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**TITLE**

Validation of an eDNA-based method for surveying fish and crustacean communities in the rivers of the French West Indies

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**CONTRIBUTIONS**

Estelle LEFRANCOIS, Marion LABELLE, Marie ROBERT and Alice VALENTINI developed the study. Estelle LEFRANCOIS, Marion LABELLE, Joévin MARQUÈS and Marie ROBERT performed the field sampling. Alice VALENTINI supervised the eDNA analysis. Estelle LEFRANCOIS, Joévin MARQUÈS and Alice VALENTINI conducted the statistical analyses and drafted the manuscript. All authors contributed to the writing of the manuscript.

## **ABSTRACT**

Freshwater biodiversity is rapidly declining worldwide, especially in tropical island environments which appear to be particularly vulnerable to alien species. Monitoring biodiversity using effective methods is crucial for conservation efforts. This study aims to examine the capacity of the eDNA-based method to provide accurate measures of fish and crustacean biodiversity, and how it can be implemented in the French West Indies. To this end, we conducted eDNA and electric fishing capture-based surveys in parallel, both upstream and downstream, in nine Guadeloupean rivers and during two different seasons. While both methods were congruent in terms of species richness and composition, the eDNA-based method generally exhibited greater efficacy and reliability in assessing biodiversity. The exception to this trend was observed during the second campaign for the crustacean community. The quantitative data (the numbers of individuals and percentages of sequences) also proved to be uncorrelated for crustaceans during the second campaign, indicating that further knowledge of crustacean biology is needed. Nevertheless, the eDNA-based method was able to detect the presence of alien, rare and cryptic species, showing that it can be used in tropical island conditions for biodiversity assessment or conservation purposes.

## **KEYWORDS**

eDNA metabarcoding; Electric fishing; Fish; Crustaceans; River; French West Indies; Alien species; Biodiversity assessment

## INTRODUCTION

Freshwater ecosystems only cover approximately 0.8% of the world's surface area but they host over 126,000 species i.e. 9.5% of known animal species, making them a significant component of global biodiversity (Dudgeon, 2019; Garcia Moreno et al., 2014). Freshwater biodiversity is rapidly declining due to accelerated human-induced disturbances and biotic homogenization caused by the extinction of native species and the widespread introduction of non-native species (Keke et al., 2021; Young et al., 2017). Tropical ecosystems harbour the most biodiversity (Harvey et al., 2020). Among these ecosystems, the Caribbean islands are part of 34 designated "hotspots", defined as areas where biodiversity is particularly rich, vulnerable and irreplaceable (Myers et al. 2000). Combined, these hotspots host more than 150,000 endemic plant species (more than 50% of all vascular plant species) and nearly 13,000 endemic terrestrial vertebrates (42% of all terrestrial vertebrates) (Mittermeier et al., 2004). The principal threats to these ecosystems are climate change, land modification (e.g. habitat destruction and pollution) and invasive alien species (Bellard et al., 2014).

Guadeloupe, a Caribbean island spanning 1,628 km<sup>2</sup> and part of the French West Indies (FWI), is no exception to the problems faced by tropical island environments and is considered a biodiversity hotspot. It is a volcanic, fertile and densely populated territory where multiple pressures impact floral and faunal communities. Its western portion, Basse-Terre (848 km<sup>2</sup>), which is dominated by the volcano La Soufrière, is covered by numerous tropical rivers and by tropical rainforest that is largely protected by its National Park status. Basse-Terre is considered the "water tower" of Guadeloupe and many surface water intake structures hinder the continuity of its rivers. This dual isolation, insular and geographical, has led to the development of unique ecosystems from which certain biological groups are absent, leaving vacant ecological niches that foster the invasion of non-native species (Soubeyran, 2008). Consequently, this part

of Guadeloupe appears to be particularly vulnerable to alien species. The discovery in March 2014 of several individuals of the *Ancistrus triradiatus* Eigenmann, 1918 species in La Ravine Borine, a stream located in the commune of Saint-Claude in the Guadeloupe National Park, has heightened concerns about alien species.

To survey the Guadeloupean freshwater communities, traditional electric fishing (TEF) protocols complying with current standards (EN 14011 et EN 14962) can be used, depending on the objectives and local specificities (river accessibility, water conductivity, studied communities etc.) (Belliard et al., 2012). Recent decades have seen the development of molecular biology tools to tackle ecological issues (Biggs et al., 2015; Civade, 2016; Ficetola et al., 2008; Herder et al., 2014). In this study, environmental DNA (eDNA) is defined as genetic material extracted directly from environmental samples, showing no discernible indications of its biological source (Thomsen & Willerslev, 2015). It has been successfully implemented for numerous and varied applications (Berry et al., 2017; Egeter et al., 2015; Monterroso et al., 2019; Nørgaard et al., 2021; Ruppert et al., 2019) such as biodiversity evaluation, invasive species monitoring (Dejean et al., 2012), species protection (Biggs et al., 2015), bioindication (Kermarrec et al., 2014; Leese et al., 2016; Pawlowski et al., 2018; Rimet et al., 2018) and intraspecific genetic diversity estimation (Tsuji et al., 2020). Moreover, eDNA-based methods are less intrusive than electric fishing which is highly disturbing (including animal electrification and trampling) and, in some cases, can lead to organism mortality (Pottier et al., 2022b). Successful use of the eDNA approach to detect invasive, rare or cryptic species, or to assess the biodiversity of an ecosystem, has been proven in several studies in both temperate (Civade, 2016; Pont et al., 2018) and tropical ecosystems (Cantera et al., 2019; Cilleros et al., 2019; Greathouse et al., 2006; Sales et al., 2021). Nevertheless, only 26% of the eDNA research articles published between 1993 and 2019 concern tropical ecosystems (Huerlimann et al. 2020) and, furthermore, the majority of eDNA-method

validation research has focused on fish species while very little has centred on crustacean species (Fediajevaite et al., 2021).

Biodiversity monitoring and the early detection of invasive species are major challenges for biodiversity managers in the FWI. The implementation of a non-invasive, less time- and labour-consuming and more accurate method would be highly advantageous. Only one study has implemented eDNA-based methods in the FWI to date: for the detection of one crustacean species, using a single-species approach (Baudry, 2022). Our study aims to employ the eDNA metabarcoding approach to study the entire fish and crustacean community for the first time in Guadeloupe, in order to determine its effectiveness in detecting local and exotic species and in an attempt to specify the conditions for its implementation. Specific inventories were carried out using both traditional (electric fishing) and eDNA-based methods. To our knowledge, this is the first *in situ* validation of an eDNA metabarcoding approach for crustacean inventories performed in parallel to a traditional survey, with the aim of confirming or refuting the species' presence. So as to include a broad range of Guadeloupean freshwater biodiversity, the samples were collected in nine different rivers in Basse-Terre, representing three different system sizes. These sampling locations have high species biodiversity. They are perennial rivers from which it is easy to obtain samples using traditional methods. In each river, two stations were sampled (upstream and downstream), as species assemblages can radically change along the river continuum. Two sampling seasons were chosen in order to evaluate the temporal variability of eDNA species detectability. The data we collected allowed us to: characterise the aquatic communities through inventory analysis, examine species diversity at each station, and explore the influence of abiotic and biotic factors on eDNA detectability (such as taxonomic group, sampling season and sampling location).

