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Metabarcoding outperforms traditional electrofishing in decapod and fish inventories, paving the way for enhanced biodiversity monitoring in the Caribbean.

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Abstract

Environmental DNA (eDNA) metabarcoding has revolutionized the biodiversity monitoring in aquatic ecosystems, giving access to taxonomic lists in a non-disruptive way. Despite limits (taxonomic resolution nor taxa quantification), the method has repeatedly proved its effectiveness and is now used as routine freshwater monitoring tool. Especially since traditional method, including electrofishing (TEF), are reputed stressful for the species, non-selective and disruptive for the ecosystem, leading to its abandonment in Martinique (F.W.I.) for regular

monitoring. The aim of this project was to explore the possibility of using eDNA metabarcoding for the detection of fish and decapods in Martinique streams, by first validating it with TEF. We selected 14 stations, a representative panel of the river diversity, and performed TEF and eDNA to compare both, based on the species richness recovered. Then, from eDNA taxonomic inventories, we assessed the ecological state of the studied stations, using different biodiversity indices (Shannon, Simpson and Bray-Curtis) and investigated how stations abiotic characteristics shape assemblages. Here, we confirmed the eDNA metabarcoding method as a reliable tool for monitoring fish and decapods, confirming most of the taxa caught by TEF and revealing the presence of additional (native and/or invasive) species. We faced some issues in discriminating some genetically close species (e.g. Sicydium sp.) potentially leading to under-representation in community assemblages, but not in functional diversity. Additional efforts are needed to raise standardized protocols, but we encourage stakeholders to join such initiative to shed light on the rich biodiversity in sometimes poorly studied regions and to face invasions.

Keywords: Biodiversity hotspot, Biodiversity indices, Ecological assessment, Environmental DNA, Martinique island, Method validation.

1. Introduction

Freshwater ecosystems are of high importance, providing habitat for at least 6% of known species (and probably many more to discover) on <0.8% of the total Earth surface (Michelet 2017). Nevertheless, unsustainable human activity (i.e. use of pesticides, urbanization, dredging and draining, for example) has had a considerable impact on these freshwater ecosystems in recent years, with an estimated loss of 84% of the biodiversity since 1970 and almost one species of three threatened with extinction, all taxa combined (Magurran 2009, WWF 2020). These losses weaken the environment by affecting the distribution and composition of native

communities, sometimes disrupting migratory patterns and associated life cycles (Magurran 2009, Engman and Ramirez 2012). Tropical islands are particularly vulnerable, characterized by low species diversity combined to high endemism (Nivet et al. 2010) on small areas (Myers et al. 2000). For instance, Martinique is a rugged island, located in Lesser Antilles archipelago, presenting a wide variety of landscapes and terrestrial ecosystems with more than 70 permanent rivers (as well as many non-permanent ones and tropical forests wetlands), justifying its place in one the 25 hotspots of biodiversity (Anadón-Irizarry et al. 2012). These disruptions also make the ecological niche much more permeable to invaders, introduced mainly via aquaristic activities and aquaculture, one of the major factors in current biodiversity loss (Gherardi et al. 2008, Nunes et al. 2015, Rodríguez-Barreras et al. 2020). For all these reasons, there is an urgent need for conservation of these freshwater ecosystems, based on environmental protection programs (Flitcroft et al. 2019). However, these programs require a good knowledge of the environment and the distribution of native and native species, which calls for inventories with accurate species identification and assessment (endemic, rare, endangered or invasive).

Biological inventories of macro-organisms in aquatic environments were initially based on traditional methods like direct capture, electrofishing, or baited traps, known to be non-selective, time-consuming and particularly disruptive for the environment (Hänfling et al. 2016, Wang et al. 2021), making them increasingly controversial. Traditional electrofishing (TEF) for example, which is largely used in freshwater environment, uses electric currents to temporarily stun fish, making them easier to capture and study (Pusey et al. 1998). While effective, this method can be stressful or even harmful to the organisms and potentially disrupting the fragile ecosystems, harboring small populations, researchers seek to understand and protect (Snyder, 2003; Dolan & Miranda, 2004). Finally, the labor-intensive and time-consuming nature of these inventories limits the scale of their use, especially in larger or more remote areas (Hense et al.

2010, Evans et al. 2017). Despite these drawbacks, traditional methods remain invaluable for certain research aims, particularly when precise biometric data or population densities are needed (Thomsen and Willerslev 2015, Evans et al. 2017).

In recent years, freshwater inventories have undergone a revolution with the emergence of monitoring techniques based on the detection of DNA shed by organisms in the water (i.e. skin cells, mucus or feces) dubbed 'environmental DNA' (Ficetola et al. 2008). This approach offers the possibility to detect targeted species even with low population density (i.e. invasive and/or rare, endangered endemic species) without the need to observe it, at any stage of life, regardless of their size or ecology (i.e. very small or even microscopic, and sometimes cryptic, living underground or in disconnected ditches) (Ficetola et al. 2008, Thomsen and Willerslev 2015). The eDNA method rapidly gained traction in the field of biodiversity assessments, representing a promising alternative or complement to traditional methods, thanks to its low disruptiveness, ease to implement on field at large scale and its ability to detect a broad spectrum of species when using metabarcoding (Valentini et al. 2016, Pont et al. 2018, Taberlet et al. 2018). However, while eDNA metabarcoding presents significant opportunities, it is not without challenges and drawbacks. First, there is a need for robust reference databases, containing at least genetic sequences for all species within the study area, and additionally, species susceptible to be introduced (Schenekar et al. 2020, Marques et al. 2021). Such reference databases, coupled with a good knowledge on the studied environment and its native communities, are essential for comprehensive and accurate biodiversity assessments (Schenekar et al. 2020, Marques et al. 2021). But even with such complete database, a resolutive barcode is needed to discriminate taxa, the most known and used being MiFish and Teleo primers (designed respectively by Miya et al., 2015; Valentini et al., 2016) and both targeting a quite short fragment (respectively 175 bp and 65 bp) in a highly conserved genetic region (16S

98 rRNA). Moreover, eDNA methods now reached high sensitivity yields and controls samples
99 (i.e. for cross-station or lab contaminations) are therefore crucial, in addition to rigorous
100 protocols, to ensure sample integrity, optimization of species discrimination and avoidance of
101 false positives.

102

103 As eDNA methods grow in popularity regarding their operationality for biodiversity
104 assessment, they are more and more integrated into legal monitoring frameworks and decision
105 making (Morisette et al., 2021; Adams et al., 2024; Kelly et al., 2024). Validation through
106 comparisons with traditional methods is crucial to ensure the reliability of eDNA in biodiversity
107 assessments. While eDNA metabarcoding has many advantages, it should complement, not
108 replace, traditional approaches, which provide key context on species abundance, age structure,
109 and health (Evans et al. 2017). By integrating eDNA with traditional techniques, researchers
110 can achieve a more holistic understanding of ecosystems. For instance, eDNA can be used for
111 rapid initial surveys to identify species presence across large areas, while traditional methods
112 can be employed for more detailed studies in key locations (Evans et al., 2017; Baudry et al.,
113 2023). This combined approach allows for both broad-scale biodiversity assessments and in-
114 depth investigations of particular species or habitats.

