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Enhancing neotropical fish monitoring using dietary DNA of detrivorous natural samplers

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Abstract

As neotropical freshwater fish face alarming biodiversity loss, there is an urgent need for more efficient and accurate biomonitoring tools that require less taxonomic expertise than traditional methods. While the analysis of water or sediment environmental DNA (eDNA) has rapidly gained popularity, a growing body of research is investigating 'natural samplers' - living organisms that aggregate eDNA through their feeding behavior - as tools for biomonitoring. Here, we investigated whether abundant and widely distributed freshwater shrimp could provide a reliable snapshot of local fish assemblages in large neotropical rivers. Multi-marker metabarcoding analysis of shrimp dietary DNA revealed as many species as an intensive 10-day inventory of the study area and nearly three times more species than gillnet-based methods commonly used in surveillance programs. The generalist and opportunistic feeding behaviour of these detritivorous organisms allow for the detection of a broad spectrum of species in terms of size, including small fish overlooked by traditional gillnet-based surveys. Furthermore, most fish taxa were identified at the species level thanks to the availability of nearly exhaustive barcoding reference databases. As the cost and speed of molecular analyses continue to decrease, the relative ease of sampling and processing makes this method particularly suitable for carrying out rapid biodiversity assessments and detecting the localized ecosystem impacts of anthropogenic disturbances, complementing observational approaches that provide data on fish abundance, biomass, or condition.

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Abstract (225 words)

As neotropical freshwater fish face alarming biodiversity loss, there is an urgent need for more efficient and accurate biomonitoring tools that require less taxonomic expertise than traditional methods. While the analysis of water or sediment environmental DNA (eDNA) has rapidly gained popularity, a growing body of research is investigating 'natural samplers' - living organisms that aggregate eDNA through their feeding behavior - as tools for biomonitoring. Here, we investigated whether abundant and widely distributed freshwater shrimp could provide a reliable snapshot of local fish assemblages in large neotropical rivers. Multi-marker metabarcoding analysis of shrimp dietary DNA revealed as many species as an intensive 10-day inventory of the study area and nearly three times more species than gillnet-based methods commonly used in surveillance programs. The generalist and opportunistic feeding behaviour of these detritivorous organisms allow for the detection of a broad spectrum of species in terms of size, including small fish overlooked by traditional gillnet-based surveys. Furthermore, most fish taxa were identified at the species level thanks to the availability of nearly exhaustive barcoding reference databases. As the cost and speed of molecular analyses continue to decrease, the relative ease of sampling and processing makes this method particularly suitable for carrying out rapid biodiversity assessments and detecting the localized ecosystem impacts of anthropogenic disturbances, complementing observational approaches that provide data on fish abundance, biomass, or condition.

Introduction

Tropical freshwater ecosystems harbour the most taxonomically diverse fish communities on the planet (Pelicice et al., 2017), which fulfill a wide range of critical functions such as nutrient recycling and seed dispersal (Lévêque et al., 2008; Toussaint et al., 2016; Vitule et al., 2017), while providing essential services like food provision and water quality regulation (Collen et al., 2014; Hoeinghaus et al., 2009; Longin et al., 2021; Pelicice et al., 2023). Despite their great ecological and societal value, neotropical freshwater fish are experiencing alarming biodiversity loss over recent decades due to multiple threats induced by human activities, including deforestation, flow regulation, overexploitation, non-native species introduction, and pollution (Antunes et al. 2016; Barlow et al. 2018). In many countries, the lack of adequate monitoring tools and reliable data hampers efforts to develop effective conservation stategies, contributing to an increasing homogenization of fish communities and modifications of species assemblages through local extinctions and species introductions (Barlow et al., 2018; Pelicice et al., 2017; Su et al., 2021).

To understand and quantify the impact of human pressures on freshwater ecosystems, it has become urgent to accuratly assess fish assemblages across various spatial and temporal scales. However, traditional methods for conducting fish inventories in tropical freshwaters (e.g. using gillnets, longlines or biocides) are costly and destructive (Allard et al., 2016; Araújo et al., 2009). They are also heavily reliant on the presence of taxonomic experts, particularly in highly diversified areas such as tropical rivers, characterized by complexes of closely related species, where morphological identification of specimens can be extremely challenging. Another strong limitation of traditional inventory net-based methods is their selectivity and limited capacity to detect small or rare species that may have a strong contribution to ecosystem functioning (Mouillot et al., 2013, 2014).

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In recent years, environmental DNA (eDNA) metabarcoding has allowed the emergence of promising non-invasive and cost-efficient tools for biodiversity monitoring (Takahashi et al., 2023). While the analysis of water, soil, or sediment eDNA is now widely adopted, researchers are increasingly investigating the potential of ≪ natural samplers ≫ for local scale biomonitoring, based on living organisms that, through feeding, aggregate the DNA of species in their immediate environment (Siegenthaler et al. 2019). Ideal natural samplers should be abundant, widely distributed, easy to collect, and feed opportunistically on a wide taxonomic range of prey. (Boyer et al. 2015). For example, several studies have used the blood DNA of hematophagous insects (Kocher et al., 2017; Massey et al., 2022) or the feces of generalist predators (Nørgaard et al., 2021) to inventory flora and fauna in terrestrial environments. In aquatic environments, filter-feeding organisms such as sponges or mussels have been investigated as natural samplers for marine biodiversity to capture a wide range of eDNA from supsended particules in the water colum (Weber et al. 2023; Mariani et al. 2019; Gallego et al. 2024). Similarly, the dietary DNA of small detritivorous invertebrates have shown promise for providing insight into local fish assemblages (Cordone et al., 2022; Siegenthaler et al., 2019).

Here, we investigated whether freshwater detritivorous shrimp can serve as "natural samplers" for assessing the local composition of highly diverse fish assemblages in neotropical riverine ecosystems, comparing their effectiveness with traditional monitoring methods. Our study focused on the fish communities of large rivers in French Guiana, a territory almost entirely covered by primary rainforest and characterized by a very dense hydrographic network that supports over 400 fish species, equivalent to the entirety of Western Europe (Le Bail, 2012). These ecosystems face increasing anthropogenic pressures, particularly from small-scale gold mining and logging activities, which have been shown to significantly alter local fish assemblages (Allard et al., 2016; Cantera, Coutant, et al., 2022; Coutant et al., 2023). In this context, French Guiana must comply with European regulations aiming at developing surveillance programs on water quality (Water Framework Directive 2000/60/EC, hereafter WFD) and requires the development of effective and non-invasive tools to complement or replace current inventory methods.

