set was created for each sample by splitting the original data set into several files using `obisplit`. Sequences shorter than 20 bp or occurring fewer than 10 times per sample were discarded. Sequences labelled as "internal" by the `obiclean` program, corresponding most likely to PCR errors, were clustered with the "head" sequences, corresponding most likely to the "true" sequence. All molecular operational taxonomic units with a frequency of occurrence below 0.001 per library in each sample were discarded, considered as tag-jumps (Schnell et al., 2015). These thresholds were empirically determined to clear all reads from the extraction and PCR negative controls included in our global data production procedure as suggested by De Barba et al. (2014) and Taberlet et al. (2018). Finally, `ecotag` was used for the taxonomic assignment of molecular operational taxonomic units.

2.5 | Detection by targeted dPCR

The framework put forward by the DNAqua-Net consortium and the MIQE guidelines was followed to validate our targeted eDNA assays (Huggett, 2020; Thalinger, Deiner, et al., 2021a). All information about primer design and specificity testing (in silico and in vitro) are presented in Tables S2 and S3.

2.5.1 | dPCR assays

The assays were performed by Ingénierie et Analyses en Génétique Environnementale, a company specialized in the detection of eDNA by dPCR. dPCR reaction mixtures were prepared in a preplate as follows. For Nanoplate 26K reactions (Qiagen, Cat.No/ID:250002), 10 µL of 4× QIAcuity Probe PCR kit (Qiagen, Cat.No/ID:250101), 1 µL of each of the three 20× sets of primers and probes (*H. lucifer*, *H.* nsp. "Makwali", and *Myloplus rhomboidalis*, total 3 µL), and 4 µL of eDNA and RNase-free water were combined to reach a final reaction volume of 40 µL.

Reaction mixtures were transferred into a QIAcuity Nanoplate and loaded onto the QIAcuity Eight instrument to perform dPCR. The amplification step was performed following this cycling protocol: 2 min at 95°C for enzyme activation, 15 s at 95°C for denaturation, and 30 s at 58°C for annealing/extension during 40 cycles. Then, an imaging step was completed by reading the following channels: green (excitation 463–503 nm; emission 518–548 nm), yellow (excitation 514–535 nm; emission 550–564 nm), and orange (excitation 543–565 nm; emission 580–606 nm), corresponding to the triplex. Data were analysed using the QIAcuity Software Suite V1.2 and expressed as copies per microlitre of reaction volume (40 µL final). A detection threshold of fluorescence intensity (RFU) specific to each species of the triplex was set using a manual threshold: 70 RFU for *H. lucifer*, 72 RFU for *H.* nsp. "Makwali", and 94 RFU for *M. rhomboidalis*. There is a positive control for each targeted species (diluted 1/1000) and negative controls (H_2O). Similar to Fossøy et al. (2020) and Wacker et al. (2019), the presence of positive partitions in negative controls was used to set a threshold to assess a sample as positive. Here, another type of negative control was added by using the *M. rhomboidalis* assay on our samples. This species is exclusively found in large rivers and thus does not occur in any of our sample sites. *M. rhomboidalis* detection on our sample testifies of the level of false positive. The highest concentration within the controls was assigned as the limit of blank. Samples with a concentration below the limit of blank setting at 0.2 copies/µL (i.e., four partitions) were considered as negative.

3 | RESULTS

3.1 | Metabarcoding versus dPCR

Concerning metabarcoding detection, *H. lucifer* was detected in five sites (S2, S3, S8, S9, and S10) and *H.* nsp. "Makwali" was detected in the sampling sites 6 and 7 (Figure 2). The number of positive PCRs was always high (more than 9 out of 12 replicates) except for the sampling site 8 (4/12 and 3/12 for the two biological replicates). For each species, the detection always occurred in both biological replicates (Figure 2 and Table S4). The dPCR assays detected *H. lucifer* in five sampling sites (S2, S3, S8, S9, and S10). *H.* nsp. "Makwali" was detected in two sites (S6 and S7). Each dPCR detection occurred in both biological replicates expected for sampling site 8 (Figure 2 and Table S4).

