



HAL
open science

Evaluating the eDNA spatial signal in natural populations and its application for the monitoring of the threatened *Harttiella* (Siluriformes, Loricariidae)

Céline Condachou, Opale Coutant, Sébastien Brosse, Jérôme Murienne

► To cite this version:

Céline Condachou, Opale Coutant, Sébastien Brosse, Jérôme Murienne. Evaluating the eDNA spatial signal in natural populations and its application for the monitoring of the threatened *Harttiella* (Siluriformes, Loricariidae). *Limnology and Oceanography: Methods*, 2024, 22 (9), pp.660-669. 10.1002/lom3.10634 . hal-04909500

HAL Id: hal-04909500

<https://hal.science/hal-04909500v1>

Submitted on 24 Jan 2025

HAL is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.



Distributed under a Creative Commons Attribution 4.0 International License

Evaluating the eDNA spatial signal in natural populations and its application for the monitoring of the threatened *Harttiella* (Siluriformes, Loricariidae)

Céline Condachou ,* Opale Coutant , Sébastien Brosse , Jérôme Murienne 

Centre de Recherche sur la Biodiversité et l'Environnement (CRBE UMR5300)—Université de Toulouse, CNRS, IRD, Toulouse INP, Université Toulouse 3 Paul Sabatier (UT3), Toulouse, France

Abstract

To face the current downward trajectory of freshwater biodiversity loss, the implementation of effective biodiversity monitoring programs is of utmost importance. Environmental DNA offers unprecedented opportunities for this aim but several challenges still need to be addressed before implementing efficient species monitoring using eDNA. One of them is optimizing the eDNA sampling scheme to maximize the eDNA detection probability. For instance, in flowing freshwaters, the transport of eDNA downstream from its source can impact the eDNA detection probability, and blur the link between eDNA detection and the local occurrence of the species. Here, we investigated the eDNA spatial range of *Harttiella lucifer* (Siluriformes, Loricariidae), a threatened neotropical siluriform fish inhabiting French Guianese mountain streams, and confined to waterfalls and fast-flowing environments. Environmental samples were collected at 11 sites from the *H. lucifer* population to 2000 m downstream. A species-specific dPCR approach was applied to quantify the amount of DNA present in each sample and evaluate the eDNA detection probability of *H. lucifer* according to the distance from its source. Results showed an accumulation of eDNA at 50 and 100 m downstream from *H. lucifer* population. The evaluation of detection probabilities revealed that 300 m downstream from *H. lucifer* population, the probability of detection drops to 50%. This study suggests that eDNA drift in neotropical small streams is limited to a few tens meters downstream. These findings demonstrate that in neotropical small streams, eDNA provides a picture of the local fish fauna rather than integrating information over large spatial scales.

Freshwaters are among the most threatened ecosystems (Albert et al. 2021), and within the 29,500 freshwater species assessed by the IUCN Red List, nearly a third are threatened with extinction corresponding to 42% of the mammals, 33% of the amphibians, and 28% of the fishes (Tickner et al. 2020).

*Correspondence: celine.condachou@univ-tlse3.fr

Author contribution statement: Conceptualization: CC, JM, and SB. Developing methods: CC, JM, and SB. Conducting the research: CC, JM, SB, and OC. Data analysis and interpretation: CC, JM, and SB. Preparation of figures and tables: CC. Writing: CC, JM, SB, and OC. Jérôme Murienne and Sébastien Brosse should be considered as co-senior authors.

Additional Supporting Information may be found in the online version of this article.

This is an open access article under the terms of the [Creative Commons Attribution](https://creativecommons.org/licenses/by/4.0/) License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

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

Freshwater ecosystems are moreover facing a global biodiversity crisis with higher population declines than in marine and terrestrial ecosystems (Dudgeon 2019; Albert et al. 2021; Living Planet Report WWF 2022). To face the current downward trajectory of freshwater biodiversity loss, the implementation of effective biodiversity monitoring programs is of utmost importance.

Among biodiversity monitoring methods, environmental DNA (eDNA) has emerged as an efficient alternative to traditional invasive sampling methods, such as toxicants, gillnets or electrofishing (Fediajevaite et al. 2021). This approach uses DNA shed by organisms in the environment that can be extracted from water without first isolating any target individuals (Taberlet et al. 2012). Environmental DNA is widely applied to the detection of freshwater species (Thomsen et al. 2012; Zinger et al. 2020), focusing mainly on exotic or threatened species (Yao et al. 2022). Combined with species-specific methods such as quantitative PCR (qPCR) or digital PCR (dPCR), eDNA has demonstrated high detection efficiency for species of conservation importance such as the Pinto Abalone (*Haliotis kamtschatkana*), the European eel

(*Anguilla anguilla*), the Smalltooth Sawfish (*Pristis pectinata*), or the Dwarf Wedgemussel (*Alasmidonta heterodon*) (Schill and Galbraith 2019; Lehman et al. 2020; Dimond et al. 2022; Fernandez et al. 2023).

Several challenges still need to be addressed before implementing efficient species monitoring. One of them is the optimization of the eDNA sampling scheme to maximize the eDNA detection probability. In flowing freshwaters, the transport of eDNA downstream from its source can impact the eDNA detection probability and thus needs to be quantified. Several studies investigated the spatial range of eDNA detection downstream from its emission source, but results were highly variable according to the species and the environment. For instance, brook trout (*Salvelinus fontinalis*) was not detected farther than 240 m downstream in a North American stream, whereas common carp (*Cyprinus carpio*) detection was achieved up to 3 km downstream in a Japanese headwater stream (Jane et al. 2015; Nukazawa et al. 2018). In the Rhone River (France), Pont et al. (2018) reported that the Whitefish (*Coregonus lavaretus*) was detected in abundance up to 60 km downstream of the lake where it was living. Similarly, in a flowing stream of the wet tropics of Queensland (Australia), eDNA transport of two frog species reached a distance of more than 20 km (Villacorta-Rath et al. 2021). Regarding the eDNA spatial signal of global fish biodiversity pattern, Civade et al. (2016) found a detection distance of 3 km in a temperate European river whereas in neotropical rivers, Cantera et al. (2022), reported a decrease of the signal over only a few hundred metres. Those discrepancies could lie in the wide array of factors that could impact the eDNA spatial range such as target species biomass, water flow rate, dilution, and DNA decay dynamics (Van Driessche et al. 2023).