115

116 In this study, we investigated the potential of eDNA metabarcoding for freshwater fish
117 and decapods monitoring in Martinique (F.W.I.), using respectively MiFish (Miya et al. 2015)
118 and MiDeca (Komai et al. 2019) primers. Indeed, such tropical freshwater ecosystems, as well
119 as their communities, are fragile and traditional surveys, like TEF, can result in high losses,
120 leading to a decrease of its use in Martinique in the recent years and so fragmented temporal
121 biodiversity data. MiFish primers have proved their reliability for fish detection in multiple

locations, recovering most of the known biodiversity at the genera or species level (Polanco et al., 2021; Macher et al., 2023). Concerning MiDeca primers, they were only used twice in the literature, for coastal biodiversity assessment in Indonesia (Madduppa et al. 2022) and freshwater inventories in Guadeloupe (F.W.I.) (Lefrancois et al., 2024, using degenerated MiDeca primers), with reliable and promising results.

2. Materials and Methods

2.1. Study area and sampling sites

Martinique is a French island of 1128 km2 belonging to Lesser Antilles (14° 39′ 00″ N and 61° 00′ 54″ W) and dominated by a rainy tropical climate, leading to a vast hydrographic network encompassing 70 main permanent rivers, fed by at least as many tributaries (Baudry et al. 2021). The presence of the Montagne Pelée volcano (1397 m height) in the Northern part of the island induces a difference in hydromorphologies, with North rivers characterized by steep slopes and strong waterflows and inversely, South rivers being larger and slow-moving.

In this study, we sampled 14 locations, spread over the territory, from the North to the South (Fig. 1), for the most accurate overview of the biodiversity in presence, probably shaped by the rivers characteristics described just before. Eight locations were selected in the Northern part (CERon, COULeuvre, CARbet, Maison ROUsse, LORrain, Fonds St-Jacques, BASsignac and GUEs) and six in the Southern part of the island (FRançois, SainT-Esprit, Petit BOUrg, LOWinsky, MADeleine and DORmante).

Each station was sampled in April 2023 by both eDNA filtration method (first, to minimize contamination risks) and TEF, following the protocols described below. At each of

the stations, the physico-chemical characteristics (pH, temperature, oxygen concentration and conductivity) (Suppl. Material S1) were measured using a Hanna® HI98129 instrument, to discuss the ecological preferences of taxa.

2.1.1. eDNA sampling

Filtration were performed on-site, before TEF as said above, through 0.45 µm nitrocellulose filters (Sartorius® 47 mm diameter), using a hand-operated vacuum pump (Nalgene™) together with a 1L-filtration unit (Nalgene™), as described in Baudry et al. (2021). Briefly, sampling occurred along a transect originating from the margin outwards, or in running parts, depending on the rivers considered, in duplicates (i.e. independent eDNA samples) for each station, until the filters clogged. They were then removed and placed (folded in quarters) into 1.5 mL tubes filled with 1 mL of absolute (99%) molecular-grade ethanol, using sterile forceps.

To avoid potential field cross-contamination, sampling material was decontaminated using 20% bleach and thoroughly rinsed using tap water after each sampling and a blank control sample (distilled water) was done. All eDNA samples were stored in a cooling bag until return to the laboratory, where they were stored at 4°C, until eDNA extraction, showing satisfactory yields if process quickly and ease of use on-field (Renshaw et al., 2015; Majaneva et al., 2018).

2.1.2. Traditional Electro-Fishing (TEF) sampling

The chosen protocol was adapted from Lefrancois et al. (2024), using a Smith-Root LR-24 backpack electrofisher, set on 250V (direct current) or 500V (pulsed current) (10 to 16 Hz and 15 to 25 A, respectively), depending on conductivity and hydrological characteristics of the

stations. Briefly, with reasonable human effort (3 or 4 operators), the objective was to detect the maximal fish and crustacean species richness, following past observations (Lim et al., 2002; Baudry et al., 2024), with a minimal habitat disturbance. Each station was surveyed using spot-fishing over an area of around 10 m2 (around 15 seconds per spot), moving upstream for approximately fifty meters. If all the species known to be present at the station (based on historical data) were not captured, additional spot-fishing was carried out, targeting certain micro-habitats that were favourable to certain species (for example, stumps and banks for eels). Once caught, the specimens (fish and crustaceans) were directly taken back to the riverbank to be identified, sorted and sampled. These operations must be carried out very efficiently, as the water in the tanks heats up quickly in such tropical climate, which can lead to significant losses. As the aim here was to verify the species presence (not to quantify), we have therefore chosen to handle the individuals as little as possible, taking no biometric measurements (except for taxa of interest such as the American eel - *Anguilla rostrata*). Individuals were therefore identified visually, down to species level (or genus level for juvenile individuals). A maximum of 5 individuals per species and per station were non-destructively sampled, by taking mucus or a fin fragment from fish, or by taking a P4 leg fragment from crustaceans. This was intended to, first, confirm the species identification and then, contribute to complete the genetic database (see sections below). The samples were then referenced (station, species and date) and preserved in absolute (99%) molecular-grade ethanol, in 1.5 mL tubes, until the DNA was extracted in the laboratory.

2.2. Lab analysis

2.2.1. (e)DNA extraction

DNA (and eDNA) extractions were performed in dedicated rooms, different from that used for PCRs preparations, with benches, tools and surfaces bleach-disinfected before processing samples. From tissue, DNA was extracted using Qiagen DNeasy Blood & Tissue Kit, following manufacturers guidelines. Concerning the extraction from filters, some minor modifications were applied, following Baudry et al. (2021): ¼ of each filter was cut into small pieces, using sterilized forceps and scissors and dried for thirty minutes (to evaporate the ethanol), into a 2 mL Eppendorf tube. Lysis reagents (450 µL of ATL buffer and 50 µL Proteinase K) were added, submerging the filter fragment, and then vortexed before incubation at 56°C for 3 hours. Following steps (washing) were done as described by manufacturer, until the elution, in 60 µL of AE buffer (instead of 200 µL), to concentrate the eDNA.

For both DNA and eDNA, the extraction yields were measured (concentration and absorbance ratios) using the Implen® N60/N50 nanophotometer (Implen GmbH, Munchen, Germany).

2.2.2. Sanger sequencing

First, to ensure the species identity of fish and crustaceans caught, the COI gene (Cytochrome C Oxydase subunit I) was sequenced, using universal primers designed by Ward et al. (2005) (FishF1-TCAACCAACCACAAAGACATTGGCAC, FishF2-TCGACTAATCATAAAGATATCGGCAC and FishR1-TAGACTTCTGGGTGGCCAAAGAATCA, FishR2-5′ACTTCAGGGTGACCGAAGAATCAGAA) and Folmer et al. (1994) (LCO1490-GGTCAACAAATCATAAAGATATTGG and HCO2198-TGATTTTTTGGTCACCCTGAAGTTTA). For each individual, Polymerase Chain Reaction (PCR) was performed following Chucholl et al. (2015) with minor modifications: 2.5 min at 95°C for initial denaturing, followed by 35 cycles of 45 sec at 95°C, 1 min at 50°C and 1 min

at 72°C, and finally 10 min at 72°C for final elongation. PCR products were purified and 1/10 diluted before sequencing, in both forward and reverse, on an Applied Biosystems SeqStudio Genetic Analyzer (Waltham, U.S.A.).