## MATERIALS AND METHODS

### Study area

Two sampling campaigns were organised in Guadeloupe (FWI): in February (during the dry season) and July 2019 (during the rainy season theoretically but without any significant rainfall before or during our sampling period). Nine rivers were selected according to the size of their watershed and the historical knowledge of their species communities. Two sample stations, upstream and downstream, were selected because different communities are typically present in each, for example, crustaceans tend to dominate upstream. The downstream stations were located near the river mouth while avoiding salt water. The upstream stations were chosen so as to be as far away as possible from the downstream stations while remaining accessible even during the rainy season (Fig. 1) (Tab. 1 in supplementary material). As tropical island rivers are usually short, sloping and have low branching complexity, downstream and upstream stations can share the same Strahler Stream Order rank. Grande Rivière de Vieux-Habitant, Rivière des Pères and Grande Rivière de Capesterre were the biggest rivers included, each with a watershed size between 2,619 and 3,862 hectares. The historical maximum species richness observed on these rivers varies from 4 to 12, taking into account fish and crustacean species. The medium-sized rivers (Rivière Beaugendre, Rivière Nogent, Rivière Moustique) each have a watershed size between 1,543 and 3,862 hectares and historically host a maximum of 9 to 20 species. The smaller rivers (Rivière Bananier, Rivière Ziotte, Rivière Baillif) each drain 235 to 736 hectare areas, and historically host a maximum of 8 to 14 species. Flows were recorded at each station using a salt dilution method and a SalinoMADD device (MADDTechnologies, Switzerland) (Tab. 2 in supplementary material).

The aquatic fauna is exclusively composed of 12 fish species, belonging to four families (Eleotridae Bonaparte, 1835, Gobiidae Cuvier, 1816, Mugilidae Jarocki, 1822 and Anguillidae Rafinesque, 1810) and



14 macrocrustacean species, essentially belonging to three decapod families (Palaemonidae Rafinesque, 1815, Atyidae De Haan, 1849 and Xiphocarididae Ortmann, 1895) (Monti et al., 2010). The overall richness of macrofauna species is low when compared with that of continental tropical rivers, or even temperate ones, and crustaceans account for 70% to 90% of the species richness in some rivers (Monti & Legendre, 2009). Except for one crab *Guinotia dentata* (Latreille, 1825), they are all diadromous. All are amphidromous and one fish, *Anguilla rostrata* (Lesueur, 1817), is catadromous (Greathouse et al., 2006; Monti et al., 2010). The individuals of this species spend their adult lives in freshwater. The amphidromous species (e.g. shrimps from the Palaemonidae, Atyidae and Xiphocaridae families, and Gobiidae and Mugilidae fish) spawn in freshwater habitats (Frotte et al., 2020; Smith & Kwak, 2014). Eggs or newly hatched larvae are passively transported towards the sea by river currents. At the post-larvae stage, juveniles leave the brackish or saline water and migrate upstream to freshwater systems where they grow and mature.

**Fig. 1 Location of upstream and downstream stations on a map of Basse-Terre. Studied rivers (black); Stations (white stars); Heart of the National Park of Guadeloupe limits (dark grey areas).**

#### **Environmental DNA sampling**

Three filtrations were performed at each station, resulting in three field replicates per station. Each filtration was done following Valentini et al.'s protocol for running waters (Valentini et al., 2016). We filtered water for 30 minutes at a rate of approximately 1L/minute, or less if suspended matter was abundant resulting in filter saturation. Civade et al. (2016) have shown that these filtration time or speed variations do not impact detection. The filtration was performed using a peristaltic pump (Vampire sampler, Burlke, Germany) connected to a single-use filtration capsule (VigiDNA 0.45 µm; SPYGEN, Le Bourget du Lac, France) by single-use tubing. The input part of the tubing was placed a few centimetres

below the river surface in zones with high water flow, as recommended by Cilleros et al. (2019). The sampling was carried out in a turbulent area (riffle hydro-morphological unit) to ensure an optimal homogenization of the DNA throughout the water column. To avoid DNA contamination across stations, all material that came into contact with water was systematically cleaned using a DNA decontamination solution (ELIM 60®) and the sampler was placed downstream from the filtration area. Moreover, environmental DNA sampling was always done before, or upstream of, the capture-based sampling. When the filtration was complete, the filtration capsule was emptied of water, filled with 80 mL of CL1 conservation buffer (SPYGEN) and stored at room temperature, for less than one month, until the DNA was extracted.

#### **Captured-based sampling with traditional electric fishing (TEF)**

The chosen protocol, adapted from the "complete fishing" method described by Belliard et al. (2012), is regularly employed by Guadeloupe National Park. This protocol had to be applicable during wet and dry seasons, on small and large rivers, and with reasonable human effort (4 or 5 operators). The objective was to detect the maximal fish and crustacean species richness for each station. First, we carried out a complete electric fishing sampling campaign on a 250 m<sup>2</sup> area. We used a 250V direct current or a 500V-1000V pulsed current, depending on the conductivity and hydrological facies of the stations (10 to 16 Hz and 15 to 25 A, respectively), using a backpack electrofisher (IG200-2 Hans Grassl Gmbh, Germany), an anode with a dip-net and 2 additional non-electrified dip-nets (4 mm mesh size). Second, if all species known to be present at the station, based on historical data, had not been captured, we sampled a maximum of ten additional small areas (2m<sup>2</sup>) upstream of the original fishing station and chose habitats specifically to capture the missing species. All the captured animals were stored in one or two tanks, depending on their abundance, of fresh oxygenated water and transported to the riverbank where, if

necessary, they were anaesthetised using a 0.2% eugenol solution. Next, the species were identified, counted and weighed. Ovigerous females and moulting individuals were recorded. When individuals were too small to be identified at a species level, they were gathered and identified at their genus level. Subsequently, the animals were put in a freshwater basin to recover and, finally, they were released back into the river.

### **The genetic reference database**

To optimise the taxonomic assignment of the fish and crustacean eDNA collected in our water samples, we built a local reference database. Fish and crustaceans were sampled from various stations and both seasons to maximise haplotypic diversity. A small number of species (e.g. *Polypterus endlicherii* (Heckel, 1847)) were bought in aquarium shops, as they could potentially be released into Guadeloupean rivers (Tab. 3 in supplementary material). A total of 149 tissue samples were collected from species known to populate the rivers (Di Mauro, 2009; Monti et al., 2010; Robert, 2016). Using sterilised instruments, a piece of fin no larger than 1 cm<sup>2</sup> was taken from fish in the field. For crustacean species, a leg was taken, or the whole individual for species whose adult size is less than 3 cm. Samples were stored in ethanol 95% at 4°C. In the laboratory, total DNA was extracted from a 10 mg tissue sample, following the protocol described in Valentini et al. (2016). The DNA was amplified with *teleo* primers (Valentini et al., 2016) or modified MiDeca primers (Komai et al., 2019: Pleo-F 5'- GGGACGATAAGACCCTATRAA -3'; Pleo-R 5'- ATNACGCTGTTATCCCTARAGTA -3', Pierre Taberlet pers. comm.)", depending on the taxonomic group, following the eDNA metabarcoding protocol described below, and was sequenced using a Miseq sequencer at Fasteris, Life Science Genesupport SA (Geneva, Switzerland). Sequence reads were analysed using programs executed in the OBITools package (Boyer et al., 2016). The forward and reverse reads were assembled with the ILLUMINAPAIREDD program, using a minimum score of 40 and retrieving only joined

sequences. Next, we assigned the reads to each sample using NGSFILTER software and a separate data set was created for each sample by splitting the original data set into several files using OBISPLIT. After this step, we analysed each sample individually before merging the taxon list for the final ecological analysis. Strictly identical sequences were clustered together using OBIUNIQ. Sequences shorter than 20 bp, with fewer than 10 reads, or labelled “internal” by the OBICLEAN program were excluded. Finally, the most abundant sequence was retrieved for the reference database construction. The genetic reference database included 20 fish and 15 crustacean species, covering all the freshwater species in Guadeloupe (Monti et al., 2010).