Currently, eDNA detection distances have mainly been investigated in temperate fast-flowing systems and remain scarce in tropical streams (Villacorta-Rath et al. 2021; Baudry et al. 2023b). Higher temperature, rainfall and stronger UV radiation characterizing tropical systems (Rourke et al. 2021; Joseph et al. 2022) may increase eDNA decay and dilution, thus reducing the spatial range of eDNA detection downstream from its source. Moreover, to date, most studies investigating fish eDNA spatial range used cage experiments to reproduce semi-natural conditions (Jane et al. 2015; Robinson et al. 2019; Wood et al. 2021; Van Driessche et al. 2023). Nevertheless, such experimental designs do not reflect natural conditions. Caged fish are subject to high levels of stress that can induce an increase in released eDNA (Pilliod et al. 2014; Klymus et al. 2015). In addition, the movement of caged fish is drastically reduced, introducing potential bias in the evaluation of eDNA spatial range. This is why evaluating the eDNA spatial range with wild fish populations is a milestone in understanding eDNA downstream drift in natural environments.

However, this is a difficult task because aquatic organisms, particularly fish, are often spread over large areas and only a few species are strictly associated with particular

environmental features. Here we investigated the eDNA spatial range of *Harttiella lucifer* (Siluriformes, Loricariidae), a threatened fish species living in French Guianese streams. This species can be used as a model species to measure the spatial range of eDNA downstream from a fish population because *H. lucifer* is strictly dependent on small streams with fast-flowing water and is only inhabiting large boulders and waterfall habitats (Mol et al. 2007; Brosse et al. 2013).

We built on the target species ecological specificity to investigate eDNA detection distance downstream from a waterfall inhabited by a population of *H. lucifer*. We collected environmental samples at 11 sites located from the waterfall inhabited by a *H. lucifer* population to 2000 m downstream. A species-specific dPCR approach was applied to quantify the amount of DNA present in each sample and evaluate the eDNA detection probability of *H. lucifer* according to the distance from its source. We hypothesized that eDNA concentration will decrease with distance from the source with a spike of detection close to the population. We also expected that eDNA spatial range will be shorter than in temperate systems due to a faster eDNA degradation. These results will shed light on the eDNA detection distance in tropical small streams.

Materials and procedures

Study design

The considered stream is a 2000m section of the Nouvelle France river (Maroni drainage basin, French Guiana; Figs. 1, 2). This section is a second-order stream according to Strahler classification. The stream is characterized by an average width of 3.05 ± 0.63 m and an average depth of 0.19 ± 0.09 m. Water physicochemistry was homogeneous throughout the studied section with a pH of 7.32 ± 0.2 ; conductivity of 24.6 ± 0.9 ; turbidity of 2.8 ± 0.6 and dissolved oxygen of 7.8 ± 0.1 mg L⁻¹. At the upstream end of the section lies a waterfall made of a 30-m-long barren rock with ca. 20% slope (see Fig. 1b,c). All the remaining part of the river is made of sandy bottom without emerging rocky areas or waterfalls. The *H. lucifer* population was located on the upstream section of the river and *H. lucifer* was never observed downstream from the waterfall despite repeated inventories. Indeed, this population was discovered in 2012 (Allard 2014), and then retrieved on the same site by Cilleros et al. (2019). Previous studies on this stream used Rotenone samples to inventory the fauna from the waterfall to the downstream area of the Nouvelle France river and confirmed that *H. lucifer* was not present on the Nouvelle France main channel downstream of the waterfall. However, two *H. lucifer* individuals were captured in a small tributary flowing to the Nouvelle France, 750 m downstream from the waterfall (Allard 2014). Here, environmental DNA samples were collected in March 2023 at 11 sites of the Nouvelle France stream, from the waterfall to 2000 m downstream (0, 50, 100, 150, 200, 300, 500, 750, 1000, 1500, and 2000 m) (Fig. 2). A last sampling site was located on the

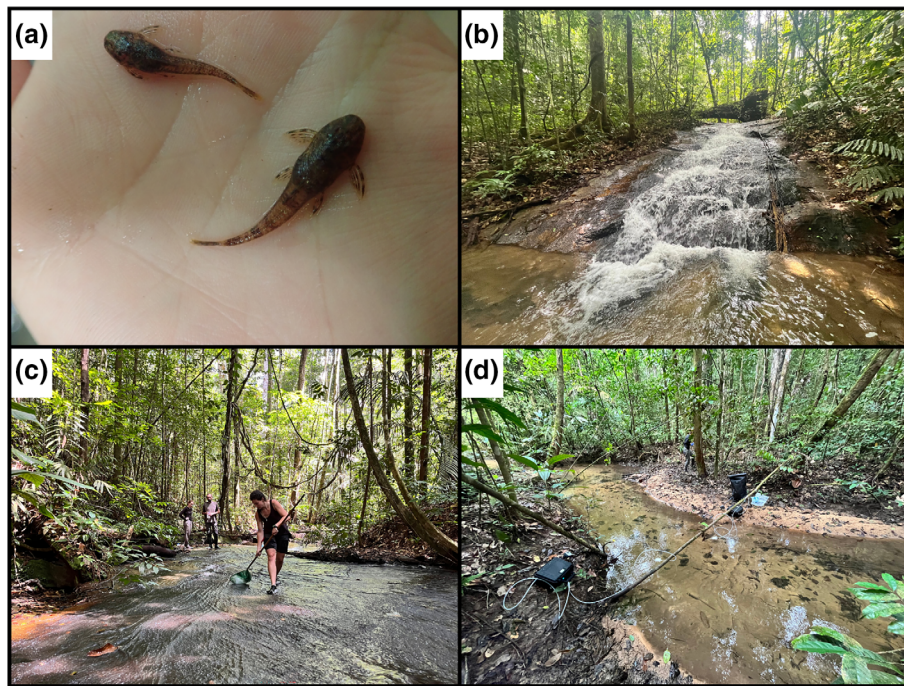


Fig. 1. Photographs of *Harttiella lucifer* collected on the Nouvelle France waterfall (a), waterfall where the *H. lucifer* population was recorded (b), specimen collection with a dipnet (c) and 200 m downstream sampling site with eDNA pumps on the banks (d). Photos by O. Coutant (a) and J. Murienne (b–d).