We developed a multi-marker metabarcoding approach to analyze the dietary DNA (dDNA, hereafter) of several abundant and widely distributed species of shrimp, which can be easily captured using traps along the riverbanks. These small scavenger crustaceans have restricted home ranges (Hirt et al., 2017) and exhibit a highly versatile feeding regime, ranging from feces to fish carcasses (da Cruz et al., 2021; Silveira De Melo & Nakagaki, 2013). As a result, they concentrate surrounding DNA in their digestive tracts, making them well suited as samplers for assessing local biodiversity. To obtain the most comprehensive and robust picture of fish assemblages, the dDNA was analysed using three mitochondrial markers (12S rRNA and cytochrome c oxidase 1 [COI] gene) that differ in taxonomic resolution and coverage (Polanco F. et al., 2021; Quéméré et al., 2021). We compared the taxonomic diversity and inferred size range of fish assemblages recovered through dDNA with those recorded after 10 days of intensive fishing with gillnets, cast nets and traps, as well as with data obtained from the standardized WFD fish monitoring protocol traditionally used in French Guiana.

Given the opportunistic feeding strategy of shrimp and their diverse microhabitats, we expected that dDNA analysis would reveal a greater number of fish species compared to traditional WFD monitoring methods, likely being more effective at identifying rare or cryptic species. Additionally, dDNA analysis is expected to be less biased toward larger species than gillnet-based fishing methods. From a methodological perspective, we also anticipated that the use of multiple markers would help overcome PCR biases and provide a more complete picture of fish diversity compared to a single-marker approach.

Material and methods

Study Area and data collection

The study was conducted on the Approuague River (French Guiana) at Saut Mathias, an area that includes both rapids and slow-flowing sections, located 90 kilometers from the river's mouth (4°18'N, -52deg34'W). Since 2008, this site has been subject to annual fishing surveys as part of a water quality surveillance program in accordance with the European WFD. These surveys use a standardized protocol involving multiple nets

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with different mesh sizes (ranging from 15 to 35mm) overnight to inventory species diversity and abundance. Over 13 years (2008-2022, excluding 2016, 2018, 2019, and 2021), WFD surveys were conducted during 11 annual campaigns at this site identifying a total of 51 fish species belonging to 20 families (Table S1). However, the average number of species recovered per year is much lower (23.3, sd = 3.17) due to a strong inter-annual variability (54.6% of species turnover between consecutive years) (Figure S1). The most recent WFD campaign (2022) recorded 23 species (13 families). To obtain the most comprehensive inventory of local fish communities, we conducted an extensive 10-day survey in October 2021 (dry season) (hereafter refereed as the 'deep inventory') using various fishing techniques and gear, including nets, traps, fishing rods, and cast nets, to capture a broad range of species (n=70, with mean body length ranging from 2 and 75 cm). Each fish captured was measured and identified by taxonomic experts.

At the same time, Freshwater decapods were collected over three days using two complementary sampling methods, conducted alongside the deep inventory. Active sampling with landing nets was used to capture*Eurhyrinchus sp., Macrobrachium brasiliense,* and*Macrobrachium carcinus* in shallow areas along the riverbank.*Macrobrachium olfersii* was collected using both landing nets and baited traps, while *Macrobrachium amazonicum* was exclusively captured using traps. In order to avoid potential DNA contamination that could interfere with metabarcoding analyses, traps were baited with commercial chicken-flavored cat food pellets. Baited traps were set at night with two collection times. This sampling strategy resulted in the collection of 116 shrimp specimens (Table 1), which were stored in ethanol at ambient temperature until analysis in the laboratory.

Laboratory procedure

Sample preparation and dietary DNA extraction

The digestive contents of the shrimps were dissected using flame-sterilized tools in a PCR-free room to avoid contamination. Of the 116 samples, 15 had empty stomachs and were discarded. DNA extraction was performed with the NucleoSpin Tissue Kit (Macherey-Nagel), following the manufacturer's instructions, with filter tips under a sterile hood. The largest samples were first crushed and mixed using sterile glass beads and a Fastprep instrument (MP Biomedicals, Eschwege, Germany) and approximatively 0.2 g of homogenized sample was used for DNA extraction. Samples were processed in batches of 24, using aerosol (empty tubes kept open) and extraction negative controls.

Library preparation and high-throughput sequencing

To assess fish diversity in shrimp dDNA, we employed three mitochondrial markers (Table S2). The first two markers were specifically designed for fish DNA metabarcoding: the 12S-Teleo (64 bp, Valentini et al. 2016, hereafter *Teleo*) and the 12S-MiFish-U (163-185 bp, Miya et al. 2015, hereafter *MiFish*). These markers target distinct short hypervariable regions of the 12S rRNA gene, and their combined use has been recommended to enhance fish species detection (Polanco F. et al., 2021). The third marker, *MG2* (Tournayre et al., 2020), targets a 133 bp region of the Cytochrome Oxidase I (COI) gene using highly degenerate primers. This marker was designed to amplify a wide range of invertebrates and vertebrates. It allows for the verification of the consumer species identity and has demonstrated its effectiveness in assessing Guyanese fish diversity when combined with other primers (Quemere et al., 2021).

Metabarcoding libraries were prepared using a two-step PCR strategy with dual-indexed combinations for tagging, following the protocol outlined in Galan et al. (2018). For each sample and marker, three independent amplicon sequencing libraries were built (PCR replicates). Multiple negative controls for extraction, PCR and indexing controls were included on each 96-well PCR plate. PCR1 (gene-specific amplifications) and PCR2 (indexing) were performed using 2X QIAGEN Multiplex Kit Master Mix (QIAGEN). PCR products were checked by electrophoresis in 1.75% agarose gel before being pooled (one pool per marker). Once pooled, non-specific PCR products and primer dimers were removed through size selection by excision on low-melting agarose gel (1.25%), followed by purification using the PCR Clean-up Gel Extraction Kit (Macherey-Nagel). Pool libraries were verified on a fragment analyzer, quantified by qPCR using the KAPA library quantification kit (Roche), and normalized to 4 nM. Sequencing was performed using a V2 500 cycle-kit reagent

cartridge (Illumina) for 2 x 200 bp paired-end sequencing on an Illumina MiSeq platform.

Bioinformatic analysis

The sequence reads were bioinformatically processed on the Genobioinfo computing cluster (GenoToul, Toulouse, France). Forward and reverse reads were assembled using the program *illuminapairend* from the*OBITools v1.2.11* package (Boyer et al., 2016) and the resulting sequences were trimmed for primers using the program *cutadapt* (Martin, 2011). Strictly identical sequences were clustered together using *obiuniq* (dereplication step). Amplicon Sequence Variants (ASVs hereafter) occurring fewer than 100 times across all samples were discarded. Additionally, sequence length filtering was applied specifically for each primer set: 40-100 bp for *Teleo* ; 140-200 bp for *MiFish* ; and >100 bp for *MG2* . To denoise the dataset, we used the *obiclean* function,keeping only sequences that were more frequently “head” or “singleton” than “internal” in the global dataset, with “internal” reads being potential PCR substitutions or indel errors (Giguet-Covex et al., 2014). The MetaBar package v1.0.0 (Zinger et al., 2021) was used to lower tag jumping and remove contaminants through detection of ASVs whose relative abundance was highest in negative controls. Finally, to remove false-positive results, we discarded not-shared occurrences among technical replicates (Robasky et al., 2014) (i.e., detected in only one of the three PCR replicates).