#### **Laboratory and bioinformatics analyses of eDNA**

The eDNA metabarcoding process, involving extraction, amplification using *teleo* primers (12 PCR replicates per sample), high-throughput sequencing and bioinformatic analysis, was performed following the procedure described in Pont et al. (2018). For crustacean species, eDNA extraction was carried out following Pont et al. (2018) before the DNA amplifications were performed in a final volume of 25  $\mu$ l of amplification mixture, using 3  $\mu$ l of DNA extract as the template. The amplification mixture contained 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems), 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.2  $\mu$ M of each primer Pleo primer and 0.2  $\mu$ g/ $\mu$ l bovine serum albumin (BSA, Roche Diagnostic). This primer couple amplifies a fragment of ca. 150 bp of the 16S gene. The primer sets were 5'-labeled with an eight-nucleotide tag unique to each sample (with at least three differences between any pair of tags), allowing the assignment of each sequence to the corresponding sample during sequence analysis. The tags for the forward and reverse primers were identical. The PCR mixture was denatured at 95°C for 10 minutes, followed by 50 cycles of 30 seconds at 95°C, 30 seconds at 50°C, 1 minute at 72°C and the final elongation step at 72°C for 7 minutes. Twelve PCR replicates were run per filtration, that is, 36

per sampling station. After amplification, the samples were quantified using capillary electrophoresis (QIAxcel; Qiagen GmbH) and purified using the MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified DNA was quantified again using capillary electrophoresis. All PCR products were pooled in equal volumes to achieve a theoretical sequencing depth of 300,000 reads per sample. In total, for the fish and crustaceans, six libraries were prepared using the MetaFast protocol and six paired-end sequences ( $2 \times 125$  bp) were carried out at Fasteris, Life Science Genesupport SA (Geneva, Switzerland) using an Illumina MiSeq sequencer and the MiSeq Flow Cell Kit v3 (Illumina, San Diego, CA, USA) as per the manufacturer's instructions. Five negative extraction controls and six negative PCR controls (three for *teleo* primers and three for *Pleo* primers) were also amplified with 12 replicates and sequenced in parallel to the samples to monitor for possible contaminants.

The sequences obtained were analysed using the ObiTools package following the same protocol described in the "genetic reference database" section. The taxonomic assignment of MOTUs (molecular operational taxonomic unit) was performed using the program ECOTAG, with the local reference database built in this study and the sequences extracted from the release 142 (standard sequences) of the ENA database (<http://www.ebi.ac.uk/ena>). To account for the incorrect assignment of a small number of sequences to the sample due to tag jumps (Schnell et al., 2015), all sequences with a frequency of occurrence  $< 0.001$  per sequence and per library were excluded. Subsequently, the data were curated for index-hopping (MacConaill et al., 2018) with a threshold empirically determined using experimental blanks (i.e. combinations of tags not present in the libraries), for a given sequencing batch between libraries.

### **Statistical analyses**

All the statistical analyses were performed in R version 4.3.1 (R Core Team, 2023).

For each station, the read number after bioinformatics filtering from the 3 field replicates and the 12 PCR replicates were summed to obtain the total number of reads per taxa and per station.

For each sampling campaign, station location on the river and taxonomic group, the species richness values (obtained with TEF and eDNA-based methods) were compared using a Wilcoxon rank sum test with continuity correction (Neuhäuser, 2011) and the R function *wilcox.test*. We also performed a Bland-Altman test (Bland & Altman, 2010; McElroy et al., 2020), using the *blandr* package (Datta, 2017). This test resulted in a scatter diagram showing, for each station, the difference between the eDNA and TEF species richness (on the ordinate) and the average of the two (on the abscissa).

For crustaceans only, a Wilcoxon rank sum test with continuity correction was performed to compare the abundance of ovigerous species and the number of ovigerous females between the two campaigns, and between the station locations. The same test was performed to compare the abundance of species with moulting individuals and the number of moulting individuals.

The Jaccard beta-diversity index between stations was calculated using the R function *beta.pair* from the *betapart* package (Baselga et al., 2023), using fish and crustacean presence/absence data separately. A distance-based redundancy analysis (dbRDA) on the beta-diversity matrices was performed using the function *capscale* from the *vegan* package (Oksanen et al., 2022), with explanatory variables chosen using the function *ordiR2step*.

To test the correlation between the quantitative data (the number of captured individuals and the proportion of sequences, for each species and at each station) we used a non-parametric correlation test. We opted for the Kendall rank correlation, which is particularly well-suited to situations involving small sample sizes and the presence of outliers (Bonett & Wright, 2000). This test provided a correlation

coefficient ( $\tau$ ) which allowed us to calculate a determination coefficient ( $\tau^2$ ). The test was carried out using the *car* (Fox & Weisberg, 2019) and *Kendall* (McLeod, 2022) packages.

## RESULTS

### Biodiversity assessment

#### Environmental DNA-based method implementation

In total, 24,128,539 reads were obtained for fish and 29,359,389 for crustaceans from the first campaign (C1) samples. For samples collected during the second campaign (C2), 31,477,444 and 11,885,133 reads were obtained for fish and crustaceans, respectively. After the bioinformatic steps, 16,600,307 reads (68.8 %) were retrieved for fish and 10,207,511 for crustaceans (34.8%) in the C1 samples, and 22,317,200 reads for fish (70.9%) and 5,371,014 for crustaceans (45.2%) in the C2 samples.

Combining both campaigns, 31 fish taxa were detected with the eDNA-based method, including 16 marine taxa assigned using the ENA nucleotide sequence database and, consequently, discarded from further data analysis (e.g. *Panulirus argus* (Latreille, 1804) and *Thunnus* South, 1845 sp.) (Tab. 4 in supplementary material). The marine species sequences could be attributed to intrusions of marine species at the river mouth, but it is more probable that they originated from malfunctioning sewage treatment facilities that release effluent into downstream rivers. Alternatively, they may have resulted from discarded fishing byproducts or individuals cleaning their fish in nearby river water. When a DNA sequence was identified at the genus level and detected with other species of the same genus at the same station, it was removed from the final data set, whether it belonged to fish or crustaceans (e.g. *Anguilla* Garsault, 1764, *Sicydium* Valenciennes, 1837, *Macrobrachium* Spence Bate, 1868 and *Atya* Leach, 1816). Two species, *Ancistrus cirrhosis* (Valenciennes, 1836) and *Ancistrus triradiatus*, cannot be distinguished using the *teleo* marker,

as well as the species belonging to the genus *Oreochromis* Günther, 1889. All the species of this latter genus were considered exotic for this territory, hence the low taxonomic resolution of this genus was not an issue in our study. However, the *teleo* marker was capable of distinguishing two patrimonial species, *Sicydium plumieri* (Bloch, 1786) and *Sicydium punctatum* Perugia, 1896. Twenty-four crustacean taxa were detected, of which 12 were assigned using the ENA database. Seven marine taxa were excluded from the subsequent analysis. The crabs *Armases roberti* (H. Milne Edwards, 1853) and *Guinotia dentata* were kept in the data set as they are freshwater species. Two haplotypes identified as *Atya lanipes* Holthuis, 1963 and *Atya margaritacea* A. Milne-Edwards, 1864, only detected using the ENA database, were considered to be possible errors in the genetic reference database and were excluded from the final data set (Tab. 4 in supplementary material). Finally, 15 fish taxa and 15 crustacean taxa were included in the eDNA results data set (Tab.1) (Tab. 5 in supplementary material).

#### **Traditional electric fishing implementation**

No mortality was observed at the time of fishing but some signs of spinal damage were noticed in a small number of *Eleotris perniger* (Cope, 1871) individuals. The TEF results data set was cleaned before analysis and interpretation. Juveniles that were unidentifiable at a species level were excluded if adults of the same genus were inventoried at the same station. When only juveniles were captured at a station and a species of this genus was detected by the eDNA-based method, the juveniles were arbitrarily counted as two individuals of this species in the TEF results for the abundance analyses. One individual belonging to the Carangidae Rafinesque, 1815 family was captured in the Rivière Beaugendre, downstream, but was subsequently discarded from the analysis because it is a marine species. One terrestrial taxon of crustacean (*Cardisoma* Latreille, 1828 sp.) was likewise discarded. Finally, 11 fish species and 14 crustacean species were captured by TEF during at least one campaign (Tab. 1) (Tab. 5 in supplementary material).