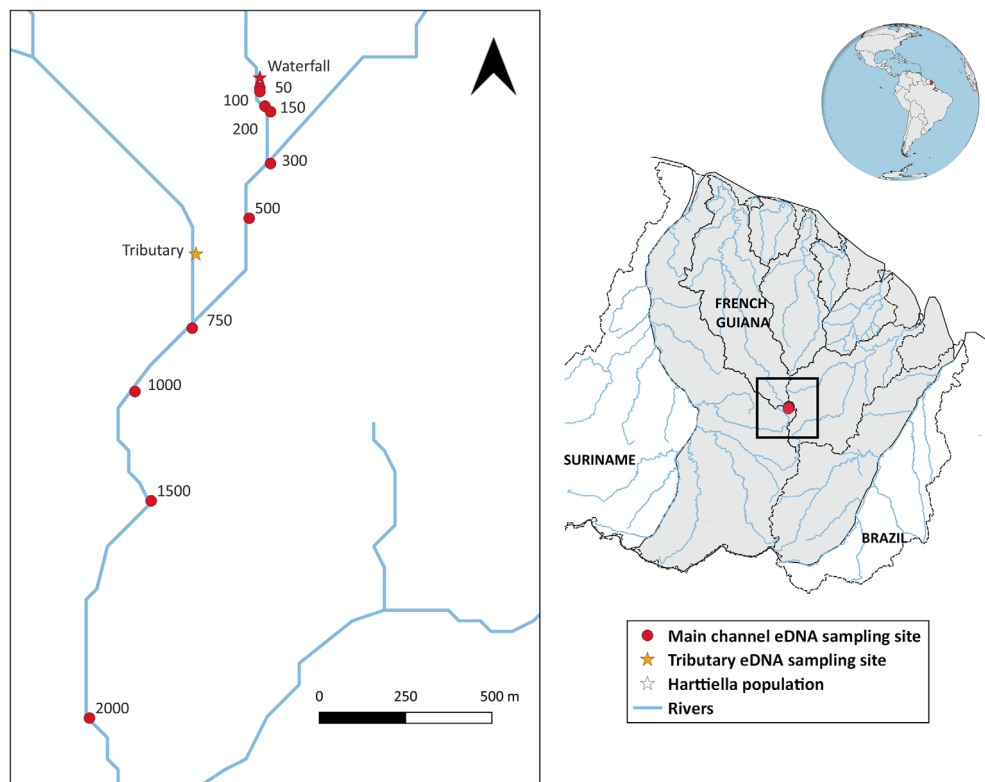


Fig. 2. Location of the 12 environmental DNA sampling sites of the Nouvelle France river, the inset map indicates French Guiana.

tributary of Nouvelle France to check for the presence of another *H. lucifer* population. We also checked for *H. lucifer* presence in each site using visual observations and dipnet captures that confirmed the presence of an *H. lucifer* population on the waterfall, but no fish were captured downstream from the waterfall nor in the tributary.

eDNA sampling

At each site, two eDNA field replicates were collected, one on each bank (Fig. 1d) by filtering water for 30 min which is equivalent to an average of 19.4 liters (± 5 liters) of filtered water across all samples. The total volume of water filtered in each sample was recorded with a flow metre (BRITA). We used a 600 cm² polyethersulfone filter with a pore size of 0.45 μm (Waterra eDNA filter). A suction-type pump was connected to the outlet of the filter and water was drawn in through the filter. To avoid DNA contamination among sites, the operator always remained downstream from the filtration area and stayed on the riverbank. The risk of contamination was also reduced by collecting samples starting from the most downstream site to the waterfall. At the end of the filtration, the cartridge was emptied of water and filled with 50 mL of preservation buffer (5 mL TRIS 1 mol L⁻¹, 5 mL EDTA 1 mol L⁻¹, 0.1 mL NaCl 5 mol L⁻¹, and miliQ water). Cartridges containing the preservation buffer were agitated for 30 s with a Waterra eDNA filter shaker. Then, the preservation buffer was retrieved from the capsule and stocked into a 50 mL tube until further lab processing.

eDNA extraction

For DNA extraction, each 50 mL tube was centrifuged for 15 min at 8500 $\times g$. The supernatant was removed, leaving 15 mL of liquid at the bottom of the tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 mol L⁻¹ sodium acetate were added to each 50 mL tube. The tubes were vortexed and stored for at least one night at -20°C . Tubes were centrifuged at 8500 $\times g$ for 15 min at 15 $^{\circ}\text{C}$, and the supernatants were discarded. After this step, 720 μL of ATL buffer (Qiagen) was added. The tubes were then vortexed and transferred to 2 mL tubes containing 20 μL of Proteinase K. The tubes were finally incubated at 56 $^{\circ}\text{C}$ for 2 h. Afterward, DNA extraction was performed using the NucleoSpin Soil kit (MACHEREY-NAGEL GmbH & Co.) starting from step six and following the manufacturer's instructions. One negative extraction control was also performed and then quantified and amplified in parallel to the field sample to check for potential cross-contamination between samples or possible laboratory contaminants. The quantity of extracted DNA was evaluated using a Qubit fluorometer.

Digital PCR detection

We performed dPCR assay using the *H. lucifer* primers and probe described in Condachou et al. (2024). Each dPCR reaction mixture contained 10 μL of 4X QIAcuity Probe PCR kit

(Cat. No/ID:250101; Qiagen), 1 μL of each of the two 20X sets of primers and probes, 18 μL of eDNA and RNase-free water were combined to reach a final reaction volume of 40 μL . Three dPCR replicates were performed per sample. Positive control (*H. lucifer* tissue DNA, diluted 1/1000) and negative control (H₂O) were included in each plate. Reaction mixtures were transferred into a 26K QIAcuity Nanoplate and loaded onto the QIAcuity One instrument. The amplification step was performed using the following cycling protocol: 2 min at 95 $^{\circ}\text{C}$ for enzyme activation, 15 s at 95 $^{\circ}\text{C}$ for denaturation, and 30 s at 58 $^{\circ}\text{C}$ for annealing/extension during 40 cycles. The imaging step was performed by reading the yellow channel (excitation: 514–535 nm, emission: 550–564 nm). Data were analyzed using the QIAcuity Software Suite V1.2 and expressed as copies μL^{-1} of reaction volume (40 μL final).

A detection threshold of fluorescence intensity (RFU) was set manually at 70 RFU after testing other thresholds (Supporting Information Fig. S1). Then a detection threshold (limit of blank) was set to 0.2 copies μL^{-1} based on Condachou et al. (2024).

Data analysis

We used the formula of Brys et al. (2021) to convert the eDNA concentration of *H. lucifer* in copies μL^{-1} to the eDNA copy number present per liter of filtered water. Occupancy models were used to quantify the uncertainty and imperfect detection associated with each eDNA level of sampling (site, field replicate, and dPCR subsample) (Fig. 4a). Indeed, even if a species is present at a site, not all the samples will necessarily contain eDNA of the species, thus leading to potential false negative detection. Moreover, following the guideline implemented by Thalinger et al. (2021), evaluating detection probabilities by statistical modeling is necessary to complete the validation process of an eDNA assay.