Reference databases and taxonomic assignment

Taxonomic assignment was performed using the *ecotag* function of OBItools package. For both *Teleo* and *MiFish* markers, taxonomic assignments were made using a comprehensive reference database of 12S rRNA barcodes for over 90% French Guiana’s freshwater fish (Brosse et al., submitted). *MG2* sequences were obtained from BOLD (Ratnasingham & Hebert, 2007) through the Gui-Bol project (Barcoding Guianese fishes, see Papa et al. 2021). To enable meaningful comparisons of fish assemblages across survey methods, we only kept assignments reaching species-level resolution and showing [?]97% sequence identity with the reference database. Sequences below this threshold were discarded, and assignments were further refined to exclude taxa never documented in the Approuague River systems, based on Le Bail (2012).

Statistical analysis

All statistical analyses were performed using R version 4.3.2 (https://www.R-project.org/). To evaluate sampling completeness and compare the detection efficiency between bio-sampler species or genetic markers, we generated species accumulation curves using the random method with 100 permutations and estimated the expected species richness using Chao2 (Chao et al., 2014), with the *vegan* package version 2.6 (Oksanen et Blanchet, 2017). Following Deagle et al. (2019), dDNA metabarcoding data were summarized as : (1) the Frequency of Occurrence (FOO), defined as the percentage of samples in which a fish species was present, and (2) the Relative Read Abundance (RRA), calculated as the average proportion of total reads assigned to the species.

Fish assemblages recovered through dDNA, WFD surveys, and deep inventory were compared using multidimensional scaling (MDS) based on Jaccard dissimilarity matrices. To assess potential size-related detection biases across monitoring methods, we compared the body length distributions of detected species using Wilcoxon rank-sum tests. For each species, we used the mean body length recorded from historical WFD surveys conducted in French Guiana. Additionally, to determine whether detection probability via dDNA metabarcoding was influenced by species abundance, we compared the catch abundances between species detected and not detected by metabarcoding using Wilcoxon rank-sum tests. Finally, we also tested whether species exclusively detected through metabarcoding exhibited different frequencies of occurrence compared to species detected by both metabarcoding and traditional surveys.

Results

Intensive survey using traditional fishing methods

During the intensive 10-day survey, taxonomic experts identified 70 fish species. Nearly half of the species (53%) were sampled using gillnets, while the remaining were captured using a combination of traps, seine

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nets, fishing lines and cast nets. The number of fish species surveyed increased rapidly in the first four days, reaching 90% of the total species by day 6, before levelling off at a clear asymptote (Figure S2).

Dietary DNA metabarcoding data analysis and comparison across markers

A total of 2,197,057, 2,499,872 and 2,245,225 raw sequence reads were obtained by HTS from *MG2* , *MiFish* and *Teleo* , respectively. Information on the numbers of ASVs and reads retained after the sequence-length filtering and denoising, and after the assignment step is summarized in Table 2. A large proportion of reads were assigned to fish (*Actinopterygii*) for 12S rRNA *Teleo* and *MiFish* (74%, and 69% respectively), whereas the percentage of fish reads assigned using *MG2* (COI gene) was much lower (3.3 %). The three markers showed very strong taxonomic discriminatory power, with the vast majority of taxa (>90%) identified at the species level but *Teleo* and *MiFish* revealed significantly more fish taxa (58 and 57 respectively) than MG2 (37). Chao2 asymptotic richness estimate (63.68 +- 5.8 for *MiFish* , 73.9 +- 10.9 for *Teleo* , 37.8 +- 1.3 for *MG2*) suggested the presence of few undetected prey taxa compared to the actual number prey detected (Figure S3).

The two 12S rRNA markers revealed similar fish assemblages, with more than 71% species shared (48 out of 67) (Figure S4). However, 27% (N=19) of the species were exclusivly detected by only one of the two makers. Species detected by all three markers (N=32) were generally those with the highest frequency of occurrence, with the top 13 most frequently detected species being consistently identified across all markers. The Frequency of Occurrences of fish in the crustacean samples (FOO) was highly correlated between the *MiFish* and *Teleo* markers (r2=0.72). Furthermore, for both markers, Relative Read Abundance (RRA) and FOO showed good correlation (r2 = 0.50 and r2 = 0.48 for MiFish and Teleo respectively), indicating that the species frequently detected also tended to have higher sequencing coverage. The*MG2* marker revealed only one species,*Saxatila aff. saxatilis sp2* , which was not detected by*Teleo* or *MiFish* , out of the 37 taxa identified (Figure S4).

The three markers collectively detected 68 species belonging to 6 different orders, 21 families and 46 genera (Table S1). The vast majority (N= 66; or 97%) were correctly assigned based on the reference databases. For the remaining two species, local wildlife experts helped in refining the taxonomic assignments.

Comparison of Biosampling Efficiency and Fish Assemblages between Crustacean Species

An average of 4.6 fish species were detected per digestive sample, with significant variation in biosampling efficiency among crustacean species (Table 1, Figure 1). The most abundant shrimp species,*Macrobrachium olfersii* and *Macrobrachium brasilience* , were the most efficient biosamplers, detecting 51 and 36 fish species respectively, with high detection rates (6.36 and 4.50 fish species per digestive sample in average). Together, they captured more than 94% of the total dDNA fish diversity (Figure S4). In comparison, the biosampling efficiency of the three other shrimp was much lower, with fewer than three fish species per specimen on average. Species accumulation curves (Figure 1) support this finding, showing faster accumulation rates for *M. olfersii* and *M. brasilience* compared to the other species.

Comparison of fish assemblages across monitoring methods

The three approaches (dDNA, deep survey, and the 2022 WFD campaign) collectively revealed a total of 91 fish species. Dietary DNA detected nearly as many species than the 10-day deep survey (68 and 70 respectively) and three times more species than the WFD campaign. Biosampler dDNA provided a fish assemblage that was broadly consistent to the deep inventory, with almost two third species shared (n=51 species) (Figure 2). However, it also detected 17 species not recorded with traditional techniques despite the use of multiple fishing techniques. Conversely, 19 species recorded in the deep inventory were not detected in shrimp digestive contents. When excluding unique species absent from the reference databases, fish not detected by dDNA metabarcoding were significantly less abundant in field surveys, with an average of 7.3 (+- 8.7) catches, compared to 21.0 (+- 12.5) catches for detected species (Wilcoxon test, p = 1e-4). Additionnally, species detected exclusively through metabarcoding (n = 16) generally showed significantly lower frequencies of occurrence (mean FO = 3.27%, SD = 2.8%) compared to species detected through both metabarcoding

and deep survey (mean FO, 8.01%, SD = 6.81%) (Wilcoxon rank-sum test, p-value = 0.0052).