Ovigerous females were observed during both campaigns and in all rivers except the Rivière Ziotte upstream and the Rivière Baillif downstream (Tab. 2 in supplementary material). A comparison of the two campaigns for crustaceans revealed no significant difference in the mean abundance of ovigerous species ( $V = 24.5$ ,  $p$ -value = 0.147) or the mean number of ovigerous females ( $V = 54$ ,  $p$ -value = 0.177). The same was true of the comparison between upstream and downstream ( $V = 58$ ,  $p$ -value = 0.750 and  $V = 60$ ,  $p$ -value = 0.276, respectively).

The presence of a limited number of moulting individuals was sporadically noted across the various stations (Tab. 2 in supplementary material). A comparison of the two campaigns revealed no significant difference in the mean abundance of moulting species ( $V = 59$ ,  $p$ -value = 0.120) or in the mean number of moulting individuals ( $V = 53.5$ ,  $p$ -value = 0.272). However, the mean abundance of moulting species observed downstream was significantly different from upstream ( $V = 5$ ,  $p$ -value = 0.040), the latter being slightly lower ( $V = 5$ ,  $p$ -value = 0.020). The mean numbers of moulting individuals upstream and downstream were not significantly different ( $V = 19$ ,  $p$ -value = 0.069).

#### **Comparison of methods based on sampling campaign**

During the first campaign, 10 fish taxa were detected by both the TEF and eDNA-based method and four taxa (*Awaous banana* (Valenciennes, 1837), Cyprinidae Rafinesque, 1815, *Oreochromis* Günther, 1889 sp. and *Rhonciscus crocro* (Cuvier, 1830)) were detected by eDNA-based method only. During the second campaign, 10 species were both caught by TEF and detected by the eDNA-based method. One species (*Microphis brachyurus* (Bleeker, 1854)) was only caught, and three taxa (*Awaous banana*, *Oreochromis* sp. and *Xiphophorus maculatus* (Günther, 1866)) were only detected by the eDNA-based method. However, one species in the first campaign (*Microphis brachyurus*) and one species in the second campaign (*Rhonciscus crocro*) were detected by eDNA and caught by TEF but not at the same station (Tab. 1).

Regarding the crustaceans, during the first campaign, 13 taxa were both detected by eDNA and TEF methods, and two species (*Armases roberti* and *Macrobrachium ohione* (Smith, 1874)) were only detected by the eDNA-based method. During the second campaign, 14 species were common to both the TEF and eDNA samples; however, *Potimirim potimirim* (Müller, 1881) was both detected by eDNA and caught by TEF but not at the same station (Tab. 1).

The most frequent and abundant species (two fish species: *Sicydium punctatum* and *S. plumieri*; and three crustacean species: *Atya innocous* (Herbst, 1792), *Macrobrachium heterochirus* (Wiegmann, 1836) and *Micratya poeyi* (Guérin-Méneville, 1855)) were well inventoried, whether by TEF or the eDNA-based method (Tab.1).

**Tab. 1 Detection capacity of traditional electric fishing and the eDNA-based method - Number of stations where each species was captured (TEF), detected (eDNA) or both, during each campaign.**

The Wilcoxon rank sum test with continuity correction indicates that the eDNA and TEF species richness medians were significantly different for the first campaign ( $V = 12$ ,  $p\text{-value} < 0.001$ ) and both campaigns together ( $V = 287$ ,  $p\text{-value} < 0.001$ ), eDNA species richness being greater than TEF species richness (C1:  $V = 12$ ,  $p\text{-value} < 0.001$ ; C1 and C2:  $V = 287$ ,  $p\text{-value} < 0.001$ ). However, the eDNA and TEF species richness medians were not significantly different for the second campaign ( $V = 126.5$ ,  $p\text{-value} = 0.080$ ) (Fig. 2).

For fish, the eDNA and TEF methods gave significantly different species richness medians for each campaign and both campaigns together (C1:  $V = 0$ ,  $p\text{-value} = 0.001$ ; C2:  $V = 0$ ,  $p\text{-value} = 0.001$ ; C1 and C2:  $V = 0$ ,  $p\text{-value} < 0.001$ ). In all three cases, eDNA species richness was greater than TEF species richness (C1:  $V = 0$ ,  $p\text{-value} < 0.001$ ; C2:  $V = 0$ ,  $p\text{-value} < 0.001$ ; C1 and C2:  $V = 0$ ,  $p\text{-value} < 0.001$ ). For crustaceans, the results depended on the campaign. The eDNA and TEF specific richness medians were significantly different (C1:  $V = 8$ ,  $p\text{-value} < 0.001$ ), with significantly greater values for eDNA ( $V = 8$ ,  $p\text{-value} < 0.001$ ) for

the first campaign. For the second campaign, eDNA and TEF medians were not significantly different ( $V = 61$ ,  $p\text{-value} = 0.977$ ), meaning that the two methods were equivalent in terms of species richness detection (Fig. 2).

**Fig. 2 Species richness obtained by TEF, eDNA or both methods, at each station and during each campaign (light grey: number of species detected by TEF only, dark grey: number of species detected by both methods, black: number of species detected by eDNA-based method only; (A) Fish species during the first campaign, (B) Fish species during the second campaign, (C) Crustacean species during the first campaign, (D) Crustacean species during the second campaign).**

The Bland-Altman scatter plot shows that the 95% confidence interval around the mean difference does not overlap with zero for fish in both campaigns or for crustaceans in the first campaign, which signifies that the difference between the DNA and TEF species richness was significant (Fig. 3). Species richness differences were not significant for crustaceans in the second campaign. The eDNA-based method outperformed the electric fishing method in terms of species richness for fish in both campaigns and crustaceans in the first campaign, while the Bland-Altman test did not demonstrate which method is the most effective for crustaceans in the second campaign.

**Fig. 3 Bland-Altman plots for (A) Fish-Campaign 1, (B) Fish-Campaign 2, (C) Crustaceans-Campaign 1 and (D) Crustaceans-Campaign 2. The dark grey band indicates a mean (wide dashed line) with 95% confidence intervals. Light and medium grey bands indicate two standard deviations (wide dashed lines) from the mean with 95% confidence intervals. No outlier (observation that lies above or below the light and medium grey bands, respectively) is observed. Positive differences indicate that the eDNA-based method detects more species than traditional electric fishing, across all panels.**

### Comparison of methods based on station location (upstream or downstream)

A comparison of the TEF and eDNA-based methods for estimating species richness based on station location, as determined by a Wilcoxon rank sum test with continuity correction, revealed that eDNA species richness significantly differed from that obtained by TEF at the upstream stations when considering either the first campaign or both campaigns combined (C1:  $V = 8$ ,  $p$ -value = 0.003; C1 and C2:  $V = 104$ ,  $p$ -value = 0.038). Indeed, eDNA species richness was greater than TEF species richness (C1:  $V = 8$ ,  $p$ -value = 0.001; C1 and C2:  $V = 104$ ,  $p$ -value = 0.019). There was no significant difference between the eDNA and TEF species richness at the upstream stations during the second campaign (C2:  $V = 45$ ,  $p$ -value = 0.655). For fish during both campaigns, eDNA species richness significantly differed from that obtained by TEF at the upstream stations (C1 and C2:  $V = 0$ ,  $p$ -value = 0.002), eDNA species richness being greater than TEF species richness (C1 and C2:  $V = 0$ ,  $p$ -value < 0.001). On contrary, no significant difference between methods was observed for crustaceans at the upstream stations (C1 and C2:  $V = 60$ ,  $p$ -value = 1). During both campaigns, TEF and eDNA species richness medians were significantly different at the downstream stations (C1:  $V = 0$ ,  $p$ -value < 0.001; C2:  $V = 27$ ,  $p$ -value = 0.035; C1 and C2:  $V = 55.5$ ,  $p$ -value < 0.001) with eDNA species richness medians being consistently higher than TEF ones (C1:  $V = 0$ ,  $p$ -value < 0.001; C2:  $V = 27$ ,  $p$ -value = 0.018; C1 and C2:  $V = 55.5$ ,  $p$ -value < 0.001). Environmental DNA species richness significantly differed from that obtained by TEF at the downstream stations for fish (C1 and C2:  $V = 0$ ,  $p$ -value < 0.001) and crustaceans (C1 and C2:  $V = 28.5$ ,  $p$ -value = 0.024), eDNA species richness being greater than TEF species richness (Fish C1 and C2:  $V = 0$ ,  $p$ -value = 0.001; Crustaceans C1 and C2:  $V = 28.5$ ,  $p$ -value = 0.012). The Bland-Altman plot shows that fish species richness means were greater downstream than upstream, except in the case of Grande Rivière de Capesterre and Rivière Baillif (Fig.3).