The probability of detecting *H. lucifer* eDNA was investigated according to the distance from the population using occupancy modeling with the “eDNA occupancy” package Dorazio and Erickson (2018). As eDNA was detected on the tributary of Nouvelle France, thus blurring the overall spatial signal, we only considered the seven sites located upstream from the confluence to run the occupancy modeling. The “occModel” function was run to fit the multiscale occupancy models for 50,000 iterations to obtain parameter estimates (the first 5000 iterations of the Markov Chain were discarded). We used the distance from the *H. lucifer* population as a covariable of eDNA occurrence in site (ψ), field replicates (θ), and dPCR replicates (ρ). Ψ represents the probability that *H. lucifer* eDNA is present in a site. θ represents the probability of detecting the species eDNA in a field replicate given that eDNA is present at the site level. Then ρ represents the probability that the species eDNA is detected in one of the dPCR replicates given that eDNA is detected in a field replicate. Comparison between the null model and the model including

distance was performed using the Posterior Predictive Loss Criteria (PPLC). Models with lower values of this criteria are favored.

Results

eDNA concentration according to the distance from the *H. lucifer* population

The highest eDNA concentration (32.0 ± 31.2 copies L⁻¹ in average on the site) was observed 50 m downstream from the *H. lucifer* population (Table 1). At 50 and 100 m downstream, an accumulation of eDNA is observed on the right bank (Fig. 3). *H. lucifer* detection also occurred (12.8 ± 14.1 copies L⁻¹ in average on the site) in the tributary of the Nouvelle France in one of the two field replicates (Fig. 3; Table 1), as well in the site located downstream from the confluence (site 1000 m). There, *H. lucifer* was detected in all dPCR replicates of the left bank (Fig. 3). Detection occurred up to 2000 m downstream of the eDNA source but at a very low concentration (2.9 ± 2.3 copies L⁻¹) (Fig. 3; Table 1). At 150, 200, 500, and 750 m downstream the *H. lucifer* population, *H. lucifer* eDNA was not detected (Fig. 3; Table 1).

eDNA occurrence according to the distance from the *H. lucifer* population

Occupancy models showed a negative effect of the distance from the *H. lucifer* population on the eDNA detection probabilities (Table 2). Estimated posterior medians of eDNA detection probabilities (ψ , θ , ρ) decreased with distance from the source (Fig. 4). Estimated detection probabilities of *H. lucifer* eDNA according to the distance showed a decreasing trend but with an overlapping of the 95% confidence intervals. The model including distance as a covariable had the smallest value (7.52) of PPLC compared to the null model (9.05). The highest detection probabilities are estimated for the site where the *H. lucifer* population was observed ($\psi = 0.833$, IC 95%: 0.290–0.998; $\theta = 0.841$, IC 95%: 0.386–0.997 and $\rho = 0.862$, IC 95%: 0.599–0.978). Three hundred meters downstream the population, the probability of detecting *H. lucifer* in a site (ψ) or in a dPCR replicate (ρ) falls to 50% ($\psi = 0.504$; IC 95%: 0.242–0.966 and $\rho = 0.501$; IC 95%: 0.174–0.834). Before the tributary (500 m downstream of the population) the probability of detecting *H. lucifer* in a site reached the lowest value of 26.1% ($\psi = 0.261$; IC 95%: 0.0003–0.996).

Discussion

Over the 11 sampling sites surveyed, 6 were positives for the detection of *H. lucifer*. Probabilities of *H. lucifer* detection showed a decreasing trend with distance from the population. From 300 m downstream, the probability of detection in a site drops to 50%. Those results therefore support the hypothesis of a short-distance detection of eDNA downstream from its emission source. The short distance decline of eDNA spatial signal was consistent with results from large tropical rivers (Maroni and Oyapock rivers in French Guiana) showing a similar distance decay of taxonomic similarity between eDNA and capture samples, interpreted by Cantera et al. (2022) as a short detection distance of eDNA. Nevertheless, this study was based on a decline of fish assemblage similarity between sites and does not provide a formal measure of downstream eDNA detection per species. Here, we quantify the eDNA concentration decline with distance and showed a tendency toward a short-distance detection in tropical small streams. This detection distance appears short compared to studies in temperate environments where the detection distance has been estimated to a few kilometers (Wood et al. 2021; Van Driessche et al. 2023). Several factors might explain our results, such as the higher water temperature that can increase the degradation of eDNA (Joseph et al. 2022), thus reducing the detection distance. Moreover, the characteristics of rivers such as the slope and the flow rate can induce changes in the eDNA transport distance (Van Driessche et al. 2023).

In tropical systems, only a few studies investigated the eDNA spatial range. Villacorta-Rath et al. (2021) found a long eDNA detection distance (up to 20 km) in an Australian tropical flowing stream for two species of frogs. Baudry et al. (2023b) demonstrated that in a Martinique stream, a fish (*Anablepsoides cryptocallus*) was still detected in two replicates 1000 m downstream of the source. Nevertheless, even in similar ecosystems, many physical factors can influence the eDNA detection distance such as the flow rate, water temperature, and oxygen concentration (Baudry et al. 2023a). It appears that previous studies focused on steep sloped rivers with marked water flow (Villacorta-Rath et al. 2021; Baudry et al. 2023a), whereas the present study relies on a gentle slopes stream (< 3.5%), thus implying a slow downstream transportation of eDNA. The biomass of the source population also seems to strongly affect the eDNA spatial range. Villacorta-Rath et al. (2021) investigated the eDNA downstream transport for two frog species and found higher detection for the most abundant species. Van Driessche

Table 1. Mean *H. lucifer* eDNA concentration (copies L⁻¹) for each field replicate according to the sampling site. “TRB” means tributary and was not a part of the main river.