The Venn diagram revealed limited overlap between species identified by the 2022 WFD survey and those detected through dDNA or during the deep inventory, with only 15 and 17 species shared, respectively. Additionally, fish detected during the 2022 WFD campaign were significantly larger (201.4 +- 104.8 mm) than those capture by dDNA metabarcoding (145.9 +- 129.3 mm, Wilcoxon test, p = 0.003 and exhibited a more restricted size range (Figure 3). In contrast, no significant difference in expected size distribution was observed between dDNA and deep inventory fish assemblages (Wilcoxon test, p = 0.46)

DISCUSSION

Strenghts and limitations of the dDNA metabarcoding approach

The dDNA approach offers several notable advantages over traditional methods used for monitoring tropical freshwater communities. First, it is cost-effective and easy to implement in the field, requiring minimum equipment. Using just a few baited traps and net hauls, we were able to collecte large numbers of shrimp (>30 specimens for *M. olferssi* and *M. brasilience*) within just a few days along riverbanks and in rapid zones. Encapsulated in the stomach, dDNA samples can be easily preserved in ethanol with minimal contamination risk until extraction in the laboratory (Siegenthaler et al., 2019). This makes it especially suitable for tropical environments, where logistical challenges often hinder extensive sampling campaigns. This method avoids the capture and handling of fish, which is particularly relevant in protected areas or when monitoring threatened species. The shrimp used as biosamplers are highly abundant and occupy basal levels of food webs, meaning that collecting a few dozen individuals likely has a limited impact on population dynamic and ecosystem stability.

Another significant strength of this method is its remarkable efficiency and low selectivity. The metabarcoding analysis shrimp dDNA enabled the detection of as many species as an intensive 10-days deep inventory of the study area. This approach uncovered nearly three times more species than the 2022 WFD survey and exceeded the total number of species recorded over the 11 years of WFD campaigns. As expected, given the generalist and opportunistic feeding behaviours of these detritivorous organisms, dietary DNA analysis detected a broad spectrum of species in terms of size. Indeed, our analyses revealed no significant difference in the size ranges of species identified through using dDNA and those recorded during our deep inventory. This suggests that the dDNA metabarcoding of detrivorous crustaceans does not present any particular size-related detection bias, in contrast to WFD gillnet-based campaigns that preferentially captured large fish. Dietary DNA analysis also detected elusive species that live in specific microhabitats and are rarely captured when using traditional sampling methods. For instance,*Cyphocharax biocellatus* and *Jupiaba abramoides* are small, abundant species inhabiting shallow waters along riverbanks or vegetation in fast-flowing zones were detected using dDNA but never during WFD campaigns using gillnets. However, nearly 20% of the species from the deep inventory were absent from the shrimp dDNA samples. Some of theses species (e.g. *Anchovia surinamensis or Moenkhausia aff grandisquamis*) are relatively common but undetected due to gaps in the reference barcoding databases. Other species are rarer, with low capture rates and increasing the sampling effort with additional biosampler specimens might help detect them. The use of easily accessible, generalist invertebrate species for large-scale monitoring has already been demonstrated in other ecosystems. For instance, dDNA analysis of brown shrimp (*Crangon crangon*) detected twice as many species than traditional net surveys in coastal marine areas (Siegenthaler et al., 2019). Similarly, molecular gut content analysis of mussels has proven effective in monitoring planktonic communities, identifying a broad range of dietary taxa that mirror the diversity in surrounding water samples (Weber et al., 2023).

The dDNA approach is also notable for its high taxonomic resolution combined with its minimal reliance on taxonomic experts. Despite the small size of the DNA barcodes, we successfully identified most fish taxa at the species level, with only minor corrections needed, primarily due to recent taxonomic updates. This is a critical point because taxonomic identification can be challenging in highly diverse environments, especially when dealing with cryptic species complexes. However, this success was greatly supported by the well-documented fish fauna in French Guiana (Le Bail, 2012) and the availability of nearly exhaustive

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barcoding reference databases. Accurate taxonomic assignment through metabarcoding depends largely on the completeness and accuracy of reference databases (Hilario et al., 2023; Keck et al., 2022) and significant gaps still exist for tropical fish species (Marques et al., 2021; Sales et al., 2018). This underscores why the development of open-acess, highly-quality and complete reference databases has been identified as the top priority for the field (Blackman et al., 2024).

Choice of markers and biosampler species

Our results emphasize the importance of combining several markers to enhance species detection and confidence in the results (Alberdi et al. 2019; Polanco et al. 2021; Tournayre et al. 2024). As expected,*MiFish* and *Teleo* showed substantial overlap, detecting more than 70% of species in common and yielding highly similar frequencies of occurrences in biosamplers. Both markers identified nearly the same number of fish species, though 10 species were detected exclusively by one or the other. These unique species were found in a small number of digestive contents, suggesting that these differences likely reflect the rarity of these species in the environment, or at least their lower consumption by crustaceans, rather than variations in primer robustness or discriminatory power. The third marker tested (*MG2*) preferentially amplified invertebrates and detected significantly fewer fish species than the 12S rRNA markers. While it appears less effective for fish monitoring, it may still prove helpful for validating the identity of the biosampler species or to inventory other taxonomic groups (Tournayre et al., 2024). Although using multiple markers can increase the time and cost of molecular analyses, laboratory-processing time can be reduced by pooling stomach contents while ensuring reliable detection through large sequencing depth.

Another important practical consideration is the choice of natural samplers based on their abundance and distribution range. For neotropical rivers, *Macrobrachium brasiliense and Macrobrachium olfersii* which are widely distributed across South America and can be easily collected in large numbers, seem to be ideal candidates, collectively detecting 94% of the total dDNA fish diversity. Understanding the spatial ecology and feeding habits of potential biosamplers can help guide the selection of species (or species combinations) that will yield the most comprehensive inventory (Calvignac-Spencer et al., 2013). This knowledge can help assess the spatial and temporal resolution of the dietary data, which depends on the home range size and digestion rate of organisms (Feller 2006; Prog, Pihl, and Rosenberg 1984). In this context, an interesting perspective is to compare scavenger crustacean dDNA and the increasingly used water eDNA approaches to determine which one provides the best insight into local assemblages in large rivers (Cantera, Decotte, et al., 2022; Cantera et al., 2023b, 2023a; Coutant et al., 2023; Zinger et al., 2020). Indeed, while water eDNA provides a snapshot of biodiversity, dietary DNA may offer a more temporally integrated signal due to gut retention time and circumvents several issues commonly encountered with water eDNA in turpid river systems, such as high sediment loads that limit filtration capacity and high concentrations of organic matter that can cause PCR inhibition.