Once species identity verified, DNA extracts were used to complete the genetic database for both fish (12S rRNA) and crustaceans (16S rRNA). The protocol used was the same as described before, with metabarcoding primers MiFish (Miya et al. 2015) and MiDeca (Komai et al. 2019) (see just below).

2.2.3. Metabarcoding amplifications

As said, eDNA amplification were performed using MiFish-U-F-GTCGGTAAAACTCGTGCCAGC and MiFish-U-R-CATAGTGGGGTATCTAATCCCAGTTTG primers targeting a 175 bp fragment of the mitochondrial 12S rRNA gene (for fish, Miya et al., 2015) and MiDeca-F-GGACGATAAGACCCTATAAA and MiDeca-R-ACGCTGTTATCCCTAAAGT primers targeting a 164 bp fragment of the mitochondrial 16S rRNA gene (for crustaceans, Komai et al., 2019). MiFish primers were selected based on their applicability and resolution for assessing fish communities (Polanco et al. 2021, Shu et al. 2021), and recently used in another French tropical island (Lagarde et al. 2021). Concerning crustaceans, MiDeca primers are the only ones designed for this taxon, recently published and only used twice in the literature (in marine environment Madduppa et al. (2022); in freshwater ecosystems Lefrancois et al. 2024).

PCR reactions were set up in a sterile room, decontaminated every night by UV-light treatment. Each eDNA was amplified four times, representing eight PCR reactions per station. PCR reactions were carried out in a 25 µL final volume containing: 12.5µL of KAPA HiFi

HotStart ReadyMix (Roche), 5 µL of each primer with index (final concentration 0.2 µM) and 2.5 µL of template. Each PCR plate contained one negative control (i.e. no-template DNA), to assess for potential contamination during the amplification and three positive mock controls. These mocks were constituted with DNA extracted from individuals (Suppl. Material S2), spiked in equimolarity (10 ng.µL^{-1}), in dominance of certain species (15 ng.µL^{-1} for dominant vs. 5 ng.µL^{-1}) and then, inversing these dominances (Suppl. Material S2). Mock samples serve as positive control and once sequenced, allow to calibrate the genetic database with high accuracy.

Amplifications programs were: activation at 95°C for 3 min followed by 35 cycles of 98°C for 30 sec, 65°C (60°C for MiDeca) for 30 sec and 72°C for 30 sec, and finally 72°C for 5 min, for final extension. PCR products were visualized on 1.5% agarose gels and then sent to PGTB sequencing platform in Bordeaux (France) for quality check (using TapeStation, Agilent, USA) and sequencing on Illumina NextSeq 2000 (U.S.A.), using 2 x 150 pb kit.

2.3. Bioinformatics and data analyses

2.3.1. Genetic reference database

First, sequences obtained from Sanger sequencing (12S rRNA and 16S rRNA) were cleaned and trimmed using Geneious Pro R10 software (https://www.geneious.com; Kearse et al., 2012). They were added to MIDORI2, a newly curated database for eukaryotic taxonomic assignments (Leray et al. 2022), giving a complete database for fish and crustaceans assignments in Martinique (Lim et al., 2002; Baudry et al., 2024). Within this database, an additional curation was done to remove sequences containing ambiguities (N with *maxambig = 0*) or of poor quality (length – *minlength = 150* - or homopolymer – *maxhomop = 10*). The

mocks were finally used to verify the reliability of the curated reference database and to determine optimal parameters for further field analyses (see below).

2.3.2. Illumina sequencing handling

Reads generated by Illumina NextSeq 2000 sequencing were handled using DADA2 package (v1.30.0; Callahan et al., 2016) implemented in R (v4.3.2; R Development Core Team, 2023). Primers were removed, reads with Ns were pre-filtered and quality profiles were inspected. Considering these profiles, especially the quality score in end sequenced reads, and expected lengths, reads were filtered and trimmed. They were then dereplicated and pair-ended merged, using the error model implemented in DADA2. Finally, chimera were removed from the final sequence table and taxonomic assignments (using the curated database) were done using mothur (Schloss et al. 2009) with RDP classifier using the *classify.seqs()* command with *method=wang*, *iters=1000* and *cutoff=75* parameters.

To limit the interpretation of low abundant erroneous DNA reads related to potential contaminants, PCR amplification or sequencing errors, we added additional filtering steps. Amplicons Sequences Variants (ASV) produced by DADA2 pipeline represented by < 10 reads in a sample were removed from the analyses. Finally, ASVs were converted in relative abundance and those representing < 0.1% of the total number of reads for a sample were not accounted in further analyses.

2.3.3. Data analyses

All statistical and graphical analyses were performed in the R environment (v4.3.2; R Development Core Team, 2023). Before each statistical treatment, when appropriate, data

normality and variance homogeneity were verified using Shapiro-Wilk and Bartlett tests, respectively. Maps were generated using QGIS 2.18 (Las Palmas) software (QGIS Team Development 2016): Martinique map was imported from the database ©IGN and the streams from BD Carthage® and BD Topo®.

We first analyzed stations characteristics and searched for correlations between physico-chemical parameters (altitude, conductivity, pH, temperature, oxygen concentration) depending on the geographical situation of the considered station, based on a principal component analysis (PCA) (FactoMineR and factoextra packages; Lê et al., 2008; Kassambara & Mundt, 2020). Contribution of each variable was visualized using *fviz_contrib()* and *fviz_pca_var()* functions and they were then projected on the factorial axes using ggplot2 (Wickham 2016).

For eDNA, after taxonomic assignment of ASVs, fish and decapods taxonomic lists were produced and taxa sorted according to their known occurrence in freshwater or marine environments. For example, *Caranx sp.* resulting from the human consumption was removed from the dataset here for species richness calculations. Then, the choice was done to pool all *Loricariidae sp.* together as they are extremely closed species, all sold for aquaria purposes (and so, invasive), sometimes without the ability to morphologically distinguish themselves. The influence of the method (TEF *vs.* eDNA) on those results (species richness) was analyzed based on an analysis of variance (ANOVA), considering a station effect. Species richness was then plotted for each station to visualize those assemblage differences individually.

From eDNA data, Simpson and Shannon indices were calculated using phyloseq package (McMurdie and Holmes 2013) and results were visualized averaged (using *geom_boxplot*) and then individually (*geom_bar*) for each station (see also Suppl. Material S1). The influence of station characteristics (altitude, conductivity, pH, temperature, oxygen concentration and North/South exposition) on these indices (species richness, Simpson and

309 Shannon) calculated from eDNA data was investigated for both fish and decapods, using a
310 linear model.

311 Finally, dissimilarity between station was tested, independently for fish and decapods,
312 based on relative abundance data measured by eDNA, using Bray-Curtis index implemented in
313 phyloseq and ape packages (Paradis and Schliep 2019). The correlation between assemblages'
314 dissimilarity and geographical distances (Suppl. Material S3) between each station was tested
315 with a Mantel's test together with a Spearman's rank correlation test, using vegan (Oksanen et
316 al. 2022) and geosphere (Hijmans 2022) packages.