	0	50	100	150	200	300	500	750	TRB	1000	1500	2000
Right bank	2.7	59.7	58.7	0	0	0.7	0	0	0	0	0	2.7
Left bank	8.8	4.3	0	0	0	2.0	0	0	25.7	5.9	0	3.1

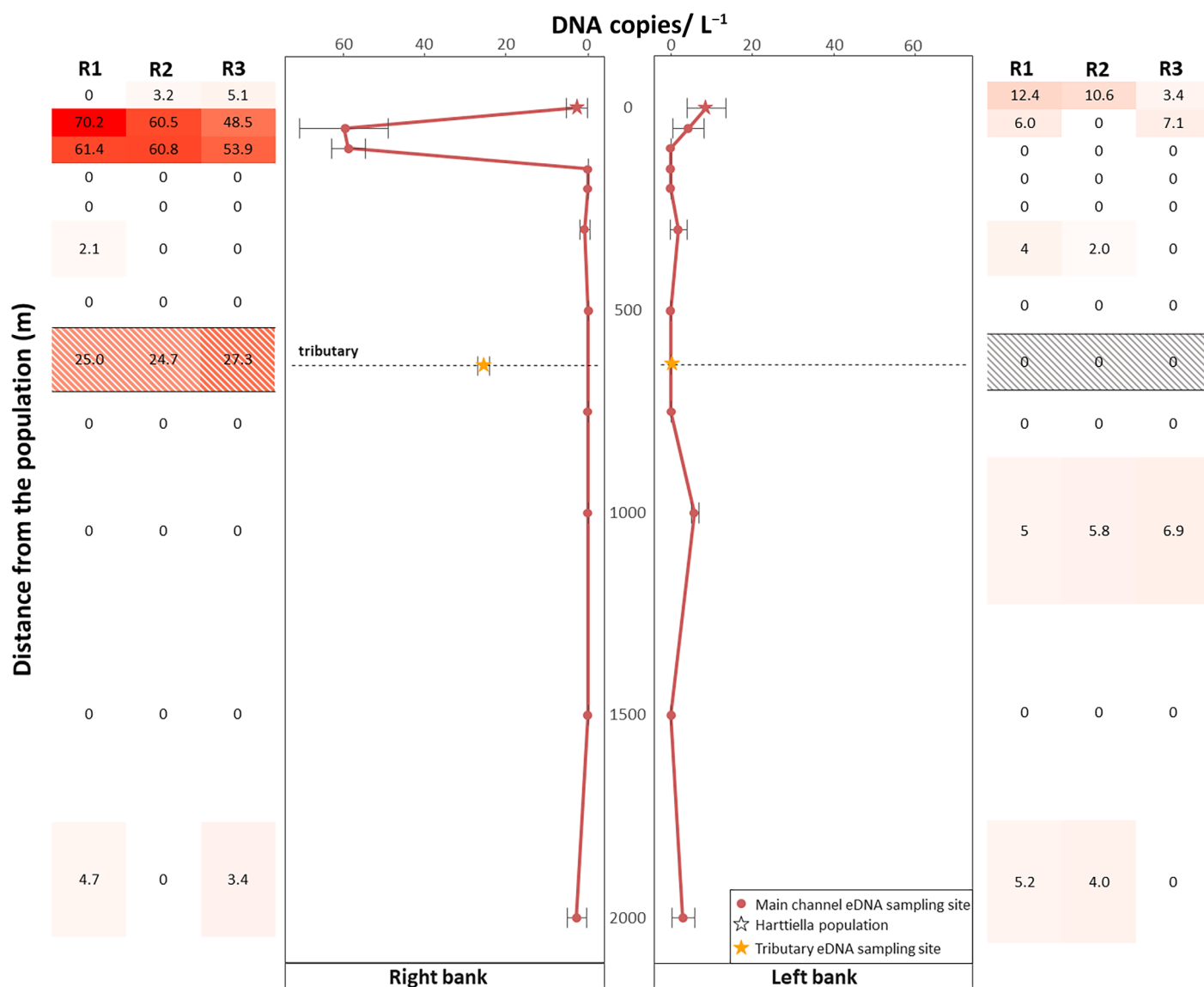


Fig. 3. Relationship between the DNA copy number per liter of filtered water (DNA copies L⁻¹) and the distance from the *Harttiella lucifer* population (m) according to the riverbank. The horizontal dotted line represents the confluence between the main river and the tributary. The dots represent the mean of the three PCR replicates. DNA concentrations of each dPCR replicate are displayed in tables following a color gradient proportional to eDNA concentrations.

et al. (2023) showed that the detection distance was extended by more than 1500 m when the source population had a higher biomass. In our case, we could not estimate the *H. lucifer* biomass, but knowing this information could help to better understand the impact of the fish biomass on the detection distance.

Another factor that can impact the eDNA spatial range is the sampling season. For instance, Baudry et al. (2023a) found higher detection probabilities of the *Austropotamobius pallipes* crayfish in summer than in fall. These differences were directly linked with the periods of the crayfish activity (hatching, molting or breeding). Water parameters can also impact the seasonal detectability of eDNA as demonstrated by Curtis et al. (2021). They found that higher flow rates decreased

Table 2. Posterior mean estimates of model parameters β , α , and δ (95% \pm credible interval) (niter = 50,000 et burnin = 5000). Ψ represents the probability that *H. lucifer* eDNA is present in a site. θ represents the probability of detecting the species eDNA in a field replicate given that eDNA is present at the site level. ρ represents the probability that the species eDNA is detected in one of the dPCR replicates given that eDNA is detected in a field replicate.

Model	β	α	δ
Ψ (distance),	-0.550	-0.246	-0.615
θ (distance),	(-1.944; 0.855)	(-1.481; 1.058)	(-1.372; 0.144)
ρ (distance)			