Conclusion and perspectives

This study demonstrated that the dietary DNA metabarcoding of freshwater scavenger shrimps offers a comprehensive and reliable snapshot of fish assemblages in neotropical rivers. As the cost and speed of molecular analyses continue to decrease, the relative ease of sampling and processing makes this method particularly suitable for carrying out rapid biodiversity assessments. Crustacean-derived dDNA likely provides fine-scale, recent insights, making it a valuable tool for detecting the localized ecosystem impacts of anthropogenic disturbances, such as overfishing, gold mining, deforestation, or dam construction, on fish communities along river networks. However, this should be seen as a complement to traditional WFD approaches, as it does not provide data on fish abundance, biomass or condition, which remain essential for effective management. Additionally, the method's ability to detect small and elusive species could make it particularly useful for early detection of invasive species or for monitoring rare native species that are difficult to capture using conventional methods.

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Conflict of interest

The authors declare no conflict of interest.

Data Accessibility and Benefit-Sharing

Supplementary data deposited in Dryad (https://doi.org/XXXX) include raw sequence data (FASTQ files), raw abundance tables and filtered abundance table including taxonomic affiliations.

Author's contributions

EQ and JMR designed the study; EQ, RV, PYLB, GL did the sampling, CC carried out the DNA extraction, quality controls and PCRs under ALB supervision; SB, JM, RC and PYLB built the reference database and barcodes. CC, EQ, HL, PYLB and BB performed the analyses and interpreted the data; BB, CC and EQ led the writing and all the authors contributed to the manuscript and gave final approval for publication.

Tables and Figures

Tables

Table 1 –Sampling metrics across crustacean biosampler species . Number of samples represents the number of digestive contents analyzed; Observed species richness refers to the total number of fish species detected; **Private species indicates fish species uniquely detected by a single shrimp species; Mean +- SE represents the average number of fish species per stomach content; Chao2 estimates the inferred total species richness, accounting for undetected species due to incomplete sampling effort

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Shrimp species	Number of samples	Observed species richness	Private species	Mean + SE number of species per sa
M. amazonicum	31	26	3	2.8 ± 2.3
M. brasiliense	16	36	8	4.5 ± 6.0
M. carcinus	10	10	1	1.9 ± 1.4
M. olfersii	36	51	8	6.4 ± 6.0
Eurhyrinchus sp.	8	9	1	2.4 ± 2.4
Total	101	68	21	4.6 ± 4.8

Table 2 – Evolution of the number of reads and ASV throughout filtering and assignment steps for each-marker

	Initial	After filtering		After assignment		
	#Reads	**#Reads**	**#ASVs**	**#Reads**	**#ASVs**	**#Species**
MG2	2,197,057	1,530,344	1,663	136,894 (8%)	56 (3.3%)	37
Teleo	2,245,225	1,192,005	163	883,070 (74%)	92 (56%)	58
MiFish	2,499,872	676,043	296	469,011 (69%)	143(48%)	57

Figures

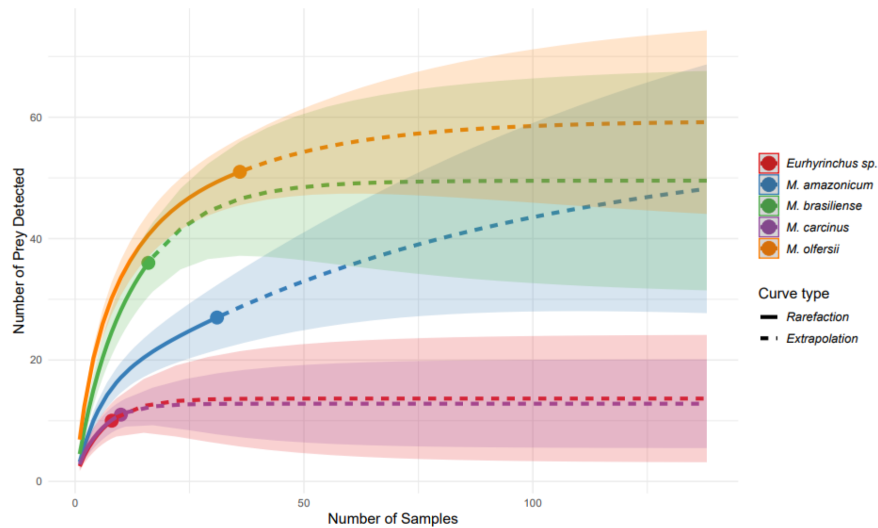

Figure 1 – Species detection accumulation curves by biosampler species . Colored points represent the observed species richness, while the dotted line segments indicates the extrapolated species richness with shaded areas showing 95% confidence intervals (extrapolation of Hill numbers using the INEXT package (version 2.0.2, Hsieh, Ma, et Chao 2016).

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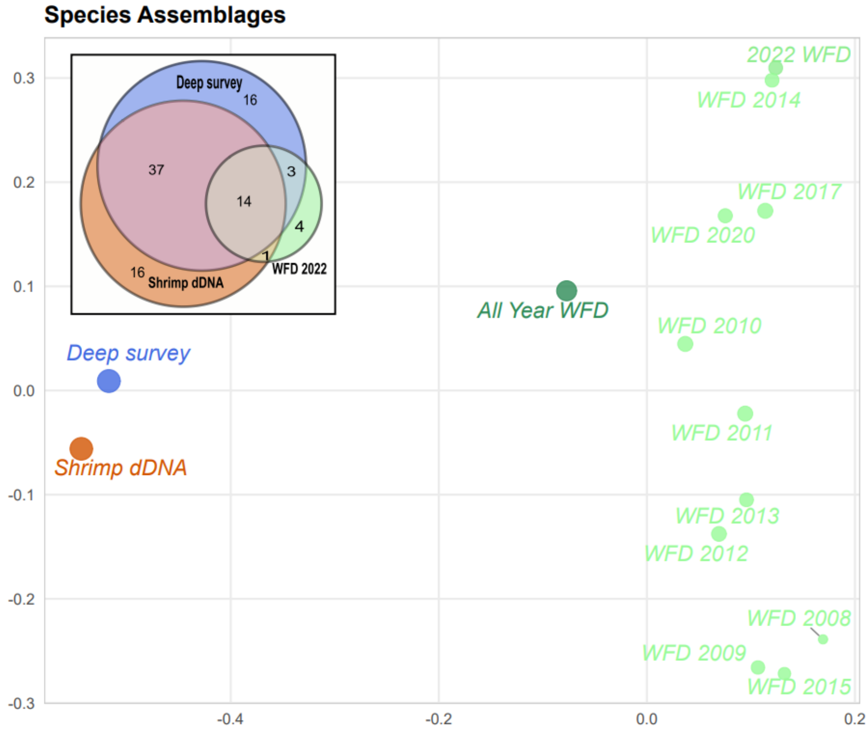