### Comparison of methods based on quantitative data

For the first campaign, the fish quantitative data (the number of captured individuals and the proportion of sequences, for each species and at each station) were significantly correlated ( $\tau = 0.496$ ,  $p$ -value  $< 0.001$ ) but the determination coefficient ( $\tau^2 = 0.246$ ) was weak, indicating that only 25% of the variance was shared between the two variables. The crustacean quantitative data from the first campaign were also significantly correlated ( $\tau = 0.305$ ,  $p$ -value  $< 0.001$ ) but the very low determination coefficient ( $\tau^2 = 0.09$ ) indicated that less than 10% of the variance was shared. For the second campaign, the fish quantitative data were significantly correlated but the variables shared only 17% of the variance ( $\tau = 0.414$ ,  $p$ -value  $< 0.001$ ,  $\tau^2 = 0.17$ ), while the crustacean quantitative data were not significantly correlated ( $\tau = -0.012$ ,  $p$ -value = 0.834).

### **Beta-diversity patterns**

The dbRDAs on fish and crustacean beta-diversity between stations showed a marked dissimilarity, although the explanatory power was relatively modest (fish) or weak (crustacean) ( $R^2 = 0.50$  and  $0.3$ , respectively) (Fig. 4). The beta-diversity of fish was found to be associated with the sampling station (upstream or downstream,  $R^2 = 0.26$ ) and the river ( $R^2 = 0.20$ ). This showed that the geographic factor had a greater influence on the structuration of the fish community detected than the survey approach or the timing of the sampling campaign. Crustacean beta-diversity was related to the sampling campaign ( $R^2 = 0.14$ ), the river ( $R^2 = 0.10$ ) and, to a lesser extent, the survey method ( $R^2 = 0.03$ ), indicating that the sampling period and the river had a greater influence on the structuration of crustacean community detected. The crustacean species composition differed between the TEF and eDNA surveys, these results are consistent with the Bland-Altman scatter plot analysis.

**Fig. 4: Distance-based redundancy analysis (dbRDA) showing the variation in fish (A) and crustacean (B) community structures between stations, according to survey method, sampling location and campaign.**

## DISCUSSION

### Environmental DNA-based method performance

Our results show that the eDNA-based method is effective at detecting fish and crustacean species in Guadeloupe. In both campaigns and at all stations, the eDNA metabarcoding method detected more fish species than the traditional method, though the species assemblage composition of the methods remained similar. This result is consistent with those of previously published comparative studies between eDNA-based and traditional methods (Fedajevaite et al., 2021; Jerde et al., 2019; McElroy et al., 2020). The eDNA-based method particularly outperformed electric fishing for four species: 1) *Anguilla rostrata* which is "endangered" in the FWI according to the International Union for Conservation of Nature (IUCN) (IUCN Comité français et al., 2021), 2) *Awaous banana* which is "near threatened" in the FWI according to the IUCN, 3) *Rhonciscus crocro*, a rare species, and 4) *Oreochromis* sp., an alien species in the FWI. The latter could not be identified at a species level using the eDNA-based method, but there are no native species of the *Oreochromis* genus in Guadeloupe. However, our results show that the eDNA-based method does not consistently outperform electric fishing for crustaceans. This is also reflected in the different species assemblages detected by the two methods. For example, *Xiphocaris elongata* (Guérin-Méneville, 1855) was more often captured than detected during the second campaign. Nevertheless, the eDNA-based method proved more effective than electric fishing for four crustacean species: 1) *Guinotia dentata*, a species endemic to the Lesser Antilles, 2) *Potimirin glabra* (Kingsley, 1878), 3) *Potimirin potimirin*, a very

small species (length < 40 mm) that is difficult to distinguish and is “vulnerable” according to the IUCN, and 4) *Macrobrachium carcinus* (Linnaeus, 1758), also “vulnerable” according to the IUCN and a patrimonial species.

The efficacy of the eDNA-based method is closely linked to the completeness of the reference database, as underlined by Mahon et al. (2023). In this study, we included sequences of the species most commonly sold in local shops (e.g. *Danio rerio* (Hamilton, 1822), *Poecilia sphenops* Valenciennes, 1846, *Polypterus endlicheri* Heckel, 1847, *Xiphophorus hellerii* Heckel, 1848, and *Cambarellus patzcuarensis* Villalobos, 1943. We also included the sequences of several species of the *Ancistrus* Kner, 1854 genus, an invasive species that is already present in Guadeloupe. *Oreochromis* sp. was also referenced as *Oreochromis* species. This species can be caught in Guadeloupe since its introduction in the 1950s and because an *Oreochromis niloticus* (Linnaeus, 1758) farm exists in Basse-Terre. There are only three other aquaculture fish farms in the region. They produce *Macrobrachium Rosenbergii* (de Man, 1879) which has never been fished in Guadeloupe (Di Mauro, 2009) and is not considered to be able to compete with other species in a natural environment (Baudry, 2022). As we did not have the resources to sequence the potentially invasive species on the international lists, we deliberately chose a localised approach to limit the risk of false negatives. The exotic species known to be present in Guadeloupe (*Poecilia reticulata* Peters, 1859, *Oreochromis* sp., *Xyphophorus helleri* Heckel, 1848 and *Xyphophorus maculatus* Günther, 1866) were detected on 12 occasions, whereas they were both caught and detected on seven occasions and only caught once (one *Poecilia reticulata* individual). Based on these findings, we believe that the eDNA-based method allows for the identification and early detection of species in the FWI and that it has the potential to be employed in extensive surveys.

Environmental DNA-based methods can detect eDNA traces coming from effluent from wastewater

treatment plants, domestic activities on the riverside, restaurants, etc. (Goldberg et al., 2016; Pont et al., 2022). In Guadeloupe, the presence of a large number of dwellings close to rivers, the prevalence of non-collective sanitation and the high proportion of non-compliant wastewater treatment plants are particularly significant issues (Office Français de la Biodiversité (OFB) et al., 2019) that certainly explain the frequency of detection of food or marine species in our results. This situation reinforces the need for the dual assignment of sequences in the local and ENA reference databases, enabling foreign eDNA traces to be identified easily while ensuring the detection of potential new species.

In terms of the quantitative data, our findings indicate a significant but relatively weak correlation between the numbers of individuals and the proportions of sequences. This correlation was observed for fish during both campaigns and for crustaceans during the first campaign only. These results are consistent with our previous conclusions but they also argue in favour of implementing and evaluating other quantitative parameters (Pont, 2022).

### **What factors should be considered when implementing the eDNA-based method in Guadeloupe?**

#### **Methodological and physical factors**

When considering the use of eDNA-based methods for species surveying in Guadeloupe, the spatial and temporal factors should be taken into account. In fact, the beta-diversity analysis demonstrated that fish assemblages upstream were different to those present downstream. In order to have a complete picture of the fish community for each river, the eDNA survey should be undertaken at different stations on the same river. Conversely, crustacean communities displayed more homogeneity across the two river stations. In fact, historical data collected from previous electric fishing surveys showed that most of the fish species are found downstream, while adult shrimps are present along the entire stream continuum according to their species habitat preferences (Frotte et al., 2020; Smith & Kwak, 2014). Species of



Sicydiinae Gill, 1860, Mugilidae, Anguillidae and macro-crustaceans, the only ones capable of crossing fast currents and obstacles to continuity (natural or otherwise), are present upstream (Lagarde et al., 2021). For crustaceans, the time of year had a greater influence on species detection and assemblages, while the fish community did not change over time. When considering the detectability of crustaceans, the sampling period and method should be chosen more carefully than for the fish community.