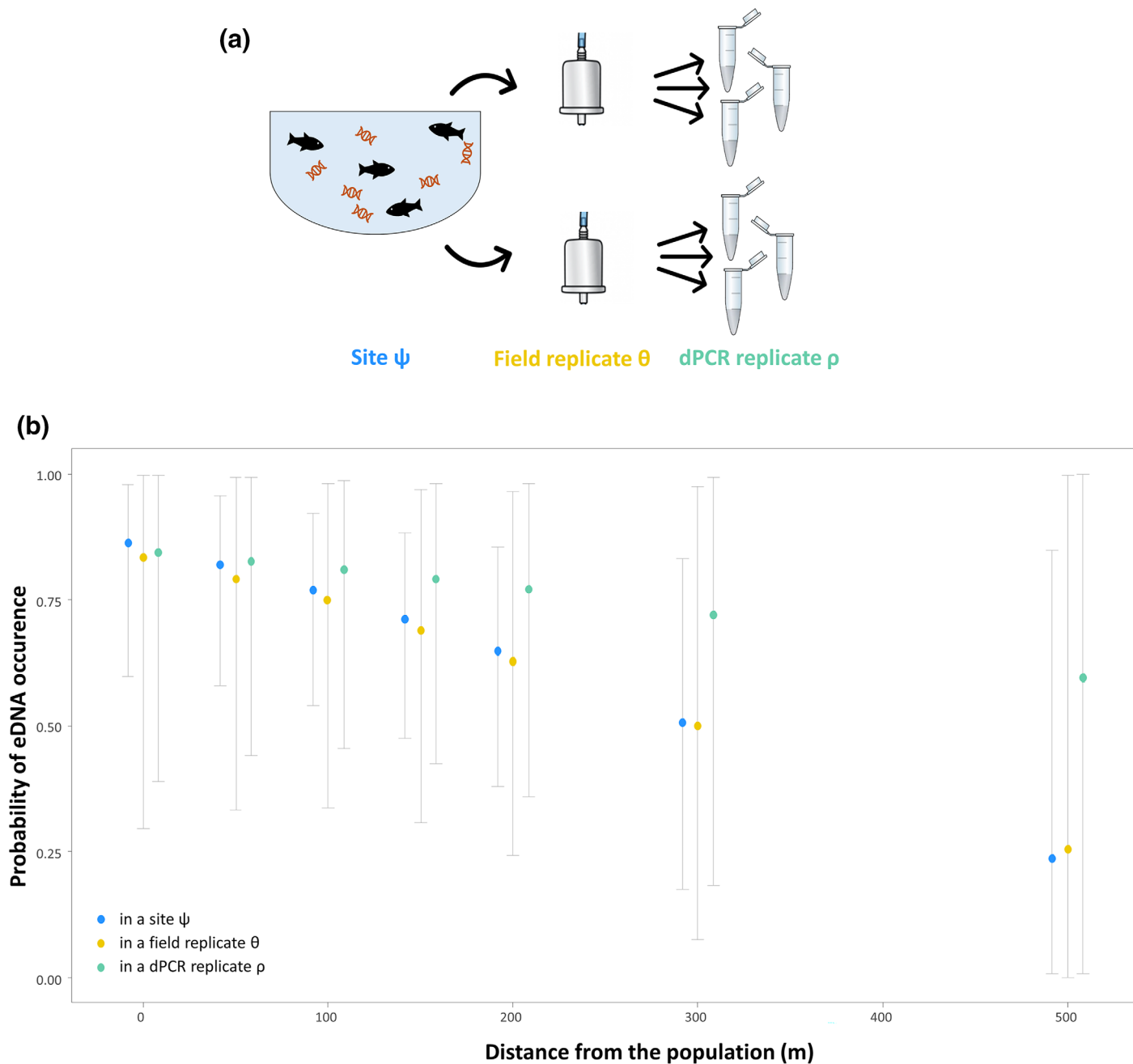


Fig. 4. Estimated detection probabilities of *Harttiella lucifer* eDNA occurrence according to the distance downstream from the population. Points represent estimates of posterior medians with 95% credible intervals. Only the sampling points before the tributary stream are represented. Ψ represents the probability that *H. lucifer* eDNA is present in a site. θ represents the probability of detecting the species eDNA in a field replicate given that eDNA is present at the site level. ρ represents the probability that the species eDNA is detected in one of the dPCR replicates given that eDNA is detected in a field replicate. **(a)** Schematic representation of the three levels of sampling used for running the occupancy models; **(b)** detection probabilities according to the distance downstream of the *H. lucifer* population.

eDNA concentration of an invasive clam (*Corbicula fluminea*) by diluting eDNA. Differences in detection across seasons were also found for amphibians with higher detection probability during the wet season (Villacorta-Rath et al. 2021). Nevertheless, the authors also showed that the eDNA downstream

transport was not impacted by the season, and detected eDNA over long distances for both seasons. Concerning tropical fish, no studies have answered the question of the evolution of the eDNA spatial range according to the sampling season. Moreover, in the particular case of *Harttiella*, little is known about

the breeding period and the overall biology of this genus. This is why caution should be taken in the implementation of the sampling design to consider potential changes in the detection distance during other seasons.

Investigating the distribution of eDNA copies according to the distance from the population is crucial to optimize the sampling design and to better understand DNA dynamics. Wood et al. (2021), described the eDNA transport as a pattern called “plume dynamics,” suggesting that eDNA is concentrated near the source and then tends to accumulate toward banks. Our results show an accumulation of DNA on the right bank at 50 and 100 m downstream of the population. In large rivers (Yangtze River, China), eDNA can be heterogeneously distributed depending on the vertical and horizontal layers of the river (Zhang et al. 2023). Nevertheless, in small streams, questions about the distribution of eDNA in water have been little addressed. To our knowledge, the study of Sakata et al. (2021), is the only one to investigate the eDNA distribution between replicate positions in a small stream (Japan). Considering the species occurrence, they have found no significant differences between replicate positions (left bank, right bank or middle). However, considering only occurrence information can hide heterogeneity in the amount of eDNA across the stream section. Indeed, our study demonstrated that in tropical small streams (average width across all sites: 3 m), eDNA is not distributed homogeneously in water. The eDNA heterogeneity between the two banks can be due to a difference in the flow rate. Indeed, in small streams, the flow rate can be variable along the horizontal transect and can induce changes in eDNA concentration as turbulent flow tends to keep particles in suspension instead of inducing sedimentation (Wood et al. 2021). The geomorphological shape of the stream can also affect the sedimentary dynamic of eDNA and induced heterogeneity within the stream. Considering the limited stream width, such findings were not expected and highlighted the importance of performing field samples integrating the overall water flow, by repeating field replicates or sampling eDNA along a lateral stream transect, to quantify all the DNA of interest, even for small streams.

In addition to the overall trend toward a short-distance detection of *H. lucifer*, small quantities of eDNA of the species are still detected 2000 m downstream from the main source population. This can correspond to traces from the affluent population or to an accumulation of traces from the two upstream *H. lucifer* populations. Another explanation could be the direct presence of some *H. lucifer* individuals downstream the head of the stream. Indeed, another small population may have been missed by the visual observation and the previous rotenone sampling. This is why using eDNA to monitor *H. lucifer* species can be an efficient method to discover new populations. Such unexpected signals should nevertheless not be considered as the proof of presence of an unknown population, but could guide further studies to check for the potential presence of a population. This is of particular importance for

this genus as all *Harttiella* species are listed as threatened by the IUCN. Moreover, the genus is targeted by a National Action Plan (2023–2032) that aims to ensure their conservation through regulatory tools and develop long-term monitoring of the populations. Condachou et al. (2024) already demonstrated that dPCR and metabarcoding provided similar results for the detection of two *Harttiella* species. Our study suggests that the optimal eDNA sampling design for monitoring such species is to use two field replicates at 50 and 100 m and three dPCR replicates per sample downstream from the source population. These findings will help managers implement efficient long-term monitoring of *H. lucifer* populations that are currently threatened by small-scale gold mining (Brosse et al. 2011). Moreover, the short downstream detection distance of DNA we demonstrated here for *H. lucifer*, deserves to be tested for other species and other streams throughout the globe. Understanding the spatial grain size of ecological measurements is indeed a prerequisite to study species habitat preferences, assess species interactions and understand the ecological processes ensuring ecosystem functioning and sustainability.

Data availability statement

All data generated or analyzed during this study are included in this published article (and its Supporting Information files) and additional information and data are available from the corresponding author upon reasonable request.

References

- Albert, J. S., G. Destouni, S. M. Duke-Sylvester, A. E. Magurran, T. Oberdorff, R. E. Reis, K. O. Winemiller, and W. J. Ripple. 2021. Scientists' warning to humanity on the freshwater biodiversity crisis. *Ambio* **50**: 85–94. doi:10.1007/s13280-020-01318-8
- Allard, L. 2014. Élaboration d'un indice de qualité des eaux basé sur la structure taxonomique et fonctionnelle des assemblages de poissons des petits cours d'eau de Guyane. These de doctorat. Toulouse 3. <https://www.theses.fr/2014TOU30321>
- Baudry, T., M. Laffitte, C. Noizat, C. Delaunay, G. Ricou, V. Vasselon, and F. Grandjean. 2023a. Influence of distance from source population and seasonality in eDNA detection of white-clawed crayfish, through qPCR and ddPCR assays. *Environ. DNA* **5**: 733–749. doi:10.1002/edn3.435
- Baudry, T., Q. Mauvisseau, A. Arqué, J.-P. Goût, C. Delaunay, H. J. de Boer, and F. Grandjean. 2023b. Environmental DNA survey to detect an endemic cryptic fish, *Anablepsoides cryptocallus*, in tropical freshwater streams. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* **33**: 325–335. doi:10.1002/aqc.3916
- Brosse, S., G. Grenouillet, M. Gevrey, K. Khazraie, and L. Tudesque. 2011. Small-scale gold mining erodes fish assemblage structure in small neotropical streams. *Biodivers. Conserv.* **20**: 1013–1026. doi:10.1007/s10531-011-0011-6

- Brosse, S., J. I. Montoya-Burgos, G. Grenouillet, and N. Surugue. 2013. Determinants of fish assemblage structure in Mount Itoupé mountain streams (French Guiana). *Ann. Limnol. Int. J. Limnol.* **49**: 43–49. doi:10.1051/limn/2013037
- Brys, R., D. Halfmaerten, S. Neyrinck, Q. Mauvisseau, J. Auwerx, M. Sweet, and J. Mergeay. 2021. Reliable eDNA detection and quantification of the European weather loach (*Misgurnus fossilis*). *J. Fish Biol.* **98**: 399–414. doi:10.1111/jfb.14315
- Cantera, I., J.-B. Decotte, T. Dejean, J. Murienne, R. Vigouroux, A. Valentini, and S. Brosse. 2022. Characterizing the spatial signal of environmental DNA in river systems using a community ecology approach. *Mol. Ecol. Resour.* **22**: 1274–1283. doi:10.1111/1755-0998.13544
- Filleros, K., and others. 2019. Unlocking biodiversity and conservation studies in high-diversity environments using environmental DNA (eDNA): A test with Guianese freshwater fishes. *Mol. Ecol. Resour.* **19**: 27–46. doi:10.1111/1755-0998.12900
- Civade, R., T. Dejean, A. Valentini, N. Roset, J.-C. Raymond, A. Bonin, P. Taberlet, and D. Pont. 2016. Spatial representativeness of environmental DNA metabarcoding signal for fish biodiversity assessment in a natural freshwater system. *PLoS One* **11**: e0157366. doi:10.1371/journal.pone.0157366
- Condachou, C., Y. Cuenot, L. Pigeyre, R. Covain, S. Brosse, and J. Murienne. 2024. Monitoring the threatened *Harttiella* (Siluriformes, Loricariidae) with environmental DNA: A comparison between metabarcoding and targeted digital PCR. *Aquat. Conserv.: Mar. Freshw. Ecosyst.* **34**: e411. doi:10.1002/aqc.4111
- Curtis, A. N., J. S. Tiemann, S. A. Douglass, M. A. Davis, and E. R. Larson. 2021. High stream flows dilute environmental DNA (eDNA) concentrations and reduce detectability. *Divers. Distrib.* **27**: 1918–1931. doi:10.1111/ddi.13196
- Dimond, J. L., B. R. Gathright, J. V. Bouma, H. S. Carson, and K. Sowul. 2022. Detecting endangered pinto abalone (*Haliotis kamtschatkana*) using environmental DNA: Comparison of ddPCR, qPCR, and conventional diver surveys. *Environ. DNA* **4**: 1397–1406. doi:10.1002/edn3.351
- Dorazio, R. M., and R. A. Erickson. 2018. eDNAoccupancy: An R package for multiscale occupancy modelling of environmental DNA data. *Mol. Ecol. Resour.* **18**: 368–380. doi:10.1111/1755-0998.12735
- Dudgeon, D. 2019. Multiple threats imperil freshwater biodiversity in the Anthropocene. *Curr. Biol.* **29**: R960–R967. doi:10.1016/j.cub.2019.08.002
- Fediajevaite, J., V. Priestley, R. Arnold, and V. Savolainen. 2021. Meta-analysis shows that environmental DNA outperforms traditional surveys, but warrants better reporting standards. *Ecol. Evol.* **11**: 4803–4815. doi:10.1002/ece3.7382
- Fernandez, S., Á. Gutiérrez, D. Deconinck, J. L. Martinez, A. Alvarez, I. Marquez, G. Machado-Schiaffino, and E. Garcia-Vazquez. 2023. Biomass quantification of the critically endangered European eel from running waters using environmental DNA. *Fishes* **8**: 279. doi:10.3390/fishes8060279
- Jane, S. F., T. M. Wilcox, K. S. McKelvey, M. K. Young, M. K. Schwartz, W. H. Lowe, B. H. Letcher, and A. R. Whiteley. 2015. Distance, flow and PCR inhibition: eDNA dynamics in two headwater streams. *Mol. Ecol. Resour.* **15**: 216–227. doi:10.1111/1755-0998.12285
- Joseph, C., M. E. Faiq, Z. Li, and G. Chen. 2022. Persistence and degradation dynamics of eDNA affected by environmental factors in aquatic ecosystems. *Hydrobiologia* **849**: 4119–4133. doi:10.1007/s10750-022-04959-w
- Klymus, K. E., C. A. Richter, D. C. Chapman, and C. Paukert. 2015. Quantification of eDNA shedding rates from invasive bighead carp *Hypophthalmichthys nobilis* and silver carp *Hypophthalmichthys molitrix*. *Biol. Conserv.* **183**: 77–84. doi:10.1016/j.biocon.2014.11.020
- Lehman, R. N., G. R. Poulakis, R. M. Scharer, K. E. Schweiss, J. M. Hendon, and N. M. Phillips. 2020. An environmental DNA tool for monitoring the status of the Critically Endangered Smalltooth Sawfish, *Pristis pectinata*, in the western Atlantic. *Conserv. Genet. Resour.* **12**: 621–629. doi:10.1007/s12686-020-01149-5
- Mol, J. H., K. W. Tong You, I. Vrede, A. Flynn, P. Ouboter, and F. van der Lugt. 2007. Fishes of Lely and Nassau Mountains, Suriname. In L. E. Alonso and J. H. Mol [eds.], RAP bulletin of biological assessment: A rapid biological assessment of the Lely and Nassau Plateaus, Suriname (with additional information on the Brownsberg Plateau). Conservation International. doi:10.1896/1-881173-98-4.107
- Nukazawa, K., Y. Hamasuna, and Y. Suzuki. 2018. Simulating the advection and degradation of the environmental DNA of common carp along a river. *Environ. Sci. Technol.* **52**: 10562–10570. doi:10.1021/acs.est.8b02293
- Pilliod, D. S., C. S. Goldberg, R. S. Arkle, and L. P. Waits. 2014. Factors influencing detection of eDNA from a stream-dwelling amphibian. *Mol. Ecol. Resour.* **14**: 109–116. doi:10.1111/1755-0998.12159
- Pont, D., and others. 2018. Environmental DNA reveals quantitative patterns of fish biodiversity in large rivers despite its downstream transportation. *Sci. Rep.* **8**: 10361. doi:10.1038/s41598-018-28424-8
- Robinson, A., Y. Paroz, M. Clement, T. Franklin, J. Dysthe, M. Young, K. McKelvey, and K. Carim. 2019. Environmental DNA sampling of small-bodied minnows: Performance relative to location, species, and traditional sampling. *N. Am. J. Fish. Manag.* **39**: 1073–1085. doi:10.1002/nafm.10344
- Rourke, M. L., A. M. Fowler, J. M. Hughes, M. K. Broadhurst, J. D. DiBattista, S. Fielder, J. Wilkes Walburn, and E. M. Furlan. 2021. Environmental DNA (eDNA) as a tool for assessing fish biomass: A review of approaches and future considerations for resource surveys. *Environ. DNA* **4**: 9–33. doi:10.1002/edn3.185