Figure 2 – Comparison of fish assemblages detected by WFD campaigns, shrimp dietary DNA and the deep survey . Multidimensional scaling ordination (MDS) plot based, on Jaccard dissimilarity matrices, illustrating differences in fish assemblages among the different monitoring methods. The Venn diagram in the insetshowed the overlap in species detection among the three methods

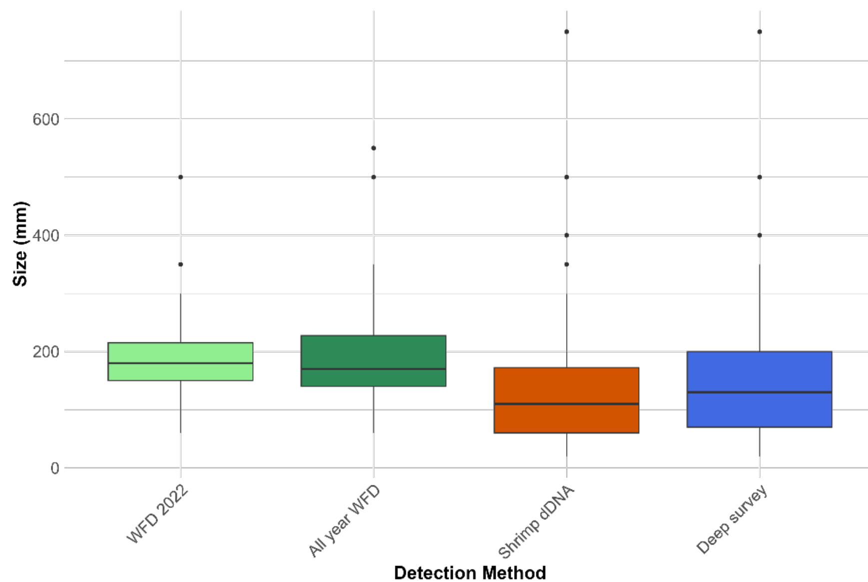

Figure 3 – Size distribution of fish species detected across monitoring methods . For all detection methods, we considered the known body length as the mean body length recorded from historical WFD

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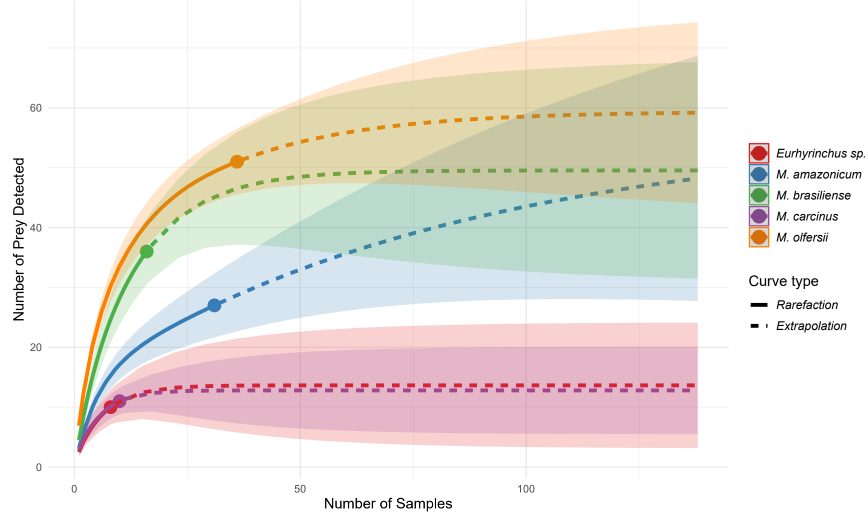
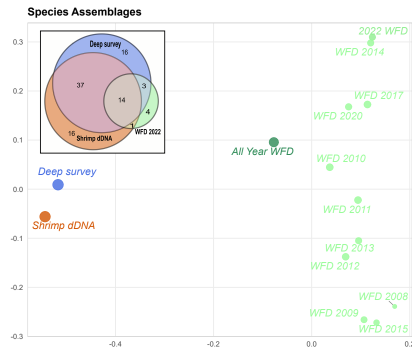
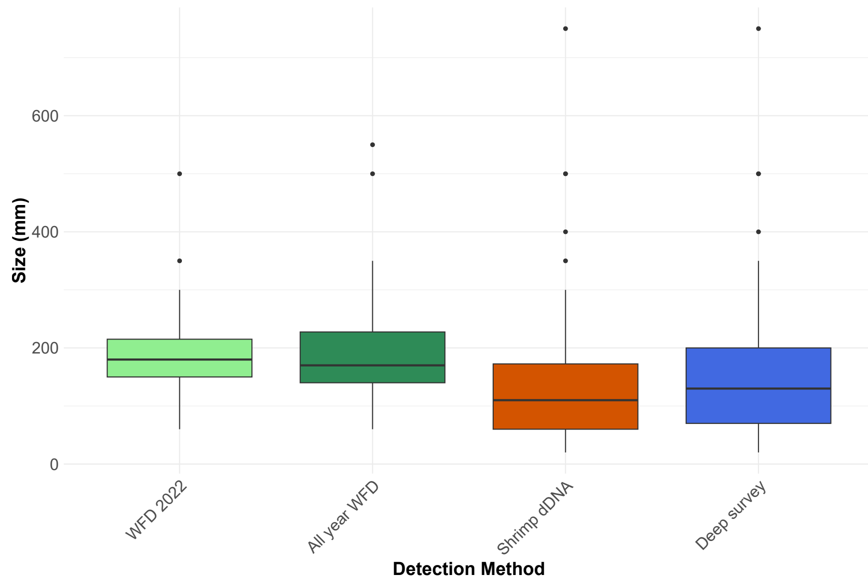
surveys conducted in French Guiana







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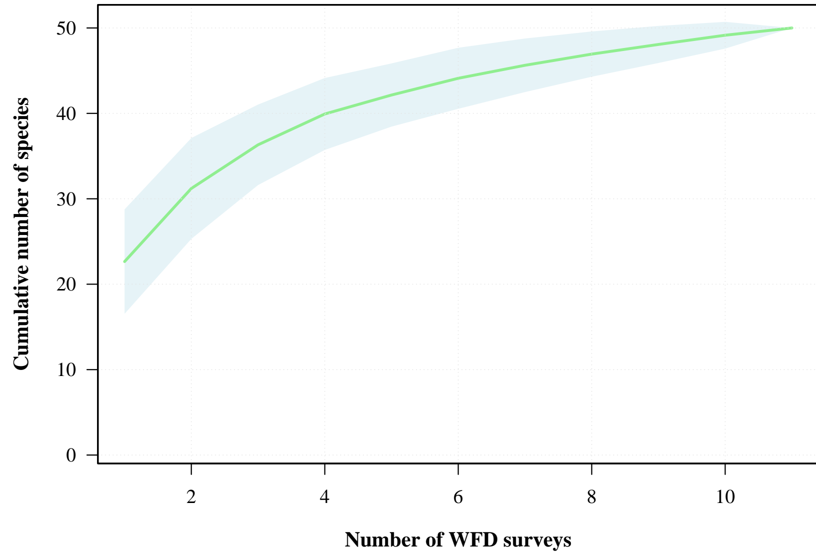

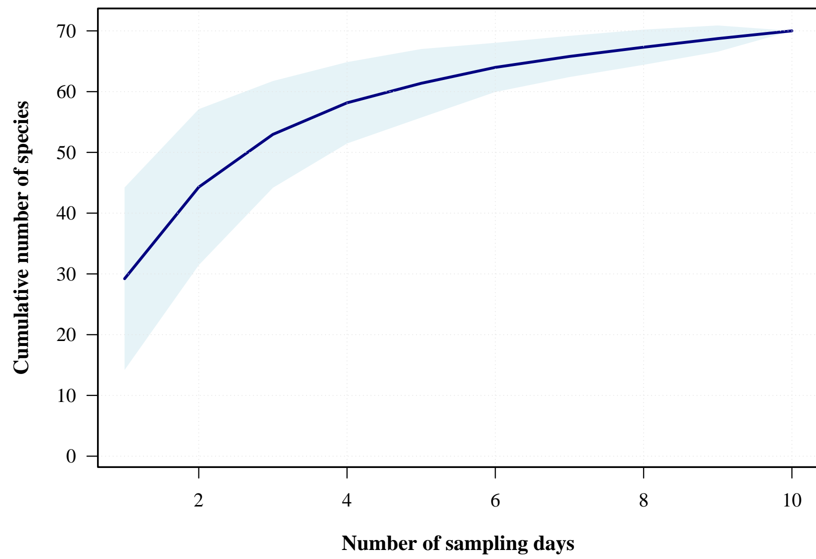

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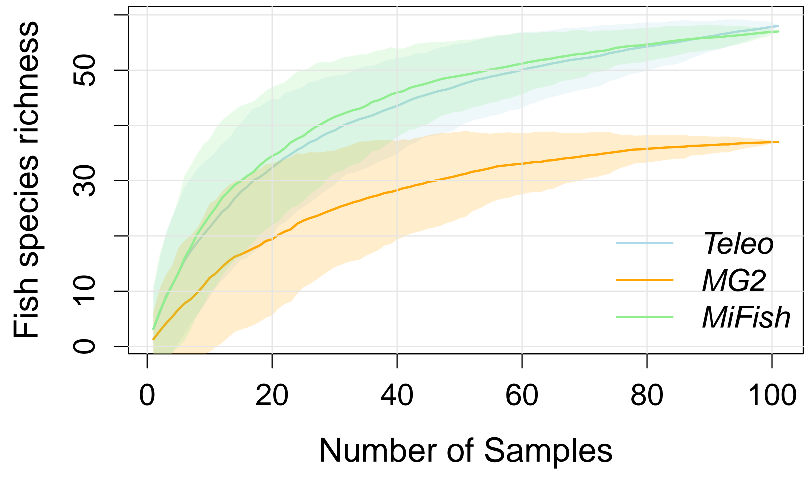

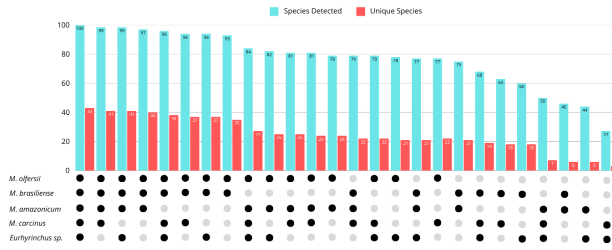

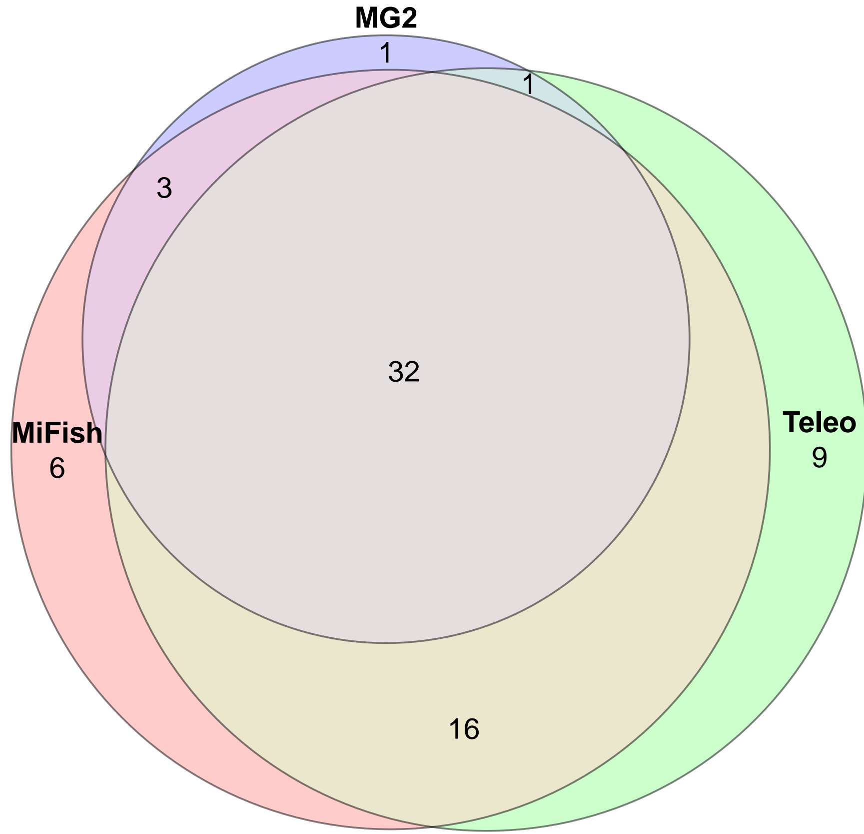

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Beloniformes
Belonidae
Pseudotylosurus microps

Characiformes

Acestrorhynchidae
acestrorhynchus microlepis
acestrorhynchus falcatus

Anostomidae
leporinus nijsseni
leporinus melanostictus
leporinus granti
leporinus gossei
leporinus acutidens

Characidae
thayeria ifati
tetragonopterus georgiae
poptella brevispina
phenacogaster wayana
moenkhausia inrai
moenkhausia hemigrammoides
moenkhausia grandisquamis
moenkhausia gr. oligolepis
moenkhausia collettii
moenkhausia chrysargyrea
moenkhausia aff. intermedia
moenkhausia aff. Grandisquamis
jupiaba meunieri
jupiaba abramoides
hyphessobrycon simulatus
hyphessobrycon copelandi
hyphessobrycon borealis
hemigrammus unilineatus
hemigrammus ocellifer
charax niger
characidium zebra
astyanax leopoldi

Chilodidae
chilodus zunevei

Curimatidae
cyphocharax spilurus
cyphocharax helleri
cyphocharax gouldingi
cyphocharax biocellatus
cyphocharax aff. spilurus sp.1
curimata cyprinoides

Doradidae
doras micropoeus

Erythrinidae
hoplias malabaricus
hoplias aimara

Gasteropelecidae
gasteropelecus marowini

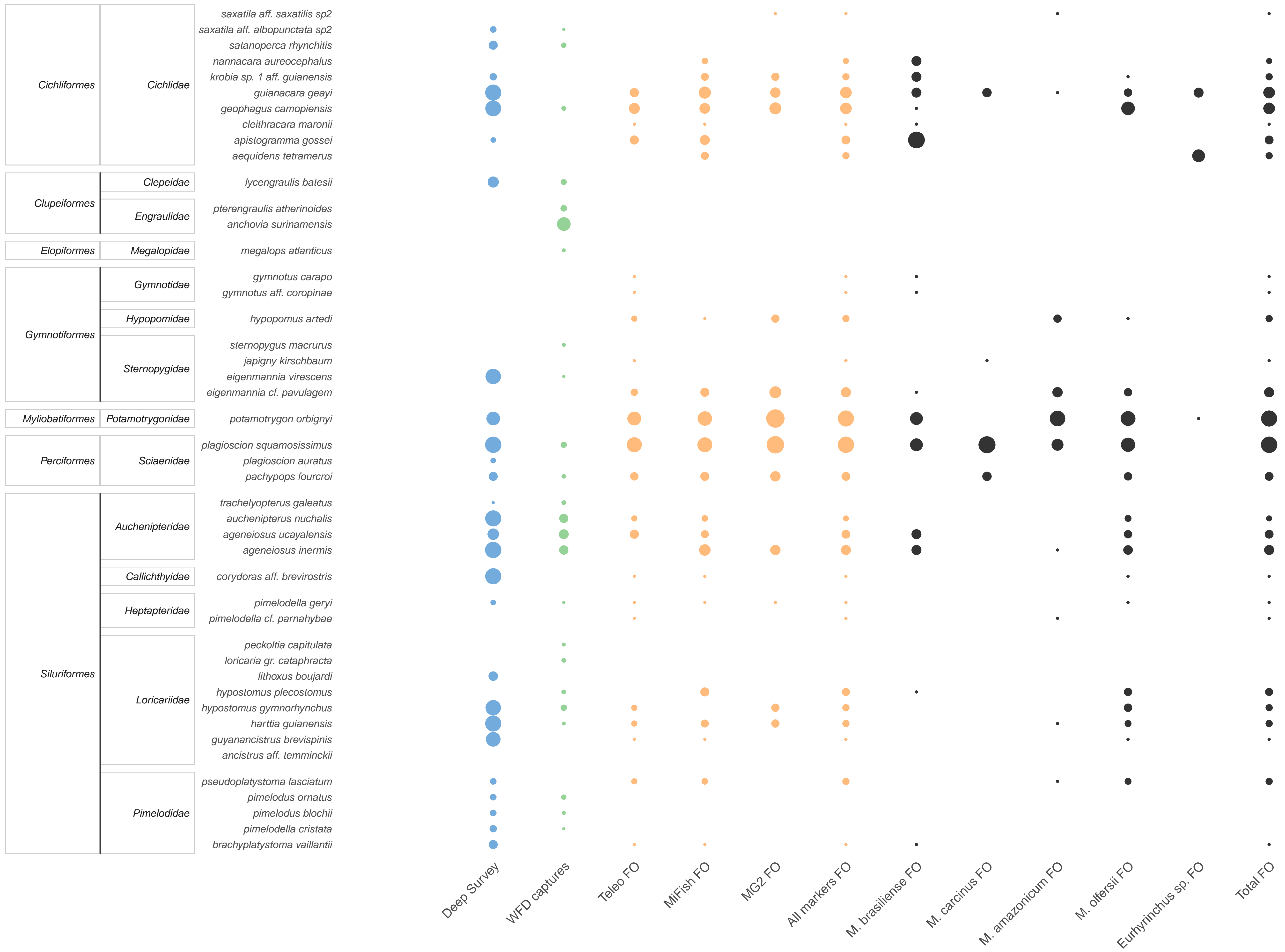
Hemiodontidae
parodon guyanensis
hemiodus gr. quadrimaculatus
hemiodus aff. unimaculatus
bivibranchia bimaculata

Iguanodectidae
piabucus dentatus
bryconops affinis
bryconops aff. melanurus sp. 2
bryconops aff. melanurus sp. 1
bryconops aff. caudomaculatus

Lebiasinidae
nannostomus bifasciatus

Serrasalmidae
serrasalmus eigenmanni
myloplus tumukumak
myloplus ternetzi
myloplus rhomboidalis

Deep Survey
WFD captures
Teleo FO
MiFish FO
MG2 FO
All markers FO
M. brasiliense FO
M. carcinus FO
M. amazonicum FO
M. olfersii FO
Eurhyrinchus sp. FO
Total FO


Cichliformes
Cichlidae
saxatila aff. saxatilis sp2
saxatila aff. albopunctata sp2
satanoperca rhynchitis
nannacara aureocephalus
krobia sp. 1 aff. guianensis
guianacara geayi
geophagus camopiensis
cleithracara maronii
apistogramma gossei
aequidens tetramerus
Clupeiformes
Clepeidae
lycengraulis batesii
Engraulidae
pterengraulis atherinoides
anchovia surinamensis
Elopiformes
Megalopidae
megalops atlanticus
Gymnotiformes
Gymnotidae
gymnotus carapo
gymnotus aff. coropinae
Hypopomidae
hypopomus artedi
Sternopygidae
sternopygus macrurus
japigny kirschbaum
eigenmannia virescens
eigenmannia cf. pavulagem
Myliobatiformes
Potamotrygonidae
potamotrygon orbignyi
Perciformes
Sciaenidae
plagioscion squamosissimus
plagioscion auratus
pachypops fourcroi
Siluriformes
Auchenipteridae
trachelyopterus galeatus
auchenipterus nuchalis
ageneiosus ucayalensis
ageneiosus inermis
Callichthyidae
corydoras aff. brevirostris
Heptapteridae
pimelodella geryi
pimelodella cf. parnahybae
Loricariidae
peckoltia capitulata
loricaria gr. cataphracta
lithoxus boujardi
hypostomus plecostomus
hypostomus gymnorhynchus
harttia guianensis
guyanancistrus brevispinis
ancistrus aff. temminckii
Pimelodidae
pseudoplatystoma fasciatum
pimelodus ornatus
pimelodus blochii
pimelodella cristata
brachyplatystoma vaillantii
Deep Survey
WFD captures
Teleo FO
MiFish FO
MG2 FO
All markers FO
M. brasiliense FO
M. carcinus FO
M. amazonicum FO
M. olfersii FO
Euhyrinchus sp. FO
Total FO