Both eDNA-based and TEF methods have methodological bias. It is difficult to measure the extent of DNA sequence upstream/downstream drift, although Cantera et al. (2022) showed that the eDNA-based method provides a fish species assessment at a limited spatial grain, similar to the one obtained using capture-based methods. Concerning electric fishing, Pottier et al. (2022b) have demonstrated that the anodic taxis depends on the electrical frequencies and shrimp species. More generally, rare, benthic or cryptic species are trickier to capture, especially in tropical, small, and rocky substrate streams. It is important to keep in mind that these methodological limitations can reinforce or erase the differences between the two methods.

Station hydro-morphology should also be considered. Increased depth or more abundant aquatic vegetation hamper electric fishing by reducing both electric field strength and operator visibility (Pottier et al., 2022a) though they do not compromise the eDNA-based method performance. Species richness and composition differences between the eDNA-based and TEF methods, observed in the Rivière Ziotte and Rivière du Bananier (downstream) can be attributed to these hydro-morphological factors. Flow can impact both electric fishing, by modifying facies, and the eDNA-based method, by DNA dilution and transport (Buxton et al., 2017). This is one of the reasons why our experimental protocol planned two campaigns, during different hydrological seasons. Unfortunately, flows measured during the second campaign were lower than during the first one, except for three stations (Grande Rivière de Capesterre,

Rivière Nogent and Rivière Baillif, all upstream) (Tab. 2 supplementary material), impeding our ability to study the hydro-morphological effect.

Environmental DNA detectability can be affected by high temperatures and ultraviolet radiation (UV) which accelerate the biological and physical degradation of DNA (Eichmiller et al., 2016; Jo et al., 2019; Strickler et al., 2015). Considering that tropical island ecosystems are characterised by high temperatures, strong UV radiation and short, and often turbulent, rivers (Monti & Legendre, 2009) with little organic matter in suspension except during flood periods, these factors may collectively pose challenges to the efficacy of the eDNA-based method (Ruppert et al., 2019). We consistently recorded higher water temperatures during the second campaign (22.1 to 28.1°C) than during the first (19.5 to 24.8°C) (Tab. 2 in supplementary material). Likewise, insolation (7.6 h/day on average), solar irradiance and UV radiation were higher during the second campaign, which took place from 1 to 24 July 2019 (Centre météorologique de la Guadeloupe - Section climatologie, 2019; <https://globalsolaratlas.info>). Hence, the temperature conditions during the second campaign may have been less conducive to eDNA detection. However, temperature and UV radiation do not explain why the eDNA-based method did not outperform TEF during the second campaign for crustaceans alone. Our results are therefore consistent with Mächler et al. (2018) who assert that there is no effect from sunlight or UV on the detectability of eDNA.

### **Biotic factors**

Environmental DNA detectability is known to be impacted by biotic factors, especially eDNA production rate which varies among taxonomic groups. For example, fish and amphibians produce abundant mucus, a significant source of DNA in the environment, whereas arthropods have a chitin exoskeleton that limits the release of DNA (Tréguier et al., 2014). Life stage is another important biotic factor, as eggs and *exuviae*

are a rich source of DNA (Tillotson et al., 2018; Tréguier et al., 2014). The density and residence time of individuals (Goldberg et al., 2013; Pilliod et al., 2014) are also crucial factors of detectability.

Varying DNA excretion depending on the biology of the species may explain the observed differences in the detection rates between taxonomic groups and between the two campaigns. Our results show that, during the second campaign (in July), the eDNA-based method did not outperform electric fishing and the quantitative data were no longer correlated, suggesting a seasonal effect for crustaceans (Tsuji et al., 2019; Xia et al., 2021). Life stage (ovigerous status in particular) may facilitate crustacean eDNA detection in the natural environment (Dunn et al., 2017). Crane et al., (2021) experimentally showed that eDNA detection rates were significantly higher for ovigerous female crabs than for non-ovigerous females or males. The presence of ovigerous females in Guadeloupe's rivers (still poorly studied) depends on the species. Most species, particularly those in the Atyidae family, have ovigerous females throughout the year. Peaks of ovigerous females, associated with rainfall in continental tropical freshwater (Barros-Alves et al., 2021; Mossolin & Bueno, 2002) are also reported in Guadeloupe, for example, from May to October for *Atya scabra* (Leach, 1816), from August to October for *Macrobrachium faustinum* (de Saussure, 1857) or from July to November for *Macrobrachium acanthurus* (Wiegmann, 1836) (Monti et al., 2010). However, as ovigerous species abundance and the number of ovigerous females were not significantly different based on sampling season or station location, this biotic factor cannot explain the eDNA detectability differences. Nevertheless, further studies would be useful to confirm or disprove whether reproductive activity, be it due to the presence of ovigerous females or the release of gametes or eggs, impacts species detectability using the eDNA-based method. The age of individuals is also a factor, as the younger the individuals the more numerous the *exuviae* which are a rich source of DNA (Tréguier et al., 2014). However, as our survey did not demonstrate any significant difference in moulting species abundance or moulting individual

number between the two sampling seasons, the age of individuals cannot be an explicative factor for the eDNA detectability differences. Even if moulting individual numbers do not differ between upstream and downstream, moulting species abundance could contribute to the good eDNA detectability observed downstream at the two seasons.

The abundance and residence time of individuals (Goldberg et al., 2013; Pilliod et al., 2014) are also crucial factors of detectability. In tropical rivers, the abundance of diadromous individuals is strongly influenced by the downstream migration of larvae and the recruitment of post-larvae, two complex phenomena (March et al., 1998; Benstead et al., 2000). Frotte et al. (2020) have shown that the post-larval recruitment period is not systematically seasonal (though it occurs mainly during the wet season) and varies depending on the river, the species and over time. Consequently, the migratory flow of diadromous species could explain the variation in crustacean detectability over time.

## CONCLUSION

This study demonstrates the effectiveness and reliability of the eDNA metabarcoding approach for evaluating fish biodiversity in Guadeloupe, regardless of the sampling period. Although the results obtained for crustaceans are very promising, the eDNA-based method does not systematically detect more crustacean species than the traditional method: the number of species detected with the eDNA-based method was higher or equal to the number of species obtained by electric fishing on 16 of the 18 stations studied during the first campaign; however, this fell to 7 stations during the second campaign. Although various abiotic and biotic factors were considered, our study did not enable us to identify which of these factors might explain the observed differences. However, as the climatic and hydrological conditions of the two campaigns were very similar it is likely that biotic factors are responsible for the results, which

highlights the need for a better understanding of the species living in Guadeloupe. Future studies using the eDNA-based method should investigate the impact of increased flow rates, and the resulting dilution during a high-water period, which our study was unable to explore. The reference database also needs to be improved in order to better integrate all the haplotypic variability of Guadeloupean species. Future studies should also investigate the quantitative aspect of the eDNA-based method. Nevertheless, our results show that the eDNA-based method enables biodiversity assessment in tropical island conditions and the detection of likely alien or invasive fish and crustacean species. This non-invasive (no electricity and less aquatic fauna disturbance) method can be used as an alternative to traditional methods to detect the presence or absence of species, or in addition to traditional methods when taxonomic uncertainties or implementation difficulties are expected.

## DATA AVAILABILITY STATEMENTS

The fish and crustacean biometric data sets (including species name, quantity, weight and size) generated and analysed during this study are available in the Karunati regional repository: <https://karunati.fr/>. Sequences for the reference databases and all Illumina raw sequences data are available on figshare, doi: 10.6084/m9.figshare.23608056 (Campaign 1), doi: 10.6084/m9.figshare.23608158 (Campaign 2) (Li, 2018).