- Sakata, M. K., and others. 2021. Determining an effective sampling method for eDNA metabarcoding: A case study for fish biodiversity monitoring in a small, natural river. *Limnology* **22**: 221–235. doi:10.1007/s10201-020-00645-9
- Schill, W. B., and H. S. Galbraith. 2019. Detecting the undetectable: Characterization, optimization, and validation of an eDNA detection assay for the federally endangered dwarf wedgemussel, *Alasmidonta heterodon* (Bivalvia: Unionoida). *Aquat. Conserv.: Mar. Freshw. Ecosyst.* **29**: 603–611. doi:10.1002/aqc.3069
- Taberlet, P., E. Coissac, M. Hajibabaei, and L. H. Rieseberg. 2012. Environmental DNA. *Mol. Ecol.* **21**: 1789–1793. doi:10.1111/j.1365-294X.2012.05542.x
- Thalinger, B., and others. 2021. A validation scale to determine the readiness of environmental DNA assays for routine species monitoring. *Environ. DNA* **3**: 823–836. doi:10.1002/edn3.189
- Thomsen, P. F., J. Kielgast, L. L. Iversen, C. Wiuf, M. Rasmussen, M. T. P. Gilbert, L. Orlando, and E. Willerslev. 2012. Monitoring endangered freshwater biodiversity using environmental DNA. *Mol. Ecol.* **21**: 2565–2573. doi:10.1111/j.1365-294X.2011.05418.x
- Tickner, D., and others. 2020. Bending the curve of global freshwater biodiversity loss: An emergency recovery plan. *BioScience* **70**: 330–342. doi:10.1093/biosci/biaa002
- Van Driessche, C., T. Everts, S. Neyrinck, and R. Brys. 2023. Experimental assessment of downstream environmental DNA patterns under variable fish biomass and river discharge rates. *Environ. DNA* **5**: 102–116. doi:10.1002/edn3.361
- Villacorta-Rath, C., C. J. Hoskin, J. M. Strugnell, and D. Burrows. 2021. Long distance (>20 km) downstream detection of endangered stream frogs suggests an important role for eDNA in surveying for remnant amphibian populations. *PeerJ* **9**: e12013. doi:10.7717/peerj.12013
- Wood, Z. T., L.-R. Anaïs, F. LeBlanc, M. Trudel, M. T. Kinnison, C. Garry McBrine, S. A. Pavey, and N. Gagné. 2021. Spatial heterogeneity of eDNA transport improves stream assessment of threatened Salmon presence, abundance, and location. *Front. Ecol. Evol.* **9**: 650717. doi:10.3389/fevo.2021.650717
- WWF. 2022. *In* R. E. A. Almond, M. Grooten, D. J. Bignoli, and T. Petersen [eds.], Living planet report 2022—Building a nature-positive society. WWF.
- Yao, M., S. Zhang, Q. Lu, X. Chen, S.-Y. Zhang, Y. Kong, and J. Zhao. 2022. Fishing for fish environmental DNA: Ecological applications, methodological considerations, surveying designs, and ways forward. *Mol. Ecol.* **31**: 5132–5164. doi:10.1111/mec.16659
- Zhang, Y., X. Zhang, F. Li, and F. Altermatt. 2023. Fishing eDNA in one of the world's largest rivers: A case study of cross-sectional and depth profile sampling in the Yangtze. *Environ. Sci. Technol.* **57**: 21691–21703. doi:10.1021/acs.est.3c03890
- Zinger, L., and others. 2020. Advances and prospects of environmental DNA in neotropical rainforests, p. 331–373. *In* A. J. Dumbrell, E. C. Turner, and T. M. Fayle [eds.], *Advances in ecological research*, v. **62**. Academic Press. doi:10.1016/bs.aecr.2020.01.001

Acknowledgments

This work was supported by “Investissement d’Avenir” grants managed by Agence Nationale de la Recherche (CEBA: ANR-10-LABX-25-01), ANR (DEBIT: ANR-17-CE02-007-01), DGTM and Office de l’Eau Guyane. This work is part of the CEBA-IQCN project.

Conflict of Interest

None declared.

Submitted 08 March 2024

Revised 07 June 2024

Accepted 17 June 2024

Associate editor: Steven W. Wilhelm