3.2 | Habitat preference and detection

According to the habitat characteristics, the sampling sites S2, S3, S6, S7, S8, S9, and S10 harboured favourable conditions for *Harttiella* (Figure 3). These sites were characterized by a high percentage of boulders (60% ± 12.9) and gravels (15% ± 14.1), high O_2 saturation rate (95.2% ± 2.5), and a high elevation (422 m ± 69) (Figure 3). On the opposite, unfavourable habitats were characterized by higher temperatures (23.2°C ± 0.7) and wider (5 m ± 2.5) and deeper (0.3 m ± 0.18) rivers with more silt (25% ± 21) and mud (23% ± 26) corresponding to the sampling sites S1, S4, S5, and S11. Detection (metabarcoding or dPCR) always occurred in sampling sites where the habitat was favourable for *Harttiella* (Figure 3). Concerning visual detections of *Harttiella*, specimens were only observed in three sampling sites (S3, S6, and S9) (Figure 2).

4 | DISCUSSION

4.1 | Congruence between metabarcoding and dPCR results

First, results were all congruent between replicates for metabarcoding detection (Figure 2). dPCR detections were also congruent between replicates except for one sampling site (S8) in which detection only occurred in one replicate. This detection corresponds to the lowest concentration observed among all sampling sites (0.4 copies/µL). The number of positive PCR (fewer than 5 positive PCRs out of 12) and number of reads (fewer than 9000) obtained with the metabarcoding approach at this same site were the lowest observed across all sites highlighting that both methods provided congruent results. Moreover, from a conservation point of view, detecting the species in one replicate is enough to validate its presence. Comparing metabarcoding and dPCR detections shows a congruent detection of *Harttiella* species, highlighting the effectiveness of both methods (Figure 2). Our

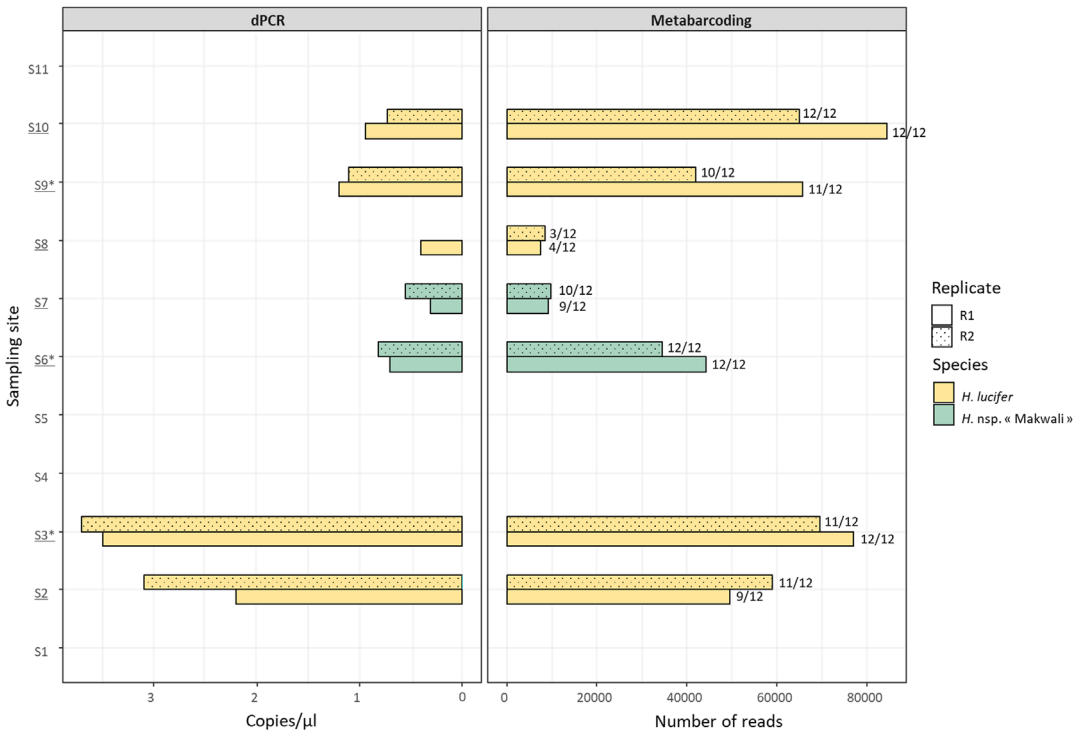

FIGURE 2 Detection of *Harttiella* species using a multispecies metabarcoding approach (right panel) and a target species digital PCR (dPCR) approach (left panel). The number of positive replicates is displayed for each sample. Sites underlined represents favourable habitat for the genus, and asterisks (*) correspond to sites where specimens were observed. Only the detections above the threshold of detection are shown.

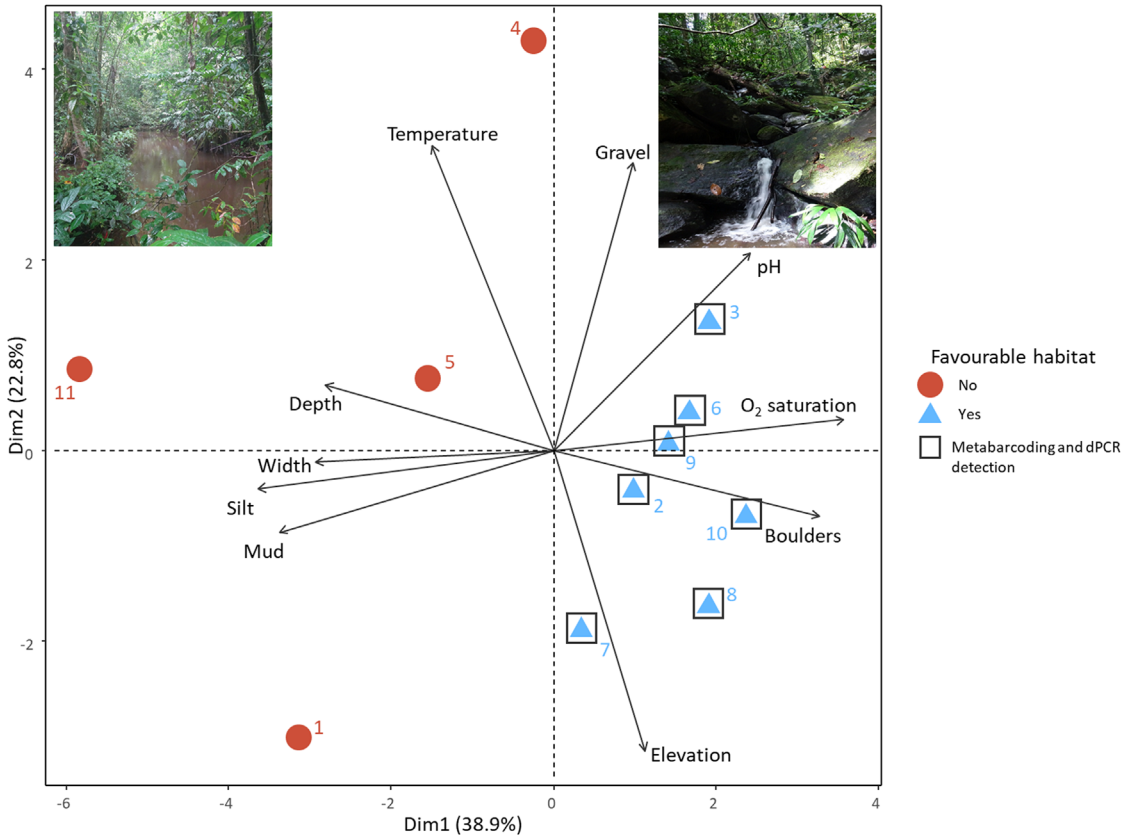

FIGURE 3 Principal component analysis showing sampling sites (triangles) ordination according to environmental features. Only the 10 environmental variables which contribute the most were represented. Blue triangles indicate favourable habitats for *Harttiella*, whereas red circles indicate unfavourable habitats. Squared triangles indicate *Harttiella* detection by digital PCR (dPCR) and metabarcoding.

results are consistent with those of Schneider et al. (2016) on invasive mosquito species, in which they found congruent results between qPCR and metabarcoding. In contrast, Bylemans et al. (2019) and Schenekar et al. (2020) have found more contrasted results for stream fishes suggesting that metabarcoding has a lower detection efficiency than targeted species methods.

Detection of *H. lucifer* and *H.* nsp. "Makwali" was congruent with the expected presence of *Harttiella* given stream habitat characteristics (Figure 3). Overall, this work has demonstrated that targeted (dPCR) and multispecies (metabarcoding) approaches can both be used for the monitoring of *H. lucifer* and *H.* nsp "Makwali" species with similar efficiency as they provide congruent results between methods. Those results were moreover consistent with the known distribution of the targeted species and detected only in local environmental contexts favourable to the species.

4.2 | Advantages and drawbacks associated with each method and their implication for conservation

In the present study, we showed that targeted and multispecies approaches provide similar detection efficiency of *Harttiella* species in French Guiana. Each of these non-invasive approaches is essential to help improve *Harttiella* conservation and to answer objectives of the French National Action Plan targeting the genus. Indeed, a major challenge with the conservation of threatened species is to be able to effectively manage populations without disturbing them.

4.2.1 | Updating *Harttiella* species distribution

One of the first priority actions of the National Action Plan is to complete the spatial distribution of the *Harttiella* species (Axis 4 of the National Action Plan). Indeed, the distribution of two *Harttiella* species (*H.* aff. *lucifer* and *H.* nsp. "Makwali") has not yet been evaluated (Plan National d'Actions des Harttiella et des Anomaloglossus de Guyane). They are only known from a single stream, without precise knowledge on their actual distribution. Although some *Harttiella* species are micro-endemics and known from a single population (*H. intermedia*, *H. janmoli*, and *H. parva*), *H. lucifer* and *H. longicauda* have a wider distribution range. Prospecting for new *H.* aff. *lucifer* and *H.* nsp. "Makwali" populations is therefore needed to update their distribution range. In the same way, prospecting for new *Harttiella* populations in the remote mountain stream of French Guiana is needed to check for new populations of known species but also to search for still unknown *Harttiella* species.

Metabarcoding is the most appropriate approach to achieve this goal as it has already been shown to provide highly reliable fish inventories in rivers and streams of French Guiana (Cantera et al., 2019; Coutant et al., 2021). It also provides the opportunity to detect new putative species from a known genera (e.g., *Harttiella*). Metabarcoding therefore offers the possibility for managers to avoid an a priori selection of target organisms, thus allowing the detection of unexpected species (Barnes & Turner, 2016). Even if metabarcoding is already largely used, further development would benefit from the improvement of this approach. For instance, until now, most of the studies interpret metabarcoding results in terms of presence/absence information. A recent review reported that only a few studies (8/63) used metabarcoding to estimate quantitative information in natural environments (Rourke et al., 2022). Contrary to controlled environments, the relationship between eDNA concentrations and abundance seems to be fuzzier in natural environments (Yates et al., 2023). Indeed, linking metabarcoding information and biomass first requires to quantify the link between the relative read abundance and the absolute eDNA concentration and then to translate eDNA concentration into fish biomass (Pont et al., 2023). Although further research efforts are needed to extract the quantitative information supported by metabarcoding data, we currently encourage environmental managers to use metabarcoding as a systematic biodiversity assessment method to update species distribution range.

4.2.2 | Updating the conservation status of *Harttiella*

Another priority of the National Action Plan is to develop monitoring protocols and implement them over the long term (Axis 3 and 5 of the National Action Plan). Managers of the National Action Plan need methods to be able to track changes in *Harttiella* populations (decline or expansion). Indeed, once populations are identified using metabarcoding techniques, their conservation status requires to be evaluated (Axis 5 of the National Action Plan). This step needs a temporal follow-up of the populations and thus requires an intensive sampling focussed on the target species. dPCR is here the most appropriate. Indeed, dPCR procedure is much faster than metabarcoding because it requires no library preparation, sequencing, or bioinformatic treatment. Moreover, our results showed that only one dPCR replicate is sufficient to give the same presence/absence information as a metabarcoding approach. In addition, at the time of this research, the cost of dPCR is low (i.e., 5€ per site and per targeted species) compared with metabarcoding (i.e., 100€ per site). Although metabarcoding cost is higher, it should be noticed that metabarcoding provides information on the entire fish community. Such information might help to consider the targeted conservation in a more integrated way encompassing accompanying species and potential predators or competitors.

The advantages of dPCR therefore allow a real-time evaluation of anthropic impacts facing *Harttiella* populations. Such temporal follow-up is particularly important because French Guiana is experiencing a drastic increase of habitat degradation due to gold mining activities (Dezecache et al., 2017; Hammond et al., 2007). Gold mining and associated deforestation have been recognized to cause harsh local biodiversity declines (Cantera et al., 2022; Timsina et al., 2022). Fish species restricted to small streams are particularly sensitive to these anthropic disturbances and have been reported to disappear from most anthropized sites (Allard et al., 2016; Brosse et al., 2011).

The next important step for an efficient monitoring of *Harttiella* with dPCR is to be able to shift from presence/absence information to quantitative information, allowing to detect early signals of population decline and set appropriate conservation actions. Targeted approaches such as dPCR are claimed to provide quantitative data (Baker et al., 2018; Lehman et al., 2020; Schweiss et al., 2020; Steiner et al., 2022), but it is still complex to link eDNA concentration to population size. Although studies have shown a positive correlation between eDNA concentration and the biomass or abundance of the targeted species in mesocosms (Doi et al., 2015), this relationship appears fuzzier in natural environments, especially in large water bodies (Yao et al., 2022). Indeed, the link between eDNA concentration and fish abundance or biomass is not straightforward as eDNA concentration directly relies on the eDNA emission (size, life stages, reproductive state, metabolic activity, …) and environmental conditions (temperature, water chemistry, flow, pH, …) (Rourke et al., 2022). eDNA concentration can also be impacted by river-specific characteristics such as river discharge or stream velocity through dilution and sedimentation effects (Thalinger, Kirschner, et al., 2021b; Van Driessche, Everts, Neyrinck, & Brys, 2023a; Van Driessche, Everts, Neyrinck, Halfmaerten, et al., 2023b; Wood et al., 2021). Given the numerous factors affecting eDNA abundance, it appears reasonable to consider that while dPCR results provide a relevant measure of DNA concentration in the water, translating it into abundance or biomass of organisms remains hazardous. We thus suggest using dPCR and metabarcoding as efficient qualitative detection tools for species management and conservation pending future investigation of the relationship between DNA concentration and species abundance/biomass. Pursuing the development of eDNA is especially important in tropic ecosystems as until now, it encompasses only a small portion of the literature (Schenekar, 2023), while facing the highest biodiversity loss.

AUTHOR CONTRIBUTIONS

Céline Condachou, Jérôme Murienne, and Sébastien Brosse conceived the ideas and design methodology; Jérôme Murienne and Sébastien Brosse collected the data; Laetitia Pigeyre, Raphael Covain, and Yves Cuenot conducted the laboratory work; Céline Condachou, Jérôme Murienne, and Sébastien Brosse analysed the data; and Céline Condachou, Jérôme Murienne, and Sébastien Brosse led the writing of the manuscript. All authors contributed critically to the drafts and gave final approval for publication.

ACKNOWLEDGEMENTS

This work was supported by "Investissement d'Avenir" grants managed by Agence Nationale de la Recherche (CEBA: ANR-

10-LABX-25-01; TULIP: ANR-10-LABX-41), ANR (DEBIT: ANR-17-CE02-007-01), DGTM, and Office de l'Eau Guyane. This work is part of the CEBA-IQCN project. The Parc Amazonien de Guyane provided logistical support for the data-collecting mission in Mont Galbao. We would like to thank Jérôme Orivel, Heidy Schimann, and Hadrien Lalagüe for field trip design and organization, Sébastien Sant for help on the field, and Céline Jezéquel for her help in creating the map.

CONFLICT OF INTEREST STATEMENT

L. P. is a research scientist at a private company specializing in the detection of environmental DNA by digital PCR.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in the supporting information of this article. The reference database sequences and Illumina raw sequence data are available on figshare (https://doi.org/10.6084/m9.figshare.22242085).

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SUPPORTING INFORMATION

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

How to cite this article: Condachou, C., Cuenot, Y., Pigeyre, L., Covain, R., Brosse, S. & Murienne, J. (2024). Monitoring the threatened *Harttiella* (Siluriformes, Loricariidae) with environmental DNA: A comparison between metabarcoding and targeted digital PCR. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 34(2), e4111. https://doi.org/10.1002/aqc.4111