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## CONFLICTS OF INTEREST

AV is a research scientist in a private company specialising in the use of eDNA for biodiversity monitoring, with some patented technologies (SPYGEN). The other authors have no competing interests to declare that are relevant to the content of this article.

## ETHICAL APPROVAL

Fieldworks were conducted under the responsibility of Marie Robert and Marion Labeille, who are permitted to practice electric fishing. All the manipulations (individual capture, electric fishing and water filtration) that were carried out in the National Park during this project were authorised by decrees:

*Nagoya protocol : décision du 5 avril 2018 portant sur l'accès aux ressources génétiques sur le territoire national et le partage des avantages découlant de leur utilisation (NOR : TREL1734890A/7).*

*Practice of electric fishing : arrêté DEAL/RN 971-2019-04-01-001 du 01 avril 2019 portant autorisation de campagnes de pêches électriques sur les cours d'eau de la Guadeloupe dans le cadre des suivis scientifiques mis en œuvre par le Parc national de la Guadeloupe.*

Destruction of invasive alien species : *arrêté DEAL/RN 971-2019-02-22-001 du 22 février 2019 autorisant le Parc national de Guadeloupe à procéder à des opérations de captures et de destructions de spécimens d'espèce exotique envahissante de la faune sauvage d'eau douce dans le cadre du projet Guad3E qui vise à tester l'efficacité de la nouvelle méthode d'inventaire par ADNe sous les latitudes tropicales.*

Protected area national park regulations : *arrêté n°18-69 relatif à des prélèvements de tissus et d'ADN de poissons et crustacés dans les cours d'eau en cœur de Parc national dans le cadre du projet Guad3E.*

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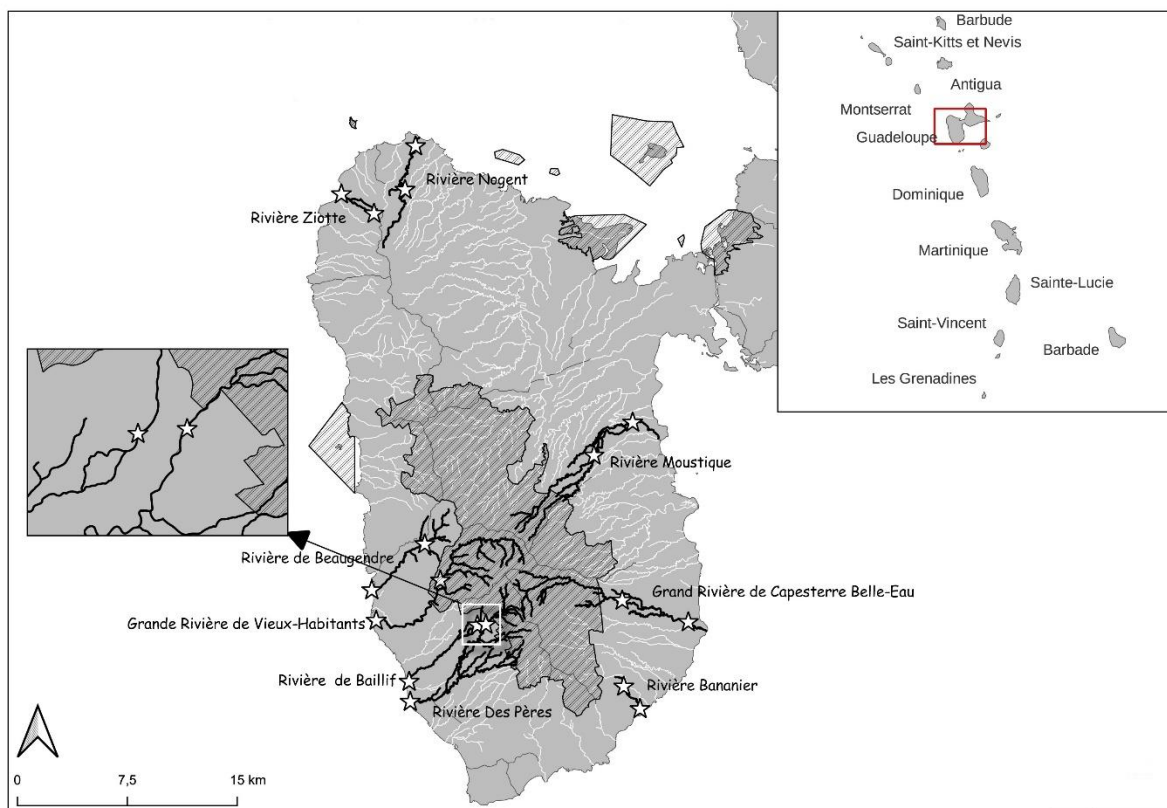


TABLE 1:

Fish taxa	Species code	Reference database	C1			C2		
			TEF only	Both eDNA and TEF	eDNA only	TEF only	Both eDNA and TEF	eDNA only
<i>Agonostomus monticola</i> (Bancroft, 1834)	AMO	Guadeloupe	0	9	2	1	8	3
<i>Anguilla rostrata</i>	ARO	Guadeloupe	0	2	5	1	4	5
<i>Arcos nudus</i> (Linnaeus, 1758)	ANU	Guadeloupe	0	8	2	0	7	1
<i>Awaous banana</i> (Valenciennes, 1837)	ABA	Guadeloupe	0	0	9	0	0	9
Cyprinidae	CYP	EMBL	0	0	2	0	0	0
<i>Eleotris perniger</i>	EPE	Guadeloupe	0	9	0	0	7	2
<i>Gobiomorus dormitor</i>	GDO	Guadeloupe	0	4	0	0	3	1
<i>Microphis brachyurus</i>	MBR	Guadeloupe	1	0	1	1	0	0
<i>Oreochromis</i> sp.	ORE	Guadeloupe	0	0	3	0	0	2
<i>Poecilia reticulata</i>	PRE	Guadeloupe	0	2	2	1	2	1
<i>Rhonciscus crocro</i>	PCR	Guadeloupe	0	0	4	1	0	5
<i>Sicydium plumieri</i>	SPL	Guadeloupe	0	14	2	0	15	1
<i>Sicydium punctatum</i>	SPU	Guadeloupe	0	18	0	1	16	0
<i>Xiphophorus hellerii</i>	XHE	Guadeloupe	0	1	2	0	2	1
<i>Xiphophorus maculatus</i>	XMA	Guadeloupe	0	0	0	0	0	1

Crustacean taxa	Species code	Reference database	C1			C2		
			TEF only	Both eDNA and TEF	eDNA only	TEF only	Both eDNA and TEF	eDNA only
<i>Armases roberti</i>	ARB	Guadeloupe	0	0	2	1	3	0
<i>Atya innocous</i>	AIN	Guadeloupe	1	11	6	1	8	6
<i>Atya scabra</i>	ASC	Guadeloupe	1	12	2	3	7	3
<i>Guinotia dentata</i>	GDE	Guadeloupe	0	2	9	0	4	6
<i>Jonga serrei</i> (Bouvier, 1909)	JSE	Guadeloupe	0	2	0	1	2	0
<i>Macrobrachium acanthurus</i>	MAC	Guadeloupe	2	1	0	2	1	0
<i>Macrobrachium carcinus</i>	MCA	Guadeloupe	0	4	9	1	3	5
<i>Macrobrachium crenulatum</i>	MCR	Guadeloupe	0	8	6	2	6	6
<i>Macrobrachium faustinum</i>	MFA	Guadeloupe	3	11	1	4	8	2
<i>Macrobrachium heterochirus</i>	MHE	Guadeloupe	0	15	3	1	11	4
<i>Macrobrachium ohione</i>	MOH	EMBL	0	0	1	0	0	0
<i>Micratya poeyi</i>	MPO	Guadeloupe	0	15	1	6	9	1
<i>Potimirim glabra</i>	PGL	Guadeloupe	1	4	8	3	1	4
<i>Potimirim potimirim</i>	PPO	Guadeloupe	2	2	1	3	0	3
<i>Xiphocaris elongata</i>	XEL	Guadeloupe	5	9	0	9	6	0