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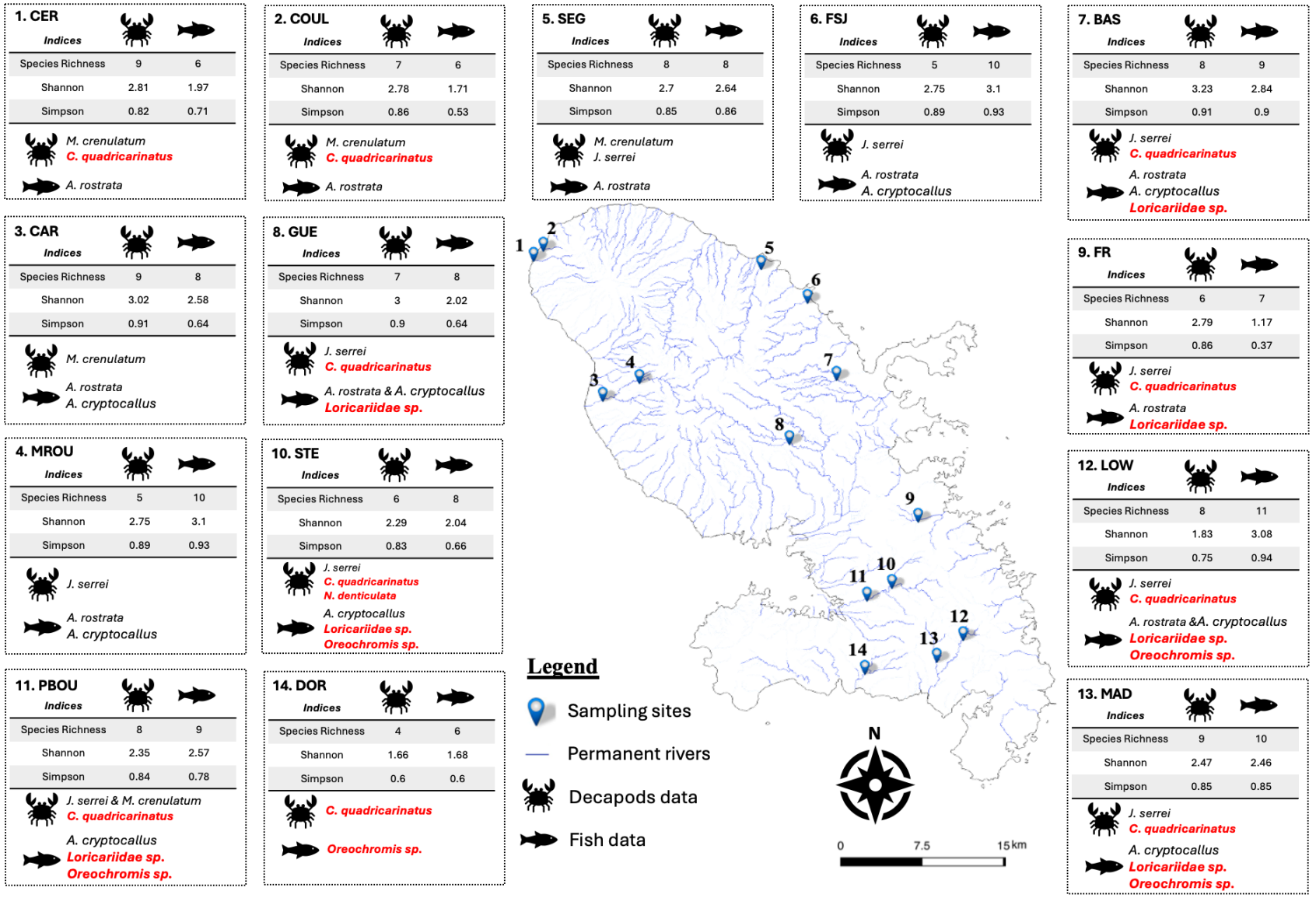

Fig 1. *The hydrological network of Martinique, in Lesser Antilles, with the location of the 14 stations sampled during this study. For each station, the indices results (Species richness, Shannon and Simpson) were given for both decapods and fish and the species of interest (endemic and invasive, the latter ones in red) were highlighted.*

3. Results

3.1. Environmental characterization of sampling sites

Conductivity and temperature were the most influential variables, contributing respectively 30.79% and 28.61% to the variation explained by the axis 1 (Fig 2A). Inversely, pH played a major role (65.48%) in shaping the axis 2 (Fig 2A). As expected, temperature and conductivity were negatively correlated with altitude, and oxygen concentration is positively correlated with altitude (Fig 2B). All stations seemed to exhibit variable pH values, but the North cluster was mainly characterized by lower temperatures and conductivity than the cluster of stations from the South (Fig 2C).

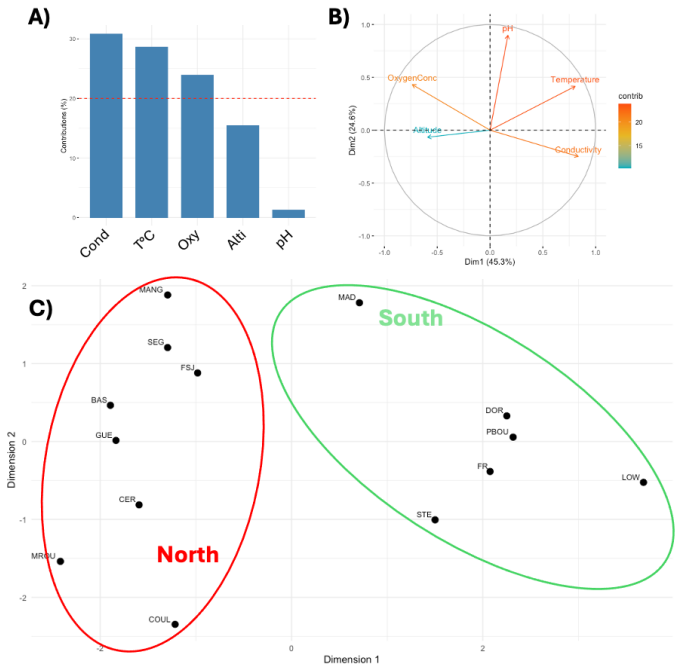

Fig 2. *Principal component analysis (PCA) on physico-chemical parameters measured (conductivity, temperature, oxygen concentration, altitude and pH) within the 14 stations studied, with (A) The contribution of each parameter to the axis 1, (B) The first factorial plane, with 45.3% of variation on the axis 1 and (C) The projection of the station, related to their exposition, in the Northern or Southern part of Martinique.*

348

349 *3.2. Bioinformatics and dataset clean-up*

350 In total, for the 14 stations studied (without the mocks), 11,656,029 reads were generated
351 for fish (mean 832,573.5 ± 142,312.1 per station) and 14,813,701 reads for decapods (mean
352 1,058,121.5 ± 408,818.2 per station). After data filtering, taxonomic assignment and curation,
353 average sample read counts per station was 570,082.9 ± 124,442.6 for fish and 963,464.9 ±
354 388726.8 for decapods (Suppl. Material S4).

355

356 *3.3. Comparison of TEF vs. eDNA*

357 Considering all stations, the eDNA method detected significantly more fish species than
358 TEF (7.93 ± 1.94 vs. 4.28 ± 1.68 using TEF; $F = 53.41$, $p < 0.001$) (Fig 3A) and more decapod
359 species (6.93 ± 1.68 vs. 5.36 ± 1.28 using TEF; $F = 6.47$, $p < 0.05$) (Fig 3B).

360 This trend was confirmed when analyzing each station separately, with for example nine
361 fish species detected by eDNA against four by TEF at BAS station, or seven species detected
362 by eDNA at FR station against only one by TEF (Fig 3C). TEF reached comparable results at
363 MROU station, with four fish species reported with each method (Fig 3C). Most importantly,
364 in a validation context, eDNA succeeded in detecting all fish species caught by TEF, so
365 sometimes reporting new species presence, not observed on field (Fig 3C). Just noted the
366 inability of eDNA to discriminate *Sicydium sp.* species (*S. plumieri* and *S. punctatum*), unlike
367 TEF.

368 For decapods detection, the results were not as clear-cut, with 10 stations (BAS, CER,
369 COUL, GUE, LOW, MAD, MANG, PBOU, SEG and STE) reporting higher species richness
370 when using eDNA (Fig 3D). TEF reached similar yields (compared to eDNA) at one station

(FR) and outperformed eDNA at three stations (DOR, FSJ and MROU) (Fig 3D). TEF appeared to be more effective in detecting species such as *Xiphocaris elongata* (absent in eDNA at DOR, FR, FSJ, MROU and PBOU) and *Macrobrachium heterochirus* (absent in eDNA at BAS, DOR, FR, GUE and PBOU) (Fig 3D). Inversely, eDNA was more effective in detecting certain hard-to-find species, such as *Jonga serrei* or *Neocaridina denticulata*, or highly invasive species *Cherax quadricarinatus* at station COUL (where it was unknown until now; Baudry et al., 2021) (Fig 3D).

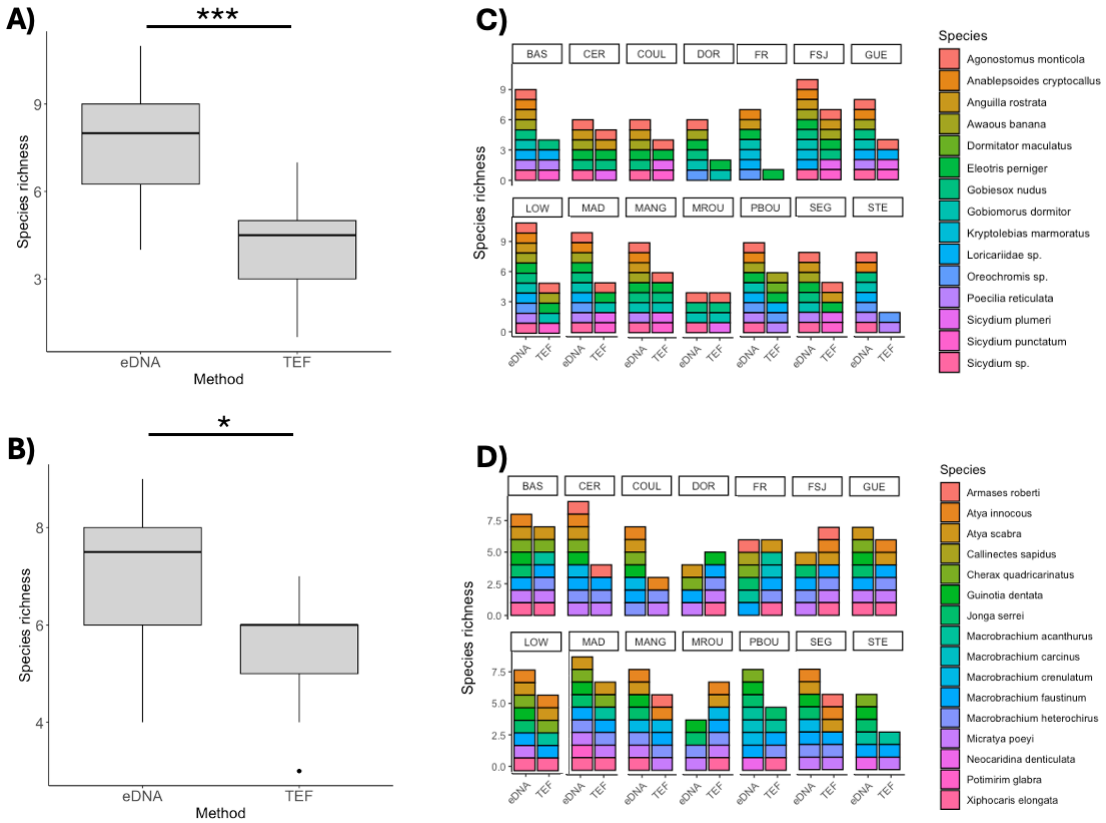

Fig 3. *Comparison of species richness between the traditional electrofishing (TEF) and the eDNA methods, all stations combined for fish (A) and decapods (B) and then independently for each of the 14 stations studied, highlighting the species detected by both methodologies for fish (C) and decapods (D). * < 0.05 and *** < 0.001*

384

385 *3.4. Biodiversity assessment using eDNA*

386 For fish, the Shannon diversity index mean value reached 2.29 ± 0.56. It ranged from
387 1.17 (FR) to 3.09 and 3.1 (respectively LOW and FSJ), indicating a greatest species diversity
388 for these latest ones (Fig 4A). The Simpson index mean value was 0.75 ± 0.17 and it seemed to
389 be linked with the Shannon index, with FSJ and LOW reaching the highest values (0.93 and
390 0.94 respectively) and highlighting them as the most evenly distributed species diversity (Fig
391 4B). Inversely, FR reported the lowest Simpson value (0.37), likely linked to the dominance of
392 certain fish species (Fig 4B). The Figure 4C provides additional information, confirming certain
393 trends: FSJ, LOW and BAS station (reporting the highest diversity scores) showed a wide
394 variety of species, with a relatively even distribution, while other stations such as GUE, CER,
395 DOR and COUL or STE, PBOU and FR, all characterized by lower diversity scores, were
396 respectively dominated by *Agonostomus monticola* or the invasive *Oreochromis sp.* species.
397 Finally, the Bray-Curtis dendrogram highlighted a pattern in four main clusters: a highly diverse
398 one (composed by FSJ, BAS and LOW), a dominance by *A. monticola* (with GUE, CER, DOR,
399 COUL and MROU), a co-dominance by *A. monticola* and *Sicydium sp.* (SEG, MAD, MANG)
400 and a dominance by the invasive *Oreochromis sp.* (STE, PBOU and FR) (Fig 4C). Interestingly,
401 these Bray-Curtis dissimilarities for fish assemblages were significatively correlated with the
402 geographical distances between stations (Mantel statistic R: 0.31; p = 0.02).

403

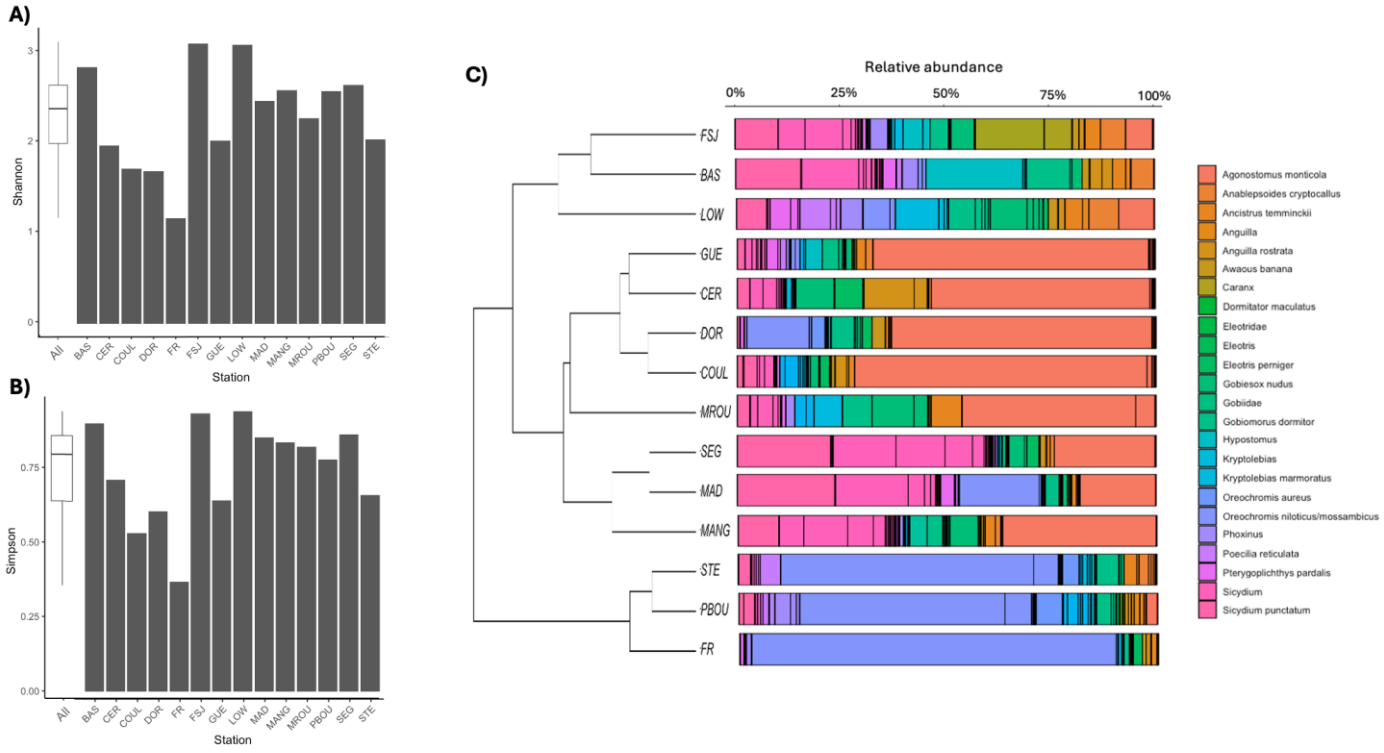

Fig 4. *Fish biodiversity assessment reported for each of the 14 stations studied (BAS, CER, COUL, DOR, FR, FSJ, GUE, LOW, MAD, MANG, MROU, PBOU, SEG and STE) using A) the Shannon index, B) the Simpson index, both calculated from the C) relative abundance of reads, clustered based on the Bray-Curtis dissimilarity index.*

Concerning the decapods, the Shannon diversity index mean value was slightly higher, reaching 2.57 ± 0.45, and ranged from 1.66 (DOR) to 3.23 (BAS) (Fig 5A). As for the fish assemblages, the Simpson index values (mean 0.83 ± 0.08) seemed to be linked with the Shannon index, with BAS reaching the highest values (0.91), closely followed by MANG and GUE stations (both 0.9) (Fig 5B). Inversely, DOR showed the lowest Simpson value (0.6) (Fig 5B). The Bray-Curtis dendrogram for decapods here exhibited a pattern in three main clusters: a dominance by *Macrobrachium sp.* (with FR and DOR), a co-dominance by *Macrobrachium sp.* and *Atya sp.* species (MAD, LOW, GUE and BAS with a noted presence of the invasive *Cherax quadricarinatus* in these stations, and SEG, MANG, COUL, CER, MROU and FSJ),

and a dominance by the invasive *C. quadricarinatus* in STE and PBOU (Fig 5C). These Bray-Curtis dissimilarities values, using decapods assemblages, were also significatively correlated with the geographical distances between stations (Mantel statistic R: 0.52; $p < 0.01$).

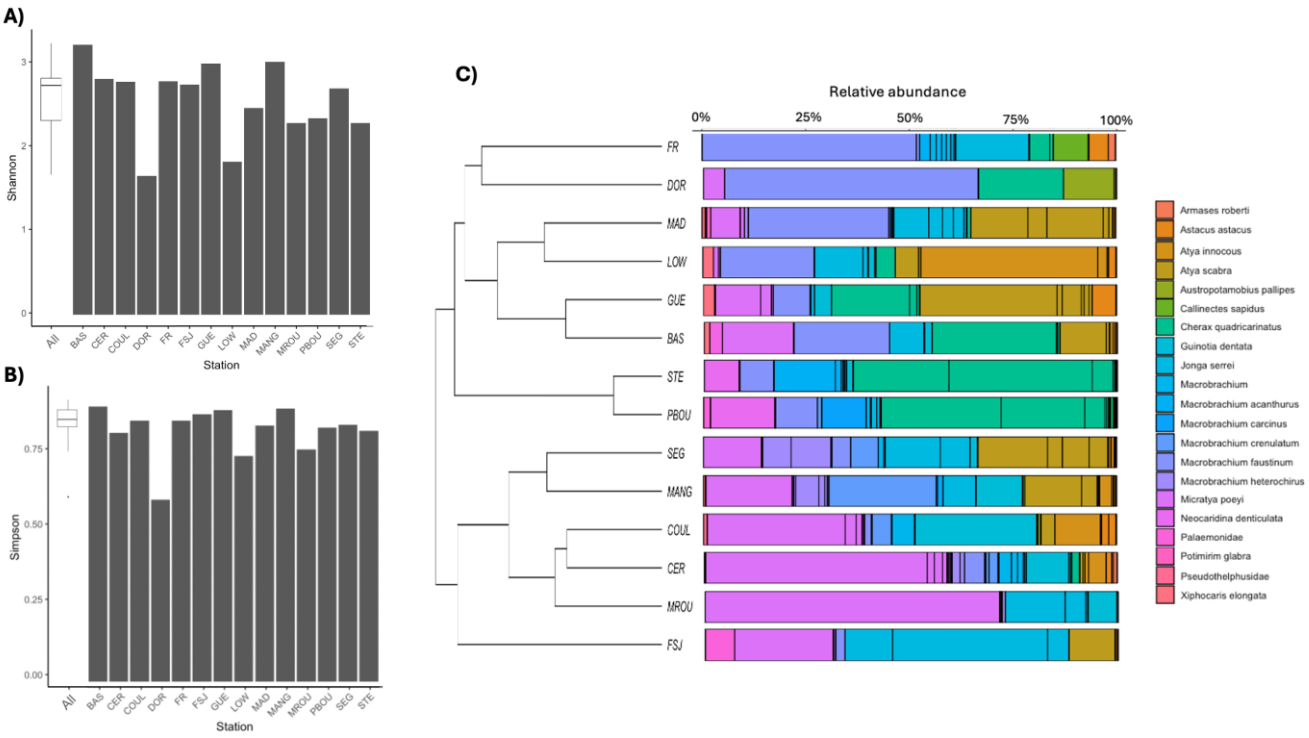

Fig 5. *Decapods biodiversity assessment reported for each of the 14 stations studied (BAS, CER, COUL, DOR, FR, FSJ, GUE, LOW, MAD, MANG, MROU, PBOU, SEG and STE) using A) the Shannon index, B) the Simpson index, both calculated from the C) relative abundance of reads, clustered based on the Bray-Curtis dissimilarity index.*

Finally, the effect of physico-chemical variables on the biodiversity indices was limited, with only the temperature playing a significatively (or closely) positive role for fish assemblages (species richness: $t = 2.13$, $p = 0.08$; Shannon: $t = 2.68$, $p = 0.036$ and Simpson: $t = 2.41$, $p = 0.05$). Only the conductivity seemed to negatively affect the Shannon ($t = -3.03$, $p = 0.02$) and Simpson ($t = -3.71$, $p < 0.01$) indices for decapods.

433

434 **4. Discussion**

435 In this study, we evaluated the operationality of the eDNA metabarcoding approach as
436 a reliable tool to monitor fish and decapods in Martinique, located in the very little studied
437 F.W.I. region in terms of eDNA. Indeed, this study represents the second of its kind in this
438 region, after Lefrancois et al. (2024) in Guadeloupe, and we showed very promising results in
439 the view to implement this metabarcoding approach as a regular biomonitoring tool. We first
440 compared the eDNA-based method and the traditional one (TEF), highlighting a higher number
441 of species detected when using the first one, and a confirmation of most of the species already
442 detected by TEF, especially for fish. However, the eDNA metabarcoding seemed to encounter
443 some issues when it comes to discriminate genetically close species (such as *Sicydium sp.* or
444 *Macrobrachium* species), possibly leading to a biased representation of some of them within
445 the assemblage. Finally, we calculated biodiversity indices, from these eDNA results, to
446 investigate how the stations and their characteristics shaped the fish and decapods assemblages
447 through the Martinique territory. Below, we discuss these two points separately, and we
448 conclude by future implications to progress on some issues and on the potential of eDNA
449 metabarcoding for regular biomonitoring.

450

451 *4.1. Validation of eDNA metabarcoding*

452 The eDNA metabarcoding for fish detection has been now largely used, representing a
453 major part of the studies led in the field of water biomonitoring (Belle et al. 2019) and giving
454 rise to many different protocols and assay testings (Miya et al., 2015; Valentini et al., 2016;
455 Macher et al., 2023). Most, if not all, of these studies highlighted a better species detection
456 when using eDNA metabarcoding for biodiversity assessments, compared to traditional

457 methods, validating it as a reliable tool for biodiversity monitoring of fish biodiversity (see for
458 instance Pont et al. (2021) for large-scale and temporal study and Lefrancois et al. (2024) for a
459 local methodological validation). Our results concorded with those ones, with almost twice the
460 number of species detected with eDNA (7.93 ± 1.94 species vs. 4.28 ± 1.68 by TEF) and most
461 importantly in this validation context, a high coherence between the species caught with TEF
462 and the ones detected by eDNA. The only downside here was the inability of the eDNA
463 methodology to confidently discriminate the two *Sicydium* species (S. punctatum and *S.*
464 *plumieri*). Indeed, despite many efforts to refine the database, the genetic similarity between
465 these two species and possibly with the closely related species *S. altum* (present in Costa Rica)
466 seems to be such that taxonomic assignment to a specific determination seems impossible. The
467 case of hybridization between these two very close species cannot be ruled out either, in which
468 case metabarcoding is known to be unable to discriminate hybrids from parental species (Di
469 Muri et al. 2020). That said, this issue does not represent a huge problem, as these two species
470 have similar morphological characteristics and ecological functions (Lim et al. 2002), so the
471 presence of one or the other makes little difference to the ecological state of the environment
472 concerned. Finally, eDNA metabarcoding methodology appeared to have a considerable
473 contribution when it comes to the early detection of invasive species, such as *Oreochromis*
474 species or several *Loricariidae* species, initially with *Hypostomus robinii* known to be the only
475 species in Martinique (Dubreuil et al. 2021) (more details below).

476

477 Even if decapods are very important species within ecosystems, their study through
478 eDNA assays was less widespread in the literature (Belle et al. 2019). This trend is even more
479 exacerbated with metabarcoding, for which the recently developed MiDeca primers, developed
480 to be the most reliable for decapods detection (Komai et al. 2019), have only been used twice
481 to date (Madduppa et al., 2022; Lefrancois et al., 2024). Both studies showed a high reliability

of the eDNA method, detecting most of the species already recorded for Indonesia in SeaLifeBase (www.sealifebase.org) and WorMS (Ahyong et al. 2024) databases (Madduppa et al. 2022) or previously caught by TEF in Guadeloupean freshwaters, outperforming the traditional method in some stations (Lefrancois et al. 2024). Martinique and Guadeloupe, distant only from hundreds of kilometers, present very similar ecosystems and species assemblages and interestingly we reached comparable and reliable results, facing the same issues for the decapods detection: a better detection by TEF for *X. elongata* but not for some hard-to-find species such as *J. serrei* or *N. denticulata* (Lefrancois et al. 2024). Firstly, this complexity in detecting *X. elongata* can be explain by lower shedding rates reported in a morphologically and genetically close species (the glass shrimp), estimated three times lower than those of fish for example (see Allan et al., 2021 and references therein). Even if such finding can be applied to *N. denticulata*, this latter (being invasive) was found in very high densities on field when present, offsetting this low eDNA shedding effect. Finally, the high morphological similarities between species from the same genera or family can led to confusion during visual taxonomy assignments, for example in *Macrobrachium sp.* young adults, presenting underdeveloped rostrum or chelae – used for species determination (Lim et al. 2002). Such misidentification can also be encountered with *Atyidae* species, which include the natives *Atya innocous, A. scabra, Micratya poeyi, Potimirim sp.* and *J. serrei* but also the introduced *N. denticulata*, all characterized by a reduced size (around 20 to 40 mm, except largest *Atya sp.* males, reaching up to 100 mm) and an almost transparent colour (Lim et al. 2002).

4.2. eDNA biodiversity assessment in Martinique

The eDNA metabarcoding method enabled to assess the ecological state of the stations studied, in a non-disruptive way, by calculating biodiversity indices from relative abundance data. Here, based on fish and decapod inventories, certain trends were highlighted: BAS, MAD,

LOW and FSJ stations are those most often found with the highest indices, and therefore seemed to present good ecological states. Inversely, MROU, FR and DOR are those with the poorest ecological states. These indices were found to be positively correlated with temperature for fish and negatively correlated with conductivity for decapods. As these two factors are inversely related to altitude, the best habitats were indicated to be located upstream for crustaceans and downstream for fish. This second observation seems somewhat surprising, given that it is well known that in Martinique, the downstream zones are the most anthropized, subject to modifications such as the creation of bridges (Baudry 2022), and this could be explained in two ways. Firstly, the eDNA method relies on the detection of a signal coming from upstream, sometimes as far as several hundred meters or even kilometers (Deiner & Altermatt, 2014; Baudry et al., 2023), even though these two studies cited rely on single-target qPCR recognized as being more sensitive than metabarcoding (Bylemans et al. 2019). The second, more plausible, would be the amphidromic reproductive characteristic of most of decapods and fish species in Martinique (Lim et al. 2002) and the preponderance of these downstream areas to be subject to biological invasions, a well-known and increasingly frequent phenomenon in islands (Li et al. 2024). In a recent introduction context, and therefore still reduced impacts on biodiversity (Strayer et al. 2017), this could contribute to increasing biodiversity indices and skewing the principle of good ecosystem state. However, long-term effects of invasive species are well known, inducing a homogenization of biodiversity at local level, especially in such fragile tropical island ecosystems (Rodríguez-Barreras et al. 2020), and FR, STE and PBOU stations dominated by the invasive tilapia (*Oreochromis sp.*) are a “good” example. This phenomenon of biological invasion should therefore be considered in such kind of analysis, while having a keen eye on the environment being studied. As an example, the MROU station seems to have quite bad results in terms of ecological state even though it represents one of the most preserved stations in Martinique, free of invasive species to date, and very little (if not at

all) polluted. It only suffers from a very particular, rugged ecosystem adapted to very few even native species (Lim et al. 2002). Finally, these biodiversity indices, particularly Simpson's, are based on eDNA emitted by species with variable physiological characteristics and therefore variable eDNA shedding rates (Allan et al. 2021), partially biasing these calculations.

Interestingly, Bray-Curtis dissimilarity index and Mantel test highlighted a clustering of station, for both fish and decapods, shaped alongside a geographical gradient. This observation particularly fitted with what is known about the Martinique territory, a northern part characterized by a very hilly landscape resulting in a torrential hydrographic network, and conversely a less rugged southern part, presenting wider and calm rivers (Baudry 2022). All this very probably plays a role in structuring communities and we can notice, despite the fact that certain species are very widespread throughout the territory (e.g. *A. monticola* or *Sicydium sp.* for fish and *M. faustinum* or *M. poeyi* among decapods), some others particularly favour certain areas: the *Atya sp.* rather the torrential zones of the North of Martinique, just like *Anguilla rostrata*, fitting with our field observations (TEF) and other studies carried out in the territory (Lim et al., 2002; Authors' unpublished data on eDNA for eels). Finally, the eDNA metabarcoding has proven to be highly valuable for the detection of rare and/or endemic species but also for the new detection or confirmation of certain invasive species. Thus, species such as *J. serrei*, reputed hard to observe due to its small size, or even *M. carcinus*, in decline in the territory, could be detected for decapods. It also confirmed the presence of the only endemic freshwater fish species of Martinique, *Anablepsoides cryptocallus*, on the GUE, BAS, STE, PBOU, STJ and FR stations (Baudry et al. 2023), but also to open up perspectives to its presence on additional stations (MAD and LOW). Interestingly, the *Kryptolebias marmoratus* cryptic species, living hidden in the mud (Taylor 2012), only known on a single station in Martinique (Baudry, Pers. Obs.), was detected here on two new stations (FR and FSJ) presenting plausible

557 characteristics for its habitat (i.e. water with high conductivity and direct proximity to the
558 mangroves). More worryingly, this study made it possible to expand the invasion zone of the
559 well-known *Cherax quadricarinatus* (COUL) (Baudry et al. 2021) or to potentially highlight
560 the occurrence of several invasive species of *Loricariidae sp.* on the territory instead of just
561 one, *H. robinii* (Dubreuil et al. 2021).

562

563 *4.3. Conclusion and implications for the future*

564 Here, we confirmed the eDNA metabarcoding approach as a reliable tool for monitoring
565 fish and decapods in Martinique, in Caribbean, often considered as an understudied region. The
566 present study, second of its kind after Lefrancois et al. (2024) in Guadeloupe, showed very
567 promising results for the implementation of this molecular method in regular biomonitoring
568 programs, confirming most of the species caught by TEF and revealing the presence of
569 additional (native or sometimes more worryingly invasive) species. This metabarcoding method
570 showed limitations in discriminating some genetically close species (e.g. *Sicydium sp.*),
571 potentially leading to under-representation of communities' assemblages when it comes to
572 calculate biodiversity indices, but not affecting the functional diversity in presence. That said,
573 such metabarcoding data allowed to appreciate the ecological state of the stations studied, in a
574 non-disruptive way, using different biodiversity indices (species richness, Shannon, Simpson
575 and Bray-Curtis), and to investigate how stations characteristics shape assemblages across
576 Martinique. Finally, even if eDNA metabarcoding offers the ability to early detect invasive
577 species or the discovery of new suitable areas for endemic and/or rare native species, traditional
578 methods, such as TEF, remains indispensable for certain aims, for example genetic studies, and
579 both methodologies could be used in a complementary way. Harmonization of eDNA protocols
580 is crucial to maximize the effectiveness of biodiversity studies and we encourage stakeholders

581 to join such initiative to shed light on the rich biodiversity occurring in poorly studied regions
582 and to facilitate the fight against invasive species, one of the leading causes of biodiversity loss.

583

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594

595 **CrediT authorship contribution statement**

596 **TB:** Conceptualization, Methodology, Data curation, Formal analysis, Investigation, Software,
597 Validation, Visualization, Funding acquisition, Writing – original draft, Writing – review &
598 editing.

599 **VV:** Validation, Software, Supervision, Writing – review & editing.

600 **CD:** Investigation, Validation, Writing – review & editing.

601 **AA:** Funding acquisition, Administration, Writing – review & editing.

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605 **FG:** Conceptualization, Validation, Supervision, Funding acquisition, Administration, Writing
606 – review & editing.

607

608 **Declaration of competing interest**

609 The authors declare that they have no known competing financial interests or personal
610 relationships that could have appeared to influence the work reported in this paper.

611

612 **Data availability**

613 All data generated or analyzed during this study are included in this published article
614 (and its supplementary information files) and additional information and data are available from
615 the corresponding author upon reasonable request. Fastq.gz data are accessible in Zenodo
616 repository (doi: *in process*)

617

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