FIGURE 1:



Accepted

FIGURE 2:

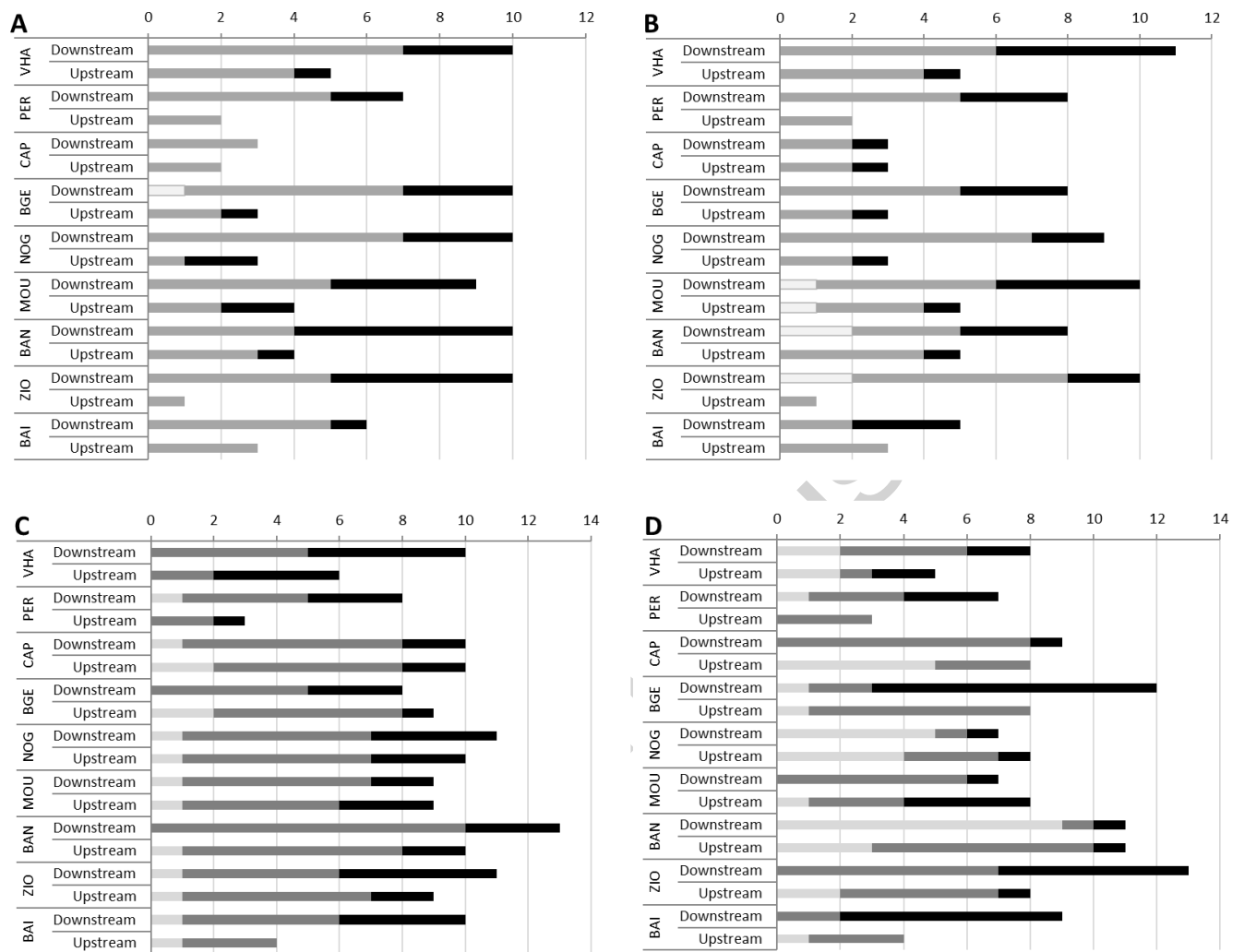


FIGURE 3:

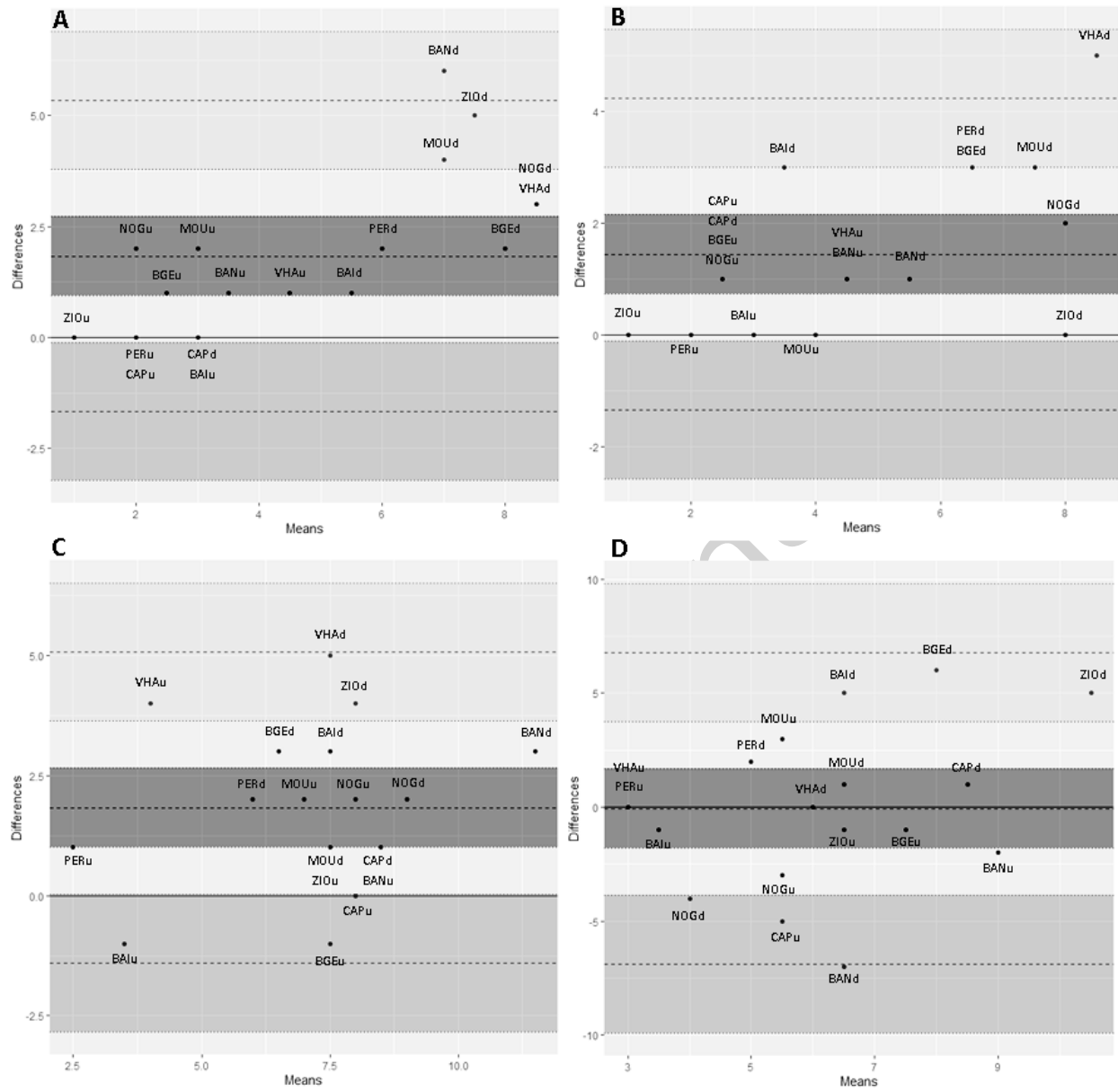


FIGURE 4:

