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Opale Coutant

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THÈSE

En vue de l'obtention du
DOCTORAT DE L'UNIVERSITÉ DE TOULOUSE
Délivré par l'Université Toulouse 3 - Paul Sabatier

Présentée et soutenue par
Opale COUTANT

Le 16 février 2023

Anthropisation et biodiversité : Impacts humains et réponses anthropiques au déclin de biodiversité des vertébrés des cours d'eau de Guyane

Ecole doctorale : **SEVAB - Sciences Ecologiques, Vétérinaires, Agronomiques et Bioingenieries**

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General Introduction

1. Freshwaters, rich ecosystems under threats

Freshwater represents only 2.5% of the Earth's water. Within this fraction, 68.7 % of freshwater is retained into icecaps, glaciers and perennial snow and 30.1% constitutes groundwater. Surface water, therefore, represents a very small portion of freshwater (0.3%) divided into swamps (11% of surface water), lakes (87% of surface water), and rivers (2% of surface water) (Garcia-Moreno et al., 2014).

Despite the small Earth's surface occupied by freshwater, it represents the most diversified realm and hosts approximately 126 000 described animal species representing 9.5% of the world's biodiversity (considering only eukaryotic organisms). Among these species, the largest groups are insects (60.4%), vertebrates (14.5%), and crustaceans (10%) (Balian et al., 2008). The density of living organisms in freshwater is among the highest. For instance, the total number of living fish species is approximately 25,000 with 58% of the species dwelling in marine ecosystems and 41% inhabiting freshwater. The remaining 1% of the species migrate to both habitats. This enormous fish diversity concentrated in such small surface makes the freshwater fish density 7,500 times greater than in oceans (Lundberg et al., 2000).

1.a. Explaining the biodiversity of freshwater ecosystems

This diversity originates from the unique particularities of freshwater ecosystems. Although it was not the core of the Theory of Island Biogeography, MacArthur and Wilson suggested that species richness of isolated continental habitats could be regulated by the same factors as oceanic islands (MacArthur & Wilson, 1967). This analogy also stands for freshwater systems. Freshwater entities such as lake or river basins are isolated, restricting the dispersal of the species and therefore promoting speciation between independent rivers, thus at least partly explaining the biodiversity of freshwater at a regional scale.

At the scale of the watershed, freshwaters such as riverine ecosystems present a hydrographic network characterised by a dendritic structure. This dendritic environment consists in a geometric pattern of arborescent bifurcations emerging from nodes and expending in one direction, forming a hierarchical network of nodes and branches (Altermatt, 2013). Small headwater patches are therefore numerous, but more isolated than larger downstream patches. In such a way, the Theory of Island Biogeography stipulates that smaller and isolated patches experience higher extinction rates and lower immigration rates than larger and more connected

patches (Harvey et al., 2018). In rivers, this is strengthened by the spatial connectivity known as the hydrologic connectivity (Pringle, 2001), which dictates a dispersal pathway from the source to the estuary, defining and restricting species movements. These processes thus promote a gradient of species diversity increasing from upstream to downstream (Altermatt, 2013). Associated to the hydrologic connectivity, the spatial heterogeneity along the dendritic network is characterised by a variation of habitats and environmental parameters. Generally, the diversity of habitats increases from upstream to downstream, giving rise to an increase in species richness toward the river mouth (Harvey et al., 2018). This is reported in the River Continuum Concept emphasizing the longitudinal dimensions of rivers. This concept states a progressive shift from headwaters to mouth, of physical gradients and energy inputs, which generates a shift in trophic organization and biological communities (Vannote et al., 1980).

Freshwater ecosystems are also tightly connected to terrestrial ecosystems, which contributes to the diversity of aquatic habitats and influences water physicochemistry (Vannote et al., 1980). For instance, the riparian vegetation reduces the primary productivity by shading and releases large amounts of allochthonous woody and leaf debris, bringing fine particles of organic matter in the water (Vannote et al., 1980). Besides, many species playing important roles in structuring riverine ecosystems such as crocodiles or hippos are dependent on both freshwater and terrestrial habitats. In such way, they link freshwater and terrestrial faunas through the trophic chain (Pringle, 2001). An example that well illustrates the connectivity between these two ecosystems is the study of Leal et al., (2020), which demonstrates that integrated terrestrial-freshwater conservation planning accounting for hydrologic connectivity can double the conservation of tropical aquatic species.

1.b. Freshwaters in the Anthropocene

With the vast diversity of species and habitats composing freshwater, this biome serves human societies in many ways, from crops irrigation, food supply to drinking water. Consequently, it undergoes multiple threats and freshwaters are among the most endangered ecosystems in the world (Dudgeon et al., 2006). They are particularly sensitive to human activities because rivers are landscape receivers and collect environmental pressures from the surrounding landscapes (Dudgeon et al., 2006; Van Rees et al., 2021). Rivers are also downstream transmitters through the hydrologic connectivity of riverine systems, the water-mediated transfer of matter, energy, and/or organisms within or between elements of the hydrologic cycle (Dudgeon, 2019; Pringle, 2001). As a result, local disturbance impacts do not only have local consequences on river biodiversity and ecosystem functioning, but also spread

to the fraction of the basin located downstream (Van Rees et al., 2021). These impacts and their large spatial repercussions severely impair biodiversity. As a consequence, a drastic drop of biodiversity is reported in freshwater, with population trend data from the living planet index showing that declines in freshwater species (4% on average, annually) are consistently greater than those on land (annual average 1.1%) (Balian et al., 2008).

A classification of the major anthropic threats to freshwaters have been established by Dudgeon, (2019) and includes overexploitation, flow regulation, pollution, land-use change, invasive species and, climate change.

The overexploitation of biological resources mainly affects fishes, molluscs and crustaceans that are used as protein sources for humans. Because species with high economic values are often large species with late maturity and low fecundity, it deeply disturbs the trophic chain in addition to deplete species biomass (Geist, 2011). For instance, Castello et al., (2013) explained the fishing-down process in the Amazon, where historical exploitations have driven a global decrease in specie mean body size through the depletion of high-value, large-bodied species. While mean maximum body length of the main harvested species was approximately 206 cm in 1895 (fish including *Arapaima spp.*, *Brachyplatoma filamentosum* but also other species such as *Trichechus inunguis* and *Podocnemis spp.*), it dropped to 79 cm in 2007 (Castello et al., 2013).

The alteration of flow is mostly the consequence of dams that critically modify the surrounding landscapes, creating reservoirs upstream and emptying the river downstream. Generally, dams altered the river hydrological cycles, the biogeochemical cycles such as sediment and nutrient transport, the ecological conditions but also obstruct species movements (Dudgeon, 2019; Wang et al., 2018). For example, the São Francisco River in Brazil has undergone a reduction of >75% of the downstream sediment load. These reductions in sediment loads, can then result in the erosion of the estuary and riverbeds, generating habitat reductions (Walling, 2006; Wang et al., 2018).

Water pollution originates from multiple sources and may be the consequence of the release of contaminants such as heavy metals but also inorganic nutrients, inducing water eutrophication. It also includes non-chemical pollution as changes in water parameters. For instance, power stations generate thermal water pollution by cooling-water discharge (Dudgeon, 2019).

Land-use change includes the partial or total removal and transformation of the vegetation and leads to cascading impacts. It generates soil erosion, water sedimentations and

pollutions, changes primary productivity and therefore water physicochemical parameters (Dudgeon, 2019).

The introduction of invasive species is another major threat in freshwater ecosystems and the main consequence is the alteration of the structure of native communities and the modification of ecosystem functioning (Bernery et al., 2022; Dudgeon, 2019). Carnivorous species such as apex-predators may be particularly detrimental because they can consume high quantities and diversity of preys as well as new, unexploited, resources (Bernery et al., 2022; Zaret & Paine, 1973). For instance, *Cichla ocellaris* was introduced to Gatun Lake in the Panama Canal Zone in 1967. The predator population spread through the lake, occasioning a dramatic reduction in almost all secondary consumers. In turn, these species reductions produced second- and third-order modifications at other trophic levels of the ecosystem (Zaret & Paine, 1973). 45 years after this introduction, the fish community has not recovered. *Cichla ocellaris* remains the most abundant predator in Lake Gatun while the mean abundance of native littoral fish is now 96% lower than it was before the introduction, with some species being even locally extinct (Sharpe et al., 2017).

Climate change is deeply modifying watercourses. The main impacts stem from the rise in temperature, but also changes in rainfall regime. The perturbation of the water cycle is particularly detrimental to riverine biodiversity because species are closely associated to hydrology (Dudgeon, 2019). 23 out of 31 ecological processes measured at multiple levels of biological organisation (organism, population, species and community levels) show signs of alteration in response to climate change in freshwater (Scheffers et al., 2016). The shift in species distribution is one of the most rapid responses to climate change. For instance, in freshwater, species have extended their cold limit edge by 19.7 km per decade (Scheffers et al., 2016).

These six threats categories interacts together and may results in combined effects that are less (antagonism), greater (synergism) or equal to the sum (additive) of their individuals effects (Craig et al., 2017; Dudgeon, 2019; Reid et al., 2019). For instance, the rise of water temperature increasing productivity combined to inorganic nutrient releases by land exploitation are responsible for synergistic effects, favouring the bloom of harmful algae and bacteria and therefore causing water eutrophication (Reid et al., 2019; Scheffers et al., 2016). In freshwater, however, a meta-analysis revealed that the interactions of multiple stressors more often gave rise to antagonistic effects (41%) than synergistic (28%) or additive (15%) effects (Jackson et al., 2016). For example, the invasive plant *Trapa natans* (waster-chestnut) in the

Hudson River denitrifies anthropogenic sources of nitrate originating from agriculture, industry and urbanization present in the watershed (Tall et al., 2011).

Humans divert $> 10\,000\text{ km}^3$ of freshwater per year for agriculture (70% of all freshwater usage), industry (20% of all freshwater usage) and domestic uses (10% of all freshwater usage) (Albert et al., 2021). The proportion of river discharge diverted for human activities is over 50% in the most populated areas of Europe and Asia (Albert et al., 2021; Grill et al., 2019). Freshwaters are also used for waste disposal, transportation or power production. People rely on freshwaters not only for water but also for other services. With the modification and alterations of freshwaters, the services they provide to human societies are expected to deteriorate, with for example a reduce capacity to provide food resources or ensure nutrient cycling (Garcia-Moreno et al., 2014).

2. Assessing biodiversity changes from a community ecology view

2.a. Communities as ecological entities

The word “ecology” as first been used by Ernest Haeckel in 1869 describing the scientific study of the interactions between organisms and their environment. With the contribution of Krebs in 1972, it was later defined as “the scientific study of the distribution and abundance of organisms and the interactions that determine distribution and abundance” (Begon et al., 2006). Ecology can be studied at different levels of biological organisations, from the cells to the biosphere. At a macroecological scale, population, community and ecosystem ecology are disciplines that investigate the relations between living organisms and the environment.

Community ecology refers to the study of patterns in the structure and behaviour of multispecies assemblages (Begon et al., 2006). The community defines an ecological entity composed by a group of species occurring together in space and time (Begon et al., 2006; Mittelbach & McGill, 2019). The limits on space and time as well as on the identity and number of species is arbitrary and delimited by the investigator, given that it is impossible to study all the species occurring together in the same place and at the same time (Mittelbach & McGill, 2019). The main rationale behind community ecology is to understand how the biodiversity is structured throughout the planet, but also what are the determinants responsible for its structuration. The answers will be dependent on the spatial scale and Robert Whittaker proposed the use of cross-scale comparisons to understand the spatial organisation of biodiversity

(Whittaker, 1960, 1972). He provided biodiversity measures that represent different facets of biodiversity, giving complementary information to describe patterns of diversity.

2.b. From local to regional diversity patterns

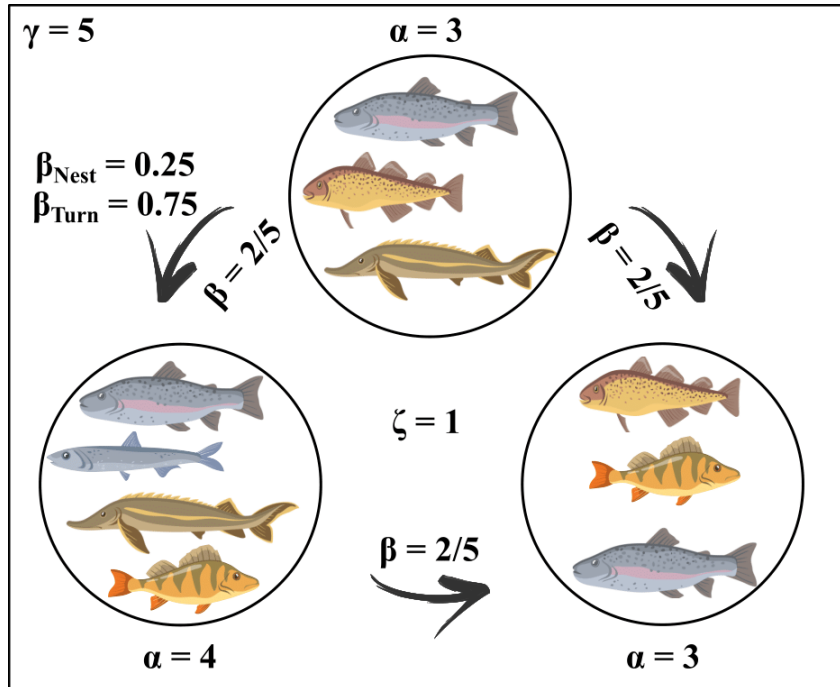


Figure 1. Biodiversity can be regarded at different spatial scales: α -diversity, β -diversity, γ -diversity and, ζ -diversity. β -diversity can be measured using different indices and decomposed into turnover and nestedness components following different frameworks. The framework presented in the figure comes from the β -diversity partitioning of Baselga, (2010) using the Jaccard index, one of the most frequently used index.

Traditionally, species diversity is expressed in three different ways. **α -diversity** is related to the assemblage diversity within a habitat or a local site and is usually expressed as the number of species (*i.e.*, species richness). **γ -diversity** is the measure of species richness in a region. **β -diversity** is a measure of difference between two or more species assemblages restricted to local habitats or sites within a region (Figure 1). The use of β -diversity allows predicting changes in biodiversity over the entire region (γ -diversity) from local biodiversity changes (α -diversity) through the measure of inter-site differences between local to regional species assemblages (Socolar et al., 2016). In other words, γ -diversity can be partitioned into α and β components using Whittaker's original multiplicative partitioning ($\beta = \gamma / \alpha$) (Whittaker, 1960). Therefore, β -diversity is in the strict sense of the definition the factor to which the diversity of a region exceeds the mean diversity of local sites within the region and is classically measured as the percentage of species shared (or not shared) between two assemblages (Figure 1) (Koleff et al., 2003). It reveals the spatial structuration of biodiversity (Baselga, 2012). The

most widely used ecological measures of β -diversity include the measures described by Jaccard, (1900) and Sorensen, (1948). In addition, β -diversity reflects two different phenomena, referred to as species turnover and species loss (or gain) (Baselga, 2010). Species turnover represents the differences in species compositions between sites independently from differences in number of species between sites (Baselga, 2010; Carvalho et al., 2012). Species loss (or gain) can be viewed as the species richness difference between sites (Carvalho et al., 2012) or as the nestedness (Baselga, 2010), a special case of an ordered pattern of differences in species richness (Carvalho et al., 2012).

Recently, a novel measure of biodiversity was proposed. The ζ -diversity measures the number of species shared by any number of assemblages or sites (Figure 1) (Hui & McGeoch, 2014). This measure was established to overcome the limitation of the measure of pairwise assemblage comparisons. In fact, because β -diversity is designed to determine pairwise difference between two assemblages, when multiple assemblages (> 2) are involved, the average of the pairwise differences is used. In this case, pairwise metrics cannot represent assemblage difference across multiple locations (Hui & McGeoch, 2014).

2.c. Multifaceted communities

Across scales, ecologists are interested in the spatial and temporal patterns of species distribution and diversity. These patterns have traditionally been conducted from a species diversity-centered approach, accounting for the taxonomic diversity (TD). This facet of biodiversity quantifies the species richness and composition of communities. However, this facet limits our comprehension of biodiversity distribution patterns because species identity provides little information on the evolutionary history and functional roles occupied by the species in the ecosystems (Swenson, 2011). This limitation suggests that a multifaceted approach of biodiversity should be more informative. Phylogenetic diversity (PD) is another facet of biodiversity that corresponds to the sum of branch lengths between root and tips for a community (Faith, 1992). The consideration of the phylogenetic facet of biodiversity allows exploring the evolutionary processes (*e.g.* biogeography history) that produce diversity patterns (Webb et al., 2002). Communities may also be described through the prism of the functional diversity (FD), which relates to the number and the diversity of functions that the species exercise in the ecosystems (Petchey & Gaston, 2006). FD is estimated from species characteristics called ‘traits’ that provide information on how species interacts with their environment (*e.g.* teeth type can indicate the position in the trophic chain) or with each other (Petchey & Gaston, 2006). PD can also approximate the FD of a community if traits

characteristics remain unchanged along the evolutionary time scales and that closely related species share ecological similarities (Devictor et al., 2010). This can be particularly useful because estimating functional diversity is difficult, as it requires selecting the most informative traits. However, PD and FD can also be decoupled in some cases such as convergent evolution, when similar characteristics appear in different lineages as, for instance, the result of a strong selection pressure (Devictor et al., 2010). Multifaceted studies of biodiversity provide complementary information on diversity patterns. For example, two communities may be equal in TD but composed of species with highly similar or different phylogenetic histories or traits (Devictor et al., 2010; Webb et al., 2002).

2.d. How are species assembled into communities

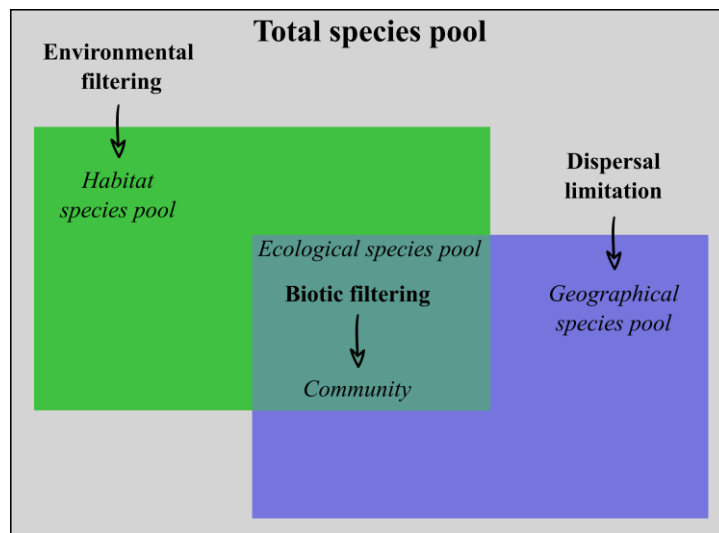


Figure 2. The different processes structuring communities. Adapted from Booth & Swanton, (2002)

MacArthur and Wilson’s theory of island biogeography was the pioneer of the modern community assembly theory, describing the dynamic of species dispersal to oceanic islands from the mainland (MacArthur & Wilson, 1967). Later, Jared Diamond first introduced the concept of assembly rules to study bird’s community in New Guinea, focusing on the role of biotic interactions in shaping locale communities, in contrast to island biogeography (Diamond, 1975).

Unifying these works, the study of community assembly (or assembly rules) systematically calls two central concepts that are ‘species pool’ and ‘filters’ integrating evolutionary and ecological concepts (Figure 2) (Kraft & Ackerly, 2014; Pearson et al., 2018). The species pool contains a suite of potential colonists for a specific community. The composition and abundance of these colonists in a community is determined by a series of hierarchical filters, representing abiotic and biotic barriers selecting the colonists based on species functional traits (Kraft &

Ackerly, 2014; Pearson et al., 2018). The different filters can be assigned to three discrete levels: (1) dispersal, (2) abiotic environment, and (3) biotic interactions (Figure 2) (Götzenberger et al., 2012). Importantly, community assembly is context-dependent and community-specific because each filter can vary in nature and strength among communities (Pearson et al., 2018).

From a total species pool, dispersal limitation determined by the geography and the biogeography history of a region shapes the geographical species pool and thus contains all the species that are able to arrive at a site. The habitat species pool contains all the species that can persist under the abiotic environments. This process is often referred to as environmental filtering. The species pool resulting from the overlapping of the geographical and the habitat species pool is the ecological species pool. It is then shaped by internal dynamics, the biotic interactions, such as competition or predation. The community is the subset of species persisting after these different levels of filtrations (Figure 2) (Booth & Swanton, 2002).

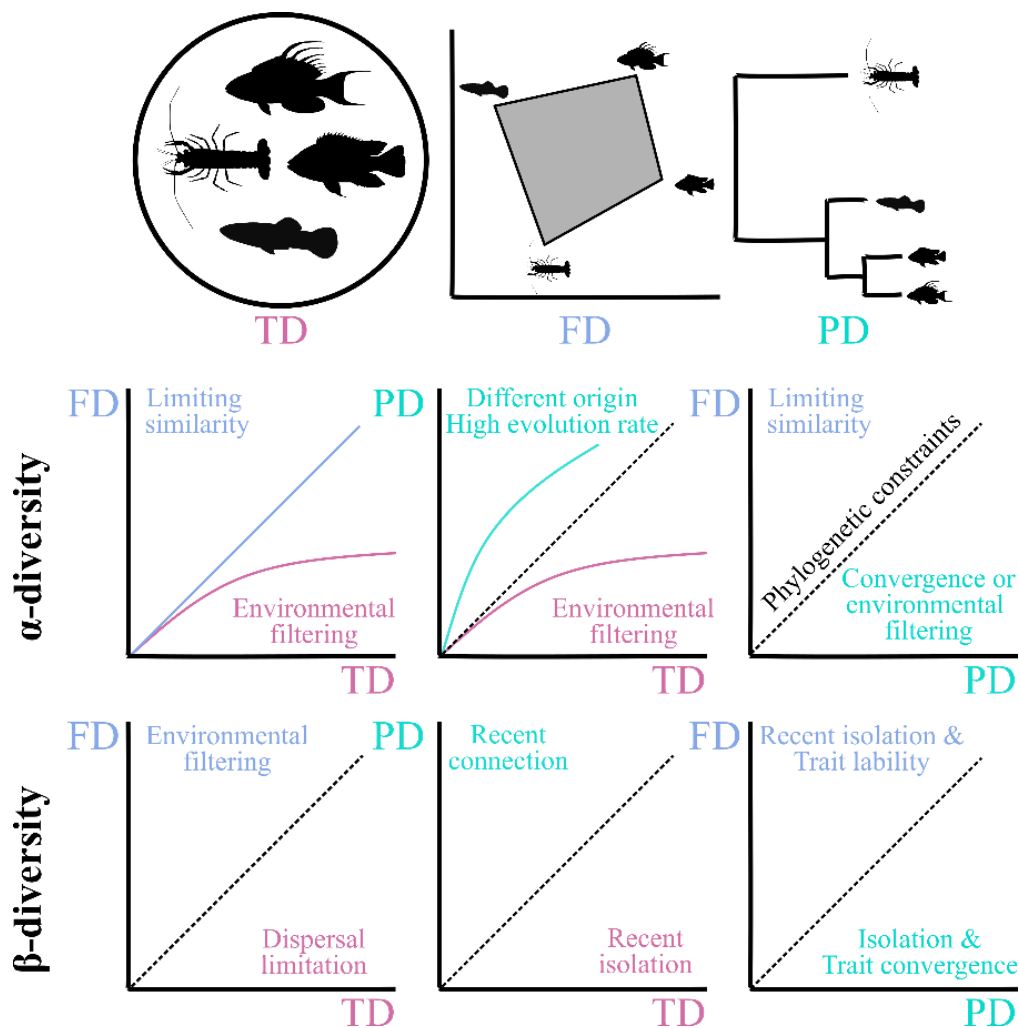


Figure 3. Comparing taxonomic, functional and, phylogenetic diversity can help to depict the assembly rules structuring communities within (α -diversity) and between (β -diversity) communities. Dominant processes are indicated on each plot.

While abiotic and biotic filters are determinist filters, there are also stochastic processes that determine assembly rules. Stochastic processes include species demography such as birth, death, immigration, speciation and, dispersal limitation and influence all level of species pools (Figure 2) (Hubbell, 2001).

2.e. Disentangling community assembly rules

Comparing the three diversity facets (TD, PD, and, FD) can help to disentangle the assembly rules that structures communities, both within and between communities. The following sections will describe the underlying processes between pairwise comparisons of biodiversity facets, but the three facets should be considered altogether to depict a complete picture of the processes defining community assembly rules.

α -diversity scale

Within communities, comparing functional and taxonomic diversity at the α -diversity scale can indicate whether assemblages are more structured by biotic interactions and particularly limiting similarity or environmental filtering. Limiting similarity occurs when species with similar characteristics are in competition, leading to competitive exclusion (Macarthur & Levins, 1967; Mouillot et al., 2007). Within an assemblage, if environmental filtering is stronger than biotic filtering, we should observe a trend toward functional redundancy resulting in a lower than expected functional diversity, given the observed taxonomic diversity, as species with similar suitable traits will persist in the community (Mouillot et al., 2007). Conversely, if limiting similarity is the dominant process, species will tend to be complementary and functional diversity will increase proportionally with taxonomic diversity (Figure 3) (Mouillot et al., 2007).

In another hand, the comparison of taxonomic and phylogenetic diversity can bring information about the species evolution history in the community. If the taxonomic diversity is higher than the phylogenetic diversity, it suggests that species derive from recent speciation events. If phylogenetic diversity is higher, it can indicate that species originates from different regions (following recolonization or introduction) or that evolutionary rate is accelerated due to strong selective pressures, barrier to gene flows or low species dispersal rates (Figure 3) (Safi et al., 2011; Xiang et al., 2004).

Finally, investigating the relationship between phylogenetic and functional diversity can also give information about the preponderance of environmental filtering or biotic filtering in

the community structuration. Generally, PD and FD are expected to vary proportionally, as it is expected that less closely related species are more functionally different. Nevertheless, a higher FD than PD can characterize communities under limiting similarity while the opposite pattern could signify a strong environmental filtering causing traits convergence, with distant species presenting similar ecological traits (Figure 3) (Safi et al., 2011).

β -diversity scale

Between communities, the main expectation is that dissimilar communities will present functional and phylogenetic differences as a consequence of isolation by distance and ecological differences. Deviation from this null hypothesis can shed light onto the dominant processes structuring communities in space. Between communities, a higher functional than taxonomic dissimilarity suggests that environmental filtering is stronger than dispersal limitation. This is the result of the selection of different sets of species in each community as a response to different environmental filters. Conversely, a higher taxonomic than functional dissimilarity evidences the dominance of a strong dispersal limitation. In this case, environmental barriers or limited dispersal abilities hampers the movement of species between communities, resulting in different sets of species, but that can be functionally similar (Figure 3) (Cilleros et al., 2016).

The comparison of phylogenetic and taxonomic dissimilarity between communities can help discriminate between dispersal limitation caused by recent or ancient isolation (Weinstein et al., 2014). If communities were historically isolated but recently connected, it should result in a higher phylogenetic than taxonomic dissimilarity between assemblages because the lineages evolved separately. Conversely, historically connected but recently isolated communities should exhibit the opposite pattern, as lineages evolved conjointly in communities but recent isolation results in speciation leading to higher taxonomic dissimilarity between communities (Figure 3) (Weinstein et al., 2014).

Lastly, if phylogenetic diversity does not reflect trait conservatism between closely related species, then it can inform on trait convergence or trait lability between communities sharing or not a common evolution of lineages. For instance, a higher functional than phylogenetic dissimilarity between communities indicates different environmental filtering in each community selecting different sets of traits. The lower phylogenetic dissimilarity reveals a recent isolation, and therefore common lineages between communities (Head et al., 2018). On the other side, a higher phylogenetic than functional dissimilarity between communities

demonstrates that there is a convergence of traits between distinct lineages (Figure 3) (Head et al., 2018).

3. Enhancing biodiversity distribution knowledge

3.a. Traditional inventory methods and biodiversity representativeness

The collection of information on species distribution throughout space and time constitutes the basis of ecological and conservation studies. Usually, the traditional methods to inventory biodiversity are specific and adapted to habitat types or species taxonomic groups. In aquatic ecosystems, a broad range of traditional methods based on the capture of individuals is employed such as toxicants, net or electrofishing to monitor fishes (Cilleros et al., 2019; Keck et al., 2022). Yet, these methods present several limitations. First, they are often time-consuming and labour-intensive and necessitates the interventions of experts for taxonomic identifications (Keck et al., 2022). Second, in aquatic ecosystems, these capture based-methods are often harmful to the fauna, leading to the death of the organisms (Cilleros et al., 2019; Fediajevaite et al., 2021; Keck et al., 2022). In small Neotropical streams for instance, electrofishing, deemed to be one of the less harmful capture-base methods, is hardly implementable due to the low conductivity of the water. Therefore, the most performant fish inventory method for small streams has long been rotenone, a natural toxin used to poison fishes (Allard et al., 2014). In addition to be destructive to the fauna, the implementations of capture-base methods is constrained by habitat types and species characteristics and are therefore species and habitat-selective (Cilleros et al., 2019; Fediajevaite et al., 2021; Keck et al., 2022). Attempts to develop electrofishing less selective than rotenone in small stream with low water conductivity have been conducted, but the protocols still require technical improvements, qualified experts to work with electricity and cannot be implemented in large courses (Pottier et al., 2020). In large Neotropical rivers, net sampling is the most frequently used methods, but only collects fish from a limited range of habitat (Cantera et al., 2019). The limits of traditional methods to inventory biodiversity have therefore encouraged the development of new methods that are more polyvalent and easily implementable, less destructive and providing a highest fauna representativeness (Cilleros et al., 2019; Fediajevaite et al., 2021; Keck et al., 2022).

3.b. Environmental DNA metabarcoding

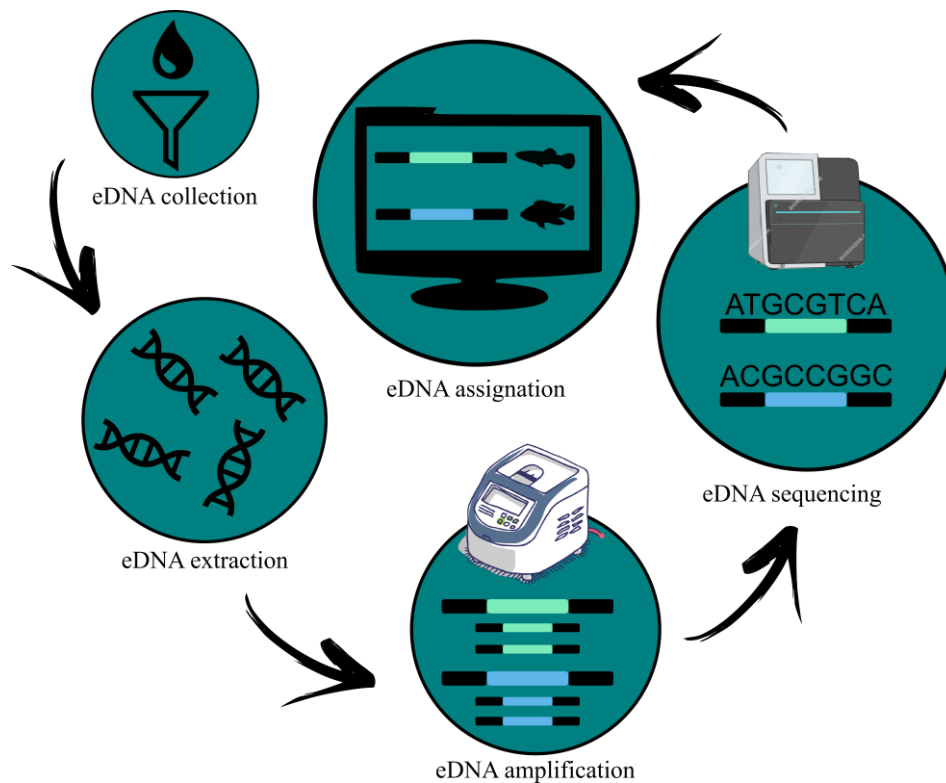


Figure 4. The different steps to build biodiversity inventories from environmental DNA metabarcoding.

Environmental DNA (eDNA) coupled to the metabarcoding approach has been intensively developed this past decade, to provide robust and representative inventories of the fauna (Taberlet et al., 2018). eDNA is the genetic material released by organisms in the environment, contained into cells, faeces, saliva or urine (Taberlet et al., 2018). The development and availability of next generation sequencing has allowed exploiting eDNA information to build multi-taxa inventories, bypassing the expensive and time-consuming step of Sanger-sequencing (Taberlet et al., 2012). The PCR-based approach for simultaneously identifying multiple taxa is named DNA metabarcoding (Riaz et al., 2011). It consists of selective amplifications, where a specific set of primers is designed to bind a region in the DNA that is conserved among all the species of interest. Conversely, the amplified region called barcode presents nucleotide variations that allow species discrimination (Taberlet et al., 2018). eDNA methods have deeply improved our assessments of biodiversity by resolving several constraints related to traditional methods and are presented as cheaper, faster, more representative, and more flexible to develop monitoring programs (Fediajevaite et al., 2021; Keck et al., 2022). Many studies comparing eDNA and traditional monitoring methods have reported an equivalent or even higher performance of eDNA to provide species presence/absence and occurrences (Cantera et al., 2019; Ficetola et al., 2015; Hänfling et al.,

2016; Hinlo et al., 2017; Mächler et al., 2016; Pilliod et al., 2013; Thomsen et al., 2012; Wilcox et al., 2016; Yamamoto et al., 2017).

Traditionally, eDNA metabarcoding involves 5 different steps: (1) The DNA is first collected from the environment. It is then (2) extracted from the samples, (3) amplified by PCR and, (4) sequenced. The last step is bioinformatic and consists in the taxonomic identification of the amplified DNA fragments by comparison with a molecular reference database (Taberlet et al., 2012; Tsuji et al., 2019) (Figure 4).

In aquatic environment, the first study describing microorganism communities from eDNA was conducted in 2004 (Venter et al., 2004). It is however later in 2008 that a study aiming at detecting the American bullfrog (*Rana castesbeiana*) brought eDNA barcoding at the vanguard of the detection of aquatic vertebrates (Ficetola et al., 2008). Since 2008, independent groups of researchers all over the world developed eDNA metabarcoding analysis techniques, contributing to methodological advances of the method (Shu et al., 2020; Tsuji et al., 2019).

eDNA collection

Riverine environments act as eDNA collectors, receiving DNA from the aquatic but also the surrounding faunas. DNA is homogenised throughout the water column and integrates biodiversity information at a wider scale than immobile environments (Valentini et al., 2016). In water, the DNA is traditionally collected using filters that retain cells or fine particles on which DNA is bound. In this step, two parameters that can critically influence the data are the type and characteristics of the filters (Eichmiller et al., 2016) as well as the volume of filtration (Mächler et al., 2016). Filters can vary in type (cartridge or disk membrane), pore size, surface of filtration or membrane constituents (Tsuji et al., 2019). Similarly, the volume of filtration used in the different studies varies greatly, from 200 mL to 360 L.

eDNA extraction

eDNA extraction is a critical step, as it determines the quantity of DNA that is further amplified and sequenced. The choice of the extraction technic has been shown to strongly affect the detection of DNA (Minamoto et al., 2016). To extract DNA, the most used method is the commercial DNA extraction kit, which represents approximately 75% of the studies since 2008. The second most used method is the liquid phase separation method, encountered in 18% of the studies since 2008 (Tsuji et al., 2019).

eDNA amplification and sequencing

In the amplification step, the choice of the primer set is widely discussed in the literature and is possibly one of the most influential parameters determining the detection probability of the targeted species (Zhang et al., 2020). Different universal primer sets have been developed for diverse taxa. They target mitochondrial DNA, which is present in largest number of copies in cells than nuclear DNA, but also possesses higher mutation rates and is often more covered in genetic databases (Goldberg et al., 2016).

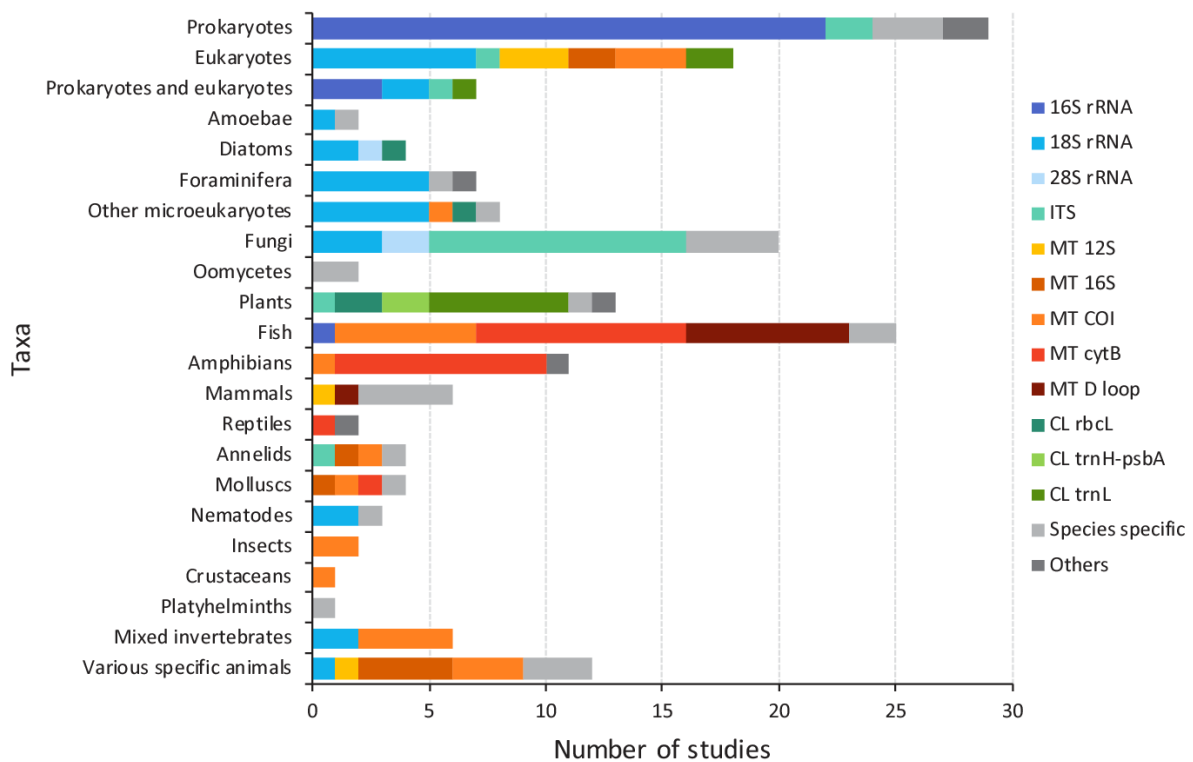


Figure 5. Summary of different primer sets used in the literature for diverse taxa. The figure comes from Lear et al., (2018). rRNA indicates ribosomal RNA genes, MT indicates mitochondrial genes, and CL indicates chloroplast genes.

There is currently no consensus about the ideal primer set and this choice is conditioned upon the availability of reference sequences but also the taxonomic group of interest as the target region in the DNA maybe more or less conserved depending on the taxonomic group (Lear et al., 2018). The number of PCR to conduct per sample can also be an important parameter and increases the detection probability of the species (Ficetola et al., 2015). In the literature, PCR replicates vary from 2 up to 12, but increasing PCR replicates inflates costs and workload and might also increase the rate of false positives while reducing the risk of false negatives (Ficetola et al., 2015).

During the sequencing stage, the sequencing depth and the sequencing platforms are additional parameters that influence taxa detection. The reproducibility of taxon coverage and composition of a sample was shown to improve with increasing sequencing depth per taxon (Smith & Peay, 2014). In the same study, illumina MiSeq platform retrieved higher species richness than 454-pyrosequencing platform.

eDNA identification

Bioinformatic processing of the amplified DNA fragments is a pivotal aspect of eDNA metabarcoding as the stringency of the sequence filtering steps influences the resulting biodiversity data (Evans et al., 2017). The different bioinformatic steps including sequence cleaning and identification are often gathered in a bioinformatic pipeline that can ensure the standardization and therefore the reproducibility of the results (Xiong et al., 2022). Bioinformatic analyses are generally performed using two distinct approaches.

The first approach uses the clustering of molecular operational taxonomic units (MOTU) that are clusters of reads differing by less than a fixed sequence dissimilarity threshold, usually 3% (Callahan et al., 2017). The pipelines based on the constructions of customary MOTU involves multiple quality assurance steps including sequence merging and trimming, removal of the low quality and short reads, removal of chimeras and singleton originating from PCR errors and then sequence dereplication and denoising. The taxonomic assignation is then achieved through a comparison of MOTU clusters against a reference database, using a similarity threshold (Xiong et al., 2022).

The second approach relies on amplicon sequence variants (ASVs), which implies inferring the biological sequences before the introduction of amplification and sequencing errors. With ASV methods, biological sequences are discriminated on the basis that they should be more observed than error sequences, as these errors are created throughout the PCR cycles and are therefore not present from the beginning (Callahan et al., 2017). In the pipeline, the number of time each sequence was read is first determined, and the data are then combined with an error model to determine the probability that a given read at a given frequency is not due to sequencer error. The sequences are then filtered using a threshold value to only keep the biological sequences. The taxonomic assignation can be achieved as for the MOTU approach, with comparisons to a reference database using a similarity threshold (Prodan et al., 2020).

Although MOTU approaches are still widely used, it has been claimed that ASVs are more practical. Unlike MOTUs, ASVs represent a biological reality existing outside the analysed data, therefore allowing comparison between different samples. In addition, ASVs can

also capture all biological variation and are not limited by the completeness of the reference databases like MOTUs (Callahan et al., 2017).

3.c. Major limitations and advances

eDNA metabarcoding is increasingly used to measure biodiversity, providing multiple benefits and sometimes outperforming traditional methods (Fediajevaite et al., 2021). First, it does not require the expertise of taxonomists as for traditional methods often relying on visual identification of the species and DNA samples may be collected by non-specialists. No specimens are needed, and the method is therefore harmless to the fauna. Second, the field step is easy to conduct compared to most traditional methods, which is particularly adapted to survey difficult to access or remote areas. Third, DNA extracted from environmental samples can simultaneously provide information about the distribution and occurrence of species across multiple branches of life (Fediajevaite et al., 2021; Lear et al., 2018).

Nevertheless, eDNA metabarcoding face several limitations. First, the method implies a succession of steps, each potentially introducing its own biases. In each of these steps, choices have to be made among different parameters that can critically influence the biodiversity information obtained. This led researchers to develop a consequent number of protocols, challenging the production of high-quality and reproducible data and restraining comparisons between studies (Zinger, Bonin, et al., 2019).

Another limitation of the method is the type of data collected. Protocols are deemed robust enough to provide presence/absence and occurrence information on species, but obtaining quantitative data such as species abundance is still at the proof-of-concept stage (Elbrecht & Leese, 2015; Pont et al., 2018). They are existing reliable methods to quantify eDNA such as quantitative PCR or Droplet Digital PCR. Nevertheless, multiple biases at the different steps of the protocols may skew the quantitative output. For instance, they can be PCR inhibitors during the collection steps, differential DNA extraction success, or different amplification rates between different species (Lamb et al., 2019). Moreover, besides methodological biases, the most important limitation lies on whether there is a relationship between eDNA quantity and species stocks (Pont et al., 2018). The DNA release in the environment may be condition upon species characteristics such as metabolic rates, body size or behaviour and a correlation between species stock and eDNA quantity may not always be verified (Lamb et al., 2019; Nichols et al., 2018).

Despite multiple limitations, methodological advances are progressively overcoming certain sources of biases in the protocols. For instance, eDNA metabarcoding is a PCR-

amplification based approach which introduces various sources of biases (Elbrecht & Leese, 2015; Nichols et al., 2018). Primer-template mismatches influence the quantity of amplified DNA, resulting in varying quantity of amplified DNA depending on the species. In addition to limit quantitative information, it can even lead to the non-detection of species present in a sample (Ficetola et al., 2008). PCR can also generate chimeric molecules or point mutations (Coissac et al., 2012). To circumvent the PCR-biases, PCR-free approach are being developed, such as shotgun sequencing or mitochondrial enrichment. These two methods remained however costly because requires high sequencing depth (Dowle et al., 2016). Hybridization capture is another promising approach. With this approach, instead of amplifying a region of interest, the targeted region is hybridized to complementary DNA or RNA probes bound to a magnetic bead while the other sequences are removed (Dowle et al., 2016; Wilcox et al., 2018). While the method is often used in human genomic research, phylogenetic and evolutionary studies, it has seldom been applied to environmental biomonitoring (Dowle et al., 2016). A recent study developed a new approach to overcome the quantity of species-specific probes that would need to be design in species rich communities (Mariac et al., 2018). They used a single probe able to capture all main fish orders of the Amazon basin, which relies on the COI of a distant species absent from the study area (*Danio rerio*). The rationale behind is that because hybridization capture can tolerate high divergence between probes and targets, a single probe that is equidistant from all the species of the study area can successfully estimate species diversity and frequencies (Mariac et al., 2018; Seeber et al., 2019).

Another promising advance targets the bioinformatic process of the amplified sequences. While laboratory molecular steps are being optimised, major technical challenges have shifted from the development of efficient sampling and laboratory protocols to the processing of large set of amplified DNA fragments into taxonomic lists (Flück et al., 2022). A recent study proposed to use deep learning and more specifically convolutional neural networks (CNNs) to speed up environmental DNA annotation (Flück et al., 2022). They showed that CNNs were able to process raw fastq files 150 times faster than with OBITools, a widely used MOTU-based bioinformatic pipeline (Flück et al., 2022).

4. Amazonian freshwaters, conservation challenges

4.a. The species diversity eldorado

The Neotropics harbour the largest concentration of biodiversity on Earth for many taxonomic groups. Amazonia is the world's most diverse rainforest, but is also the region on

the American continent that contributes the most to its total biodiversity (Antonelli et al., 2018). This is particularly true for freshwater ecosystems that cover 14 to 29% of the 5,500,000 km² Amazonian surface (Castello et al., 2013). The total catchment extends over 7.05 million km² and stream/river surface area cover 93,800 km², including the Amazon and Orinoco basins but also smaller coastal basins of the Guianas (Albert et al., 2020). These waters are home to approximately 6,080 fish species, although dozens of new species keep being described each year. It represents 40% of all known freshwater fishes (Albert et al., 2020).

The exceptional Amazonian fish diversity can be explained by the action of different processes. At the scale of the basin, fish assemblages in the Amazonian region are characterized by a high α -diversity and β -diversity. A high α -diversity can reflect, from a functional point of view, the coexistence of many complementary species determined by biotic interactions and notably limiting similarity. A high β -diversity indicates species sorting based on traits among habitats and therefore reflecting environmental filtering (Albert et al., 2020; López-Delgado et al., 2020). Across river basins, biogeographical processes such as the dynamic of connexion and isolation of river basins are also at stakes. Movements and dispersal processes of freshwater fishes are limited by the dendritic structure and the isolation of rivers. Consequently, speciation events have been occurring independently in each basin, generating a high taxonomic diversity (Carvajal-Quintero et al., 2019).

4.b. A threatened reservoir of resources and services

Because of the unique habitats and species diversity, Amazonia provides irreplaceable services to human societies. Amazonian freshwater ecosystems are connected to atmospheric, terrestrial, and oceanic ecosystems via the hydrologic cycle (Castello et al., 2013). Other key services include water quality and irrigation or food and fibre production (Castello & Macedo, 2016).

These ecosystems are also a reservoir of resources, heavily and increasingly exploited (Figure 6). Water is exploited for the production of energy as well as agricultural and cattle ranching activities. The water demand has driven the proliferation of dams, with currently 154 hydroelectric dams and 277 dam projects (these counts do not include small dams) (Figure 6) (Castello et al., 2013). The aquatic biodiversity, and notably fishes, represent an important source of food and some species are overharvested. Amazonian freshwater ecosystems also possess minerals such as gold. Gold mining activities have known a surge after the years 2000 due to an increase of 360% of gold prices (Castello & Macedo, 2016).

There are important conservation issues around Amazonian ecosystems as they face a consistent increase of threats and alterations, but also because our knowledge of the biodiversity inhabiting this region and its distribution is limited. Understanding the determinants and the processes structuring this diversity is more complex than in other regions. Consequently, estimating how biodiversity responds to the increase of anthropogenic pressures and conceiving adapted conservation strategies is laborious.

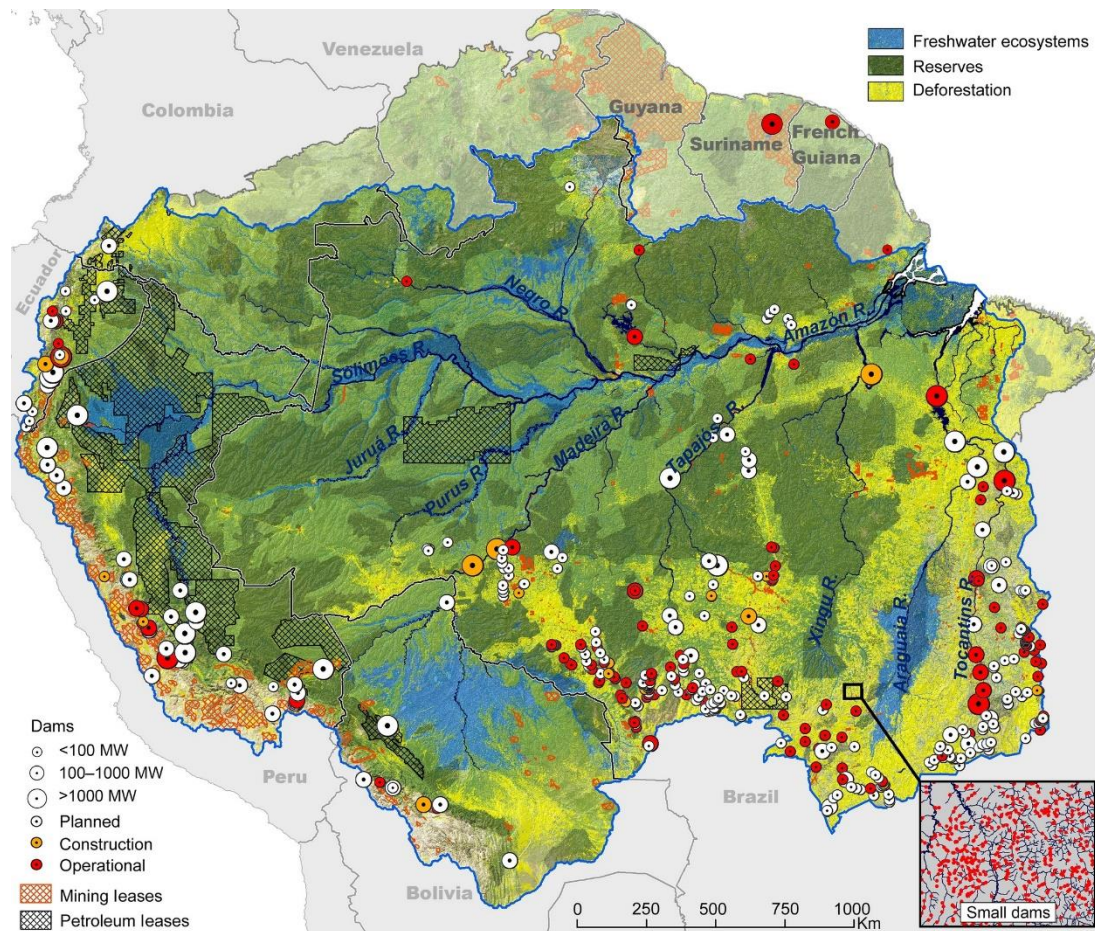


Figure 6. The diversity of anthropogenic threats driving hydrological alterations in Amazon freshwaters. Figure from Castello & Macedo, (2016).

4.c. French Guiana, a case of study

French Guiana is an overseas French department, located in Amazonia in the Guiana Shield. It is bordered by Suriname and Brazil and occupies a surface of 83,846 km². In January 2019, French Guiana was inhabited by 281,678 people with a very weak population density of 3.4 people per km² (INSEE, 2020). One of the unique feature of French Guiana is its single forest block expending over more than 90% of the territory. Another particularity is its water resources with a hydrographic network extending over 112,000 km split in seven river basins.

This department is ranked among the world's top three countries in terms of water resources per capita.

French Guiana is home to a fauna extremely diversified and harbours more than 1500 vertebrates species. For example, it hosts approximately 350 described strict freshwater fish, although new species are regularly discovered but remained undescribed (Melki, 2016). Among them, 87 species are considered endemic to the territory (Le Bail et al., 2012). This tremendous diversity is probably related to the paleogeographic history of the Guiana Shield, with the prevailing hypothesis suggesting that this area was a quaternary glacial refugium offering a stable forest during periods of aridity.

Despite being one of the most preserved Amazonian region (de Souza et al., 2020), the territory faces a rapid increase of anthropogenic pressures. This is notably due to one of the highest demographic growth of South-America (2.4%) (Roser et al., 2013), generating an economic growth and consequently, an increase of anthropic activities. However, the most severe threat remains gold mining. In French Guiana, it represents approximately 40% of all deforested surfaces (Cantera, Coutant, et al., 2022). The first gold deposits were discovered in 1855, but the prospections did not last over time due to the harsh environmental constraints. The first important gold rush started at the end of the 1800s, but remained non-mechanised and declined with the rise of the gold price in the years 1930-1960. A second gold rush started in the 1970s with the prospection campaigns led by the BRGM (Bureau de Recherches Géologiques et Minières) and with a global inflation of the gold price (Transler et al., 2006) (Figure 7). Gold mining generates drastic alterations of the environment. The technics employed to extirpate gold from the riverbed remobilise the natural mercury contained in the soils. In its natural state, mercury does not affect biodiversity, but it becomes harmful when it is methylated. In addition, illegal gold miners also introduce exogenous mercury to extract gold. During gold extraction, these two sources of mercury are methylated and accumulates along the trophic chains. Gold mining also generates ecological impacts such as deforestation, riverbed erosion, changes of water chemistry such as critical increase of turbidity. It results in a cascade of impacts altering diverse biodiversity compartments not only restricted to aquatic biodiversity (Aertgeerts et al., 2018; Transler et al., 2006). Besides affecting biodiversity, local human populations that are strongly dependent on freshwater resources are at the forefront of the impacts generated by this industry. In French Guiana, there are two ethnic groups (the Amerindians and the Bushinengués¹) settled along the rivers and relying on subsistence hunting

¹ The Bushinengués are the descendants of enslaved Africans that escaped from the plantations and settled in the inlands.

and fishing. This territory thus presents important local and global environmental conservation challenges because it hosts a rich diversity that is being increasingly affected, but it is also home to local indigenous populations that are heavily dependent on these biodiversity resources.

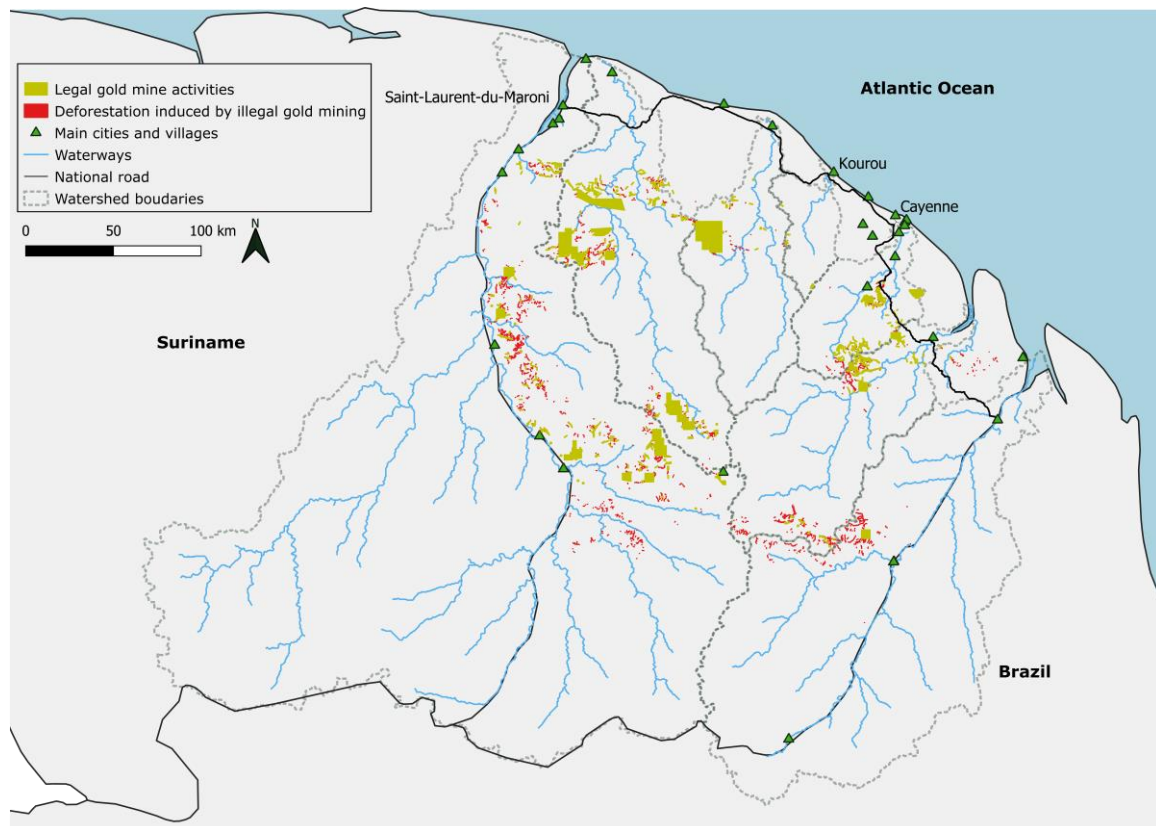


Figure 8. Map of the areas harbouring legal and illegal gold mines in French Guiana. For deforestation resulting from illegal gold mining, each red square is a 1 km square in which a mine was censused. Information on illegal gold mining activities are provided by the Parc Amazonien de Guyane and the Observatoire de l'Activité Minière (OAM). Legal gold mining activities are shared by the DEAL Guyane (2020)

5. Thesis objectives

This PhD provides comprehensive knowledge on the impacts of anthropogenic disturbances on freshwater biodiversity, but also the impacts of a modified biodiversity on local human populations. This PhD thus aims at investigating the consequences of anthropogenic disturbances on the vertebrate diversity that inhabit freshwaters, mainly fishes but also mammals. To understand these impacts and predict biodiversity responses, it is also necessary to investigate the spatial structuration of fish biodiversity at the scale of the territory and quantify the contribution of the different determinant shaping this biodiversity. The PhD also aims at encouraging the development of multidisciplinary approaches between social and ecological science to understand how indigenous societies are affected by the alteration of their resources that is biodiversity. To answer this question, biodiversity inventories will be realised

using environmental DNA metabarcoding. An innovative method to assess biodiversity that will also be optimised during the PhD, mainly by testing its robustness and by extending its application field. The PhD is divided into three chapters:

1) Optimising and extending the application of environmental DNA

This chapter is articulated in two parts. In the first part, we investigated the robustness of our environmental DNA metabarcoding approach by comparing fish community richness and composition of 12 streams and 3 rivers from French Guiana, sampled with two distinct protocols commonly used in aquatic eDNA studies. Testing the robustness of datasets to protocol variations is crucial to valorize old data, time series, or data collected in difficult to access locations. Here, I show that ecological results are poorly influenced by the differences between protocols, therefore underlining the robustness of our method.

In the second part of this chapter, we tested a new application of the sampling methods. We used environmental DNA collected from running water to detect aquatic, semiaquatic, terrestrial and arboreal mammal fauna along three rivers of the Amazonian biome. Water acts as a DNA collector and receives eDNA from the surrounding landscape. Moreover, as it is a mobile substrate, it integrates biodiversity information at a larger scale than immobile substrates such as soil. To estimate the reliability of this application, we used a comparative analysis between aquatic eDNA metabarcoding and standardized visual faunistic inventories classically used for mammal monitoring in French Guiana. Here, I demonstrate that aquatic eDNA metabarcoding provide relevant information on mammal distribution, complementary to that obtained with traditional method.

2) Investigating biodiversity distribution patterns at local and regional scales to assess anthropogenic impacts on biodiversity

This chapter gathers two studies with different research questions, but remains centred around the assessment of anthropogenic impacts on freshwater fish and mammal communities. In the first study, we used environmental DNA metabarcoding to evaluate the relationships between vertebrate biodiversity (fish and mammals) and disturbance intensity in two Amazonian rivers. Given the hydrologic connectivity of river networks, the fauna inhabiting river catchments may be affected by remote disturbances via the water-mediated downstream transfer of matter, energy, and/or organisms. We measured the spatial extent and the strength of anthropisation on biodiversity by analysing the relationships between the diversity of local

fauna and disturbance intensity measured at multiple scales. We show that low levels of anthropisation are responsible for drastic declines of fish and mammals species. In addition, we demonstrate that these declines are better explained by the integration of deforested surfaces over an area of 30 km upstream the sites than the deforested surfaces in the vicinity of the sampling sites, therefore illustrating the spread of anthropogenic disturbances along the river.

In the second part of this chapter, we shifted to a regional investigation of biodiversity by quantifying the relative contribution of geographical, environmental and anthropic factors to taxonomic and functional diversity distribution patterns. We then realised biodiversity predictions to map spatial biodiversity over the entire French Guianese hydrographic network and assess the representativity of sites surveyed within the EU Water Framework Directive and highlighted areas that should host unique freshwater fish assemblages. Here, I emphasize the contribution of the major determinants of taxonomic and function fish diversity. While the basin isolation mainly explained the taxonomic fish distribution between basins, I show that fish are also structured by limited dispersal capacities within basins. Contrastingly, I demonstrated that environmental sorting and anthropogenic activities mainly explain the functional distribution. Importantly, modifications of functional assemblages generated by human activities are directed towards fish species that possess unique functional attributes in the assemblages.

3) Human responses to biodiversity modifications

The last chapter of the PhD is exploratory. We used a multidisciplinary approach combining sociological and ecological sciences to understand how human population are affected by the modification of biodiversity and how they adapt to the depletion of the resources. To answer this question, we sampled different sites along a river facing different level of anthropogenic pressures. In parallel, at the same sites, we realised social surveys of biodiversity perception with local Amerindians from the Wayãpi and Teko ethnic groups. Here we demonstrated that despite possessing accurate knowledge of biodiversity distributions, the biodiversity perceptions of Amerindian populations is influenced by the presence of gold mining activities, by subsistence hunting and fishing, and by environmental factors involved in the structuration of fish and game species diversity.

Chapter I. Optimising and extending the application of environmental DNA metabarcoding

1. Part 1

Detecting fish assemblages with environmental DNA: Does protocol matter? Testing eDNA metabarcoding method robustness

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Abstract

Environmental DNA (eDNA) metabarcoding has recently gain much attention to assess aquatic environment biodiversity. A great variety of protocols have been developed to collect, extract and analyse eDNA, some of which are continuously evolving and optimized with technological improvements. Such technological shifts might deprecate the biological data produced with earlier protocols, leading to a loss of biological knowledge. Here we investigated the robustness of an aquatic eDNA metabarcoding method through the comparison of two biodiversity datasets generated by two optimized protocols with different collection and extraction steps. To this end, we compared fish community richness and composition of 12 streams and 3 rivers from French Guiana, sampled with two distinct protocols commonly used in aquatic eDNA studies. Although samples collection with each protocol were not achieved the same year, our results show that species richness and species composition were only slightly affected by the protocol choice, both protocols producing similar fish assemblages at each sample site. Both protocols had a higher replicability in streams than in rivers, strengthening the importance to adapt sampling effort to waterbody type as rivers host a larger number of species than small streams. Despite the need for a standardized approach in eDNA metabarcoding studies, testing the robustness of datasets to protocol variations remains crucial to valorise old data, time series or data collected in difficult to access locations.

Introduction

Environmental DNA (eDNA) methods have recently emerged as non-invasive powerful methods to inventory biodiversity in a wide range of ecosystems (Taberlet et al., 2018; Zinger et al., 2020). The recent striking progress in DNA sequencing technologies allowed to shift from single species detection (eDNA barcoding) (Ficetola et al., 2008) to the simultaneous detection of entire species assemblages (eDNA metabarcoding) (Taberlet et al., 2018; Valentini et al., 2016). A particular interest has been devoted to the development of eDNA-metabarcoding studies in aquatic environments (Rees et al., 2014) as water acts as a recipient for DNA, allowing an integrative assessment of aquatic biodiversity (Valentini et al., 2016; Zinger et al., 2020). Among those studies, eDNA fish inventories were particularly effective and often outperform traditional sampling methods (Cantera et al., 2019; Fujii et al., 2019; Pont et al., 2018). Indeed, Cilleros et al., (2019) showed that eDNA fish inventories are not biased by distinct detection probabilities among species or environments whereas traditional capture

methods are species selective (e.g. size-dependence for gillnets) or inefficient in some habitats (e.g. deep or encumbered areas for electrofishing and seine nets, respectively) (Fujii et al., 2019). The flourishing development of eDNA-based studies led to the development of various eDNA sampling and laboratory protocols but robust experimental designs allowing between-studies comparisons are still lacking (Bylemans et al., 2018; Dickie et al., 2018; Zinger, Bonin, et al., 2019; Zinger et al., 2020).

The methods developed for aquatic eDNA metabarcoding studies generally follow three major steps: (i) eDNA collection and preservation; (ii) eDNA extraction; and (iii) eDNA detection (Tsuji et al., 2019) with strong variations in each step depending on the studies. In fact, researchers developed various strategies for aquatic eDNA-based studies adapted to field specificities and protocols are rapidly evolving with technology and molecular biology advances. For instance, the protocol of Valentini et al., (2016) for running waters has widely been used from 2014 to 2016, before being replaced by the protocol of Pont et al., (2018) since 2016 (Figure 1A). The differences between those protocols lie primarily in the eDNA collection method. Both above cited protocols use filtration with enclosed filters to collect eDNA, this filter type being recognised as more efficient than “open filters” such as disk membranes (Li et al., 2018; Spens et al., 2017). Nevertheless, enclosed filter membrane can vary in surface, porosity and composition. For instance, filters with larger pore size and membrane surface are less sensitive to clogging and allow filtering larger water volumes than those with smaller pore sizes, but might also be less efficient to capture eDNA. Importantly, such variations can affect the probability of species detection as they are influencing the amounts of captured eDNA (Capo et al., 2019; Li et al., 2018; Takahara et al., 2012; Turner et al., 2014). In addition, the membrane composition also influences the quantity of eDNA retrieved (Deiner et al., 2018; Hinlo et al., 2017; Majaneva et al., 2018; Shu et al., 2020). The extraction step appears more consensual, with most studies processing eDNA with commercial DNA extraction kit (Lear et al., 2018; Tsuji et al., 2019). In the same way, most studies agree that during the amplification process, the number of PCR replicates is important to minimise missing detection of taxa that are present (false negatives) (Ficetola et al., 2015), with 3 PCR replicates being the lower limit to get relevant data (Pont et al., 2018; Rees et al., 2014; Valentini et al., 2016) and up to 12 replicates when detection probabilities are low (Ficetola et al., 2015). Similarly, sequencing depth was also claimed to be an important parameter to increase the detection probability of rare taxa (Smith & Peay, 2014). Aquatic eDNA-metabarcoding studies hence exhibit considerable variations in the protocols. Considering that those variations can impact species

detection probability, it can be expected that different protocols will produce distinct biodiversity measures for the same sampled fauna.

Here we investigate the robustness of an aquatic eDNA metabarcoding method through the comparison of two biodiversity datasets generated by two different protocols. Those two protocols differ mainly in the eDNA collection step, each protocol using a distinct filter, with different membrane composition, surface and pore size, leading also to different volumes of filtered water and in the extraction step, with two different extraction kits and different sample preparation prior to the extraction (Figure 1A). Those two protocols were used in many rivers and streams throughout the world including Europe, South America and, Asia (Cantera et al., 2019; Cilleros et al., 2019; Civade et al., 2016; Dufresnes et al., 2019; Lopes et al., 2017; Milhau et al., 2019; Pont et al., 2019; Sasso et al., 2017; Spitzen - van der Sluijs et al., 2020; Vimercati et al., 2020). It is therefore important to test the robustness of eDNA results to such variations in protocols to determine if the data acquired using distinct protocols deserve to be merged and ultimately if they can, or not, be used jointly for biodiversity and conservation studies. This is of particular importance for the studies replicated over years with temporal changes in the protocols, as well as for data sampled in remote and difficult to access locations.

Materials and Methods

1. eDNA collection

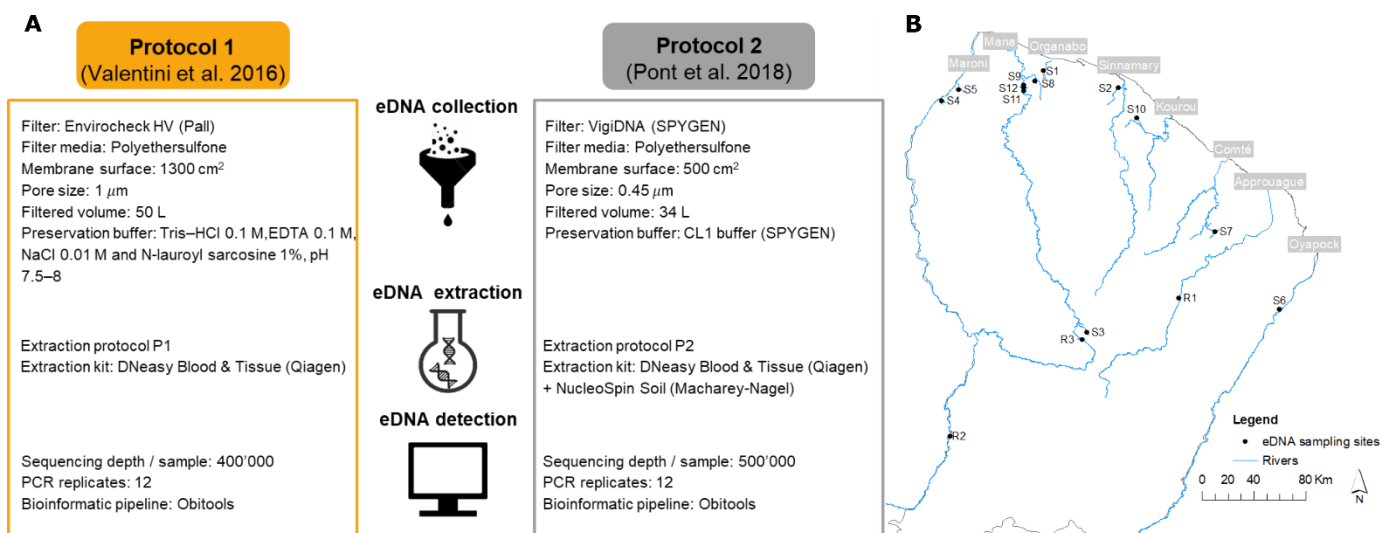


Figure 1. eDNA workflow for the two considered protocols (A) and sampling site locations (B).

This study was conducted in French Guiana (Latitude 2 to 6°N; Longitude 51 to 54°W). This 80, 000 square kilometre territory is part of the Guiana shield and bordered by Brazil to

the South and East and by Suriname to the West. Climate is equatorial with annual rainfall ranging from 3,600 mm (North-East) to 2,000 mm (South and West), and most of the territory (90%) is covered by primary forest. French Guiana also possesses a dense hydrographic network constituted of six major watersheds and several coastal rivers. Altogether, these freshwater bodies constitute a hotspot of freshwater fish diversity and endemism with at least 367 strictly freshwater fish species (Keith et al., 2000). eDNA collection was achieved using two different sampling protocols, protocol 1 (P1) described in (Valentini et al., 2016) and protocol 2 (P2) described in (Pont et al., 2018). These protocols were developed by SPYGEN and optimised with large volumes of filtered water, high number of PCR replicates and with the amplification of short fragments. P1 and P2 were used to inventory fish fauna from 15 rivers and stream sites in French Guiana (Figure 1B and Table S1). P1 was used to collect eDNA in 2014 and 2015 by Cilleros et al., (2019). 15 sampled sites were selected for which the bioinformatic pipeline was reprocessed to perform taxonomic assignments based on an updated reference database (containing 24 species more than the one used in Cilleros et al., (2019)). P2 was used to collect eDNA from 2016 to 2019 on the same 15 sites and the taxonomic assignments were performed based on the same updated reference database. Differences between P1 and P2 are provided in Figure 1A and the general framework of the sampling was as follows. All the samples were collected in November (dry season) and none of the sites experienced environmental or anthropogenic changes during the study period. The 15 sites (3 large rivers and 12 streams) were sampled using encapsulated filtering cartridges (Figure 1B and Table S1). In each site, one field replicate was performed by filtering a water volume of c. 50L for P1 (Envirocheck HV, PALL, USA) and c. 34L for P2 (VigiDNA, SPYGEN, France). A peristaltic pump (Vampire sampler, Burlke, Germany) and disposable sterile tubing were used to pump the water through the encapsulated filtering cartridges. The input part of the tube was held a few centimetres below the surface in rapid hydromorphologic units to allow a better homogenisation of the DNA in the water column. When filters began to clog, the pump speed was decreased to avoid material damages. To minimize DNA contamination, the operators remained downstream from the filtration either on the boat or on emerging rocks. After filtration, the capsules were filled with a preservation buffer and stored in the dark at room temperature for less than a month before DNA extraction. The three river sites (R_1 to R_3) were wider than 30 meters and deeper than 1 meter. The 12 stream sites (S_1 to S_{12}) were less than 15 meters wide and 1 meter deep. The sites belong to different watersheds (Maroni (R_2 , R_3 , S_4 , S_5), Oyapock (S_6), Sinnamary (S_2), Organabo (S_1), Mana (S_3 , S_8 , S_9 , S_{11} , S_{12}), Approuague (R_1), Comté (S_7) and Kourou (S_{10})), and account for various environmental conditions and fish

species diversities (Figure 1B and Table S1). Among sites turbidity varied from 0.28 to 16 NTU, pH from 4.78 to 7.64, temperature from 22.3 to 31.1 °C, conductivity from 15 to 54 μS^1 , and O₂ saturation from 45.1 to 111 %, encompassing most of the range of freshwater conditions encountered in French Guiana.

2. eDNA extraction

Extraction protocols followed different procedures for P1 and P2 (Figure 1A). Detailed extraction procedures for each protocol are as follows.

For P1, extraction was performed according to Civade et al., (2016)'s instructions. Filtration capsules were left at 56°C for 2 hr, agitated manually for 5 min and then emptied into three 50 mL tubes. In total, ~120 mL was divided among three tubes that were centrifuged for 15 min at 15,000g. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of each tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50 mL tube. The three tubes were centrifuged at 15,000g for 15 min at 6°C, and the supernatant was discarded. After this step, 360 μL of ATL Buffer of the DNeasy Blood & Tissue Extraction Kit (Qiagen) was added to the first tube, the tube was vortexed, and the supernatant was transferred to the second tube (Tréguier et al., 2014). This operation was repeated for all tubes. The supernatant of the third tube was finally transferred to a 2 mL tube, and the DNA extraction was performed following the manufacturer's instructions. Four negative extraction controls were also realised, amplified and sequenced in the same way as the field samples to highlight possible contaminations.

With P2, the extraction procedure was achieved according to Pont et al., (2018)'s instructions. For DNA extraction, each filtration capsule was agitated for 15 min on an S50 shaker (cat Ingenieurbüro™) at 800 rpm and then emptied into a 50-mL tube before being centrifuged for 15 min at 15,000 \times g. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of the tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50-mL tube and stored for at least one night at -20 °C. The tubes were centrifuged at 15 000 \times g for 15 min at 6 °C, and the supernatants were discarded. After this step, 720 μL of ATL buffer from the DNeasy Blood & Tissue Extraction Kit (Qiagen) was added. The tubes were then vortexed, and the supernatants were transferred to 2-mL tubes containing 20 μL of Proteinase K. The tubes were finally incubated at 56 °C for two hours. Afterwards, DNA extraction was performed using NucleoSpin® Soil (MACHEREY-NAGEL GmbH & Co., Düren Germany) starting from step six and following

the manufacturer's instructions. The elution was performed by adding 100 μ L of SE buffer twice. Four negative extraction controls were also realised, amplified and sequenced in the same way as the field samples to highlight possible contaminations.

3. eDNA detection

3.a. Amplification and sequencing

DNA amplification and sequencing protocol was the same for P1 and P2. The samples were first tested for inhibition using qPCR (Biggs et al., 2015). Then, the samples were diluted 5-fold before the amplification if they were considered inhibited. DNA amplification was performed in a final volume of 25 μ L including 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10 mM of Tris-HCl, 50 mM of KCl, 2.5 mM of MgCl₂, 0.2 mM of each dNTP, 0.2 μ M of "teleo" primers (Teleo R 5'-CTTCCGGTACACTTACCATG-3' and Teleo F 5'-ACACCGCCCGTCACTCT-3', (Valentini et al., 2016) and 3 μ L of DNA template. 4 mM of human blocking primer for teleo primers and 0.2 mg/mL of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland) were also added to the mixture. 12 PCR replicates per field sample were performed. The forward and reverse primer tags were identical within each PCR replicate. The PCR mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C and a final elongation step at 72 °C for 7 min. The amplification step was realized in a dedicated room with negative air pressure and physical separation from the DNA extraction rooms (with positive air pressure). The four negative extraction controls and the three PCR negative controls (also 12 replicates) were sequenced in parallel.

3.b. Bioinformatic processing

The purified PCR products were then pooled in equal volumes to reach a sequencing depth of 400,000 (P1) or 500,000 reads (P2) per sample before library preparation. Five libraries were prepared using the Metafast protocol (<https://www.fasteris.com/metafast>), a PCR-free library preparation, at Fasteris facilities (Geneva, Switzerland). Sequencing were performed using an Illumina HiSeq2500 (2 \times 125 bp) (Illumina, San Diego, CA, USA) and a HiSeq SBS Kit v4 (Illumina, San Diego, CA, USA) following the manufacturer's instructions at Fasteris facilities (Geneva, Switzerland).

For both protocols, the sequence reads were analysed using the functions of the OBITools package (<http://metabarcoding.org/obitools>) following the protocol described in

Valentini et al., (2016). The ecotag function was used for the taxonomic assignment of molecular operational taxonomic units (MOTUs) using a threshold of 98% of identity with the reference database updated from Cilleros et al., (2019), which references 255 Guianese fish species. The MOTUs occurring with a frequency below 0.0003 per library sample were considered as tag-jumps and discarded (Schnell et al., 2015). These thresholds were empirically determined to clear all reads from the extraction and PCR negative controls included in our global data production procedure as suggested in De Barba et al., (2014).

4. Data analyses

According to the river continuum concept, the biodiversity markedly differs along the upstream-downstream gradient (Vannote et al., 1980). This has also been demonstrated in tropical regions and particularly in Guianese streams (Cilleros et al., 2017). As our sites represented a large range of watercourse types and sizes, we ordered the sites using the Strahler stream order. The Strahler stream order defines stream size based on their hierarchy of tributaries, with increasing orders from the source to the estuary (Strahler, 1957). Among the different taxa obtained, only taxa assigned to the species level were considered. Species-by-site matrices with presence/absence data were created separately for the sites sampled with P1 (P1-samples) and P2 (P2-samples) (Table S2 and Table S3). The differences in species richness and species occurrence (the number of sites where each species was detected) between the P1- and P2-samples were tested with a Mann–Whitney U-tests for non-parametric and unpaired samples (both protocol having been conducted at different years). The dissimilarity (β -diversity) was calculated between sites within each protocol for each habitat separately (for each protocol: 66 sample pairs for the streams, 3 sample pairs for the rivers), and between P1-P2 sample pairs (15 pairs) of each site with the Jaccard dissimilarity index (β_{jac}) using the betapart package (Baselga & Orme, 2012) and the functions *beta.pair* and *beta.temp*. The two components of β_{jac} , nestedness (β_{nes}) and turnover (β_{turn}) as well as their individual contribution percentage (P_{turn} and P_{nes}) were then calculated for the dissimilarity values between P1-P2 site pairs as follows: $P_{turn}/\beta_{jac} \times 100$ and $P_{nes}/\beta_{jac} \times 100$ (Villéger et al., 2014). This quantifies the extent to which the species composition (turnover) and richness (nestedness) contribute to differentiate the communities of each sample pair for each site (Baselga et al., 2018).

Protocol comparisons were led by considering β_{jac} dissimilarity values at two different levels (Figure 2): (i) between sites within each protocol and, (ii) between P1 and P2 sample pairs of each site. The dissimilarity values of the first level (i) were compared between both

protocols using Mann–Whitney U-tests (Figure 2A) to test if the two protocols present replicability differences in both habitats (stream and river considered separately). Likewise, the dissimilarity values of the first level (i) were also compared between habitats using Kruskal-Wallis tests (Figure 2B) to determine if both protocols have the same performance in stream and river (protocols considered separately). Finally, the dissimilarity values of the second level (ii) were compared between both habitats using Kruskal-Wallis tests (Figure 2C) to determine if sample dissimilarity between P1 and P2 is dependent on habitat type. In addition, a unilateral one sample Z test was performed to test if turnover contribution to β_{jac} dissimilarity was higher than 50% (habitat type considered separately), and therefore if turnover and nestedness relative contribution to P1-P2 samples dissimilarity is dependent on habitat type.

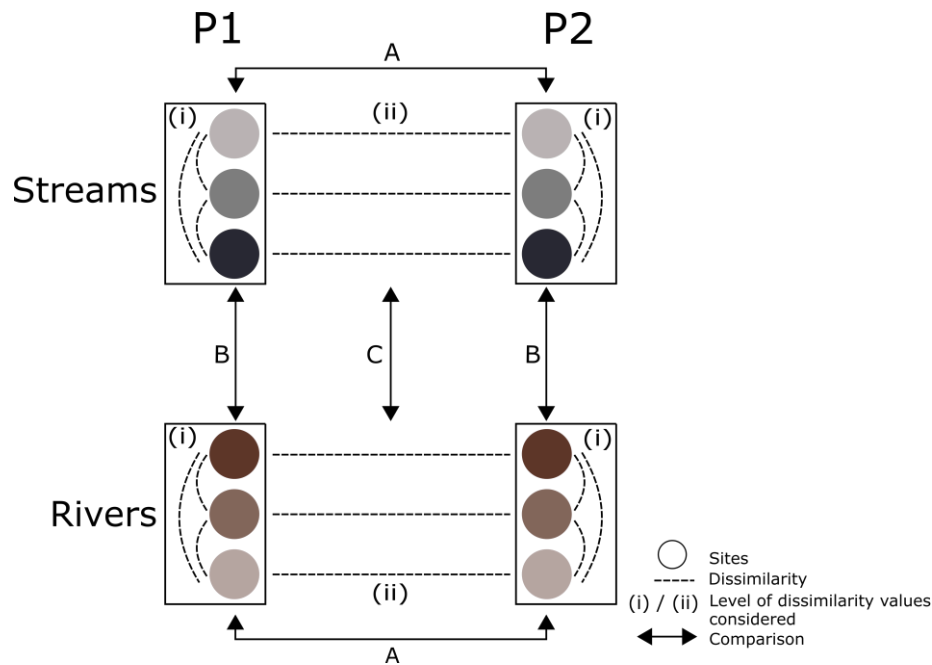


Figure 2. The different levels of dissimilarity comparison. (i) Between site dissimilarities obtained with P1 were rivers were tested separately (A); between stream site dissimilarities were compared to between river site dissimilarities, P1 and P2 were tested separately (B). (ii) P1 and P2 sample pair dissimilarity of streams were compared to that of rivers (C). Circles with the same colour refer to the same sites sampled with P1 or P2, dashed lines represent the dissimilarities considered, (i) / (ii) indicates the level at which dissimilarity values were considered, arrows show the different comparisons (A, B and C; see Methods).

As the β -diversity and the α -diversity are interrelated, the β_{jac} is influenced by the number of species present in each pair of samples. To address this issue, a null model using the Raup crick metric (β_{rc}) was performed to determine if the pairs of samples were more similar than expected under stochastic effects. This metric expresses the dissimilarity between two communities relative to the null expectation of randomly assembled communities by estimating

the probability that two randomly drawn communities have the same number or more species in common than the two observed communities. The β_{rc} metric was calculated between sites within each protocol for each habitat separately (for each protocol: 66 sample pairs for the streams, 3 sample pairs for the rivers), and between P1-P2 sample pairs of each site following (Chase et al., 2011). For each site, 9,999 mock communities were simulated for each sample pair by randomly selecting species from the regional species pool (all the species detected in the samples). Each simulated community contained the same species richness to that of the observed community and the presence probabilities of each species was weighted by its among site occupancy. The dissimilarity was calculated for each pair of simulated communities and the observed β -diversity was then compared to the null distribution, e.g. $\beta_{rc} = \beta_{obs} - E(\beta_{null})$ where β_{obs} is the observed dissimilarity of the observed sample pair communities and $E(\beta_{null})$ is the mean dissimilarity of the simulated sample pair communities. The resulting value represents a β -diversity metric ranging from -1 to +1. A negative β_{rc} value stands for communities more similar than expected by chance while a positive value refers to communities less similar than expected by chance. A null value indicates that community assembly is highly stochastic.

The β_{rc} dissimilarity values were considered at the same first level (i) and compared with the same statistical tests as detailed above for the β_{jac} analysis (Figure 2). The second level (ii) of comparison was investigated using a permutational analysis of variance (PERMANOVA) (Anderson, 2001) to test for differences in species composition after checking for homogeneous dispersion among P1- and P2-samples with a permutation dispersion (Anderson et al., 2006). Non-metric multidimensional scaling (NMDS) were then performed on the Jaccard and Raup-Crick indices to visualize the stochastic and non-stochastic effects explaining sample pair dissimilarities. All the analyses were performed using R software version 3.6.1 (2019-07-05) (R Core Team, 2019).

Results

A total number of 2,251,622 and 2,430,314 reads were retained after bioinformatic screening for the sites sampled with P1 and P2, respectively. They respectively represented 48.09% and 51.91% of the 4,681,936 total reads retained. After the bioinformatic filtering step, reads were detected in all our 360 PCR replicates while no reads were found in the PCR and extraction controls. Among all the samples, we detected 160 species out of the 255 species referenced in our database. We obtained 779 occurrences in the 15 sites and all the species detected were consistent with their known distribution.

1. Protocol effect on species richness and occurrence

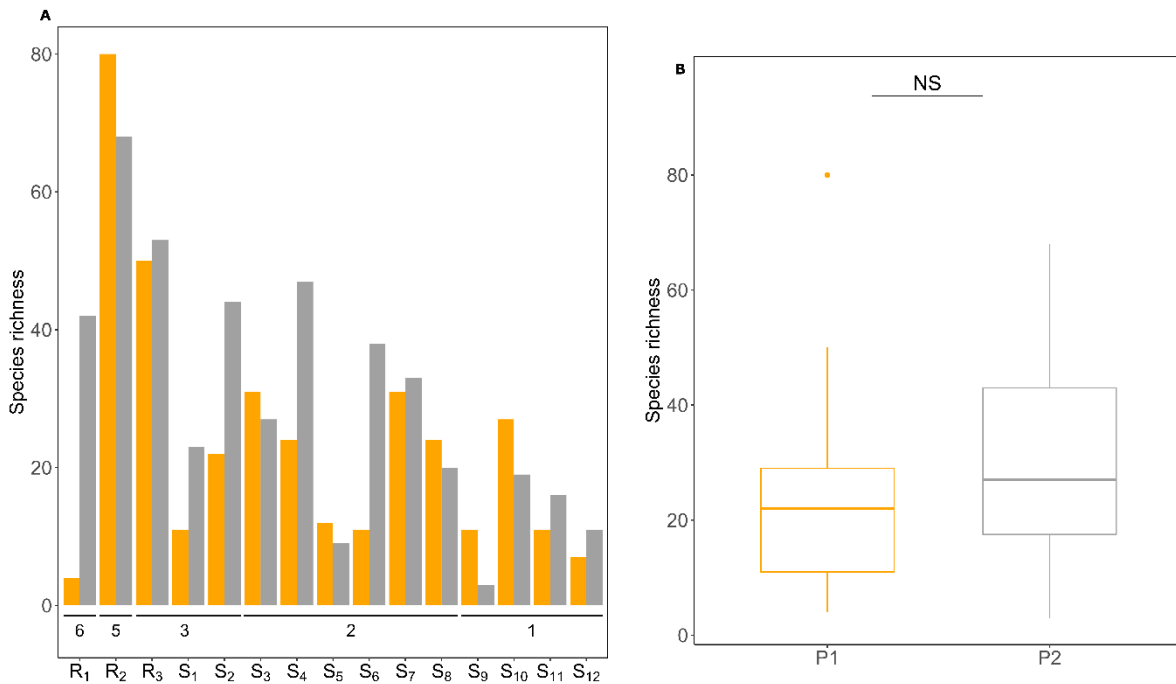


Figure 3. Site species richness obtained with protocol 1 (P1) and protocol 2 (P2) (A) and averaged across sites for each protocol (B). The Strahler stream orders are indicated below the bars of panel (A), and the sites are ordered by decreasing stream order (indicating the size of the river/stream, with larger ones having higher Strahler orders). Bright orange and dark grey bars indicate the sites sampled with P1 and P2, respectively. Species richness of P1 and P2 samples were compared using a Mann-Whitney U-test, NS = non-significant ($p > .05$).

Among the 15 sites, we detected 139 species with P1 and 148 species with P2. The species richness detected tended to increase with the watercourse size of the sites, regardless of the protocol (Figure 3A), emphasizing the difference in species richness between streams and rivers. The low species richness detected in the P1-sample of the R₁ site is suspicious and may reveal an issue during the sampling or laboratory treatment (Figure 3A). Nevertheless, excluding site R1 from our data did not change our conclusions (results not shown). Among the 15 sites, the number of detected species with P1 ranged from 4 to 80 (median = 22) and from 3 to 68 (median = 27) with P2. The number of species detected with P2 was higher than with P1 in 9 out of the 15 sites, but the average species richness obtained among sites did not significantly differ between protocols (Mann-Whitney U rank test, $U = 86.5$, $p = .289$, $n = 15$) (Figure 3B).

Among the 779 occurrences obtained across all the sites, 341 and 438 occurrences were detected in the P1-samples and the P2-samples, respectively. Moreover, among the 160 species detected, 12 species were only detected in the samples collected with P1 while 21 species were

only found with P2. Among the 12 species recovered only with P1, 10 occurred in a single site and 2 species were present in 2 sites. Among the 21 species found only with P2, 16 occurred in a single site, 4 in two or three sites and one species was detected in 4 sites. Regarding the 127 species detected with both protocols, most of the species occurred in 1 or 2 sites, representing 69.29% of the P1- and 59.06% of the P2-species, respectively (Figure 4A). Few of the shared species occurred in more than half of the sites with higher occurrences detected with P2, representing 7.09% and 9.45% of the P1- and P2-species, respectively. The species obtained with both protocols represented 79% of the total fauna detected. The species occurrence ranged from 1 to 10 (median= 1) in the P1-samples while it ranged from 1 to 14 (median = 2) for the P2-samples. Overall, there was a significant difference in the average number of species occurrence between protocols, with P2 detecting from 0 to 4 more occurrences than P1 (Mann–Whitney U rank test, $U = 15056$, $p = .004$, $n = 160$) (Figure 4B).

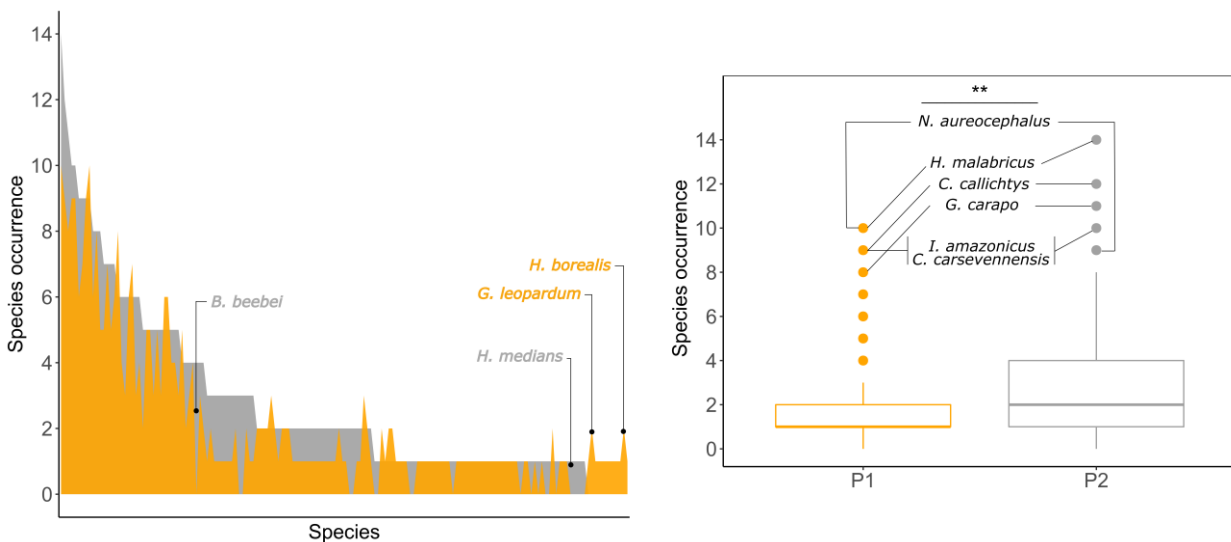


Figure 4. Species occurrence obtained with P1 and P2 for each species (a) and averaged among species for each protocol (b). Species occurrence stands for the number of sites where each species was detected. Species occurrences in panel a were ordered in descending order for the sites sampled with P2 and plotted as a dark grey shaded area. The P1 species occurrences (orange area) were then superimposed to the P2 area while maintaining the same species order. Species names in orange and grey are examples of species only detected with P1 and P2 respectively in panel a. Species name are indicated for the 6 most occurrent species in panel b. The number of species occurrences from each protocol were compared using a Mann-Whitney U-test, (** = $p < .01$).

2. Protocol effect on community composition

Table 1. Median values and range (inside brackets) of β_{jac} and β_{rc} dissimilarity values within P1- and within-P2-samples and between P1- and P2-samples.

Dissimilarity index	Total		Rivers		Streams	
	β_{jac}	β_{rc}	β_{jac}	β_{rc}	β_{jac}	β_{rc}
Within- P1-samples	0.86 (0.48 – 1)	-0.63 (-1 – 1)	0.99 (0.77 – 1)	0.88 (0.8 – 1)	0.79 (0.48 – 0.97)	-0.96 (-1 – 0.74)
Within- P2-samples	0.83 (0.38 – 1)	-0.8 (-1 – 1)	0.77 (0.74 – 0.84)	0.99 (0.68 – 1)	0.76 (0.38 – 0.98)	-0.97 (-1 – 0.69)
Between P1- and P2-samples	0.83 (0 – 1)	-0.8 (-1 – 1)	0.98 (0.79 – 0.99)	0.67 (0.45 – 1)	0.76 (0 – 0.97)	-0.99 (-1 – 0.69)

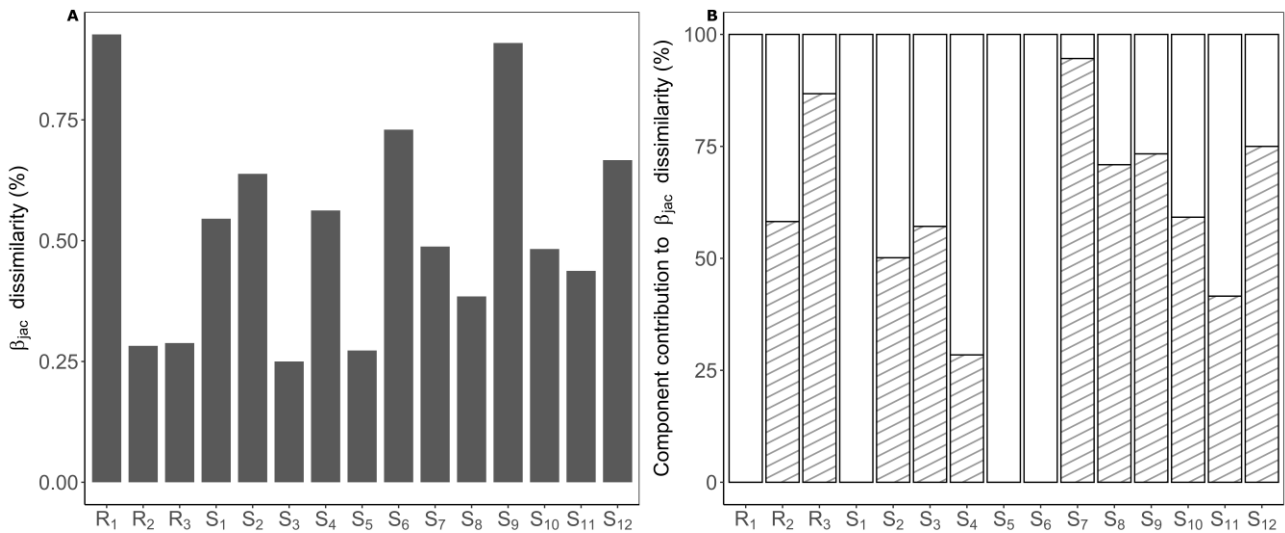


Figure 5. β_{jac} dissimilarity (A) and nestedness and turnover contribution to β_{jac} dissimilarity (B) of sample pairs for each site. White and striped bars represent nestedness and turnover, respectively. Sample pairs refer to samples collected with P1 and P2 in the same site.

Considering the first level (i) of comparison (between site dissimilarities within each protocol), the β_{jac} and the β_{rc} dissimilarity of the within- P1-samples did not significantly differ to that of the within- P2-samples neither for the rivers nor for the streams (Figure 2A and Table 1). This indicates that the degree of species dissimilarity among sites is not significantly different between protocols in both habitats.

Moreover, the β_{jac} dissimilarity of the within- P1- stream samples did not significantly differ to that of the within- P1- river samples (Figure 2B, Kruskal-Wallis rank sum test, $\chi^2 = 3.28$, $df = 1$, $p = .07$, $n = 69$). Likewise, the β_{jac} dissimilarity of the within- P2- stream samples did not significantly differ to that of the within- P2- river samples (Figure 2B, Kruskal-Wallis rank sum test, $\chi^2 = 0.15$, $df = 1$, $p = .70$, $n = 69$). This indicates that both protocols perform in

the same way in rivers and in streams when considering both nestedness and turnover components of β -diversity. However, the β_{rc} dissimilarity values of the within- P1- and the within- P2 stream samples were both significantly smaller than that of the within- P1- and the within- P2 river samples, respectively (Figure 2B, Kruskal-Wallis rank sum test, $\chi^2 = 8.76$, $df = 1$, $p = .003$, $n = 69$ for P1; $\chi^2 = 8.89$, $df = 1$, $p = .003$, $n = 69$ for P2) (Table 1). This demonstrates that in both protocols, the turnover in species among river samples is higher than among the stream samples.

Considering the second level (ii) of comparison, the dissimilarity difference between P1-P2 stream sample pairs of each site and P1-P2 river sample pairs of each site, the β_{jac} dissimilarity ranged from 0.28 to 0.93 (median = 0.29) in the three river sites and from 0.25 to 0.91 (median = 0.52) in the twelve stream sites. There was no significant difference between the dissimilarity of the P1–P2 river sample pairs and the P1–P2 stream sample pairs (Figure 2C, Kruskal-Wallis rank sum test, $\chi^2 = 0.08$, $df = 1$, $p = .87$, $n = 15$) (Figure 5A). In addition, nestedness contribution to sample pair dissimilarity ranged from 13.24 to 100% (median = 41.78) for the river sample pairs while it ranged from 5.38 to 100% (median = 46.36) for the stream sample pairs. For the sites R₁, S₁, S₅, and S₆, nestedness contributed to 100% of the total sample pair dissimilarity (Figure 5A and 5B). Turnover contribution to the dissimilarity between sample pairs for each site ranged from 0 to 86.76% (median = 58.22) for the river sample pairs while it ranged from 0 to 94.62% (median = 53.64) for the stream sample pairs. However, the turnover contribution to site dissimilarity was not significantly greater than 50%, thus implying no significant difference between the nestedness and turnover contribution to β_{jac} dissimilarity (one sample Z test, $z = 3.60$, $p = 1$, $n = 15$). Moreover, the turnover contribution as well as the nestedness contribution did not significantly differ between river and stream sample pairs (Kruskal-Wallis rank sum test, $\chi^2 = 0.05$, $df = 1$, $p = .83$, $n = 15$, Figure 5B).

The stress of the NMDS based on β_{jac} and β_{rc} was lower than 0.2 for both metrics, indicating that the first two axes of each NMDS provided a good two-dimensional representation of the sample pairs according to their community composition. The β_{jac} -NMDS displays sample pair distance resulting from differences in species richness and composition. Stream sample pairs and river sample pairs formed two distinct clusters. Moreover, the R₁ samples were the most distant samples belonging to the same site due to the large difference in species richness obtained between P1 and P2. The sample pairs of the S₆ and S₉ sites were also more distant from each other than the other sample pairs (Figure 6A). This is consistent with the previous results demonstrating that the S₆ sample pair presented the highest dissimilarity

due to nestedness and that the S_9 samples exhibited the highest β_{jac} dissimilarity among stream sites (Figure 5A and Figure 5B). The use of the null model allowed correcting for the difference in α -diversity and therefore only account for compositional differences in the communities between sample pairs. The resulting β_{rc} index indicated that in all sites, the P1- and P2-samples presented homogeneous dispersion (BETADISPER, $F_{(1,28)} = 0.037$, $df = 1$, $p = .85$, $n = 15$) and there was no significant compositional difference among samples collected with P1 and P2 (PERMANOVA, $F_{(1,28)} = 0.0536$, $df = 1$, $p = .807$, $n = 15$). While controlling for species richness differences in the β_{rc} NMDS, the river samples remain more distant from each other than the stream sites. Specifically, the closeness between R_1 , S_6 and S_9 sample pairs in the β_{rc} NMDS indicate that their strong dissimilarity identified by the Jaccard's index, was mainly due to differences in the number of detected species, while species turnover remained low (Figure 6B).

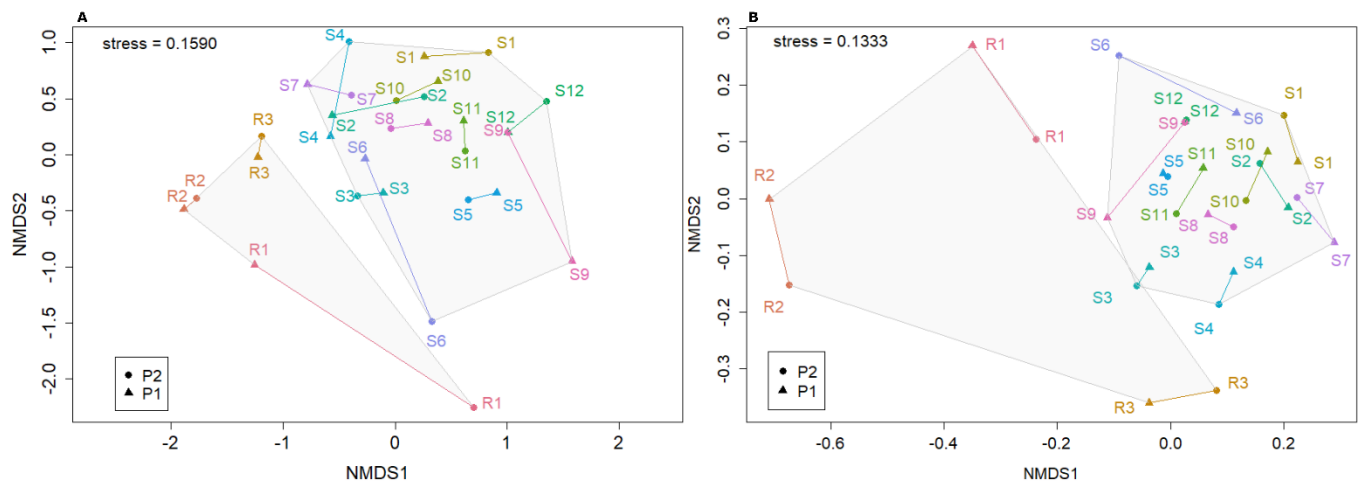


Figure 6. Non-metric multi-dimensional scaling (NMDS) ordinations based on β_{jac} (a) and β_{RC} (b) indices. The segments connect each pair of samples from the same site collected with P1 and P2. River sites and stream sites are clustered with a grey area. Communities that are closer together, using modified Raup-Crick dissimilarities, are more deviant from the null expectation, whereas communities that are farther apart are less deviant from the null expectation.

Discussion and Conclusion

The variety of protocols developed for aquatic eDNA-based metabarcoding studies represents a hindrance to study comparisons (Minamoto et al., 2016; Shu et al., 2020). Moreover, because protocols are continuously improved and optimized with scientific advances, heterogeneous datasets are produced, some of which may become deprecated, for either technical or financial reasons (Deiner et al., 2015). Understanding the extent to which different protocols produce different outputs by comparing community species richness and

composition is therefore a key question to determine if merging data obtained with distinct protocols is possible, or if the biodiversity estimates from distinct protocols require to be considered separately. The two protocols compared in this study followed the same general optimized workflow but presented marked differences during the data collection and the extraction step.

Overall, the two protocols produced similar measures of richness in species. Although P2 allowed, on average, to detect more species per site than P1, such differences remained low and non-significant when considering the sites altogether. From an ecological point of view, the observed richness obtained with the two different protocols remains consistent with the expected richness of fish fauna across Guianese rivers (Le Bail et al., 2012). Moreover, the increasing richness pattern along the upstream-downstream continuum revealed by both protocols supports the river continuum concept, which describes changes in species assemblages along the upstream-downstream gradient in response to variability in the environment and a gradual increase of the stream size offering more habitats (Vannote et al., 1980). This richness pattern also echoes Cilleros et al., (2017) findings that demonstrated an increase in local species richness with stream size from the upstream to the downstream of the same Guianese rivers using traditional sampling data. The weak richness of the R₁ sample collected with P1 might thus reveal an issue during the sampling or the laboratory processes as it is the only river sample presenting a lower richness than the stream samples.

The known patterns of species occurrences were conserved in the samples regardless of the protocol, even if P1 was slightly less efficient at detecting the most frequent species known to be widespread in Guianese streams and rivers such as *Copella carsevennensis*, *Hoplias malabaricus* or *Gymnotus coropinae* (Planquette et al., 1996). Species inhabiting most of the small streams such as *Nannacara aureocephalus* and *Callichthys callichthys* (Cilleros et al., 2017; Keith et al., 2000) were also rightly retrieved among the most occurring species in both P1 and P2- stream samples. Moreover, *Ituglanis amazonicus*, a species dwelling sandy habitats and encountered in various watercourse sizes, is not catchable by classical methods due to its elongated body and burrower habits, but was among the most occurring species in our samples. On the other hand, the species only detected with P1 or P2 presented few occurrences (from one to four). Those differences in species detections were omission errors as they were all rightly detected in the expected river basins but not systematically detected with both protocols. Among these species, some are known to occur in most sampled basins such as *Brachyhypopomus beebei* (only detected with P1) or *Glanidium leopardum* and *Hyphessobrycon borealis* (only detected with P2) but other species occurred only in one basin

such as *Hemiancistrus medians* (only detected with P2). Contrastingly, the less occurring species detected with both protocols were species presenting limited geographical distribution and that do not colonise all the basins.

Overall, the occurrence patterns obtained with both protocols were congruent with the known proportion of fish with large and small distribution encountered in Guianese rivers. Indeed, we recovered around two third of fish species with restricted distribution (occurring in one or two sites) and one third of fishes with wider geographical distribution, paralleling previous studies on the spatial distribution and species composition of the Guianese fish fauna (Cilleros et al., 2016; Le Bail et al., 2012). More generally, our occurrence gradients are in line with the hyper-diversified tropical regions recognised to present a majority of species with a restricted spatial distribution and only a limited number of widely distributed species (Colwell et al., 2004).

P1 and P2 also performed similarly on retrieving patterns of beta-diversity. Both β_{jac} and β_{rc} dissimilarities of within- P1-samples were not significantly different to that of within- P2-samples in both streams and rivers indicating that the protocols allowed for similar replicability in both habitats. In addition, the protocols provided similar species inventories for each site regardless of habitat type, as no significant compositional difference among samples collected with P1 and P2 was found. Moreover, the absence of a significant difference in β_{jac} dissimilarity between river and stream samples within each protocol suggested that both protocols performed as well in streams as in rivers. This was also supported by the absence of significant difference in turnover contribution to β_{jac} dissimilarity between P1-P2 stream sample pairs and P1-P2 river sample pairs of each site.

However, the smaller β_{rc} dissimilarity values in within-P1 and within-P2 stream sample pairs than in within-P1 and within-P2 river sample pairs, respectively, indicated that river samples presented more differences due to species composition than stream samples. This was also emphasized by the larger size of the cluster formed by the river samples on the β_{rc} NMDS than on the β_{jac} NMDS. This demonstrates that when considering only species turnover and fixing for the nestedness effect, P1 and P2 protocols allow the same sample replicability but they both perform better in streams than in rivers. This is in line with Cantera et al., (2019) results showing that an eDNA sample collected with P2 captures in average 67 % of the fauna of the river sites and 87% of the fauna of the stream sites. Therefore, different sets of species could have been retrieved from the P1 and P2 samples in each site and this is likely to be more important in large watercourses that shelter a rich biodiversity with many rare species. The sampling stochasticity (or species hardly detected with this method) may explain a part of the

species turnover obtained between our site sample pairs, and its contribution was higher in the river samples where a single sample captured a lower proportion of the species from the site than in the stream sites. Moreover, P1- and P2-samples were not collected in the same year and this could have involved temporal heterogeneity, consisting in another source of turnover between P1- and P2-samples. Nevertheless, all the samples were collected during the dry season and no environmental changes were detected during the entire study period. We nevertheless cannot exclude such inter-annual effects in the species turnover between P1 and P2.

Despite some differences in species inventories between P1 and P2 in some sites, both richness and species composition trends remained similar among protocols. This demonstrates the robustness and the replicability of the method, regardless of the differences between the two protocols, and therefore testifies that the two datasets could be analysed together. The use of enclosed filters might have played an important role in the robustness of the method, because the filters used in both protocols had a larger surface of filtering membrane than most of the open filters used in the literature reviewed in Shu et al., (2020) and Tsuji et al., (2019), allowing filtering a large volume of water (more than 30L per sample). This also permitted us to overpass potential bias due to membrane clogging as we did not face clogging issues constraining the amount of filtered water. Moreover, the smaller membrane surface and sampling effort with P2 might have been compensated by the smaller filter pore size (0.45 μ m) compared to P1 (1 μ m). This suggests that a smaller volume of filtered water by smaller pore size may reach equivalent eDNA capture than a larger volume filtered by larger pore size, echoing Eichmiller et al., (2016)'s results on the detection of carp (*Cyprinus carpio*) in mesocosms using different eDNA protocols. In addition, both protocols use a large number of PCR replicates per sample (12), which might also contribute to the robustness of the method. Indeed, Ficetola et al., (2015) emphasized the importance of adjusting the number of PCR replicates depending on the features of the study system and suggested to realise at least 8 PCR replicates when dealing with taxa with low detection probabilities (e.g. rare species). Our large number of PCR replicates was therefore adapted to tropical environments containing rich species assemblages with a majority of rare species.

The general call for developing a standard approach for aquatic eDNA-based metabarcoding studies is challenging because of the specificity of each environment (Deiner et al., 2018). Still, while it has been proven that the protocols and the material used have an impact on the total quantity of DNA recovered (Capo et al., 2019; Thomas et al., 2018), it does not necessarily implies that the different datasets generated by distinct protocols are strongly affected by those protocol variations (Djurhuus et al., 2017). In fact, protocol variations may

not produce heterogeneous datasets if the achieved sampling effort provide a valuable representation of the communities. Despite the evident need to search for a unique standardized approach offering the best biological results, testing the robustness of existing methods remains a way to potentially rescue biological data collected using deprecated protocols. This is of particular importance to consider time series data, or biological inventories gathered in remote locations difficult to access.

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Author Contributions

I.C., K.C., A.V., T.D., O.C., S.B., and J.M. conceived the ideas and designed methodology; I.C., K.C., and S.B. collected the data; A.V. and T.D. conducted the laboratory work; A.V. and T.D. conducted bioinformatic analyses; O.C. analysed the data; O.C., J.M., and S.B. led the writing of the manuscript. All authors contributed critically to the drafts.

Supplementary Materials

See the online version of the article

2. Part 2

Amazonian mammal monitoring using aquatic environmental DNA

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Abstract

Environmental DNA (eDNA) metabarcoding has emerged as one of the most efficient method to assess aquatic species presence. While the method could in theory be used to investigate non-aquatic fauna, its development for inventorying semi-aquatic and terrestrial fauna is still at its early stages. Here we aimed at investigating the potential of aquatic eDNA metabarcoding for inventorying mammals in Neotropical environments, be they aquatic, semi-aquatic or terrestrial. We collected aquatic eDNA in 96 sites distributed along three Guianese watersheds and compared our inventories to expected species distributions and field observations derived from line transect located throughout French Guiana. Species occurrences and emblematic mammalian fauna richness patterns were consistent with the expected distribution of fauna and our results revealed that aquatic eDNA metabarcoding brings additional data to line transect samples for diurnal non-aquatic (terrestrial and arboreal) species. Aquatic eDNA also provided data on species not detectable in line transect surveys such as semi-aquatic, aquatic and nocturnal terrestrial and arboreal species. While wise application of the eDNA method to inventory mammals still needs some developments to optimize sampling efficiency, it can now be used as a complement to traditional surveys.

Introduction

Mammal biodiversity is currently impacted by various factors, mainly habitat modification and loss and/or illegal and unregulated hunting (Bowyer et al., 2019). Monitoring the state of biodiversity has thus become vital to assess trends and set priorities for conservation programs (Visconti et al., 2016). Among the methods used to inventory fauna, environmental DNA has been recently developed and is increasingly used (Taberlet et al., 2018). eDNA consists in collecting DNA fragments from environmental samples (such as soil, water, faeces, or air) to detect organisms (Taberlet et al., 2018). eDNA metabarcoding surveys in aquatic environments are under active development because water acts as a collector for DNA, allowing an integrative assessment of biodiversity from a locality (Valentini et al., 2016). However, although most of previous studies have focused on assessing aquatic species or communities (Bylemans et al., 2018; Civade et al., 2016; Ding et al., 2017; Fujii et al., 2019; Lopes et al., 2017; Tréguier et al., 2014), the approach could in theory be used to investigate non-aquatic species (Rodgers & Mock, 2015). In fact, water also receives DNA from non-aquatic organisms during bathing (Ushio et al., 2017), drinking (Rodgers & Mock, 2015) swimming or when

mammals defecate in the water (Harper et al., 2019), but also potentially through soil drainage by rain.

Several studies aimed at detecting mammals or other non-aquatic vertebrates with aquatic eDNA. Early research focused on small water bodies that are expected to be intensively visited by terrestrial animals. Rodgers & Mock, (2015) successfully retrieved captive coyote (*Canis latrans*) DNA in drinking water samples. Later on, Ushio et al., (2017) tested a metabarcoding approach on eDNA collected from zoo drinking water and on small natural ponds. They detected ten out of the 13 species present in the zoo enclosure, while they retrieved from 15 to 89% of forest mammalian sequences in the pond samples. Similarly, Klymus et al., (2017) surveyed vertebrate species in uranium mine containment ponds and retrieved 18 terrestrial species including hard to observe taxa such as the tiger salamander (*Ambystoma tigrinum*). Egeter et al., (2018) detected four species out of the ten expected species in drinking water bodies in Sahara desert. Waterholes left by African megafauna were also used as eDNA collectors, allowing the detection of 16 species (Seeber et al., 2019). Given the demonstrated high potential of eDNA to detect terrestrial fauna in small water bodies that are expected to be more saturated in eDNA than larger water bodies (Harper et al., 2019), studies then focused on collecting eDNA from larger water bodies. Harper et al., (2019) evaluated eDNA metabarcoding of pond as a tool for monitoring semi-aquatic, ground dwelling and arboreal mammals. They led a comparative study on how animal behaviour affects the release of eDNA in artificial versus natural environments. While mammal life habits and behaviour did not influence eDNA detection in artificial ponds, it played a major role in natural systems. Attempts to detect mammals in natural aquatic systems remain scarce and to date, only few studies explored the reliability of eDNA metabarcoding to detect non-aquatic species in rivers and streams. Among those studies, Sales et al., (2020) retrieved 14 mammal families in the Amazon river and nine mammal families in the Brazilian Atlantic forest from aquatic eDNA. While this study highlights the potential of aquatic eDNA metabarcoding to detect non-aquatic species, the reliability of the method remained to be tested by investigating the spatial concordance between the species occurrences and their expected distribution. Indeed, one of the greatest challenges is that non-aquatic species are not in permanent contact with the water, potentially resulting in smaller amounts of DNA released in the water (Harper et al., 2019; Sales, McKenzie, et al., 2020). Consequently, false negatives (i.e. missing detections when species are present) may thus be more frequent than for aquatic fauna, particularly in large water bodies (Harper et al., 2019; Seeber et al., 2019).

We here investigate the potential of aquatic eDNA metabarcoding to detect aquatic, semi-aquatic, terrestrial and arboreal mammal fauna along three rivers of the Amazonian biome. To this purpose, we led a comparative analysis between aquatic eDNA metabarcoding and standardized visual faunistic inventories classically used for mammal monitoring. We then discuss the spatial concordance between the observations of several emblematic Amazonian mammals retrieved with aquatic eDNA metabarcoding and their expected distribution.

Materials and Methods

1. Study rivers

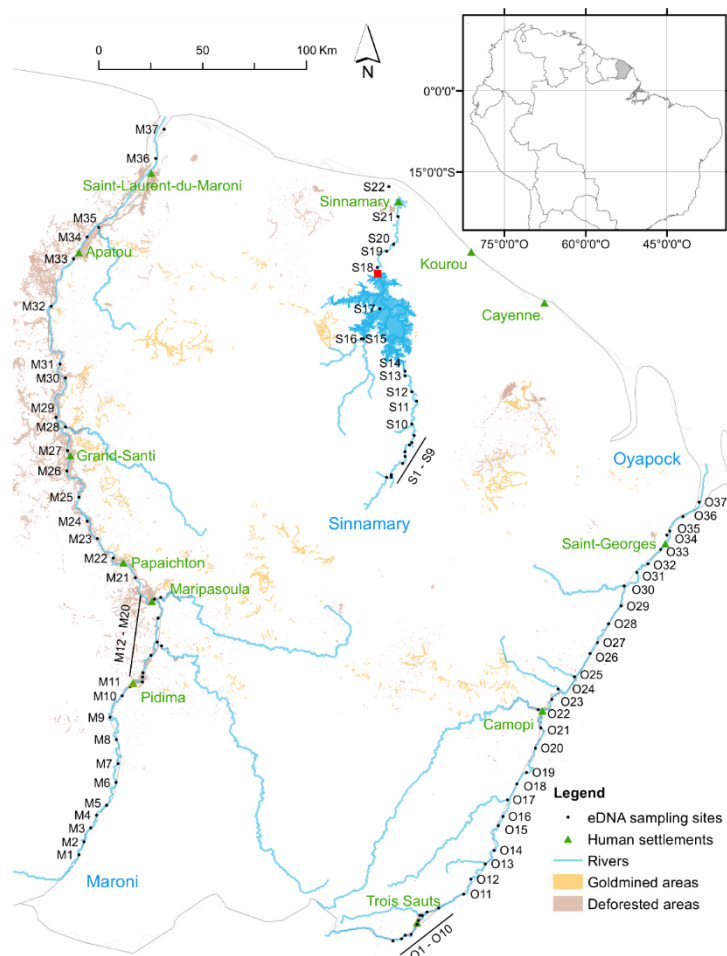


Figure 1. eDNA sampling sites. M1 to M37 indicate the sites sampled on the Maroni river, S1 to S22 the sites sampled on the Sinnamary river and, O1 to O37 those sampled on the Oyapock river. Information about gold-mined surfaces was compiled by the WWF using Landsat satellite images of deforestation due to gold-mining in 2015 (WWF, 2016). This dataset represents the most recent information available on gold-mining over the Guianese territory. Forest loss surfaces were extracted using the Global Forest Change dataset (M. C. Hansen et al., 2013). This dataset identifies the areas deforested from 2001 to 2017 using global Landsat satellite image at 30 meters spatial scale. The red rectangle on the Sinnamary river represents the dam location. Inset map indicates the location of French Guiana in South America.

We collected aquatic eDNA in three large French Guianese rivers (Figure 1): the Maroni river (612 km in length), which watershed extends over Suriname and French Guiana; the Oyapock river (404 km in length), which watershed extends over Brazil and French Guiana; and the Sinnamary river (262 km in length) situated within the territory of French Guiana. The three river basins are characterized by an equatorial climate with annual rainfall ranging from 3,600mm (north-east) to 2,000mm (south and west). These three rivers face different levels of anthropogenic pressures unevenly distributed along the watercourses as most people are concentrated in the coastal area. The Maroni river is the most inhabited with approximately 83 000 habitants (INSEE, 2020) unevenly distributed from Saint-Laurent-du-Maroni to Pidima village, which constitutes the most upstream human settlement on the Maroni river (Figure 1). The Maroni river is the most affected by human activities, mainly legal and illegal gold mining, which represented 8,058 ha of deforestation (0.37% of the catchment area in 2014) spanning from Saint-Laurent-du-Maroni to upstream of Maripasoula (Gallay et al., 2018). Only the most upstream part of the Maroni river (upstream from Pidima, Figure 1) is not impacted by human activities. The Oyapock river is more preserved with only three villages and approximately 6 000 habitants (INSEE, 2020). Gold mining is much less developed than on the Maroni drainage, and represented 1,547 ha of deforestation in 2014 (0.06% of the catchment area), mainly concentrated near the village of Camopi (Gallay et al., 2018). The Sinnamary river is not exploited for gold but the building of a large hydroelectric dam (Petit saut dam) in 1994-1995 has severely modified the landscape. 365 km² of primary rain forest were flooded, leaving hundreds of islands of various sizes covering a total area of 105 km² (Vié, 1999). Several human settlements are located downstream from the dam while the upstream part of the river remains free from human settlements, with only occasional activities of recreational fishing. Hunting activities also occur along the watercourses, subsistence hunters being frequent in remote isolated areas. In small rural villages or gold mining camps, hunting for meat represents a non-negligible disturbance to large vertebrate fauna (Richard-Hansen & Hansen, 2004). The hunting impact on wildlife populations is nevertheless concentrated on small superficies around human settlements and access paths (Richard-Hansen et al., 2019). The Sinnamary river being the least populated, it presents the weakest hunting pressure. Moreover, its upstream course belongs to the core area of the Guianese National Park (Parc Amazonien de Guyane) where access is restricted and hunting totally prohibited. Hunting is also prohibited in the Petit-Saut dam area. Contrastingly, hunting pressure is important along the Maroni course, and only the upstream areas remain hardly influenced by hunting because of their distance to human settlements. Along the Oyapock river, hunting pressure is lower than on the Maroni due to a lower human

population density, but is expended all along the watercourse, because human settlements, although concentrated in three main villages, are dispersed all along the watercourse, including the most upstream areas (Figure 1).

2. Water collection and sampling

The eDNA sampling was conducted in November (dry season) 2017 for the Maroni river, November 2018 for the Oyapock river and November 2019 for the Sinnamary river. 96 sites were sampled using VigiDNA 0.45 μm filters (SPYGEN, le Bourget du Lac). These encapsulated filters possess a 500 cm^2 membrane surface made of polyethersulfone and can process up to 50 L of water (Coutant et al., 2020). Following Cantera et al., (2019), two samples were taken per site, with 34 litres of water filtered per sample during 30 minutes. A peristaltic pump (Vampire sampler, Burlke, Germany) and a single-use tube were used to pump the water through the encapsulated filtering cartridges. The input part of the tube was held few centimetres below the water surface and sampling was achieved in rapids where eDNA is continuously homogenized in the water column. To avoid DNA contamination, the operators remained downstream from the filtration either on the boat or on emerging rocks. After the filtration, the capsules were emptied and filled with 80 mL of CL1 conservation buffer (SPYGEN) and stored in sterile individual plastic bags in the dark. The samples were kept at room temperature until the DNA extraction, performed within a month.

3. eDNA laboratory and bioinformatics

Each filtration cartridge was agitated for 15 min on an S50 shaker (cat Ingenieurbüro™) at 800 rpm, emptied into a 50 mL tube and then centrifuged for 15 min at 15'000 g. The supernatant was then discarded with a sterile pipette leaving 15 mL of liquid at the bottom of the tubes. After the addition of 33 mL of ethanol and 1.5 mL of 3 M sodium acetate, the 50 mL tubes were stored at 20 °C during at least one night. The tubes were subsequently centrifuged at 15'000 g for 15 min at 6°C and the supernatants were removed. 720 μl of ATL buffer from the DNeasy blood & Tissue Extraction Kit (Qiagen) were added to the tubes. The tubes were vortexed and the supernatants were transferred to 2 mL tubes with 20 μL of Proteinase K. The tubes were incubated at 56 °C for two hours. After this step, the DNA extraction was led with the NucleoSpin® Soil (MACHEREY-NAGEL GmbH & Co., Düren Germany) beginning at step six and following the manufacturer's instruction.

After the extraction step, the samples were tested for inhibition (Biggs et al., 2015). Briefly, quantitative PCR were performed in duplicate for each sample. If at least one of the replicates showed a different Ct (Cycle threshold) than expected (at least 2 Cts), the sample was considered inhibited and diluted 5-fold before the amplification. DNA amplification was performed in a final volume of 25 μ L including 1 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA), 10 mM of Tris-HCl, 50 mM of KCl, 2.5 mM of MgCl₂, 0.2 mM of each dNTP, 0.2 μ M of 12S-V5 vertebrate marker (12S-V5 R 5'-TTAGATACCCCACTATGC-3' and 12S-V5 F 5'-TAGAACAGGCTCCTCTAG -3', Riaz et al., 2011) and 3 μ L of DNA template. 4 mM of human blocking primer for 12S-V5 (5'-CTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACCTGCT -C3 - 3' (De Barba et al., 2014) and 0.2 mg/mL of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland) were also added to the mixture.

The choice of the primer set used to identify the targeted fauna is critical as it may impact the composition of the inventories if eDNA markers do not have the same taxonomic resolution across clades (Zinger et al., 2020). We here used the “12S-V5” marker Riaz et al., 2011) as it presents interesting features to identify the fauna considered in this study. Even though it was originally designed as a generic vertebrate marker, we have previously shown that it is very well suited to study the local mammal fauna (Kocher, de Thoisy, Catzeflis, Valière, et al., 2017; Kocher, de Thoisy, Catzeflis, Huguin, et al., 2017). The local reference database is nearly exhaustive while the marker provide a very good accuracy (99.6% of the assignments are correct) and very high specificity (90% of the assignment are done at the species level). The 12S-V5 binding sites are extremely conserved for Amazonian mammals, thus minimising mismatches that could lead to a lack of amplification of rare species. Finally, the marker presents a short size (96-103 bp) well suited for the amplification of degraded DNA (Kocher, de Thoisy, Catzeflis, Huguin, et al., 2017).

We performed 12 PCR replicates per field sample. In order to assign the sequences to the appropriate sample, the forward and reverse primers were 5'-labelled with a unique eight-nucleotide tag for each PCR replicate. Both forward and reverse primers used an identical tag in order to minimize tag-switching issues (Schnell et al., 2015). The PCR mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C and a final elongation step at 72 °C for 7 min. Running 50 cycles as in Valentini et al., (2016) and Cilleros et al., (2019), represents a compromise between the detection power of rare species and the risk of generating artefacts. The amplification step was performed in a dedicated room with negative air pressure and physical separation from the DNA extraction rooms (with

positive air pressure). The purified PCR products were then pooled in equal volumes to reach a sequencing depth of 500,000 reads per sample before the libraries preparation. Library preparation was performed at Fasteris facilities (Geneva, Switzerland) using Metafast protocol (www.fasteris.com/metafast). Two libraries were sequenced using an Illumina HiSeq 2500 (2x125 bp) (Illumina, San Diego, CA, USA) on a HiSeq Rapid Flow Cell v2 using the HiSeq Rapid SBS Kit v2 (Illumina, San Diego, CA, USA), three using a MiSeq (2x125 bp) (Illumina, San Diego, CA, USA) and the MiSeq Flow Cell Kit Version3 (Illumina, San Diego, CA, USA) and three using a NextSeq (2x150 bp+8) (Illumina, San Diego, CA, USA) and the NextSeq Mid kit (Illumina, San Diego, CA, USA). The libraries ran on the NextSeq were equally distributed in four lanes. The sequencing were performed at Fasteris (Geneva, Switzerland). Fourteen negative extraction controls and four negative PCR controls (ultrapure water, 12 replicates) were amplified per primer pair and sequenced in parallel to the samples to monitor possible contaminants.

The EMBL-EBI vertebrate database was downloaded from the European Nucleotide Archive (ENA) (<http://ftp.ebi.ac.uk/pub/databases/embl/release/std/>, release 134 for Maroni river sample, 138 for Oyapock and 140 for Sinnamary samples). The three releases were compared and the new mammalian species incremented in each new version did not belong to French Guiana. Our results were therefore uninfluenced by EMBL release number. We extracted from this database the relevant metabarcoding fragment using EcoPCR (Ficetola et al., 2010) and OBITools (Boyer et al., 2016). Our reference database thus includes the local database of French Guianese mammals (Kocher, de Thoisy, Catzeflis, Huguin, et al., 2017) which references 576 specimens of 164 species as well as all the vertebrate species available in EMBL.

The analysis of marker-gene data has long resorted to the construction of operational taxonomic units (OTUs): clustering of reads sufficiently similar to a sequence in a reference database (i.e. Closed-reference methods) or as a function of the read pairwise sequence similarities (i.e. *de novo* methods) (Callahan et al., 2017). Recently, amplicon sequence variant methods (ASVs), which discriminate sequencing errors from biological sequences without relying on the dissimilarity threshold defining the OTUs, has gained a considerable attention (Callahan et al., 2017). In our case, we used a nearly exhaustive local reference database lacking only extremely rare species not even found in specimens collections (Kocher, de Thoisy, Catzeflis, Huguin, et al., 2017). In addition, the marker used provide a very good accuracy and specificity. Both OTUs and ASV methods should provide similar results in this situation.

The sequence reads were analysed using the functions of the OBITools package following the protocol described in Valentini et al., (2016). Briefly, forward and reverse reads were assembled using *illumina-paired-end* program. Subsequently, the *ngsfilter* program was used to assign the sequences to each sample. A separate dataset was created for each sample by splitting the original dataset in several files using *obisplit*. Sequences shorter than 20 bp, or occurring less than 10 times per sample or labelled “internal” by the *obiclean* program, corresponding most likely to PCR errors, were discarded. The *ecotag* function was used for the taxonomic assignment of molecular operational taxonomic units (MOTUs). Taxonomic assignments from *ecotag* were also corrected to avoid over-confidence in assignments: species-level assignments were validated only for sequence identity with the reference database higher or equal than 98%. The MOTUs occurring with a frequency below 0.001 per library sample were considered as tag-jumps and discarded (Schnell et al., 2015). These thresholds were empirically determined to clear all reads from the extraction and PCR negative controls included in our global data production procedure as suggested in De Barba et al., (2014). For the samples sequenced with the NextSeq, only species present in at least two lanes were retrieved.

4. Line transects data

86 line transects were realised between 1998 and 2018 (See Figure S1). The line transect surveys were conducted as explained in de Thoisy et al., (2008) and in Richard-Hansen et al., (2015). Briefly, the line transect sampling consisted in visually recording fauna by walking slowly (0.8–1.3 km/h) on linear forest tracks measuring 3-5 km, presenting the same forest structure (census (Guitet et al., 2015)), but including various local habitats (i.e. hill, stream). Depending on the study, there was a single forest track (Thoisy et al., 2008) or four tracks per site (Richard-Hansen et al., 2015). The surveys were repeated daily until a cumulated sampling distance of more than 100 km was reached in each site. Those inventories were conducted during the day (from 07:00 to 18:00), and hence, strictly nocturnal species were not observed.

5. Data analysis

Because we use the “12S-V5” vertebrate marker (Riaz et al., 2011) for the amplification, we obtained broad observations for various vertebrate taxonomic groups. Data were thus first sorted to only keep mammal taxa and MOTUs assigned to the species level, thus retrieving 78

mammal species (see Table S1 and S2). Non-mammal species (amphibians, birds, reptiles) were discarded from this study because reference databases are still largely incomplete for these taxa.

To compare eDNA results with known spatial distribution of species, we used the Faune-Guyane database (GEPOG, 2020). It gathers citizen science data and observation data from scientific monitoring and constitutes the most detailed information on vertebrate distribution (excluding fishes) in French Guiana. We used the Faune-Guyane database to identify “emblematic mammalian fauna” used to conduct the comparative analysis with line transects and to discuss the consistency of the observations with the expected species distributions. “Emblematic mammalian fauna” included large (adult body mass > 1kg) mammals, but excluded the rarest species, those occurrences in French Guiana being not sufficient to draw a relevant distribution area. “Emblematic mammalian fauna” therefore excluded small mammals (adult body mass < 1 kg) such as Chiroptera, Rodentia and Didelphimorphia which are not easily identifiable without animal capture as well as medium and large mammals (adult body mass > 1 kg) considered as very rare or rare (see Table S2 for species list). This index of rarity was based on the ratio between species observation number and total number of observations, and was adjusted by experts to consider species that are difficult to observe but not necessarily rare. After the exclusion of the less documented species (47 species excluded), we focussed on 31 fairly well studied species, hereafter referred to as “emblematic mammalian fauna” to conduct the comparative analysis with line transects and to discuss the consistency of the observations with the expected distributions.

Line transects and aquatic eDNA metabarcoding survey methods are not directly comparable since they focus on different habitats/microhabitats, making site by site comparisons unrealistic. To investigate the relationship between these two methods, we used what we hereafter refer to as the *observation frequency*. Observation frequency represents the total number of sites where a species has been observed by a sampling method (line transects or aquatic eDNA metabarcoding) divided by the total number of sites. This metric differs from the species detection probability as it does not intend to define the probability of encountering at least one individual of a species present on a surveyed area (Boulinier et al., 1998), but it highlights the proportion of sites where a given species has been observed by aquatic eDNA metabarcoding or line transects across the considered region (i.e. at the scale of French Guiana). The comparison of the observation frequency obtained with both methods allow to investigate to what extent they provide similar inventories but also indicates if the observation frequency ranking is conserved between both methods. Although eDNA metabarcoding and line transect samplings were not conducted at the same sites and on the same time, both samplings cover a

substantial part of the Guianese territory and include most habitat types, levels of threats and anthropization, making relevant the broad comparison between eDNA metabarcoding and line transect sampling (Figure 1 and Figure S1). This comparison between eDNA and line transect observation frequencies should nevertheless be considered with caution as it is only meaningful over the entire studied region (French Guiana) and cannot be used to compare local areas within this region.

The observation frequency metric was computed using species by site matrices from eDNA metabarcoding and line transect data (Table S3 and S4). The species observation frequencies were calculated for both survey methods and compared with Mann Whitney U tests after species were classified as aquatic, terrestrial, arboreal, nocturnal and/or diurnal (Emmons & Feer, 1997; E. Hansen et al., 2000) to determine the effect of mammal habitat and ecology on observation frequency. Simple linear regressions were then performed to test for a linear relationship between the species observation frequencies calculated for both survey methods. To estimate the spatial consistency of the species observations with their expected distributions in French Guiana, we displayed the species occurrence patterns of several emblematic mammals with fairly well known ecologies, as well as the species richness pattern of the 31 emblematic mammal species considered in the study. All the analyses were computed in R software version 3.6.1 (2019-07-05) (R Core Team, 2019) and the maps were edited with ArcGis software.

Results

A total of 152,546,060 sequences were obtained from the eDNA samples and 99,492,637 reads were kept after bioinformatics processing. Overall, we obtained 4,524,515 reads after the removal of non-mammal species, corresponding to 78 mammal species across the 96 river sites. The mammal species retrieved belonged to 72 genera, 33 families and 11 orders (Table S1).

Among the 78 species, five species classified as very rare were detected using eDNA: the Emilia's gracile opossum (*Gracilinanus emiliae*), the Guianan white-eared opossum (*Didelphis imperfecta*), the rufous mouse opossum (*Marmosa lepida*), the white-faced spiny tree rat (*Echimyus chrysurus*) and the bush dog (*Speothos venaticus*). Moreover, among the 31 species detected and referred to as emblematic mammalian fauna, six are listed in the French-Guianese IUCN red list (IUCN France et al., 2017): the giant otter (*Pteronura brasiliensis*, endangered), the West Indian manatee (*Trichechus manatus*, endangered), the lowland tapir

(*Tapirus terrestris*, vulnerable), the jaguar (*Panthera onca*, nearly threatened), the puma (*Puma concolor*, nearly threatened) and, the white-lipped peccary (*Tayassu pecari*, nearly threatened).

1. Patterns of emblematic mammalian fauna observation frequency

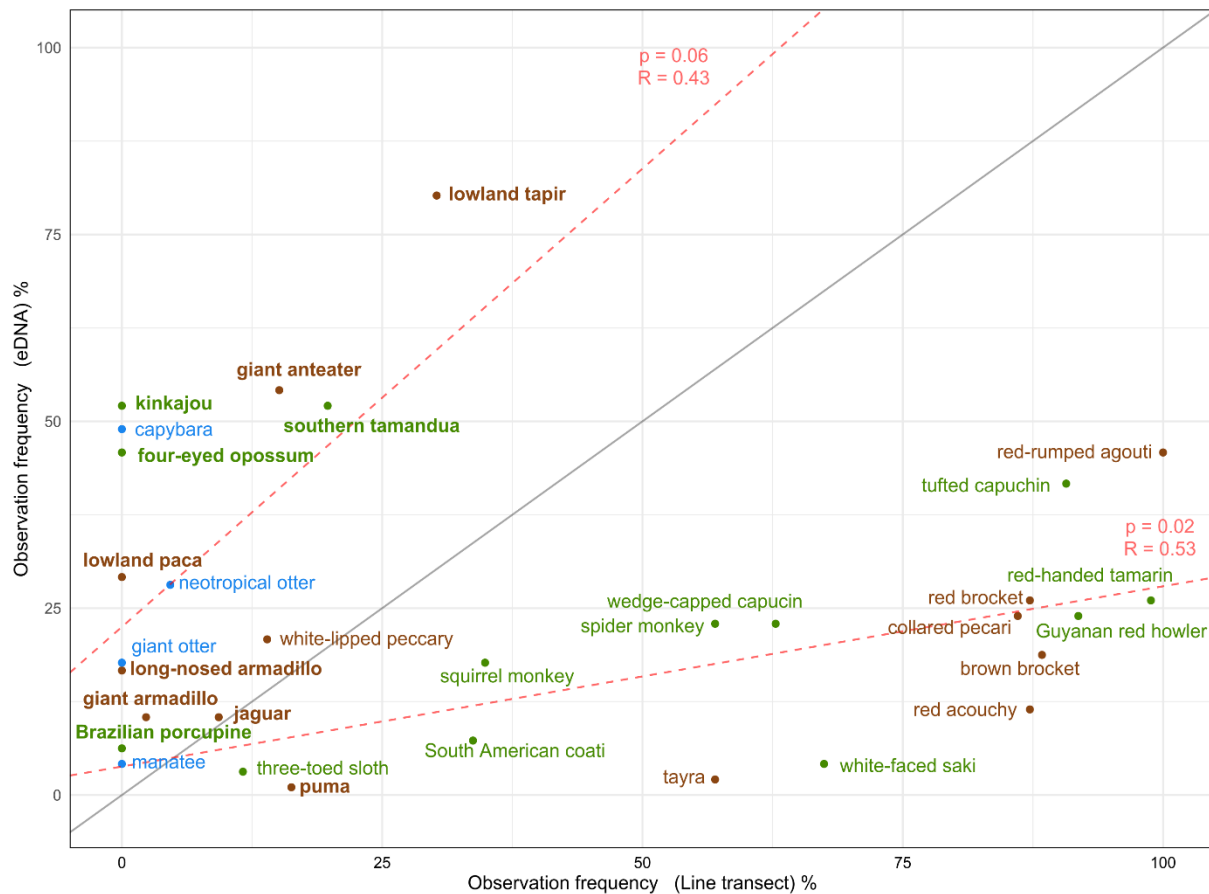


Figure 2. Observation frequency (%) of emblematic mammalian fauna obtained with aquatic eDNA metabarcoding and with line transects. Species in blue are aquatic or semi-aquatic, species in green are arboreal and species in brown are terrestrial. Bold species names refer to nocturnal species while regular font corresponds to diurnal species. The black solid line represents the 1:1 line. The red dotted lines above and beneath the 1:1 refer to the linear regressions between the observation frequency obtained with eDNA and line transects for nocturnal or aquatic/semi-aquatic species and for diurnal or non-aquatic species, respectively. p-value (p) and correlation coefficient (R) are indicated in red.

Eight species were only observed with aquatic eDNA metabarcoding (observation frequency in parentheses): the nocturnal kinkajou (*Potos flavus*, 52.08%), four-eyed opossum (*Philander opossum*, 45.83%), lowland paca (*Cuniculus paca*, 29.17%), long-nosed armadillo (*Dasypus kappleri*, 16.67%), Brazilian porcupine (*Coendou prehensilis*, 6.25%), the semi-aquatic capybara (*Hydrochoerus hydrochaeris*, 48.96%), giant otter (17.71%), and the aquatic West Indian manatee (4.17%) (Figure 2).

The lowland tapir presented the highest observation frequency with aquatic eDNA metabarcoding and was observed in 80.21% of the sites, while it was observed in only 30.23% of the line transect sites. Similarly, the southern tamandua (*Tamandua tetradactyla*) and giant anteater (*Myrmecophaga tridactyla*) as well as the neotropical otter (*Lontra laugicaudis*) were observed in 52.08%, 54.17%, and 28.13% of the sites with aquatic eDNA metabarcoding while they were observed in 19.77%, 15.12%, and 4.65% of the sites with line transect surveys, respectively. Finally, the jaguar was only slightly more observed with eDNA metabarcoding (10.42%) than with line transects (9.30%) (Figure 2).

In contrast, the observation frequency of primates including the spider monkey (*Ateles paniscus*), the wedge-capped capucin (*Cebus olivaceus*), the tufted capuchin (*Sapajus apella*), the red-handed tamarin (*Saguinus midas*), the Guianan red howler (*Alouatta macconnelli*), the white-faced saki (*Pithecia pithecia*), and the squirrel monkey (*Saimiri sciureus*) did not exceed 41.67% with aquatic eDNA metabarcoding while it ranged from 34.88% to 98.84% with line transect surveys. Similarly, the observation frequency of the common diurnal rodents including the red-rumped agouti (*Dasyprocta leporina*) and the red acouchy (*Myoprocta acouchy*) as well as the ungulates collared pecari (*Pecari tajacu*), red brocket (*Mazama americana*), and grey brocket (*Mazama nemorivaga*) and the tayra (*Eira barbara*) ranged from 56.98% to 100% with line transects while it ranged from 2.08% to 45.83% with aquatic eDNA metabarcoding (Figure 2).

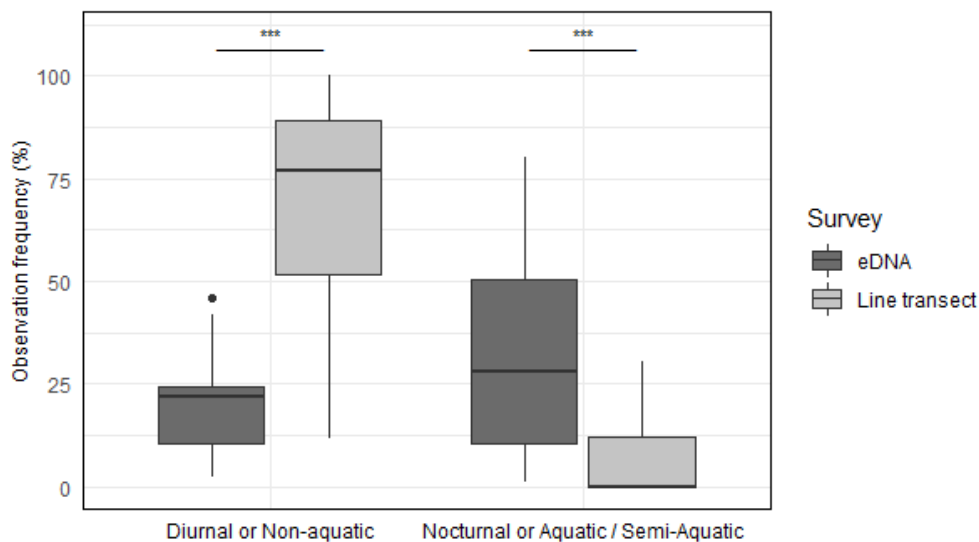


Figure 3. Observation frequency of emblematic mammalian fauna categorized into diurnal or nonaquatic and nocturnal or aquatic / semi-aquatic. Observation frequency obtained with line transects and aquatic eDNA metabarcoding were compared using a Mann-Whitney U-test, *** ($p < .001$).

Overall, aquatic/semi-aquatic and nocturnal fauna were significantly more observed with eDNA metabarcoding than with line transects (Mann Whitney U test, $U = 193$, $p < .001$, $n = 15$; Figure 2 & 3). Observation frequency of nocturnal and aquatic/semi-aquatic species ranged from 1.04 to 80.21% (median= 28.13) with aquatic eDNA metabarcoding while it ranged from 0 to 30.23% (median = 0) with line transects (Figure 3). Contrastingly, diurnal non-aquatic fauna was more observed with line transects than with aquatic eDNA metabarcoding (Mann Whitney U test, $U = 26$, $p < .001$, $n = 16$). Observation frequency of diurnal non-aquatic species ranged from 2.08 to 45.83% (median = .87) with aquatic eDNA metabarcoding while it ranged from 11.63 to 100% (median = 76.74) with line transect (Figure 3). The linear regression revealed a marginally significant linear relationship between the observation frequency of the two survey methods for aquatic/semi-aquatic and nocturnal species ($F_{(1,13)} = 4.20$, $p = .06$, $R = 0.43$, slope = 1.23). Observation frequency of the aquatic/semi-aquatic and nocturnal species was on average 4.7 times higher with aquatic eDNA metabarcoding than with line transects (Figure 2 & 3). On the contrary, there was a significant linear relationship between the observation frequency of the diurnal non-aquatic species obtained with the two survey methods ($F_{(1,14)} = 6.73$, $p = .02$, $R = 0.53$, slope = 0.24). Observation frequency of diurnal terrestrial and arboreal fauna was on average 3.4 times lower with aquatic eDNA metabarcoding than with line transects (Figure 2 & 3).

2. Species occurrence patterns of emblematic mammalian fauna

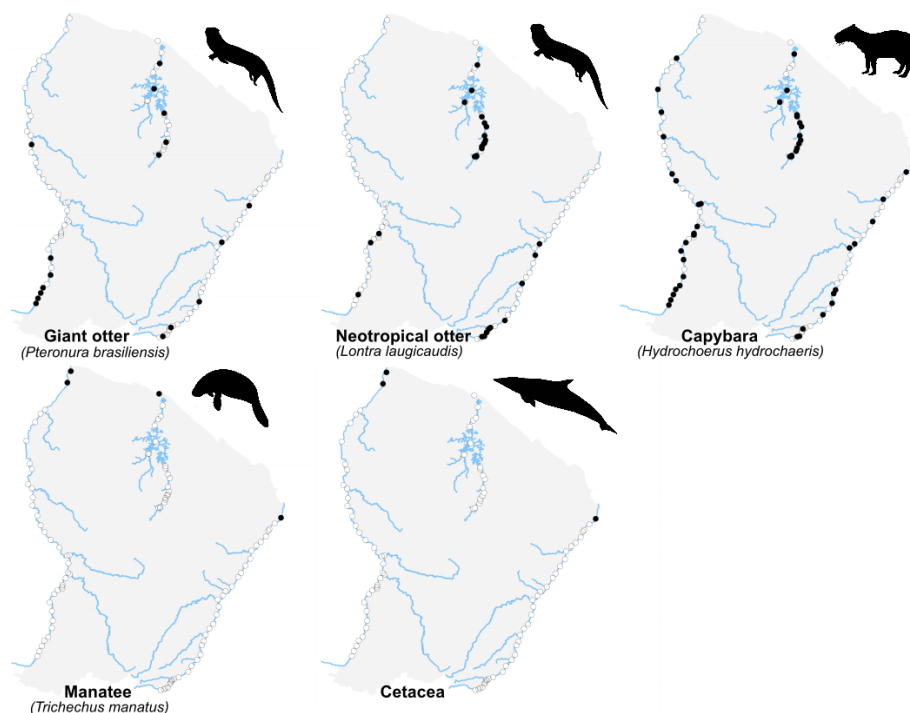


Figure 4. Species occurrences of several aquatic and semi-aquatic species. Presence (black dots) or absence of observations (white dots) in aquatic eDNA sampling sites are indicated on the maps.

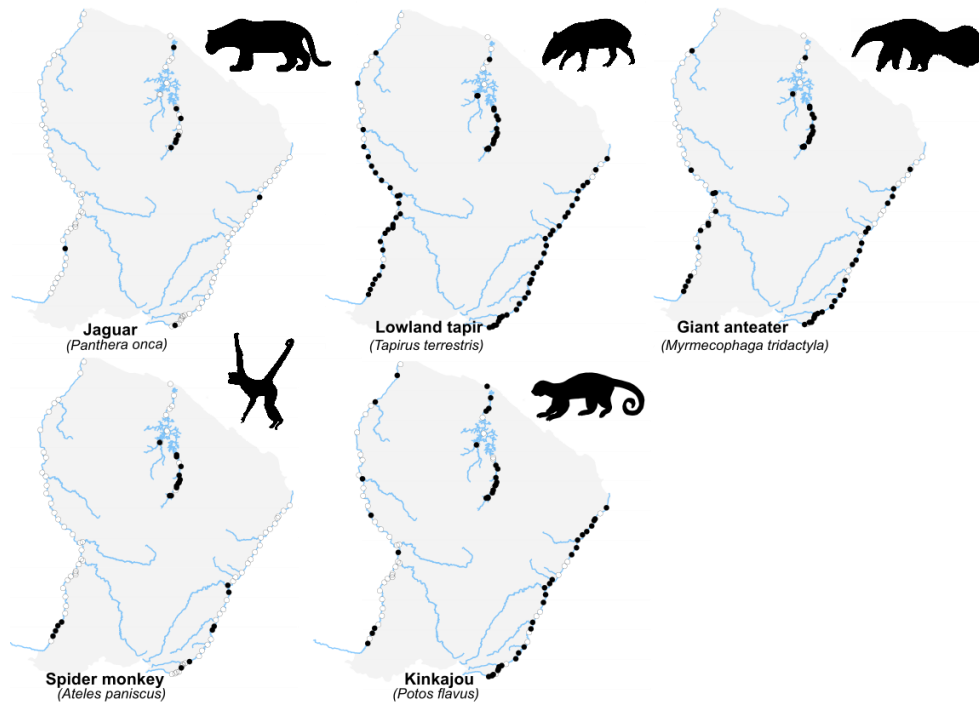


Figure 5. Species occurrences of several terrestrial or arboreal species. Presence (black dots) or absence of observations (white dots) in aquatic eDNA sampling sites are indicated on the maps.

Mammal species with a restricted distribution area were retrieved in their known habitat. The West Indian manatee was indeed observed in all three estuaries (sites M36, M37, S22 and O37). Similarly, Cetacea, although not identified to the species or genera level were observed in estuaries using eDNA metabarcoding (sites M36, M37 and O37; Figure 4), which is consistent with their known distribution (contrary to the nearby Amazon drainage, freshwater dolphins do not occur in French Guiana).

More widespread species, that inhabit the entire Guianese territory were also retrieved in a large part of the eDNA sites, or are clustered in the least anthropized areas for the species known as sensitive to human disturbances. eDNA metabarcoding observations of the capybara, the giant anteater, the kinkajou and the lowland tapir extended from the upstream to the downstream of the three rivers (excepted for the sites located at the estuaries) (Figure 4 & 5). In contrast, the giant otter, the neotropical otter and the spider monkey presented similar spatial pattern of distribution and were mostly observed at the upstream of the rivers (Figure 4 & 5). To the exception of site M5 and M7, the giant otter was observed in the six most upstream sites of the Maroni river. On the Oyapock river, this species was observed in five sites distributed all along the watercourse while it was retrieved in four sites located upstream the dam, and in one site located downstream the dam of the Sinnamary river. The neotropical otter was observed in two sites located at the upstream and in one site located near the upstream human settlements on the Maroni river. On the Oyapock river, the species was also observed in nine sites located

all along the watercourse while the species was observed in 14 sites located in the upstream part of the Sinnamary river and in a single site located downstream from the ‘Petit Saut’ dam (Figure 4). Similarly, the spider monkey observations occurred in four sites on the upstream of the Maroni river, in six sites distributed along the Oyapock river and in nine sites located upstream the Sinnamary dam (Figure 5). The jaguar observations were scarce, notably on the Maroni river with only one observation at the upstream and two observations at the upstream and the downstream of the Oyapock river. On the Sinnamary, the species was observed in six upstream sites and in one site downstream the dam (Figure 5).

3. Richness pattern of emblematic mammalian fauna

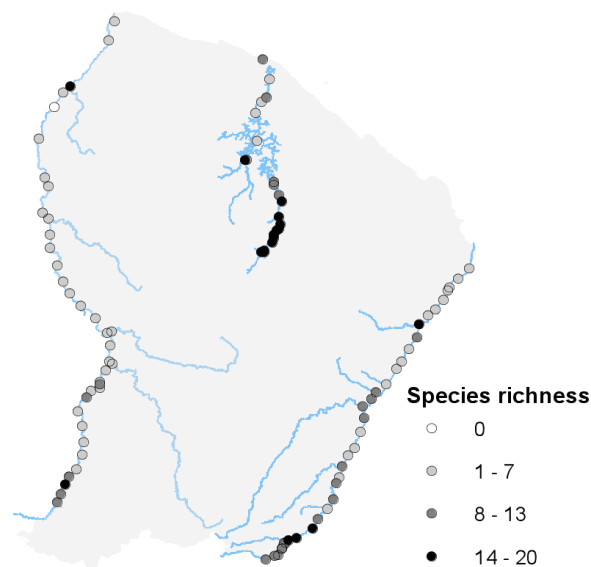


Figure 6. Emblematic mammalian fauna richness observed at each aquatic eDNA sampling site.

Out of the 31 emblematic mammals considered, 27, 28 and 31 mammal species were observed using eDNA metabarcoding on the Maroni, the Oyapock and the Sinnamary rivers, respectively. On the Maroni river, the site species richness ranged from 0 to 14 (median = 5) while it ranged from 1 to 17 (median = 8) and from 2 to 20 (median = 14) for the Oyapock and the Sinnamary river, respectively. The site species richness along the Maroni river was heterogeneous with the most upstream sites being richer than the sites located downstream from the Maripasoula village, to the exception of site M35. One site located at the downstream of the Maroni river (M33) did not provide any emblematic mammal species observation (Figure 6). Along the Oyapock river, the site species richness was distributed more homogeneously along the watercourse, the sites O9, O10, O11 and O30 being the richest (from 14 to 17). The upstream

of the Sinnamary river (S1 to S11, S15) presented the sites with the highest species richness (14 – 20), which were concentrated upstream of the dam (Figure 6).

Discussion and Conclusion

Although aquatic eDNA metabarcoding has widely been used to inventory aquatic fauna, the method is raising new interests to inventory non-aquatic species. To date, the method remains exploratory as several challenges still need to be addressed. The reliability of this survey method has already been investigated by comparing the inventoried fauna to that obtained with other methods such as camera trapping (Sales, Kaizer, et al., 2020; Sales, McKenzie, et al., 2020). Here, comparing aquatic eDNA metabarcoding inventories to line transect observations over the Guianese territory revealed consistent patterns between the expected species distributions and eDNA detections, making eDNA a promising tool to inventory both aquatic and terrestrial fauna.

1. Observation frequencies between aquatic eDNA metabarcoding and line transects

Comparing eDNA metabarcoding observations to those of traditional line transects revealed that nocturnal and aquatic species were more often observed in eDNA samples than in line transects, whereas diurnal terrestrial and arboreal species were more often observed using line transects. We nevertheless detected a marginally significant relationship between the observation frequency of nocturnal and semi-aquatic species and a significant relationship between the diurnal terrestrial and arboreal species from both methods indicating that eDNA metabarcoding retrieved a similar pattern of observation ranking compared to line transects. Although the observation frequency with aquatic eDNA metabarcoding is on average 3.4 times lower than that of line transects for diurnal terrestrial and arboreal mammals, sampling eDNA in a site is achieved in less than an hour. We therefore believe that aquatic eDNA metabarcoding can constitute a useful complement to line transect samples (or other sampling methods) for terrestrial and diurnal mammals given that the eDNA collection by water filtrations can be rapidly achieved during survey campaigns. A less stringent pattern was found for nocturnal and aquatic species, with giant anteater and tamandua being more frequently observed by both methods than the rare giant armadillo (Carter et al., 2016; Catzefflis & Thoisy, 2012) or the elusive jaguar which has a large individual home range and low population densities (Petit et al., 2018). For those species, observation frequency was fourfold higher using aquatic eDNA

metabarcoding than line transects. Together with the eight species only observed with eDNA metabarcoding, this testifies to the capacity of this method to detect nocturnal and aquatic species rarely or not observed in line transect inventories.

These relationships remain however dependent on the species considered as the observation frequency of some species can be biased by different parameters. Indeed, aquatic eDNA metabarcoding may be sensitive to particular mammal's behaviour (Harper et al., 2019). For instance, the tapir was observed in 80% of the eDNA sites regardless the proximity of villages or the land use. Yet, the tapir is an appreciated game species due to its size and the quality of its meat and is therefore under pressure in areas accessible to hunters (Richard-Hansen et al., 2019; Tobler et al., 2014). This high observation frequency was already observed by Sales, Kaizer, et al., (2020) and may be explained by the high affinity of tapir for the water, combined with its habit of defecating in the water (Tobler et al., 2010). Despite such species presenting particularities hindering fine-scale observations, aquatic eDNA metabarcoding could constitute a valuable complement to traditional samples, as it allows extending the range of species and habitats to be inventoried, while saving time for biodiversity inventories.

2. Species occurrence patterns

Detailing the occurrences of the West Indian manatee illustrated the capacity of aquatic eDNA metabarcoding to detect species only in their area of distribution. The West Indian manatee was observed in all the three estuaries sampled and in no other site, estuaries being the typical habitat of this species (de Thoisy et al., 2016). The Cetacea observations were also exclusively retrieved at the estuarine sites. Those consistent observations to the distribution area of the species thus constitute a proof of absence of false positives (observation of the species outside their distribution area) for those species.

The occurrences of the mammals inhabiting all the territory showed that some species were observed regardless of human proximity. Among them, the capybara and the kinkajou were observed in half of the sites (50.52%). They are known as tolerant to human presence, kinkajou being a discrete nocturnal and arboreal species disregarded by hunters; and capybara being a generalist species not appreciated by hunters because of the strong taste of its meat (hunting surveys show that they represent only 0.5 and 1.5% respectively out of 14,570 mammals hunted, Richard-Hansen et al., 2019). Other species recognised to be negatively impacted by anthropogenic activities such as the spider monkey or the neotropical and giant otter (de Thoisy et al., 2005; Rheingantz et al., 2014; Richard-Hansen et al., 2019) were

preferentially observed in the upstream part of the rivers, which is free from dense human settlements or activities. For some observations, we cannot exclude that aquatic eDNA comes from the butchering of hunted animals (animals are hunted far away, brought back and butchered in the villages), as the observations of the spider monkey near the Trois-saut and Camopi villages are consistent with the hunting habits of Wayapi and Teko people, researching spider monkey for its meat (de Thoisy et al., 2009; Richard-Hansen et al., 2019).

3. Richness patterns

Overall, inventories of the emblematic mammalian fauna using aquatic eDNA metabarcoding revealed strong species richness variations between the three rivers, with the Sinnamary river presenting a high species richness in a large part of its course whereas the Maroni shows rich assemblages only in restricted areas. This gradient is consistent with the human presence on these rivers, the Maroni being the most inhabited and the Sinnamary river being much less occupied by humans, with a human population density approximately 10-fold lower on the Oyapock than on the Maroni (de Mérona et al., 2012; Gallay et al., 2018).

We also outlined a trend toward highest mammal richness in the upstream part of the studied rivers, which are the least impacted by mining activities and the least densely populated by humans (de Thoisy et al., 2010; Stach et al., 2009). Maximal species richness values were indeed detected in the upstream part of the Sinnamary river, which is free from any human settlement and integrally protected as part of the core area of the Parc Amazonien de Guyane. Upstream Maroni is also free from human settlements, and traditional hunting activities by local people remain limited due to the difficulty to access these areas. On the contrary, the upstream part of the Oyapock river hosts around 1700 inhabitants which rely on local fishing and hunting as sources of proteins (Richard-Hansen & Hansen, 2004). Subsistence hunting and deforestation remains however scarce (only slash-and-burn subsistence agriculture) and this is consistent with the upstream site species richness remaining higher compared to the most downstream sites, despite hunted species abundances were shown to be locally reduced by Richard-Hansen et al., (2019).

4. Challenges and applications

Although we globally retrieved consistent patterns of species distribution/richness that are comparable to line transects, aquatic eDNA metabarcoding for assessing non-aquatic species has some limitations. Among those limitations, false negative (i.e. missing observations

of present species) is a common challenge encountered in most (if not all) survey methods (Tyre et al., 2003). While with aquatic communities such as fish, the species detectability may be conditioned by species relative abundance or species morphology and physiology (Hunter et al., 2019; Lacoursière-Roussel, Rosabal, et al., 2016), false negatives may be more frequent when assessing non-aquatic fauna as those species are less (or not directly) in contact with the water. The heterogeneous liberation of DNA in the water is then dependent on species density, species morphological and physiological characteristics but also species behaviour and water affinity (Harper et al., 2019), probably influencing the detectability of species. Moreover, site characteristics and environmental conditions may also influence the quantity of eDNA retrieved and therefore impact the false negatives rate and the inventories (Lacoursière-Roussel, Côté, et al., 2016; Lacoursière-Roussel, Rosabal, et al., 2016; Rees et al., 2014; Roussel et al., 2015). In our study system, Cantera et al., (2019) demonstrated that for a same sampling effort, fish community inventories were significantly less exhaustive in large compared to small watercourses. We therefore cannot exclude such sampling effect between small and large watercourses in our study as well.

Moreover, the spatial signal of eDNA (spatial extent of the downstream transport of eDNA) defining the spatial grain of the inventories may also be a determining parameter to consider when assessing the presence of species (Hauger et al., 2020). In our systems, Cantera, Decotte, et al., (2022) demonstrated that the downstream detection of eDNA was short (not exceeding few kilometres) but it might already be enough to observe vulnerable species in areas where the hunting pressure is concentrated on a small spatial extent (from 2 up to 5 km in the periphery of the river, Richard-Hansen et al., (2019)).

These limits interrogate to what extent should aquatic eDNA metabarcoding be used for biodiversity monitoring and particularly species of concern including invasive, pathogenic, threatened, endangered and other vulnerable species. In our study, incidental detections (unanticipated detection of species of concerns) may be precious to improve knowledge on species distributions, but the lack of regularity and exhaustivity of the method may represent a risk if used as the sole method to assess the presence of such species or to monitor the state of biodiversity (Darling et al., 2020).

Despite those limits, aquatic eDNA metabarcoding provides an efficient way to complement and extend traditional inventories. Although eDNA only provide presence data without information on species abundance, it allows to detect rare, and endangered species as illustrated by the detection of six species of IUCN concern and of five species classified as very rare in the Faune Guyane database. Moreover, aquatic eDNA metabarcoding provides presence

data for species not detectable in traditional surveys, be they aquatic or nocturnal. For instance, the widespread distribution of kinkajou revealed by eDNA strikingly contrasts with the rarity of visual observations, but coincides with local camera trap experiments revealing its local commonness (Séguigne et al., 2022). Aquatic eDNA metabarcoding therefore offers a way to extend our knowledge on mammal occurrences. Despite a lower observational frequency than the traditional line transect method for diurnal and terrestrial fauna, the sampling effort needed to collect an eDNA sample (no more than a couple of hours for a single person) makes it easily implementable together with line transects or other survey methods to complement and extend inventories. In addition, although eDNA-based methods using terrestrial substrates may be more appropriate to survey nonaquatic mammals, eDNA shed by organisms disperse less easily on the ground than in the water. Consequently, sampling strategies have significant impacts on inventories because the information collected in a single ground sample has a very restricted spatial definition (Valentin et al., 2020; Zinger, Taberlet, et al., 2019). Contrastingly, aquatic eDNA based-methods use water bodies that passively aggregate eDNA shed from target species at a wider scale than the sampling point (Deiner et al., 2016; Zinger et al., 2020). However, these methods may have limited on-field applications as they require the presence of waterbodies in the surveyed areas. Nevertheless, the lack of efficient methods to collect terrestrial derived eDNA is a major limitation in the eDNA-based terrestrial biodiversity monitoring. We therefore believe that mammal inventories performed with aquatic eDNA metabarcoding methods and adapted from protocols already well established to assess aquatic communities present a great potential to survey species rich environments such as Neotropical ecosystems, and thus complement quantitative, but more taxa specific and time consuming traditional mammal surveys using line transects.

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Author Contributions

O.C., S.B., J.M., C.R.H., and B.d.T conceived the ideas and designed methodology; O.C., S.B., J.M., J.B.D., and R.V. collected the data; A.V. and T.D. conducted the laboratory work; A.V. and T.D. conducted bioinformatic analyses; O.C. analysed the data; O.C., S.B., J.M., C.R.H., and B.d.T led the writing of the manuscript. All authors contributed critically to the drafts.

Supplementary Materials

See the online version of the article

Chapter II. Investigating biodiversity distribution patterns at local and regional scales to assess anthropogenic impacts on biodiversity

1. Part 1

Low level of Anthropization linked to harsh vertebrate biodiversity declines in Amazonia

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Abstract

Assessing the impact of human activity on ecosystems often links local biodiversity to disturbances measured within the same locality. However, remote disturbances may also affect local biodiversity. Here, we used environmental DNA metabarcoding to evaluate the relationships between vertebrate biodiversity (fish and mammals) and disturbance intensity in two Amazonian rivers. Measurements of anthropic disturbance -here forest cover losses- were made from the immediate vicinity of the biodiversity sampling sites to up to 90 km upstream. The findings suggest that anthropization had a spatially extended impact on biodiversity. Forest cover losses of <11% in areas up to 30 km upstream from the biodiversity sampling sites were linked to reductions of >22% in taxonomic and functional richness of both terrestrial and aquatic fauna. This underscores the vulnerability of Amazonian biodiversity even to low anthropization levels. The similar responses of aquatic and terrestrial fauna to remote disturbances indicate the need for cross-ecosystem conservation plans that consider the spatially extended effects of anthropization.

Introduction

The current decline in global biodiversity must be addressed proactively to protect and restore ecosystems (Leclère et al., 2020). A coalition of environmental organisations proposed the protection of at least 30% of the Earth's surface by 2030 with a final target of 50% by 2050 (Dinerstein et al., 2019; Pimm et al., 2018). These targets were based on the conservation of the spatial ranges of 85% of all species. However, they did not consider how human disturbances in surrounding unprotected areas will affect the biodiversity of the protected areas.

Given the hydrologic connectivity of river networks, the fauna inhabiting river catchments may be affected by remote disturbances via the water-mediated downstream transfer of matter, energy, and/or organisms (Pringle, 2001). Catchment-scale variables that affect the local conditions of rivers through hydrologic connectivity have been acknowledged (Leal et al., 2020; Leitão et al., 2018; Lindberg et al., 2011; McCluney et al., 2014; Pringle, 2001), as well as the downstream impact of large dams, cities, and land use alterations on river ecology (Anderson et al., 2018; McManamay et al., 2017). Theoretical efforts to account for the impact of hydrologic connectivity on freshwater conservation planning have been conducted (Fagan, 2002). However, such plans have been poorly implemented due to a lack of theory and tools to resolve this in a systematic conservation planning framework (Linke et al.,

2007), in part because the spatial extent of the influence of upstream disturbances on downstream biodiversity has not been addressed.

Moreover, the spatial extent of disturbances must be considered both for aquatic and terrestrial fauna as ecological land/water linkages result in a lateral connectivity between rivers and terrestrial habitats. Disturbances mediated by hydrologic connectivity may hamper the movement of terrestrial species, the recolonization of defaunated areas, seed dispersal, and pollination (Leal et al., 2020; Tewksbury et al., 2002). The importance of cross-realm connections between adjacent aquatic and terrestrial systems was recently underscored by Leal et al., (2020), showing that freshwater-focused conservation programmes also benefit nearby terrestrial ecosystems. Therefore, determining whether aquatic and terrestrial fauna respond comparably to disturbances may foster the design and implementation of spatially explicit conservation plans that integrate cross-ecosystem ecological connections (Tulloch et al., 2021). Similar downstream extents of disturbances between aquatic and terrestrial fauna could promote the design of efficient cross-ecosystems conservation programmes (Leal et al., 2020). In contrast, different responses of freshwater and terrestrial organisms to remote upstream disturbances would necessitate independent and separate conservation designs.

To tackle the foregoing issues, we here measured the spatial extent and the strength of anthropization on biodiversity by analysing the relationships between diversity of local fauna and disturbance intensity measured at multiple scales. We calculated the disturbance intensities at different spatial extents from the immediate vicinity of the biodiversity sampling sites (0.5 km) to a radius of 90 km upstream from the sites (Figure 1). For each spatial extent, the disturbance intensity was represented by the percentage of deforested surfaces, which served as an integrative measure of anthropization consisting of logging, mining, urban settlements, and conversion of land for agricultural uses (Curtis et al., 2018; Watson et al., 2018). On the basis of the hydrologic connectivity of the river systems, we expected that deforestation would have far-reaching effects on the downstream freshwater biodiversity because it modifies water flow, sediment transport, water quality, and aquatic food webs (Castello & Macedo, 2016; Mello et al., 2018; Paula et al., 2018). Moreover, the water-mediated transfer of disturbances caused by deforestation could also alter the linkages between aquatic and terrestrial ecosystems and, by extension, affect the terrestrial fauna.

We applied the foregoing to the Maroni and Oyapock Rivers (Figure 2) in the Northern Amazonian region (Guiana Shield). Their watersheds have undergone slight anthropization; 0.67% of the basins of both rivers have deforested surfaces. These two rivers are situated in one of the most ecologically intact areas worldwide (Su et al., 2021; Watson et al., 2018).

Nevertheless, they are under unprecedented threat as they are being subjected to increasing deforestation for gold mining. This anthropic activity disperses pollutants and sediments in the rivers and severely disturbs aquatic and terrestrial fauna (Dezécache et al., 2017; Hammond et al., 2007). The recent development of aquatic environmental DNA (eDNA) (Ficetola et al., 2008; Taberlet et al., 2018) has enabled us to build simultaneous inventories of aquatic and terrestrial vertebrate fauna along the main river channels and tributaries of the two rivers. We then evaluated the extent to which upstream disturbances are linked to fish and mammal species downstream. As freshwater conservation schemes may also benefit adjacent terrestrial ecosystems (Leal et al., 2020), we applied the same framework to both terrestrial and semi-aquatic mammals and determined whether the spatial extent of freshwater disturbances also applies to terrestrial ecosystems.

Here we found that forest cover losses of <11% in areas up to 30 km upstream from the biodiversity sampling sites are linked to reductions of >22% in taxonomic and functional richness of both terrestrial and aquatic fauna. The absence of some mammal predators and fish detritivores and herbivores downstream from deforested areas suggests that slight deforestation

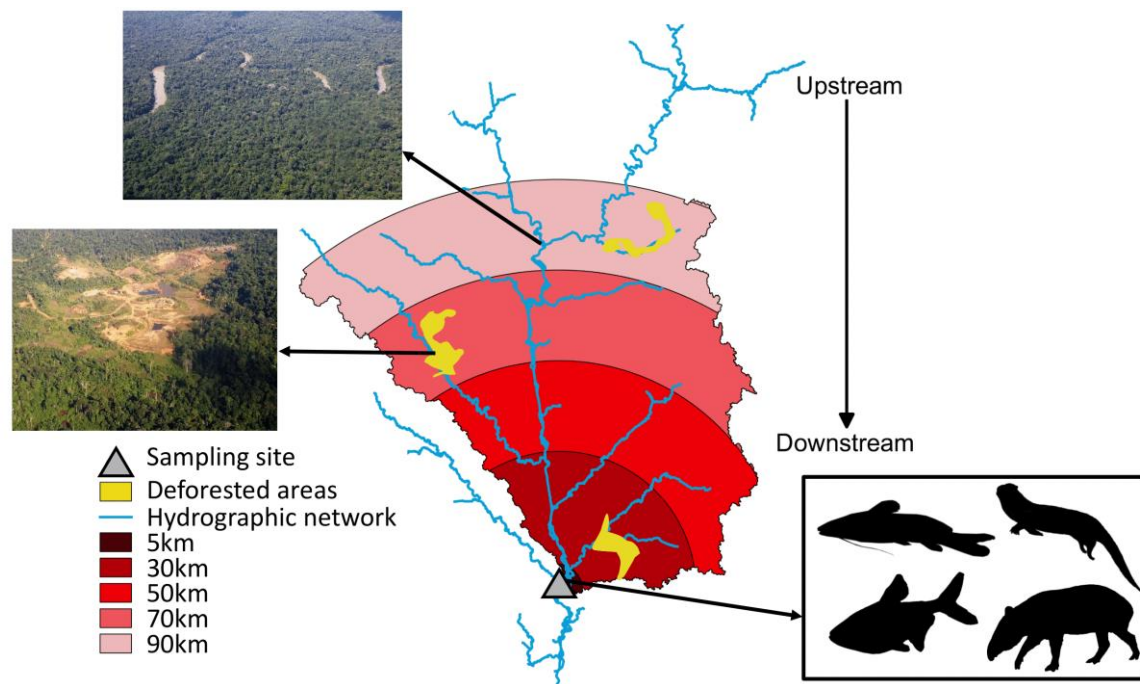


Figure 1. Measurement of the percentage of deforestation upstream from each biodiversity sampling site. For clarity, only five of the 14 spatial extents are illustrated here. Spatial extents are represented by the surface area of the river drainage basin between the biodiversity sampling site and 5, 30, 50, 70, and 90 km upstream from the site. River basin boundaries are indicated by black continuous lines. For each site, the percentage of deforested area for each spatial extent was calculated. For instance, a 30 km extent of disturbance measures the percentage of deforested area within the river basin from the biodiversity sampling site to a maximal distance of 30 km upstream from this site

due to mining, logging and agriculture causes remote decreases in biodiversity of downstream fauna, that could impact terrestrial and aquatic ecosystem functioning in the Northern Amazonian region. Moreover, the consistent response of terrestrial and aquatic fauna to upstream deforestation highlights the importance of cross-ecosystem conservation actions accounting for local and distant impacts of disturbances and for cross-ecosystem connectivity.

Materials and Methods

1. Study area

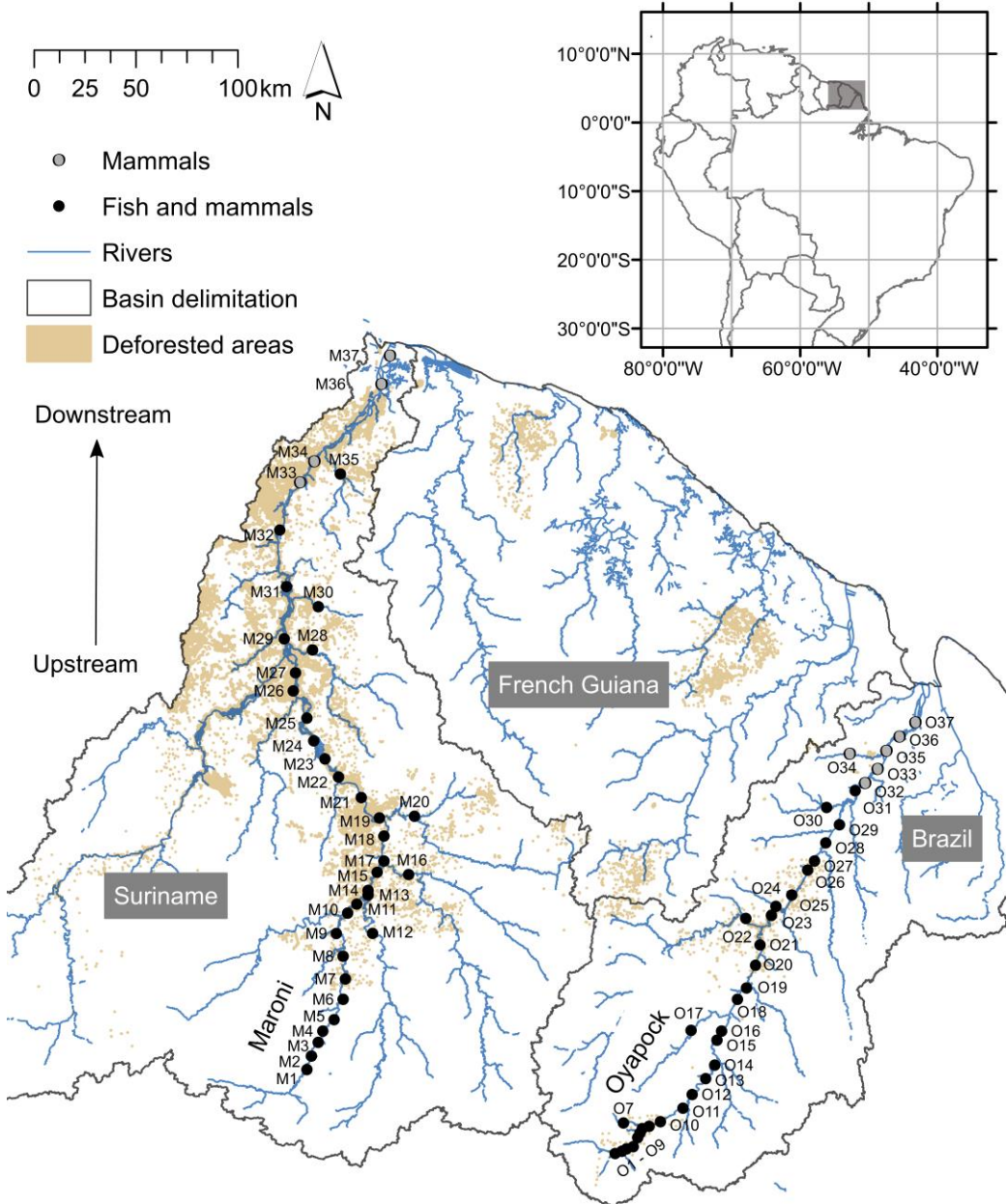


Figure 2. Map of the study area and biodiversity sampling sites. The 64 fish sampling sites are shown in black. Mammals were sampled at all 74 sites. The highlighted grey area in the inset at the upper right indicates the study area in South America.

The study was conducted on two rivers in north-eastern Amazonia *sensu lato*, including the Guiana Shield and the Amazon River drainage (Figure 2). The climate of the entire study area is homogeneous and the region is covered by dense, uniform lowland primary rainforest (A. Hansen et al., 2019). The altitude is in the range of 0–860 m a.s.l. The regional climate is equatorial, and the annual rainfall ranges from 3,600 mm in the northeast to 2,000 mm in the southwest. The Maroni River is 612 km long from its source to its estuary, and its watershed covers a surface of >68,000 km² in Suriname and French Guiana. The Oyapock River (length, 404 km; area, 26,800 km²) is located in the state of Amapa in Brazil and in French Guiana.

The foregoing river basins host nearly 400 freshwater fish species and more than 180 mammal species (Albert & Reis, 2011; Emmons & Feer, 1997). Most of the mammal species have a large distribution range, covering the entire study area (Emmons & Feer, 1997). The fish species have a less homogeneous distribution and a distinct upstream-downstream composition gradient (Le Bail et al., 2000; Planquette et al., 1996). Here, only large rivers were considered and most fish species were widespread over the whole study area. As habitat availability increases with river size, species richness is expected to increase upstream to downstream (Altermatt, 2013; Muneeppeerakul et al., 2008). The Oyapock and Maroni river basins are among the last remaining wilderness areas on Earth (Watson et al., 2018). Nevertheless, ecological disturbances are increasing there because of a growing human population and the development of small-scale gold mining activity. These disturbances have caused limited but diffuse deforestation (Dezécache et al., 2017; Rahm et al., 2017). The deforested areas currently comprise 0.67% of all Maroni and Oyapock catchments.

2. Sampling

Environmental DNA (eDNA) was collected from water samples at 74 locations (hereafter, sites) along the main channel and the large tributaries of the Maroni and Oyapock rivers (Figure 2). Thirty-seven sites were sampled at each river basin. The minimum and maximum distances between adjacent sites were 1.07 km and 50.20 km, respectively. The mean and median distances between adjacent sites were 10.18 km and 9.14 km, respectively, and the standard deviation (SD) was 7.79 km. The sites were located from sea level to 157 m a.s.l. At all sites, the river was wider than 20 m and deeper than 1 m (Strahler orders 4–8; Supplementary Figure 5). The physicochemical properties of the water slightly varied among sites. The temperature, pH, and conductivity were in the ranges of 28.4–33.2 °C, 6.5–7.6, and 16.9–54.6

$\mu\text{S/cm}$, respectively, at all sites except two estuarine locations where the conductivity was relatively high because of seawater incursion (Supplementary Data 2).

The eDNA samples were collected during the dry seasons (October–November) of 2017 and 2018 for Maroni and Oyapock, respectively. At both rivers, the sites were sequentially sampled from downstream to upstream at a rate of 1–4 sites per day depending on the distance and travel time between sites. Following the protocol of Cantera et al., (2019), we collected the eDNA by filtering two replicates of 34 L of water per site. A peristaltic pump (Vampire Sampler; Buerkle GmbH, Bad Bellingen, Germany) and single-use tubing were used to pump the water into a single-use filtration capsule (VigiDNA, pore size 0.45 μm ; filtration surface 500 cm^2 , SPYGEN, Bourget-du-Lac, France). The tubing input was placed a few centimetres below the water surface in zones with high water flow as recommended by Cilleros et al., (2019). Sampling was performed in turbulent areas with rapid hydromorphologic units to ensure optimal eDNA homogeneity throughout the water column. To avoid eDNA cross-contamination among sites, the operator remained on emerging rocks downstream from the filtration area. At the end of filtration, the capsule was voided, filled with 80 mL CL1 preservation buffer (SPYGEN), and stored in the dark up to one month before the DNA extraction. No permits were required for the eDNA sampling and the access to all sites was legally permitted. The study complies with access and benefit permits ABSCH-IRCC-FR-246820-1 and ABSCH-IRCC-FR-245902-1, authorizing collection, transport and analysis of all environmental DNA samples used in this study.

3. Laboratory procedures and bioinformatic analyses

For the DNA extraction, each filtration capsule was agitated on an S50 shaker (Ingenieurbüro CAT M. Zipperer GmbH, Ballrechten-Dottingen, Germany) at 800 rpm for 15 min, decanted into a 50 mL tube, and centrifuged at $15,000 \times g$ and 6 °C for 15 min. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of the tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50 mL tube, and the mixtures were stored at -20 °C for at least one night. The tubes were then centrifuged at $15,000 \times g$ and 6 °C for 15 min, and the supernatants were discarded. Then, 720 μL of ATL buffer from a DNeasy Blood & Tissue Extraction Kit (Qiagen, Hilden, Germany) was added. The tubes were vortexed, and the supernatants were transferred to 2 mL tubes containing 20 μL proteinase K. The tubes were then incubated at 56 °C for 2 h. DNA extraction was performed using a NucleoSpin Soil kit (Macherey-Nagel GmbH, Düren, Germany) starting

from step six of the manufacturer's instructions. Elution was performed by adding 100 μ L of SE buffer twice. After the DNA extraction, the samples were tested for inhibition by qPCR following the protocol in . Briefly, quantitative PCR were performed in duplicate for each sample. If at least one of the replicates showed a different Ct (Cycle threshold) than expected (at least 2 Cts), the sample was considered inhibited and diluted 5-fold before the amplification.

For the fish, the “teleo” primers (Valentini et al., 2016) (forward: 3'-ACACCGCCCGTCACTCT-5'; reverse: 3'-CTTCCGGTACACTTACCATG-5') were used as they efficiently discriminated local fish species (Cilleros et al., 2019; Coutant et al., 2020). For the mammals, the 12S-V5 vertebrate marker (Riaz et al., 2011) (forward: 3'-TAGAACAGGCTCCTCTAG-5'; reverse: 3'-TTAGATACCCCACTATGC-5') was used as it also effectively distinguishes local mammal species (Coutant et al., 2021; Kocher, Thoisy, Catzefflis, Huguin, et al., 2017). The DNA amplifications were performed in a final volume of 25 μ L containing 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 μ M of each primer, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM of each dNTP, and 3 μ L DNA template. Human blocking primer was added to the mixture for the “teleo” (Valentini et al., 2016) (5'-ACCCTCCTCAAGTATACTTCAAAGGAC-C3-3') and the “12S-V5” primers (De Barba et al., 2014) (5'-CTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAACCTGCT-C3-3') at final concentrations of 4 μ M and 0.2 μ g/ μ L bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland). Twelve PCR replicates were performed per field sample. The forward and reverse primer tags were identical within each PCR replicate. The PCR mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 30 s at 55 °C for the “teleo” primers and 50 °C for the 12S-V5 primers, 1 min at 72 °C, and a final elongation step at 72 °C for 7 min. This step was conducted in a dedicated room for DNA amplification that is kept under negative air pressure and is physically separated from the DNA extraction rooms maintained under positive air pressure. The purified PCR products were pooled in equal volumes to achieve an expected sequencing depth of 500,000 reads per sample before DNA library preparation.

For the fish analyses, 10 libraries were prepared using a PCR-free library protocol (<https://www.fasteris.com/metafast>) at Fasteris, Geneva, Switzerland. Four libraries were sequenced on an Illumina HiSeq 2500 (2 \times 125 bp) (Illumina, San Diego, CA, USA) with a HiSeq SBS Kit v4 (Illumina), three were sequenced on a MiSeq (2 \times 125 bp) (Illumina) with a MiSeq Flow Cell Kit Version3 (Illumina), and three libraries were sequenced on a NextSeq (2 \times 150 bp + 8) (Illumina) with a NextSeq Mid kit (Illumina). The libraries run on the NextSeq were equally distributed in four lanes. Sequencing was performed according to the

manufacturer's instructions at Fasteris. For the mammal analyses, eight libraries were prepared using a PCR-free library protocol (<https://www.fasteris.com/metafast>) at Fasteris. Two libraries were sequenced on an Illumina HiSeq 2500 (2 × 125-bp) (Illumina) using a HiSeq Rapid Flow Cell v2 and a HiSeq Rapid SBS Kit v2 (Illumina), three libraries were prepared on a MiSeq (2 × 125-bp) (Illumina) with a MiSeq Flow Cell Kit Version3 (Illumina), and three libraries were prepared using a NextSeq (2 × 150-bp +8) (Illumina) and a NextSeq Mid kit (Illumina). The libraries run on the NextSeq were equally distributed in four lanes. As different sequencing platforms were used (MiSeq and NextSeq for the Maroni and HiSeq 2500 and MiSeq for the Oyapock; Supplementary Figure 6 and Supplementary Data 3), the possible influences of the platforms on the sequencing results were verified. To this end, we compared the differences in species numbers between the sample replicates assigned to the same platform (accounting for replicate effect only) against those of the sample replicates assigned to different platforms (accounting for replicate and platform effects). As there were more sites with their two replicates sequenced with the same platform than sites with their replicates sequenced with different platforms (see Supplementary Figure 6), sites with replicates on the same platform were randomly selected for the comparisons. We repeated this procedure 50 times. The number of species between replicates sequenced on the same platform and those sequenced on different platforms did not differ for >98.5% of all fish and mammal samples (Supplementary Figure 7 and Supplementary Note 2). Similar to these results, a previous study on 16S rRNA amplicon has shown that the samples were not influenced by the Illumina sequencing platform used (Caporaso et al., 2012).

To monitor for contaminants, 13 negative extraction controls were performed for each of the primers (“teleo” and “12S-V5”); one control was amplified twice. All of them were amplified and sequenced by the same methods as the samples and in parallel to them. Therefore, for the negative extraction controls, 168 amplifications were prepared with the “teleo” primers (13 negative controls; one amplified and sequenced twice) and 156 amplifications with the “12S-V5” primers (13 negative controls). Fourteen negative PCR controls (ultrapure water; 12 replicates) were amplified and sequenced in parallel to the samples. Eight were amplified with the “teleo” primers and six were amplified with the “12S-V05” primers. Thus, for the PCR negative controls, there were 96 amplifications with the “teleo” primers and 72 amplifications with the Vert01 primers. Sequencing information for the controls is shown in Supplementary Data 3c.

An updated version of the reference database from Cilleros et al., (2019) was used. There were 265 Guianese species for the fish analyses (Cantera, Decotte, et al., 2022). The

GenBank nucleotide database was consulted, but it contained little information on the Guianese fish species. Most of the sequences were derived from Cilleros et al., (2019). For the mammal analyses, the vertebrate database was built using ecoPCR software (Ficetola et al., 2010) from the releases 134 and 138 of the European Nucleotide Archive (ENA), for the Maroni and Oyapock river samples, respectively. The two releases were compared, and it was established that the new mammal species added to each version did not originate from French Guiana. Hence, the results were not influenced by the EMBL release number. The relevant metabarcoding fragment was extracted from this database with ecoPCR and OBITools (Boyer et al., 2016). Therefore, the reference database comprised the local database of French Guianese mammals (Kocher, Thoisy, Catzeflis, Huguin, et al., 2017), which references 576 specimens from 164 species as well as all available vertebrate species in EMBL.

The sequence reads were analysed with the OBITools package according to the protocol described by Valentini et al., (2016). Briefly, the forward and reverse reads were assembled with the `illumina-paired-end` programme. The `ngs-filter` programme was then used to assign the sequences to each sample. A separate dataset was created for each sample by splitting the original dataset into several files with `obisplit`. Sequences shorter than 20 bp or occurring less than 10 times per sample were discarded. The `obiclean` program was used to identify amplicon sequence variants (ASVs) that have likely arisen due to PCR or sequencing errors. It uses the information of sequence counts and sequence similarities to classify whether a sequence is a variant (“internal”) of a more abundant (“head”) ASV (Boyer et al., 2016). After this step, we matched the ASV with the reference database to obtain the taxonomic assignment for each ASV. Sequences labelled by the `obiclean` programme as “internal” and probably corresponding to PCR errors were discarded. The `ecotag` programme was then used for taxonomic assignment of molecular operational taxonomic units (MOTUs). The taxonomic assignments from `ecotag` were corrected to avoid overconfidence in assignments. Species-level assignments were validated only for $\geq 98\%$ sequence identity with the reference database. Sequences below this threshold were discarded.

4. Measuring disturbance intensity using GIS data

In riverine systems, the disturbances may accumulate because of hydrologic connectivity, which is the downstream transfer of matter and pollutants (Pringle, 2001). Hence, the upstream sub-basin drainage network was considered to determine the size of the upstream sub-basin affecting local biodiversity (Figure 1). The sub-basins were delineated by applying a

flow accumulation algorithm to the SRTM global 30 m digital elevation model (NASA JPL, 2013). Deforestation was measured over 14 upstream spatial extents with radii of 0.5, 1.5, 3, 5, 10, 15, 20, 30, 40, 50, 60, 70, 80, and 90 km for each sampling site. Then, these 14 upstream spatial extents were intersected with the sub-basin drainage network. In addition, mammals and fish can also be affected by disturbances other than those mediated by hydrologic connectivity. Thus, deforestation was also measured upstream and downstream from the eDNA sampling sites using the same foregoing 14 radii.

At each sampling site, deforestation intensity was quantified for each of the 14 spatial extents. We summed upstream (only accounting for disturbances mediated by river hydrologic connectivity) or upstream and downstream (not only considering disturbances mediated by hydrologic connectivity) deforested surfaces from Landsat satellite image data sets. Forest loss surfaces were obtained from the Global Forest Change dataset (M. C. Hansen et al., 2013). The Global Forest Change dataset identifies areas deforested between 2001 and 2017 on a 30 m spatial scale. To incorporate deforested areas prior to 2000, tree canopy cover data for that year were also used. Except for river courses, all pixels with <25% canopy closure were regarded as deforested. Finally, surfaces deforested by gold mining activity in French Guiana, Suriname, and Northern Brazil were also included (Rahm et al., 2017).

Forest loss and gold-mined surfaces were significantly positively correlated for each spatial extent (Supplementary Table 3). We thus merged those datasets to create an integrative disturbance variable that quantifies the deforestation around the sampling sites, for each spatial extent. Here, deforestation intensity around each eDNA sampling site was considered an integrative measure of human-mediated environmental disturbances called here anthropization, which includes gold mining, logging, agriculture, and human settlements (Supplementary Table 3).

The absolute deforested surfaces are dependent on the surface area measured at each spatial extent, making the absolute value of deforestation dependent on the spatial extent considered. Similarly, within each spatial extent, the area of the considered upstream river basin varies with the shape of the river, making again the absolute deforestation surface dependent on the area considered. For this reason, deforestation was calculated as a percentage of the absolute deforested surface area divided by the surface area considered instead of an absolute deforested surface. We nevertheless ran separated models (see the *Species and functional richness models* section for details on model structure) using the percentage, the absolute measures of deforestation and the scaled absolute measures of deforestation. Absolute deforestation did not provide informative results (Supplementary Figure 8) because it depends

on the surface considered. Using the scaled absolute measures of deforestation (Supplementary Figure 9) increased the proportion of variance explained by the models but it remained lower than the explained variance obtained with percentages of deforested areas. Additionally, The assessment of biodiversity responses to deforestation percentages measured upstream and downstream from the eDNA sampling disclosed only weak or non-significant relationships between deforestation and biodiversity (Supplementary Figure 10, see the *Species and functional richness models* section for details on model structure). For instance, the models that yielded significant ($p < 0.05$) results with upstream and downstream deforestation as explicative variable explained only $<6\%$ of the variance for both fish and mammals (Supplementary Figure 10). Hence, we used the percentage of upstream deforestation as a measure of anthropization for the main analyses.

Based on the biodiversity sampling sites, the upstream deforestation intensity was, on average, $<5\%$ for all spatial extents considered (Supplementary Figure 2; Supplementary Table 1). At reduced spatial extents (0.5–10 km), deforestation was in the range of 0–39.21% (median, 0.54%). At larger extents, however, the deforestation intensity was in the range of 0–16.38% (median, 0.33%) (Supplementary Figure 2; Supplementary Table 1). The intensity and the variability of upstream deforestation thus decreased with increasing spatial extents. All the spatial analyses were performed on ArcGIS 10.8.

5. Biodiversity measures

The collected fish and mammal eDNA was amplified to build species inventories (see Supplementary Data 4 for the numbers of reads and detected species per sample). For the freshwater fish communities, 64 strictly freshwater sites were regarded (Figure 2). Estuarine areas were not considered for fish because the molecular reference database did not support detection of marine or estuarine fish species. Detecting more than 70% of the site's expected fish fauna in another study, the sampling protocol used here was shown to provide similar or more complete inventories to those derived from gill-netting in other large rivers within the study region (Cantera et al., 2019). Moreover, recent work on the same rivers showed that eDNA describes local fish communities and generates a spatial signal comparable to that of capture-based methods describing fish species over a few hundred metres (Cantera, Decotte, et al., 2022). Mammal communities were considered for all 74 sites (Figure 2).

The collected DNA supported the detection of 158 fish species with 5–90 (mean 58 ± 1.9 SE) species per site and 46 mammal species with 1–20 (mean 8 ± 0.54 SE) species per site.

Twenty-two species (9 mammals and 13 fish) are classified as Threatened according to the (UICN France et al., 2017). Mammal species with limited or poorly known distributions such as the West Indian manatee and Chiroptera were excluded. All mammals detected in the present study including five semi-aquatic, 15 terrestrial, and 26 arboreal species were reliably inventoried within the same study area by the sampling method used here (Coutant et al., 2021).

The biodiversity of each taxon at each site was measured via species and functional richness. Species richness was the number of species detected from two eDNA samples collected at each site. This sampling effort has been shown to provide relevant local fish and mammal inventories (Cantera et al., 2019; Coutant et al., 2021). Functional diversity was measured using morphological and ecological traits available from the literature. Supplementary Figure 4 shows the spatial patterns of the fish and mammal species and functional richness along the upstream-downstream gradients of the two rivers studied here.

Both the morphological and ecological traits of the fish were used as they complement functional diversity measurements for freshwater fish (Villéger et al., 2017). For the morphological traits, 12 measurements were made using side-view pictures collected over the past decade to compute 10 unitless ratios (hereafter, traits) reflecting food acquisition and locomotion (Toussaint et al., 2018; Villéger et al., 2017) (Supplementary Table 4a). The morphological traits were measured for as many individuals as possible (1–20 depending on the species) and the averages of all measurements per species were used. Intraspecific variability in morphological traits was not considered because a recent study using the same dataset demonstrated that it was negligible (Toussaint et al., 2018). The maximum body length of each species obtained from FishBase (www.fishbase.org) represented the maximum body size for the species and was regarded as a synthetic functional trait (Villéger et al., 2017). Therefore, 11 continuous traits were used to characterise fish morphological diversity. For the ecological traits, six qualitative traits related to trophic level, behaviour, and habitat preference were selected (Supplementary Table 4a) and collected from FishBase (www.fishbase.org) and the literature (Keith et al., 2000; Planquette et al., 1996).

For the mammals, the morphological traits were compiled from different databases to maximise the number of traits and minimise the missing values. Longevity, gestation length, litter or clutch size, and adult body mass were selected from the Amniote database (Myhrvold et al., 2015). Activity cycle, habitat and diet breadth, trophic level, and terrestriality were taken from the Pantheria database (Jones et al., 2009). Type of habitat (re-categorised from terrestrial, marine, freshwater, and aerial binary variables) and diet (re-categorised from proportions of

vertebrates, invertebrates, and plants in the diet) were derived from the Phylacine database (Faurby et al., 2018) (Supplementary Table 4b).

The morphological traits presenting a correlation coefficient <0.7 (see Supplementary Figure 11 for the trait correlograms), and the categorical ecological traits were combined to build functional spaces and assess functional diversity. Gower's functional distances between species were calculated for each taxon. This parameter considers categorical and continuous traits, standardises them, and handles missing data. The distance matrices (one per taxon) were ordinated into multidimensional spaces by a principal coordinate analysis (PCoA), which generates coordinates for all species within a global functional space per taxon. To calculate functional richness (Faurby et al., 2018), the first five PCoA axes for fish and the first two PCoA axes for mammals were retained. This configuration maximised functional space quality (Maire et al., 2015) and minimised data loss, as sites must have more species than the number of axes selected to compute functional richness. The resulting measure is the convex hull volume occupied by co-occurring species at each site in the functional space and is in the range of 0–1. Higher values reflect high volume occupation and, therefore, high functional diversity.

6. Species and functional richness models

For each spatial extent, a specific model was constructed to analyse the effects of deforestation on species and functional richness. Generalised linear mixed models (GLMM) with Poisson's distributions were used for species richness as species richness is a count variable. Linear mixed models (LMM) were used for functional richness as this variable is continuous and ranges between 0 and 1. As few sites had high upstream deforestation values and several sites had deforestation values close to 0, upstream deforestation was square-root transformed to down-weight the few high deforestation values. Those models were implemented for each taxon and for each diversity facet. This resulted in 56 models [two taxa \times two diversity facets \times 14 spatial extents] for the main analyses. River basin identity and site position in the upstream-downstream river network (Strahler order; Supplementary Figure 5) were included as random effects in the models because site position determines the river size, and therefore, the hosting capacity of aquatic species (Altermatt, 2013; Muneeppeerakul et al., 2008). Basin identity accounts for biogeographical processes shaping diversity. The models were built using the *lmer* function in the lme4 package of R.

The significance and the variance explained per model were calculated using a coefficient of determination (R^2). The aim was to establish which spatial extent best predicts

the relationship between local biodiversity and deforestation intensity. R^2 was calculated using the *r.squaredGLMM* function in the MuMIn package of R. Marginal R^2 values, which account only for the variance explained by fixed variables, were used to identify the pure effects of deforestation. The spatial extent associated with highest R^2 or stabilisation of R^2 with <5% change in R^2 between successive spatial extents was taken to be the most relevant spatial extent in the assessment of the effects of deforestation on biodiversity. The slope of the model at the optimal spatial extent was used to evaluate the strength of deforestation. Model validity was assessed by checking the absence of residual patterns and by testing the normal distribution of the residuals with Shapiro tests (Supplementary Figure 1).

Sampling sites were located along two upstream-downstream gradients and were, therefore, not independent of each other. Spatial autocorrelation was evaluated using Moran's *I* test on the GLMM and LMM residuals for the fish and mammal species and functional richness across all sites to test for unforeseen associations between nearby sites. After accounting for basin identity and position in the watercourse, we determined that species and functional richness of both taxa were not influenced by spatial autocorrelation (Moran's *I* test; fish species richness, observed = 0.04, $p = 0.15$; fish functional richness, observed = 0, $p = 0.69$; mammal species richness, observed = 0.04, $p = 0.17$; mammal functional richness, observed = 0, $p = 0.84$). Furthermore, the robustness of the findings was tested by performing a sub-sampling analysis on subsets of sites with increasing minimal distance (range: 2–50 km) between sites. For each minimal distance between sites, 50 site subsets were randomly built with the same GLMM and LMM model analyses to those applied for the entire dataset. This site subset analysis yielded results similar to those obtained using the entire dataset (Supplementary Data 5). Hence, the results were robust and were not influenced by the distances between adjacent sites. R code to compute generalized linear mixed models is provided as Supplementary software 1.

7. Functional structure analysis

Within the optimal spatial extent, sites were classified by deforestation level into deforested sites (deforested area exceeding 0.33%) and non-deforested sites (deforested area <0.33% explained by natural forest turnover or tree fall). This threshold was determined by measuring natural deforestation at 100 randomly selected sites in areas without human settlement, human activity, or anthropogenic deforestation. Half-circle spatial extents with a radius of 30 km were generated for each site, representing surfaces similar to those of the spatial

extents delimited for the sampling sites, and deforestation percentages were then calculated. The highest deforestation percentage was regarded as a threshold of natural deforestation (i.e. natural forest turnover, hereafter called non-deforested sites) and anthropic-mediated deforestation (i.e. deforested sites). Applying this threshold to the sampling sites and considering deforestation over a 30 km upstream spatial extent from the sampling sites, yielded 34 and 35 non-deforested sites and 30 and 39 non-deforested sites for fish and mammals, respectively.

The *envifit* function in the *vegan* package of R was used to fit the variables (traits) onto the PCoA ordination and identify any correlations between the traits and the ordination axes (Supplementary Table 2). The determination coefficients R^2 were calculated to assess the strengths of the correlations between the axes and the traits. Traits with high R^2 were strong ordination predictors. P-values were computed by comparing the observed and simulated R^2 based on 999 random data permutations. To quantify the trait contributions, the continuous variables were transformed onto vectors directed according to their correlation with the axes. Their lengths were proportional to the strengths of the correlations between the ordinations and the traits (R^2) (Supplementary Table 2b). For the categorical variables, the average ordination scores were computed for the scores of all species belonging to each factor level to locate categories in the functional spaces (Supplementary Table 2c). Convex hulls of each community at each site were represented within the global functional spaces for fish and mammals and for both habitat types. Species were included in the functional spaces and rated Threatened if they were classified as Critically Endangered (CR), Endangered (EN), Vulnerable (VU), or Near Threatened (NT) according to the IUCN Red List (IUCN France et al., 2017). The percentages of species occurrences (number of occurrences of each species divided by the sum of all occurrences of all species, Supplementary Data 1a, b) were calculated and displayed in the functional spaces. R code to compute functional spaces is provided as Supplementary software 1.

Results

The relationship between biodiversity loss and percentage of deforestation was strongest when upstream deforestation was evaluated over an upstream extent of ≥ 30 km from the biodiversity sampling sites even under low deforestation intensities (Figure 3a, c, e, g; see

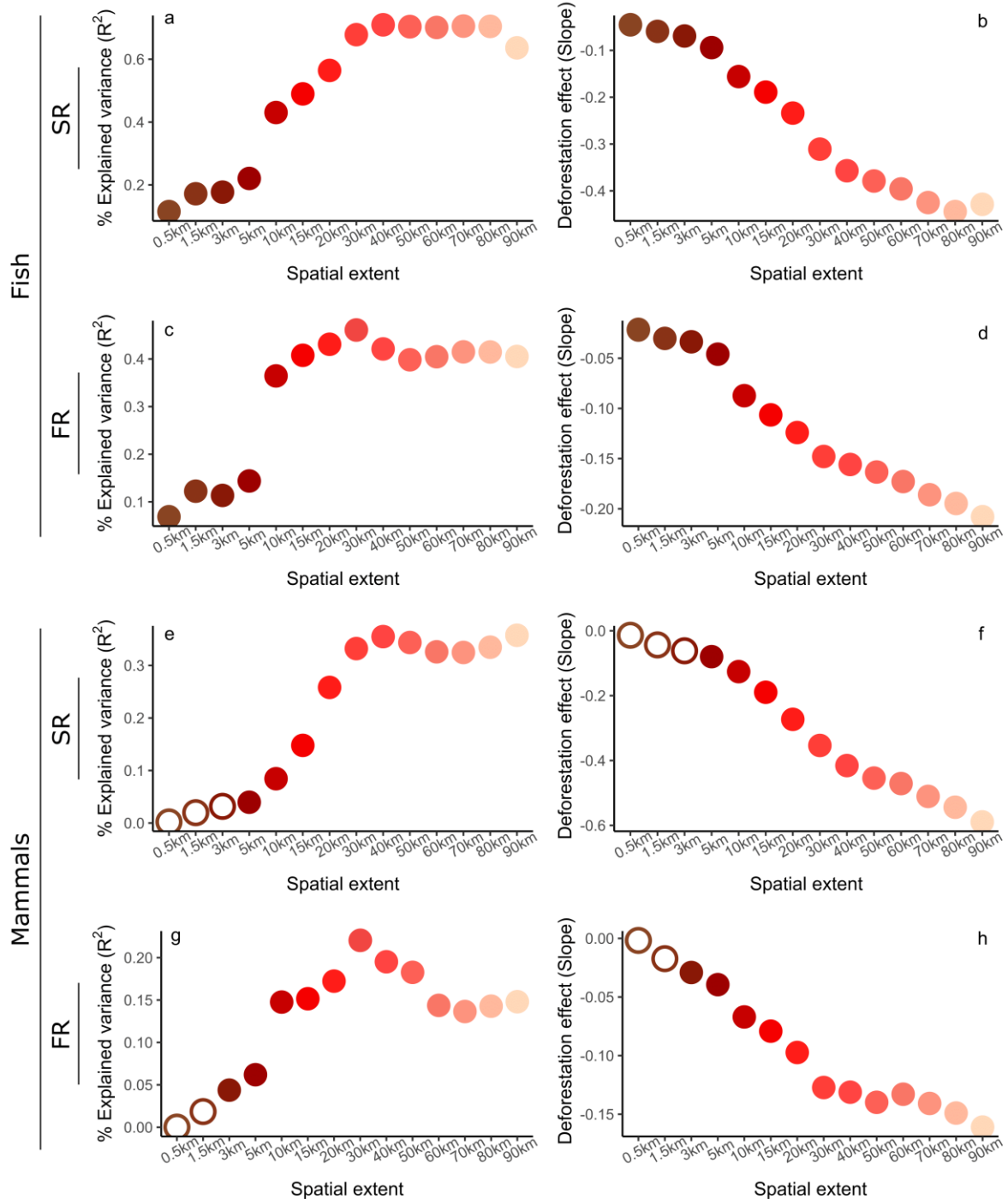


Figure 3. Identification of the relevant spatial extent to measure deforestation impact on biodiversity. Left panels indicate variance explained by mixed models (R^2) for each spatial extent. Right panels indicate the strength of deforestation effect on biodiversity (slopes). Spatial extents account for deforested areas upstream from eDNA sampling sites. (a, b) Fish species richness (SR) models. (c, d) Fish functional richness (FR) models. (e, f) Mammal species richness models. (g, h) Mammal functional richness models. For each spatial extent, a specific generalized linear mixed model accounting for site network position and basin identity as random effects was built for each biodiversity facet. Significant ($p < 0.05$) and non-significant ($p > 0.05$) models assessed with Wald's tests are indicated by filled and open circles, respectively (Supplementary Table 1). Fish: $n=64$ sites and mammals: $n=74$. Colour shades are consistent with spatial extents. Grey vertical bars indicate models with highest R^2 or R^2 reaching a plateau, with less than 5% variation between successive spatial extents.

Supplementary Table 1 for p-values, slopes and R^2 values). Indeed, the variance explained by the linear mixed models assessing the links between upstream deforestation and local species and functional richness increased with spatial extent for both fish and mammal species. This was paired with an increase in the negative link between deforestation and both fish and mammals taxonomic (Figure 3b, f; Supplementary Table 1) and functional diversity (Figure 3d, h; Supplementary Table 1).

For both taxa, the models were significant for most of the spatial extents considered in our study (Figure 3). The models considering upstream deforestation within 30 km of the sampling sites were highly significant ($p < 0.001$) and explained $>22\%$ of the model variance (Figure 3, Supplementary Table 1, see Supplementary Figure 1 for model validity assessment based on residual distributions).

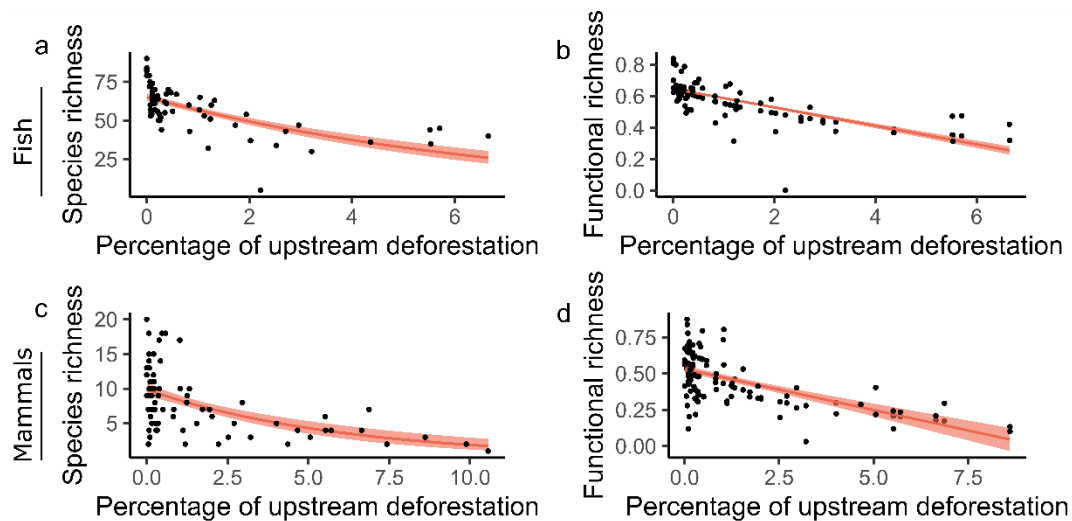


Figure 4. Effects of upstream deforestation intensity on biodiversity. Panels represent species richness (a, c) and functional richness (b, d) of fish (a, b) and mammal (c, d) communities. Red solid lines indicate the fitted values of mixed models accounting for site network position and basin identity. Light red shades indicate 95% confidence intervals. Deforestation corresponds to the percentage of deforested area at the most relevant spatial extent (30 km). $n=64$ sites and $n=74$ sites for fish and mammal models, respectively.

The models correlating biodiversity with the percentages of deforestation measured within 30 km upstream from the fish and mammal sampling sites provided the best prediction of functional and species richness as the variance they explained stabilised or reached a maximum (Figure 3a, c, e, g; Supplementary Table 1). At this spatial extent, the deforestation intensities were in the ranges of 0–6.6% and 0–10.6% for the fish and mammal sites, respectively (Supplementary Table 1a, b; Supplementary Figure 2). Along the deforestation

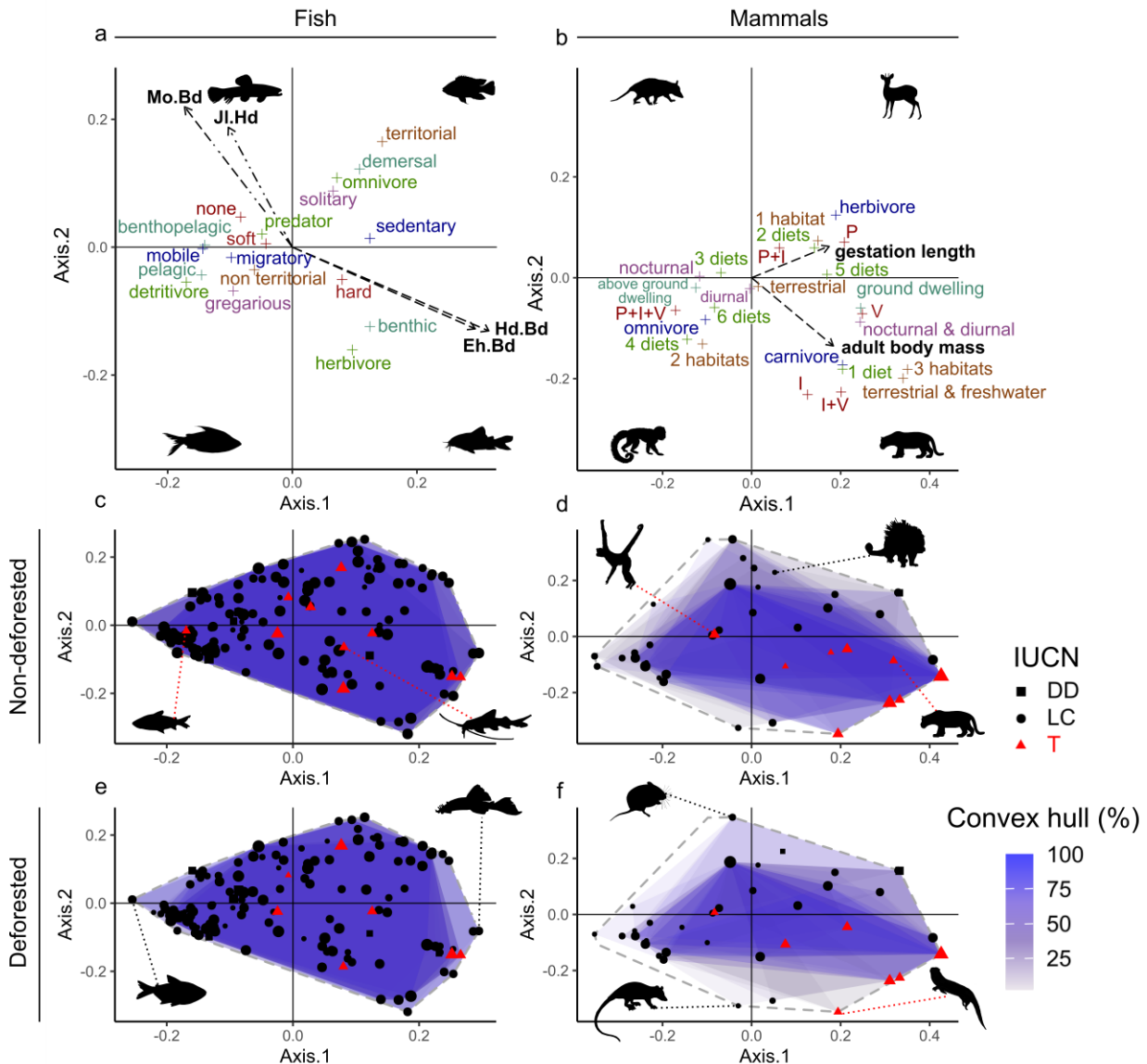


Figure 5. Functional spaces for fish and mammals in non-deforested and deforested sites. Loadings of functional traits on two first axes of principal coordinate analysis for fish (a) and mammals (b). Only significant and most highly correlated quantitative traits with axes are represented with black dotted lines. Qualitative traits are displayed in colour. For each site, the functional space of each community is represented by a convex hull. Convex hulls of each site were superimposed for each category (fish non-deforested, 34 sites; mammals non-deforested, 35 sites; fish deforested, 30 sites; mammals deforested, 39 sites). Blue shade intensity increases with the percentage of superimposed convex hulls from 0% (white) to 100% (dark blue). The Grey dotted line represents global functional space considering all species in both non-deforested and deforested habitats. Species symbol size is proportional to species occurrence percentages. In IUCN lists, DD is Data-Deficient species; LC is Least Concern species, and T is Threatened species. Sites were considered non-deforested for percentage deforestation < 0.33% (see methods) within the identified relevant spatial extent (30 km). Silhouettes illustrate functional characteristics of species in each quadrant (a, b), emblematic species only found in non-deforested sites (or rarely occurring in deforested sites for spider monkey) (c, d) and extreme functional strategies (e, f). See Supplementary Data 1 for species occurrences and coordinates in functional spaces. For panel b, P (Plants in the diet), I (Invertebrates in the diet), V (Vertebrates in the diet), diet (Number of dietary categories), habitat (Number of habitat layers) (Supplementary Table 4).

gradients, species and functional richness markedly decreased for fish (Figure 4a, b) and mammal communities (Figure 4c, d) despite marked site-specific variability at low deforestation intensity. A comparison of deforested and non-deforested sites (deforestation intensity <0.33%, see methods) revealed significant lower taxonomic and functional diversity at the deforested sites (Kruskal-Wallis test; $\chi^2 = 18.4$ and $p < 0.01$ for fish species richness; $\chi^2 = 11.7$ and $p < 0.01$ for mammal species richness; $\chi^2 = 13.8$ and $p < 0.01$ for fish functional richness; and $\chi^2 = 9.1$ and $p < 0.01$ for mammal functional richness). On average, there was 34% less species richness (26% and 41% for fish and mammals, respectively) and 28% less functional richness (22% and 33% for fish and mammals, respectively) in deforested sites than in non-deforested ones. This corresponded to average losses of 13 and 4 species for fish and mammal communities, respectively, in deforested sites.

The fish functional structure was represented by the two first axes of a principal coordinate analysis (PCoA) of the life history and morphological traits of the species. The first and second axes of the PCoA explained 37% and 24% of the variance and reflected locomotion and swimming/feeding strategies, respectively (Figure 5a; Supplementary Table 2). In most non-deforested sites, the functional richness of the fish communities (the size of community convex hulls) was high but relatively lower in the deforested sites. The lower functional richness at the deforested sites was the result of the absence of small species with extreme feeding strategies and located on the edge of the functional space (Figure 5c, e). These species included detritivores and invertivores such as *Moenkhausia* sp. and *Cyphocharax* sp. as well as benthic herbivores of the Loricariidae (Figure 5e; Supplementary Data 1a). Moreover, certain endangered species such as *Cyphocharax punctatus* and *Pimelodella procera* were never detected in deforested sites (Figure 5c, e; Supplementary Data 1a).

The mammal functional structure was represented by the two first axes of a PCoA. Axis 1, explaining 38% of the variance, displayed positive gradients of body mass and gestation time, whilst axis 2, explaining 26% of the variance, accounted for differences in diet and habitat (Figure 5b; Supplementary Table 2). Marked declines in the most extreme functional strategies were observed for the mammal communities at the deforested sites (Figure 5d, f) including terrestrial (small rodents and large predators), semi-aquatic (water opossum, *Chironectes minimus*), and aquatic (giant otter, *Pteronura brasiliensis*) species (Supplementary Data 1b). As for fish, the lower mammal functional richness observed in most deforested sites was the result of the absence of certain threatened and emblematic species, such as the spider monkey (*Ateles paniscus*) and the jaguar (*Panthera onca*) (Figure 5d, f; Supplementary Data 1b).

Discussion

The present study suggests that slight anthropogenic disturbances can cause remote decreases in the biodiversity of downstream faunas. Forest cover losses of <11% of the total area located up to 30 km upstream from the biodiversity sampling sites were significantly linked to declines of >22% in the taxonomic and functional richness of terrestrial and aquatic fauna, suggesting that anthropization had a cumulative effect on biodiversity over large spatial extents. The link between deforestation and biodiversity reached a maximum when deforestation was measured up to 30 km upstream from the sampling sites, accounting for small deforested surfaces scattered over the upstream areas. The foregoing findings suggest that previous studies linking anthropization to biodiversity without considering the effects mediated by hydrologic connectivity might have overlooked the impact of anthropization on fauna. For instance, studies measuring the local effects of gold mining and forestry-induced deforestation on Guianese fish diversity failed to detect the decline in fish species diversity associated with these activities (Allard et al., 2016; Brosse et al., 2011). We obtained similar results when we only considered deforestation over small spatial extents. Therefore, we assume that strong impacts of anthropization would have been detected in the previous studies if larger spatial extents were considered. Moreover, deforestation in the Northern Amazon region and the Guiana Shield is mainly the consequence of small-scale gold mining that reduces water quality through pollutant (Castello & Macedo, 2016; Hammond et al., 2007). Gold mining was responsible for about 40% of the deforested surfaces in the area (Supplementary Table 3), while other disturbances such as slash-and-burn agriculture and logging were spatially correlated with gold mining activity (Supplementary Table 3). Thus, mining, agriculture, and logging act together to alter the water quality and physical structure of the rivers in the region (Leitão et al., 2018), which has negative local effects on fish (Allard et al., 2016; Mol & Ouboter, 2004). According to our results, downstream dispersal of these disturbances can also markedly influence fish species over long distances. Those results also hold when considering only terrestrial mammals (Supplementary Figure 3, Supplementary Note 1). The spatially extended effects of anthropization on terrestrial mammal diversity might be explained by the upstream degradation of riparian vegetation. Through hydrologic connectivity, anthropization affects the structural dynamics of water flow and physico-chemistry, as well as aquatic habitats at the catchment scale (Bleich et al., 2014). Alterations in freshwater ecosystems may, in turn, affect the downstream structure and composition of the riparian vegetation and the terrestrial biodiversity it supports (Bleich et al., 2014). Moreover, human settlements established in remote areas in

response to the development of small-scale gold mining lead to subsistence hunting along the rivers. This practice also constitutes a non-negligible disturbance to terrestrial fauna (Benítez-López et al., 2019).

The biodiversity modifications reported here point out a severe negative impact of anthropization on local biodiversity. When comparing deforested and non-deforested sites, we observed average rates of spatial decline in mammal and fish species richness of 26% and 41%, respectively. This accounts for an average loss of 13 fish and 4 mammal species in anthropized sites, compared to non-anthropized sites. Consequently, the upstream-downstream positive gradient in fish species richness was not verified for the two river basins studied here. This almost universal pattern reflects the roles of fish dispersal and spatial heterogeneity of habitats on fish diversity along river networks (Altermatt, 2013; Harvey et al., 2018; Muneeppeerakul et al., 2008). The loss of species at the anthropized sites, mainly in the downstream parts of the rivers, reversed the expected distribution pattern of fish diversity, resulting in a higher species richness upstream than downstream. This trend was obvious for the Maroni River (Supplementary Figure 4), which had undergone more severe anthropization than the Oyapock River. Those alterations in the upstream-downstream species richness gradient were paired with functional alterations linked to the absence of some detritivores and algae browsing fishes in anthropized sites. These species play vital roles in aquatic ecosystems by controlling algal biomass and nutrient cycles (Flecker et al., 2002; Taylor et al., 2006). Concerning mammals, the absence of the largest and smallest mammal species at most of the anthropized sites parallel the trends reported elsewhere in the world (Carmona et al., 2021). The absence of top predators such as jaguars, giant otters, and bush dogs (*Speothos venaticus*) in anthropized sites could disturb top-down trophic chain control (Gorzynski et al., 2021) and thus can deeply impact terrestrial and aquatic ecosystem functioning (Estes et al., 2011). Consequently, the ecological roles performed and the ecological services provided by these taxa are jeopardised.

The remote impacts of even slight anthropization on biodiversity suggested by our results require that future conservation plans account for the hydrologic dispersal of human disturbances. This reckoning is of paramount importance even for the slightly affected forest ecosystems that support much of the global terrestrial biodiversity (Watson et al., 2018). These wilderness areas continue to shrink and are being fragmented by small and scattered deforestation patches (Watson et al., 2016, 2018). Though these ecosystems apparently remain intact, studies have indicated that they suffer from “silent” human impacts such as hunting of large mammals which reduces animal biodiversity as well as their key roles in seed dispersal and food web control (Benítez-López et al., 2019; Watson et al., 2018). This situation is

probably similar for aquatic ecosystems as well because the control that fish exerts on nutrient fluxes and food webs is a strong determinant of ecosystem functioning (Estes et al., 2011; Taylor et al., 2006). These “silent” effects could explain the disproportionate biodiversity loss observed in low deforested areas (Betts et al., 2017). Here, we suggest that, besides hunting and fishing, distant deforestation also has a “silent” effect on ecosystems by reducing species richness and the functional range of the surviving species. Hydrologically mediated effects must therefore be factored into the design and execution of terrestrial and freshwater conservation plans. For instance, the IUCN could implement the foregoing information to define protected areas and categories that do not consider hydrologically mediated effects (Dudley, 2018). Indeed, the most protected areas (IUCN categories Ia and Ib) are virtually free of human activity but may nonetheless suffer from hydrologically mediated effects caused by alterations remote from the conservation zones. Considering distant disturbances could lead to a re-evaluation of the wilderness in these protected areas and facilitate the determination of the optimal sizes of the buffer zones around them.

Here, both terrestrial and aquatic fauna were related similarly to deforestation over a spatial extent of 30 km upstream from the sampling sites. Thus, cross-ecosystem conservation designs should be beneficial to both terrestrial and aquatic fauna (Leal et al., 2020). Nevertheless, spatially explicit, integrated conservation strategies that account for multiple threats and cross-ecosystem connectivity have seldom been developed (but see Tulloch et al., (2021)) for marine and freshwater ecosystems). Terrestrial and aquatic biodiversity inventories are usually constructed using different methods. Moreover, they are time-consuming and require the participation of specialists in each organism group . However, overreaching biodiversity inventory methods such as eDNA metabarcoding are facilitating biodiversity assessments as well as realistic inventories of both aquatic and terrestrial biodiversity within the same water samples (Taberlet et al., 2018). In fact, a single eDNA sampling session can provide fish species inventories that are equivalent and even more complete than those obtained after several years of net samples (Cantera et al., 2019). Moreover, the eDNA methods generated mammal species distribution patterns consistent with traditional line-transect samplings over the Guianese territory (Coutant et al., 2021). Nevertheless, the dissemination of eDNA-based biodiversity inventories remains limited because of uncertainty regarding the detection distance of eDNA that is being transported downstream through the river network. Although measuring the exact detection distance is complex and multifactorial (Barnes & Turner, 2016), recent studies suggest a short detection distance of eDNA in slow-flowing rivers. In those studies, eDNA samples provided inventories and spatial patterns comparable to those

of local samples using capture and observation methods (Cantera, Decotte, et al., 2022; Coutant et al., 2021), testifying for local detections of species, which do not exceed a few kilometres. These short detection distances might be explained by the accelerated eDNA degradation at warm water temperatures (26–30 °C) (Eichmiller et al., 2016) and the gentle topography of Maroni and Oyapock (average slope 0.04% and 0.05%, respectively), which restrict downstream eDNA transport (Pont et al., 2018). The situation is more complex in fast-flowing rivers where eDNA may be transported far downstream (Pont et al., 2018). However, recently developed spatial eDNA drift modelling can now discriminate local and regional effects (Carraro et al., 2020). The spread of eDNA technology will therefore constitute an asset to determining the spatial extent and strength of remote human disturbances on biodiversity. Although we here show a consistent response of terrestrial and aquatic faunas, enabling thereby cross-ecosystems conservation actions (Leal et al., 2020), our framework deserves to be applied in other regions and to other disturbances. It will contribute to designing efficient conservation plans considering both local and distant impacts of disturbances on the aquatic and terrestrial biodiversity associated with riverine ecosystems.

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Author Contributions

IC, OC and SB conceived the ideas, designed methodology and led the writing of the manuscript; SB, JBD, JM and RV collected the data; IC, OC and CJ analyzed the data; AV, TD

and AI conducted the laboratory work and bio-informatic analyses. All authors contributed critically to the drafts.

Supplementary Materials

See the online version of the article

2. Part 2

Environmental DNA reveals a mismatch between diversity facets of Amazonian fishes in response to contrasting geographical, environmental and anthropogenic effects

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Abstract

Freshwater ecosystems are among the most endangered ecosystem in the world. Understanding how human activities affect these ecosystems requires disentangling and quantifying the contribution of the factors driving community assembly. While it has been largely studied in temperate freshwaters, tropical ecosystems remain challenging to study due to the high species richness and the lack of knowledge on species distribution. Here, the use of eDNA-based fish inventories combined to a community-level modelling approach allowed depicting assembly rules and quantifying the relative contribution of geographic, environmental and anthropic factors to fish assembly. We then used the model predictions to map spatial biodiversity and assess the representativity of sites surveyed in French Guiana within the EU water framework directive and highlighted areas that should host unique freshwater fish assemblages. We demonstrated a mismatch between the taxonomic and functional diversity. Taxonomic assemblages between but also within basins were mainly the results of dispersal limitation resulting from basin isolation and natural river barriers. Contrastingly, functional assemblages were ruled by environmental and anthropic factors. The regional mapping of fish diversity indicated that the sites surveyed within the EU water framework directive had a better representativity of the regional functional diversity than taxonomic diversity. Importantly, we also showed that the assemblages expected to be the most altered by anthropic factors were the most poorly represented in terms of functional diversity in the surveyed sites. The predictions of unique functional and taxonomic assemblages could therefore guide the establishment of new survey sites to increase fish diversity representativity and therefore improve this monitoring program.

Introduction

While accounting for less than 1% of the earth's surface, freshwaters harbour more than 6% of the described species (Dudgeon et al., 2006). For instance, 17,000 fish species inhabit freshwaters and account for 25% of all vertebrates (Van der Laan, 2020). Yet, freshwaters are also among the most imperiled ecosystems on earth, with more than 50% of the world rivers experiencing human-mediated biodiversity declines (Su et al., 2021), threatening more than 22% of the freshwater fish species across the globe (Albert et al., 2021).

Both local environmental factors (at the habitat scale) and large-scale variables (*e.g.* climate, land cover) are recognized as natural determinants of fish species assemblages (Benone

et al., 2020; López-Delgado et al., 2020). At a large spatial scale, freshwater fish distributions are mostly determined by the historical connections among basins during the quaternary low-sea-level period, promoting the dispersal of species between basins (Carvajal-Quintero et al., 2019). Within basins, freshwater ecosystems are characterized by a dendritic network structure in which hydrologic connectivity dictates a dispersal pathway from upstream to downstream (Vannote et al., 1980). This dispersal pathway has been recognized as a major driver of freshwater fish distribution, generating a distribution pattern reported in the river continuum concept, which predicts an increase in species richness from upstream to downstream (Harvey et al., 2018; Vannote et al., 1980). Freshwater fish distribution is thus mediated by multiple environmental factors operating at different spatial scales (Benone et al., 2020). This distribution pattern is determined by a combination of biotic and abiotic factors including species dispersal capacities, physical characteristics of the river such as dispersal barriers (*e.g.* rapids or waterfalls) or habitat hosting capacity and diversity (*e.g.* upstream parts are smaller than downstream parts), which all vary longitudinally along the network (Carvajal-Quintero et al., 2019). Moreover, the two spatial major drivers that are position in the river network and historical connections between river basins suggest that connectivity is a crucial parameter of freshwater fish distribution (Carvajal-Quintero et al., 2019; Harvey et al., 2018). Besides natural determinants, anthropic activities can also determine fish biodiversity patterns, especially in highly connected ecosystems, where altering habitat connectivity and quality modify ecological processes and patterns. For instance, Zeni et al., (2019) demonstrated that deforestation led to a biotic homogenisation of stream habitats, which was responsible for an increased functional redundancy in fish assemblages across tropical regions of the globe. Freshwaters are deeply impacted by global changes but often remain at the edge of the discussions (Albert et al., 2021). Yet, these ecosystems are strongly tied to human wellbeing as they provide multiple essential ecosystem services including the maintenance of hydro-climatic regimes, human food, energy production, transportation, recreation, as well as waste disposal and remediation (Albert et al., 2021; Van Rees et al., 2021). Consequently, quantifying the relative role of natural (historical and environmental) and anthropogenic factors in structuring biodiversity distribution is pivotal to design adequate conservation strategies.

Measuring the contribution of historical, environmental and anthropic factors in freshwater fish assembly rules requires understanding how processes shaping diversity apply and vary from local to regional scales (Socolar et al., 2016). This question can be addressed by measuring community dissimilarity between sites within a region (β -diversity), which can reveal the spatial structure of biodiversity (Whittaker, 1960) and predict changes in biodiversity

over the entire region (γ -diversity) from local biodiversity changes (α -diversity) (Socolar et al., 2016). β -diversity approaches have frequently been conducted on the taxonomic facet of biodiversity, treating all the species as functionally equivalent (Roa-Fuentes et al., 2019). However, taxonomy alone is not sufficient to understand community assembly while multi-faceted assessments of biodiversity provide complementary views on the different processes acting on communities (Roa-Fuentes et al., 2019; Villéger et al., 2013). In fact, taxonomic and functional facets can disclose mismatch patterns. For example, a pair of assemblages exhibiting taxonomic differentiation can actually show a low relative functional β -diversity if the species from each assemblage are functionally equivalent (Villéger et al., 2013). This has significant conservation implications because biodiversity facets may respond differently to environmental pressures and across scales (Devictor et al., 2010).

The environmental DNA (eDNA) metabarcoding method (Taberlet et al., 2012) enables rapid and efficient biodiversity inventories at multiple sites. It has been proven particularly efficient for fast biodiversity assessments in species rich tropical rivers, where traditional fish survey methods, be they observational or capture-based, are time-consuming, can be invasive and/or of limited efficiency (Cantera et al., 2019; Dickie et al., 2018; Lear et al., 2018; Shu et al., 2020). To date, the use of eDNA-based inventories to map biodiversity at large scales (*e.g.* catchment scale for freshwater fish) remains scarce, probably because efforts have been so far directed towards the development and optimisation of robust eDNA metabarcoding protocols (Coutant et al., 2020). Recently, eDNA has nevertheless been proved efficient in delineating conservation areas within whole river catchments over a 200,000 km² territory (Blackman et al., 2021). It has also been used to develop a hydrology-based modelling framework revealing the spatial patterns of aquatic insects over an entire river catchment (Carraro et al., 2020).

Here, using information of fish distribution over three river basins of French Guiana, we investigated the structuration of fish taxonomic and functional diversity and questioned whether different processes drive these two biodiversity facets. We hypothesised that (1) geographical processes mainly drive regional fish taxonomic facet while we expect that (2) fish functional facet is mostly governed by local environmental and anthropic factors. The regional taxonomic diversity should therefore root in the historical connections between basins while the functional diversity should be shaped by the regional variations of environmental and anthropic factors. To answer these research questions, we used eDNA-based inventories from 85 sites located along three rivers (Maroni, Sinnamary and Oyapock). These rivers are considered among the most preserved areas worldwide (Su et al., 2021) but are facing an unprecedented rise of threats linked to deforestation and gold mining, altering water

physicochemical properties and generating strong disturbances to aquatic biodiversity (Cantera, Coutant, et al., 2022; Castello et al., 2013). In addition, as unravelling the assembly rules and spatial patterns of taxonomic and functional diversity will constitute a benchmark for developing conservation strategies in such high biodiversity regions, we used Generalized Dissimilarity Models (GDM) to map and predict β -diversity over seven river basins from French Guiana, extending over 112,000 km (Maroni, Oyapock, Sinnamary, Mana, Comté, Kourou and Approuague rivers). We then asked the extent to which the sites surveyed in French Guiana within the EU Water Framework Directive are representative of the biodiversity encountered in the entire region and highlighted areas that should host unique freshwater fish assemblages.

Material and Method

1. Study area

Sampling was conducted on three river basins of the Guiana Shield, in the north-eastern Amazonian biome (Figure 1). Altogether, the three basins cover a surface of 101,365 km². The Maroni River is 612 km long from its source to its estuary, and its basin covers a surface of \pm 68,000 km² in Suriname and French Guiana. The Oyapock River (length, 404 km; area, 26,800 km²) is located in the state of Amapa in Brazil and French Guiana. The Sinnamary River (length, 262 km; area, 6,565 km²) is situated within the territory of French Guiana. The climate of the entire study area is relatively homogeneous and the region is covered by dense, uniform lowland primary rainforest. The altitude is in the range of 0–860 m a.s.l. The regional climate is equatorial, and the annual rainfall ranges from 2,000 mm in the southwest to 3,600 mm in the northeast. These rivers face different levels of anthropogenic pressure unevenly distributed along the watercourses. The Maroni river is the most inhabited with *c.* 83,000 habitants (INSEE, 2020) unevenly distributed from Saint-Laurent du Maroni to Pidima village, which constitutes the most upstream human settlement on the Maroni river (Figure 1). The Maroni river is also the most affected by human activities, mainly legal and illegal gold mining, which represented 8,058 ha of deforestation (0.37% of the catchment area in 2014) spanning from Saint-Laurent du Maroni to upstream of Maripasoula (Gallay et al., 2018). Only the most upstream part of the Maroni river (upstream from Pidima, Figure 1) has not been impacted by human activities. The Oyapock River is more preserved with only three villages and *c.* 6,000 habitants (INSEE, 2020). Gold mining is much less developed than in the Maroni basin and represented 1,547 ha of

deforestation in 2014 (0.06% of the catchment area), mainly concentrated near the village of Camopi (Gallay et al., 2018). The Sinnamary river is not exploited for gold but the building of a large hydroelectric dam (Petit Saut dam) in 1994–1995 has severely modified the landscape: 365 km² of primary rainforest were flooded, leaving hundreds of islands of various sizes covering a total area of 105 km² (Vié, 1999). Several human settlements are located downstream from the dam, while the upstream part of the river remains free from human settlements, with only occasional recreational fishing.

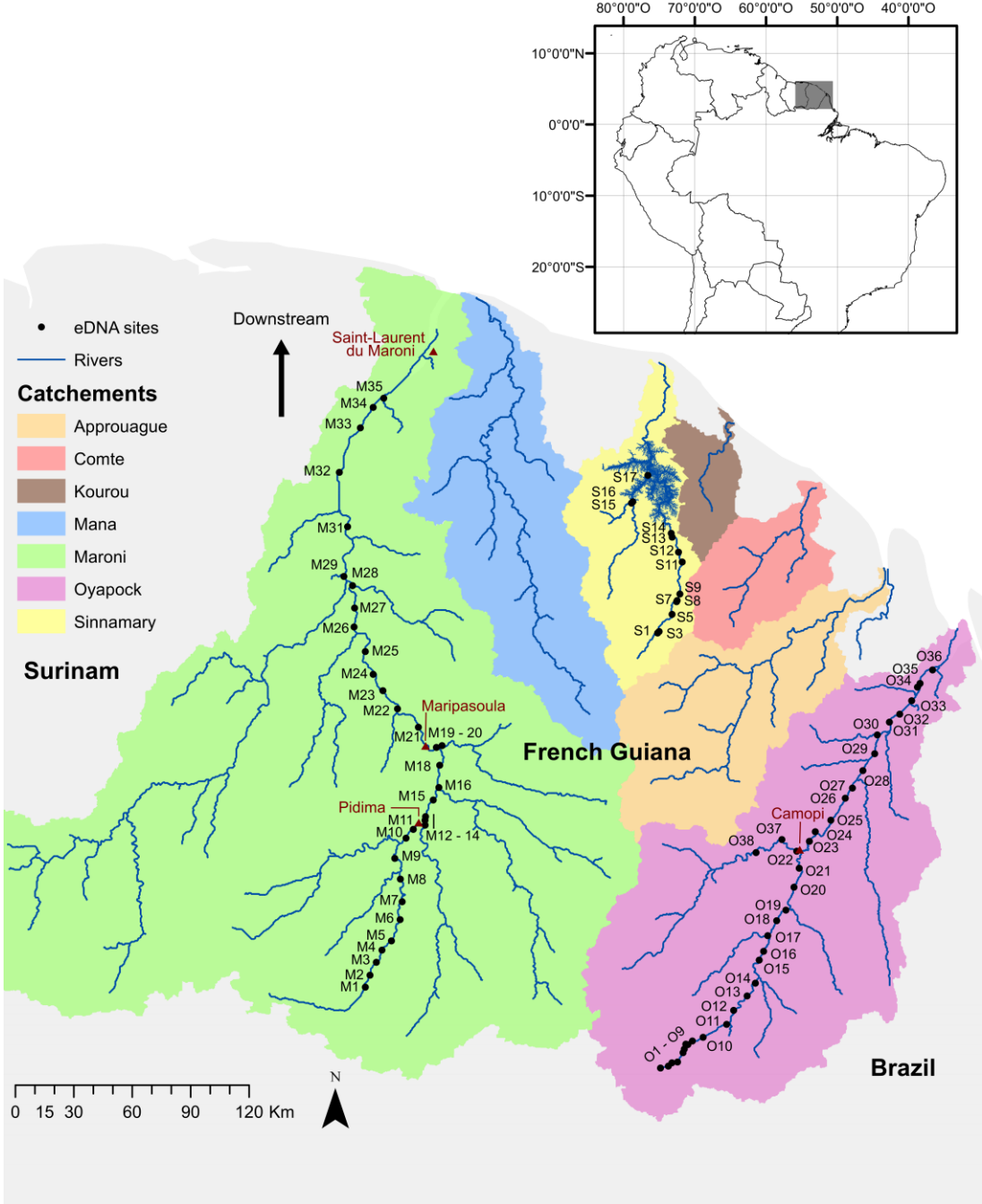


Figure 1. Map of the study area and the 85 biodiversity sampling sites. Inset map on the right indicates in grey the location of the study area in South America.

2. Fish sampling

2.a. Water sampling

Environmental DNA (eDNA) was collected from water samples at 85 locations (hereafter, sites) along the main channel and the large tributaries of the Maroni, Oyapock and Sinnamary rivers (Figure 1). All the metadata associated with the samples are described in Muriene et al., (2019) and available on the CEBA geoportal (<http://vmcebagn-dev.ird.fr>) and in Table S1. At all sites, the river was wider than 20 m and deeper than 1 m (Strahler orders 4–7; Figure S2). At all rivers, the sites were sequentially sampled from downstream to upstream. Following the protocol of Cantera et al., (2019), we collected the eDNA by filtering two replicates during 30 minutes and resulting approximately in 30L of water per site. A peristaltic pump (Vampire Sampler; Buerkle GmbH, Bad Bellingen, Germany) and single-use tubing were used to pump the water into a single-use filtration capsule (VigiDNA, 0.45 μm ; SPYGEN, Bourget-du-Lac, France). The tubing input was placed a few centimetres below the water surface in zones with high water flow as recommended by Cilleros et al., (2019). Sampling was performed in turbulent areas to ensure optimal eDNA homogeneity throughout the water column. To avoid eDNA cross-contamination among sites, the operator remained on emerging rocks downstream from the filtration area. At the end of filtration, the capsule was emptied, filled with 80 mL CL1 conservation buffer (SPYGEN), and stored in the dark up to one month before the DNA extraction.

2.b. eDNA laboratory and bioinformatics

For the DNA extraction, each filtration capsule was agitated on an S50 shaker (Ingenieurbüro CAT M. Zipperer GmbH, Ballrechten-Dottingen, Germany) at 800 rpm for 15 min, decanted into a 50 mL tube, and centrifuged at $15,000 \times g$ and 6°C for 15 min. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of the tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50 mL tube, and the mixtures were stored at -20°C for at least one night. The tubes were then centrifuged at $15,000 \times g$ and 6°C for 15 min, and the supernatants were discarded. Then, 720 μL of ATL buffer from a DNeasy Blood & Tissue Extraction Kit (Qiagen, Hilden, Germany) was added. The tubes were vortexed and the supernatants were transferred to 2 mL tubes containing 20 μL Proteinase K (Macherey-Nagel GmbH, Düren, Germany). The tubes were then incubated at 56°C for 2 h. DNA extraction was performed using a NucleoSpin Soil kit

(Macherey-Nagel GmbH, Düren, Germany) starting from step six of the manufacturer's instructions. Elution was performed by adding 100 μ L of SE buffer twice. After the DNA extraction, the samples were tested for inhibition by qPCR (Biggs et al., 2015). Briefly, quantitative PCRs were performed in duplicate for each sample. If at least one of the replicates showed a different Ct (Cycle threshold) than expected (at least 2 Cts), the sample was considered inhibited and diluted 5-fold before the amplification.

We used the “teleo” primer pair (Valentini et al., 2016) (forward: 3'-ACACCGCCCGTCACTCT-5'; reverse: 3'-CTTCCGGTACACTTACCATG-5') which targets a 60 bp marker located at the 5' end of the 12S ribosomal gene, a region presenting a high variability across fish species. The performance of the “teleo” primer pair has been investigated by Polanco F. et al. (2021), who showed that it can efficiently discriminate fish species of the Maroni river, the river with the highest species richness in French Guiana. The “teleo” primer has thus been used in previous studies focused on French Guianese fish fauna (Cantera, Coutant, et al., 2022; Cantera, Decotte, et al., 2022; Cantera et al., 2019; Cilleros et al., 2019). The DNA amplifications were performed in a final volume of 25 μ L containing 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 μ M of each primer, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM of each dNTP, and 3 μ L DNA template. Human blocking primer was added to the mixture for the “teleo” primers (Valentini et al., 2016) (5'-ACCCTCCTCAAGTATACTTCAAAGGAC-C3-3') at final concentrations of 4 μ M and 0.2 μ g/ μ L bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland). Twelve PCR replicates were performed per field sample (85 sites x 2 field replicates x 12 PCR replicates, 2040 PCR replicates in total). The forward and reverse primer tags were identical within each PCR replicate. The PCR mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, and a final elongation step at 72 °C for 7 min. This step was conducted in a dedicated room for DNA amplification that is kept under negative air pressure. The success of the amplification was verified using capillary electrophoresis (QIAxcel; Qiagen GmbH) and the samples were purified using a MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified PCR products were quantified using capillary electrophoresis and then pooled in equal volumes to achieve an expected sequencing depth of 500,000 reads per sample before DNA library preparation.

For the fish analyses, 10 libraries were prepared using a PCR-free library protocol (<https://www.fasteris.com/metafast>) at Fasteris, Geneva, Switzerland. Four libraries were sequenced on an Illumina HiSeq 2500 (2 \times 125 bp) (Illumina, San Diego, CA, USA) with a HiSeq SBS Kit v4 (Illumina), three were sequenced on a MiSeq (2 \times 125 bp) (Illumina) with a

MiSeq Flow Cell Kit Version3 (Illumina), and three libraries were sequenced on a NextSeq (2 × 150 bp + 8) (Illumina) with a NextSeq Mid kit (Illumina). The libraries run on the NextSeq were equally distributed in four lanes. Sequencing was performed according to the manufacturer's instructions at FASTERIS.

The sequence reads were analyzed with the OBITools (Boyer et al., 2016) according to the protocol described by Valentini et al., (2016). Briefly, the forward and reverse reads were assembled with the `illumina-paired-end` programme. The `ngsfilter` programme was then used to assign the sequences to each sample. A separate dataset was created for each sample by splitting the original dataset into several files with `obisplit`. Sequences shorter than 20 bp or occurring less than 10 times per sample were discarded. Sequences labelled by the `obiclean` programme as “internal” and probably corresponding to PCR errors were also discarded. The `ecotag` programme was used for taxonomic assignment of molecular operational taxonomic units (MOTUs). An updated version of the reference database from Cilleros et al. (2019) was used. This database is a local database of French Guianese freshwater fish referencing the 12S mtDNA fragments of 367 species, representing 92% of the 400 freshwater fish species described in French Guiana. Among these referenced species, 9 (2.5%) cannot be discriminated at the species level with the “teleo” primer pair. For the 33 (8%) species missing in the database, we were not able to collect DNA. The GenBank nucleotide database was consulted, but it contained little information on the Guianese fish species. Most of the sequences were derived from Cilleros et al., (2019). Species-level assignments were validated only for ≥ 98% sequence identity with the reference database. Sequences below this threshold were discarded. We discarded all MOTUs with a frequency of occurrence below 0.001 per library in each sample, considered as tag-jumps (Schnell et al., 2015). These thresholds were empirically determined to clear all reads from the extraction and PCR negative controls included in our global data production procedure as suggested by De Barba et al., (2014); Taberlet et al., (2018). For the samples sequenced on a NextSeq platform, only species present in at least two lanes were kept. These bioinformatic analyses provided a species by site matrix with read number per species (Table S2).

3. Predictors variables

3.a. Environmental and anthropogenic variables

Satellite-based environmental variables (n = 41) were extracted from the Near Global Freshwater-specific Environmental variables for biodiversity analyses at 1 km resolution

(Domisch et al., 2015). When there was no data available at the location of the sampling sites, the nearest pixel value was assigned. In addition, four water hydro-chemical parameters measured in the field for each site were used: conductivity, water temperature, pH and, turbidity (Table S3).

For anthropogenic variables, we quantified deforestation using the Global Forest Change database (M. C. Hansen et al., 2013). This dataset identifies areas deforested between 2001 and 2017 at a 30 m spatial scale. To incorporate deforested areas before 2000, tree canopy cover data for that year were also used. Except for river courses, all pixels with <25% canopy closure were regarded as deforested. Finally, surfaces deforested by gold mining activity in French Guiana, Suriname, and Northern Brazil were also included (Rahm et al., 2017). We merged those datasets to create an integrative disturbance variable that quantifies the deforestation around the sampling sites. Here, deforestation intensity around each eDNA sampling site is considered an integrative measure of human-mediated environmental disturbances including gold mining, logging, agriculture, and human settlements (Cantera, Coutant, et al., 2022).

We calculated the mean percentage of deforestation upstream (along the sub-basin drainage network) and upstream and downstream of the sampling sites (Table S3; Figure S1). Indeed, we previously showed that 30 km represented the most relevant upstream spatial extent to investigate biodiversity responses to deforestation in our system (Cantera, Coutant, et al., 2022). We considered the sites' upstream sub-basin drainage network areas because it takes into account the hydrologic connectivity of rivers and the associated water-mediated downstream transfer of matters and pollutants. We also considered circular areas around sampling sites because it integrates the hydrologic connectivity of rivers but also the potential influence of downstream impacts on sampling sites (Figure S1). We delineated sub-basins by applying a Flow Accumulation algorithm to the SRTM Global 30 m Model Elevation (Becker et al., 2009). Then, for each sampling site, we quantified deforestation intensity by summing upstream or upstream and downstream deforested surfaces and dividing this sum by the area of the delineated spatial extent to obtain percentages of deforestation (Figure S1).

3.b. Geographical effect

Three pairwise distance variables were computed: (i) the Euclidean distances, (ii) the site distances following the actual riverine dendritic network and, (iii) the paleo-distances between sites, which are the distances following the river network from the last glacial

maximum (-20 000 years) when the sea level was at -120m. This allowed accounting for connections between basins that no longer exist.

For the actual and the paleo-distances, the distances between different basins were calculated following the coastline. Distances were calculated in ArcGIS, using the network analyst extension and the OD cost matrix function. The distance matrices were used as input in a non-metric multidimensional scaling (NMDS) analysis to transform the matrices into coordinates on a two-dimensional space. The coordinates obtained through NMDS were then used to calculate Euclidean distances in Generalized Dissimilarity Models (see GDM section).

3.c. Predictor spatialization

We spatialized the predictors using a 1km spatial resolution to be consistent with the spatial scale of the inventories. We previously demonstrated that environmental DNA does not represent an integrative measure of biodiversity across the whole upstream river basin but provides a relevant picture of local fish assemblages (Cantera, Decotte, et al., 2022). We first modelled the hydrographic network applying a Flow Accumulation algorithm to the SRTM Global 1km Model Elevation using the ArcHydro tools from ArcGis. Spatialization was conducted with different procedures depending on the variables: geographic distance, field variables, environmental variables or anthropic variables. For geographic distance, we calculated the pairwise paleo-distances between all pairs of pixels and implemented these distances in a NMDS to compute NMDS-based coordinates. For field variables, we interpolated the data along the hydrographic network using the Inverse distance weighting (IDW) method. The anthropic variable was spatialized using the same methods as for sampling sites. Considering each pixel of the SRTM Global 1 km Model Elevation as outlets, we delineated 30km upstream sub-basin areas by applying a Flow Accumulation algorithm to the SRTM Global 1km Model Elevation. We then calculated percentages of deforestation for each pixel within the delineated areas (upstream areas or upstream – downstream areas) (Table S3, Figure S1). Environmental data from the Near Global Freshwater Environmental variables were already provided at a 1 km resolution, therefore we only modified the hydrographic network to consider the same network as the one used for anthropic and field variables (considering only the streams with Strahler order from 4 to 7, Figure S2).

4. Response variables

4.a. Taxonomic β -diversity

We calculated fish taxonomic β -diversity based on the Jaccard's dissimilarity coefficient using the *formatsitepair* function of the GDM package (Fitzpatrick et al., 2022). Using the Baselga framework implemented in the betapart package (Baselga & Orme, 2012), we also computed the turnover (β_{tu}) and nestedness (β_{ne}) contribution to taxonomic β -diversity (β_{jac}) to understand if β -diversity is mostly the results of difference in species compositions between sites (Baselga, 2010; Carvalho et al., 2012). Disentangling the underlying processes of β -diversity delivers fundamental insights about the mechanisms shaping communities. For instance, if β -diversity results from spatial turnover, it may signify that environmental sorting and/or geographical constraints govern fish assembly (Qian et al., 2005).

4.b. Functional β -diversity

Morphological and ecological traits were used to functionally characterize each fish species. For the morphological traits, 12 measurements were made using side-view pictures of mature fish to compute 10 unitless ratios (hereafter, traits) reflecting food acquisition and locomotion (Villéger et al., 2017) (Table S4). The morphological traits were measured for as many individuals as possible (1–28 depending on the species) and the averages of all measurements per species were used. For the ecological traits, six qualitative traits related to trophic, behaviour, and habitat preference were selected and collected from FishBase (<http://www.fishbase.org>) and the literature (Table S4). Morphological and ecological traits were missing for 18 species (6.7% of the considered fish). For these species, we assigned the average values of the morphological measures and the ecological traits of the closest related species. Using these fish functional, morphological and ecological attributes, we calculated a species dissimilarity matrix using the Gower's distance, which can handle mixed data of numeric and class variables while standardizing them. We then implemented the dissimilarity matrix into a multidimensional space using a Principal Coordinates Analysis (PCoA) and applying the Cailliez correction (Cailliez, 1983) for negative eigenvalues to build a functional space. This analysis provides coordinates for all the species in a global functional space (Table S5). We retained the four first PCoA axes, which was the best compromise to maximize functional space quality and minimize data loss (to compute site functional β -diversity, sites must possess a higher number of species than the number of selected axes (Maire et al., 2015).

Using species coordinates, we finally calculated a site functional β -diversity (β_{jac}) matrix and partitioned it into turnover (β_{tu}) and nestedness (β_{ntu}) matrices based on the Jaccard dissimilarity coefficient. These analyses were realised with the `betapart` package, with the functions `functional.beta.core` to create a `betapart` object and `functional.beta.pair` to compute the matrices.

4.c. Generalized dissimilarity modelling

GDM is a method that relates the dissimilarities of a response variable with ecological distances (Ferrier et al., 2007). It is based on the regression of a dissimilarity matrix and accommodates nonlinearities often encountered in ecological datasets using I-spline coefficients (monotonic cubic splines function) for each explanatory variable. Higher coefficients indicate higher rates of change of the response variable along the gradient of a given explanatory variable. Nonlinearities occur for two reasons: (i) there is a curvilinear relationship between ecological separation and compositional dissimilarity because most dissimilarity metrics range from 0 to 1. Therefore, once no species are shared between sites, the dissimilarity value takes on an asymptotic value of 1 while the ecological separation keeps increasing. (ii) There is a variation of the rate of compositional changes along ecological gradients.

4.d. Variable selection

The GDMs implemented with the paleo-distances had the highest explanatory power. Thus, this distance measure was used as a geographic proxy in all the GDMs (See Table S6 for model comparisons with the three different geographic measures).

To obtain the most relevant predictors, we gathered a large number of predictor variables from different databases (Table S3). Because they provided redundant information in certain cases, we conducted a preliminary analysis following a variable selection protocol using the collinearity diagnostic VIF (variance inflation factor). We used the `vifstep` function from the `USDM` package (Naimi, 2015), which excludes the highly correlated variables from the set through a stepwise procedure. We built two sets of predictors, each including one of the two anthropic variables (30km upstream deforested areas or 30km upstream and downstream deforested areas). This step removed 27 satellite-based environmental variables from the initial set. Fifteen satellite-based variables and five field variables were kept for further analyses. Table S7 shows the selected variables and Table S8 contains the two different sets of used

predictors. The two sets were not identical because correlation coefficients between anthropic and environmental variables changed depending on the spatial extent considered.

4.e. GDM implementation

GDMs were fitted using the *gdm* package (Fitzpatrick et al., 2022). For each biodiversity facet (taxonomic and functional) and for β -diversity and its turnover component, we first formatted the data using the *formatsitepair* function. We then ran GDMs using the *gdm.varImp* function (one model for each set of predictors), including the site paleo-distances as the geographic variable (see section 4). We did not run GDMs with the nestedness component because nestedness was too low and the models did not converge. We used the three I-spline basis function and the significance of each model was calculated by matrix permutation, by comparing the deviance explained by the original model with the distributions of the deviance calculated to all permutations while conducting a backward elimination process to select the best predictors. The selection of the best set of predictors for each biodiversity facet was performed by choosing the model minimising the deviance and maximising the percentage of deviance explained (Table S8). Then, the best predictors within the selected set were retained only if they were significant and explained more than 1% of the deviance (percentage of deviance explained by each variable is determined by summing the coefficients of the I-splines from the GDM). Table S9 shows the model results with the two sets of predictors for each biodiversity facet. Deviance partitioning was used to calculate the unique and shared contributions of the best-selected set of environmental, anthropic and geographic variables, using the function *gdm.partition.deviance* of the *gdm* package.

5. Taxonomic and functional predictions of β -diversity

We predicted β -diversity and turnover over seven rivers (From west to east: Maroni, Mana, Approuague, Sinnamary, Kourou, Comte and, Oyapock, see Figure 1) from the best GDMs. Predictions were only computed for the main tributaries as only fish communities sampled on stream orders of 4 - 7 were considered (Figure S2). To generate spatially explicit GDM predictions, the *gdm.transform* function from the *gdm* package was first used to create layers embodying the parameters of the fitted models. More precisely, the spline functions computed for each predictor from the fitted GDMs were applied to spatialized predictors covering the seven rivers to create transformed layers displaying how β -diversity varies in space according to historical, environmental and anthropic variables. Then, the GDM-transformed

layers were used to assess the fish representativeness of sites currently surveyed since 2000 within the EU Water Framework Directive (WFD, Water Framework Directive 2000, https://ec.europa.eu/environment/water/water-framework/index_en.html). We calculated the predicted average similarity of each location (each 1km raster pixel) to a set of 27 sites where fish communities are monitored every year as part of WFD. Additionally, we computed the uniqueness of each location (each 1km raster pixel) by calculating the mean similarity between each location and all the locations across the region. These two analyses were conducted following Mokany et al., (2022).

Results

1. Taxonomic and functional β -diversity between and within basins

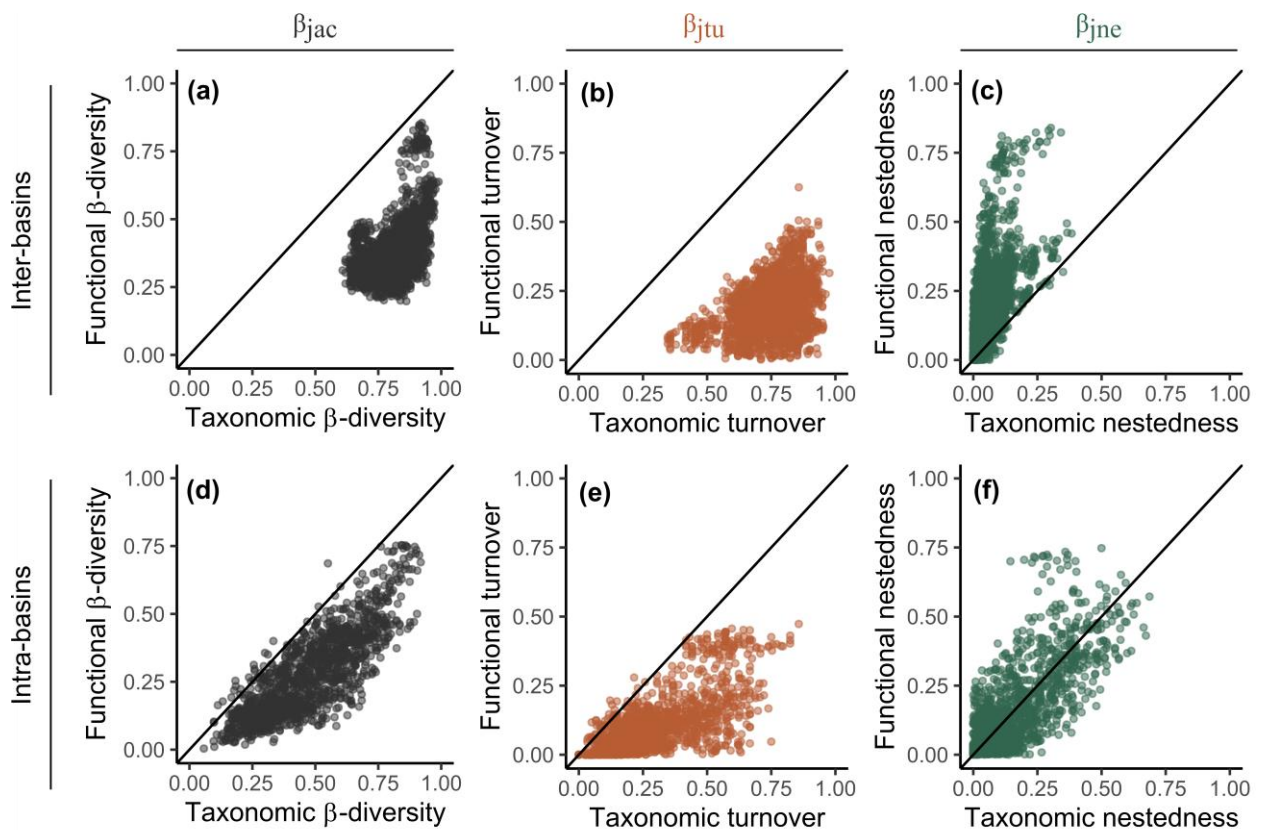


Figure 2. Relationships between taxonomic and functional β -diversity (β_{jac}) and its turnover (β_{jtu}) and nestedness (β_{jne}) components for pairwise comparisons of fish assemblages. (a, b, c) Inter-basin comparisons, (e, f, g) Intra-basin comparisons.

In the 85 eDNA sampling sites, 235 fish species were detected with site species richness ranging from 20 to 134 species (median = 68). The fish species belonged to 13 orders, 42 families and 139 genera.

Inter-basin β -diversity - The mean functional β -diversity was significantly lower than that of taxonomic β -diversity (Mann–Whitney U rank test, $U = 4994284$, $p < .001$, $n = 2248$, Figure 2a; Table 1) and the mean functional turnover was significantly lower than that of taxonomic turnover (Mann–Whitney U rank test, $U = 5050823$, $p < .001$, $n = 2248$, Figure 2b; Table 1). Contrastingly, the mean functional nestedness was significantly greater than that of taxonomic nestedness (Mann–Whitney U rank test, $U = 1072523$, $p < .001$, $n = 2248$, Figure 2c; Table 1). On the one hand, the contribution of taxonomic nestedness to β -diversity was low and β -diversity was mainly driven by taxonomic turnover (Mann–Whitney U rank test, $U = 5053465$, $p < 0.001$, $n = 2248$) (Figure 2a, b, c; Table 1). On the other hand, functional turnover contributed slightly more to functional β -diversity than functional nestedness (Mann–Whitney U rank test, $U = 3011049$, $p < 0.001$, $n = 2248$), but both components jointly shaped functional β_{jac} (Figure 2a, b, c; Table 1).

Intra-basin β -diversity - The mean functional β -diversity was significantly lower than that of taxonomic β -diversity (Mann–Whitney U rank test, $U = 1389066$, $p < 0.001$, $n = 1322$, Figure 2e; Table 1) and the mean functional turnover was significantly lower to that of taxonomic turnover (Mann–Whitney U rank test, $U = 1475102$, $p < 0.001$, $n = 1322$, Figure 2f; Table 1). The mean taxonomic nestedness was slightly greater than that of the functional nestedness (Mann–Whitney U rank test, $U = 920404$, $p < 0.02$, $n = 1322$, Figure 2g; Table 1). Overall, taxonomic turnover contributed significantly more to taxonomic β -diversity than taxonomic nestedness (Mann–Whitney U rank test, $U = 1288227$, $p < 0.001$, $n = 1322$) (Figure 2d, e, f; Table 1). Functional nestedness contributed significantly more to functional β -diversity than functional turnover (Mann–Whitney U rank test, $U = 723161$, $p < 0.001$, $n = 1322$) (Figure 2d, e, f; Table 1).

Table 1. Mean \pm SD taxonomic and functional values for pairwise combinations of sites located in different basins (Inter-basin β -diversity) and for pairwise combinations of sites located within the same basins (Intra-basin β -diversity).

	Taxonomic			Functional		
	β -diversity	Turnover	Nestedness	β -diversity	Turnover	Nestedness
Inter-basin β -diversity	0.81 \pm 0.08	0.75 \pm 0.11	0.06 \pm 0.06	0.37 \pm 0.11	0.19 \pm 0.09	0.18 \pm 0.16
Intra-basin β -diversity	0.48 \pm 0.18	0.31 \pm 0.18	0.17 \pm 0.14	0.28 \pm 0.15	0.11 \pm 0.11	0.17 \pm 0.16

2. Drivers of taxonomic and functional fish diversity

For all the GDMs, the paleo-distances between sites was the best geographic proxy. For the taxonomic β -diversity, the GDM with the highest explanatory power was the model considering deforestation over a 30 km upstream sub-basin area. This model explained 84.4% of the total deviance in β -diversity (Table S10). The most important predictor was the geographic distance; its sole effect explained 54.4% of the deviance. The two environmental predictors (elevation range and water temperature) explained together 5.9% of the deviance while the anthropic variable only accounted for 3.3% of the deviance (Figure 3a). Taxonomic β -diversity increased with site paleo-distances and was maximal between the most distant sites (Figure 4a). It also steadily increased with site difference in water temperature, and exhibited

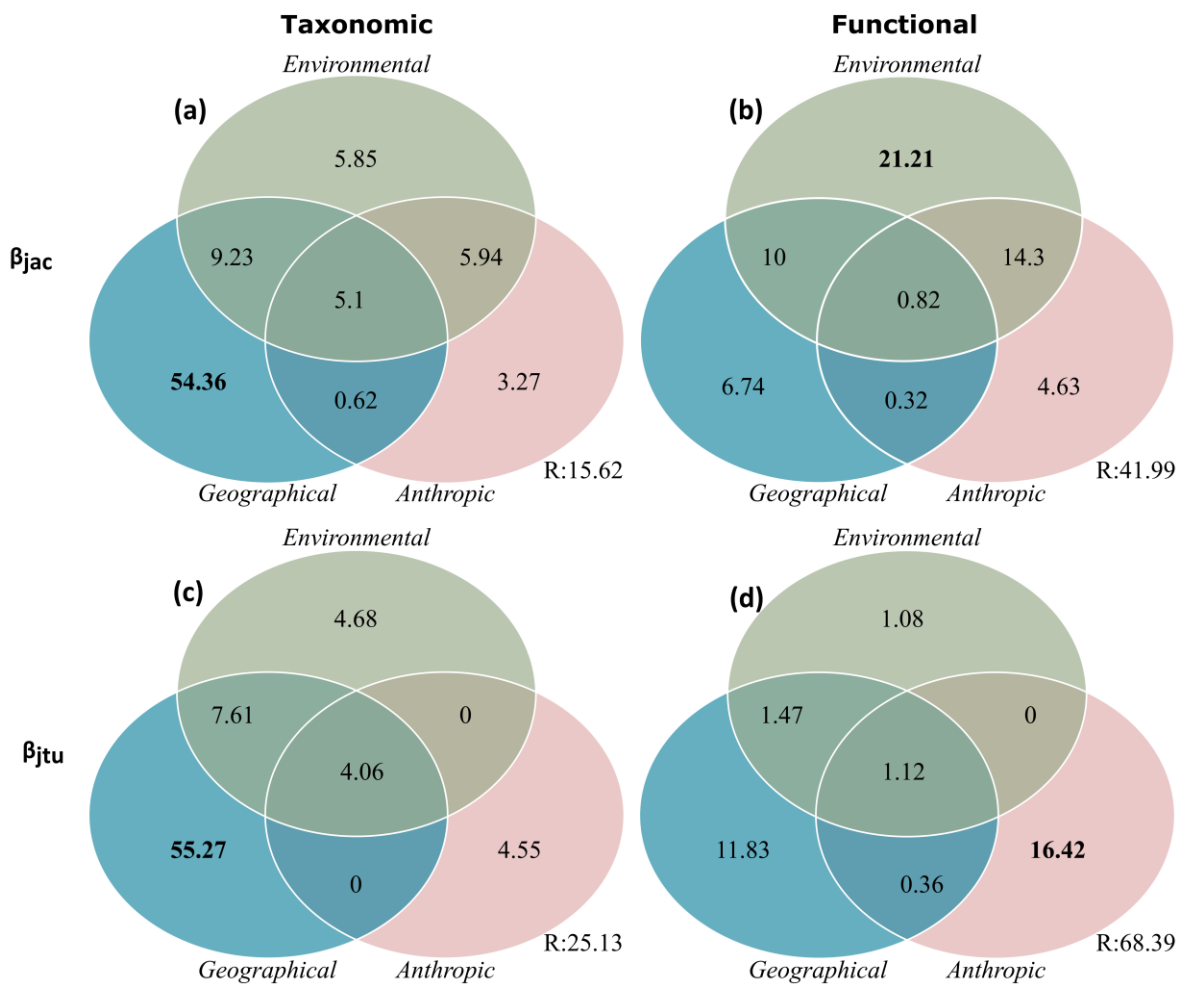


Figure 3. Partitioning of generalized dissimilarity model deviance of fish taxonomic and functional β -diversity (β_{jac}) and turnover (β_{jtu}) into geographical (blue), environmental (green) and anthropic (pink) variables across the 85 sampling sites. Values represent percentage of deviance explained. Venn diagrams represent unique and shared contribution of each variable. R refers to the model residual deviance (unexplained deviance). Bold values display the variables with the highest contributions.

an abrupt increase with site elevation range between 600 and 800m (Figure 4b and c). Finally, taxonomic β -diversity consistently increased along the site deforestation gradient with maximal values of β -diversity reached between the less and most deforested sites (Figure 4d).

For the taxonomic turnover, the GDM with the highest explanatory power was the model considering deforestation over a 30 km upstream-downstream circular area. This model exhibited similar results to that of the taxonomic β -diversity and explained 74.9% of the total deviance in turnover (Table S10). The most important predictor remained geographic distance with a contribution of 55.3% of the total deviance. The environmental predictor (water temperature) explained 4.7% of the deviance while the anthropic variable accounted for 4.6% of the deviance (Figure 3c). Taxonomic turnover displayed similar increases to taxonomic β -diversity along the site distance, water temperature and deforestation gradients (Figure 4e, f, g).

For the functional β -diversity, the GDM with the highest explanatory power was the model considering deforestation over a 30 km upstream sub-basin area. The functional GDM computed with paleo-distances explained 58% of the deviance in β -diversity (Table S10) and

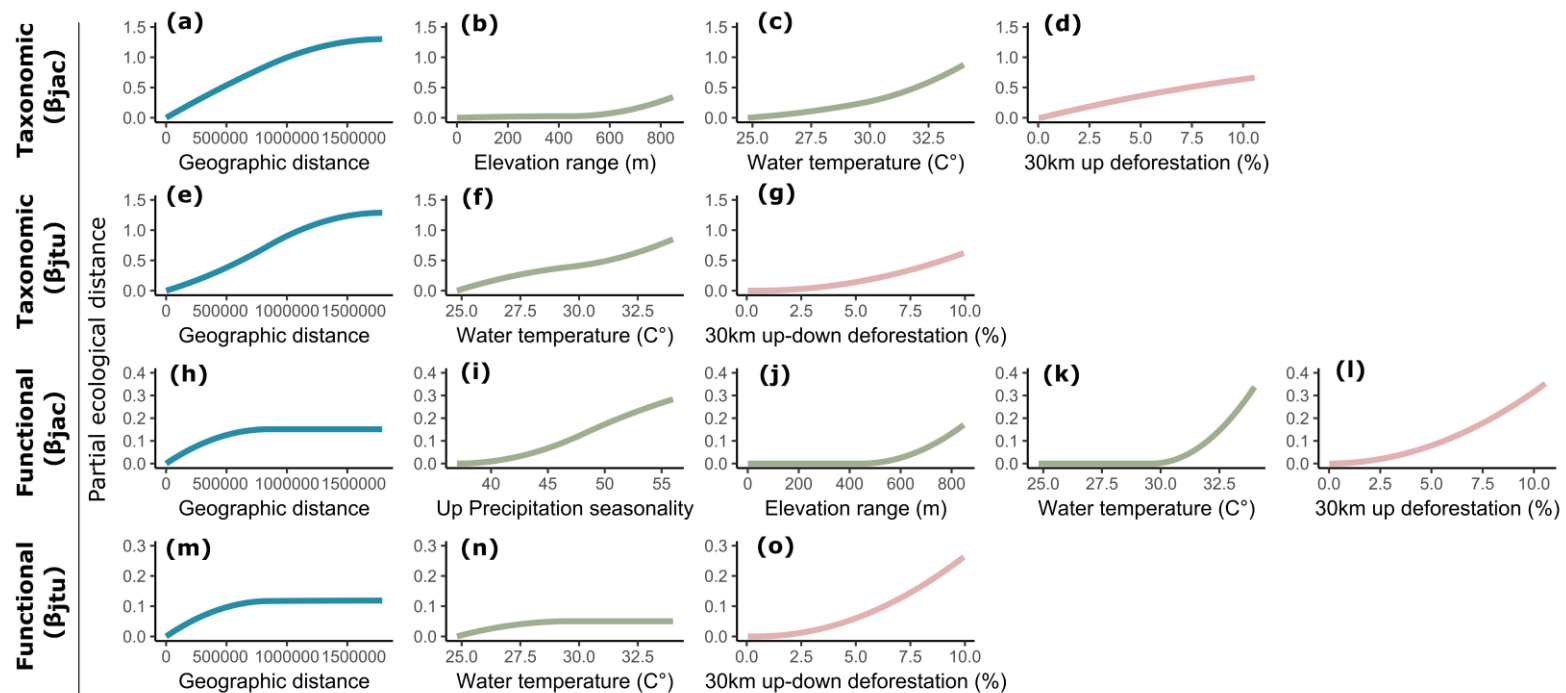


Figure 4. I-splines generated for geographic (blue), environmental (green) and anthropic (pink) variables from the final GDMs (Table S10). The maximum height reached by each function indicates the total amount of β -diversity associated with the gradient of the variable concerned, holding all other variables constant. The slope of each function displays the rate of β -diversity and its variation along the gradient concerned. Up = Upstream, Up-down = Upstream – downstream. Geographic distance unit comes from NMDS-coordinates.

showed contrasting results to those revealed by the taxonomic β -diversity and turnover. The major contributors were environmental variables (elevation range, upstream precipitation seasonality and water temperature) accounting together for 21.2% of the deviance. The geographic and anthropic predictors had a low contribution, explaining 6.7% and 4.6% of the deviance, respectively. The shared contribution of environmental and anthropic predictors explained 14.3% of the deviance (Figure 3b). The functional β -diversity did not increase along the entire range of site distances and stabilized between sites exhibiting geographic distances ranging from 50000 to 1,788,391 NMDS-based distance unit. Similarly, functional β -diversity did not increase along the 0–420m elevation range gradient and the 25–30°C water temperature gradient. Contrastingly, functional β -diversity steadily increased with upstream precipitation seasonality and deforestation rates (Figure 4h, j, k).

For the functional turnover, the GDM with the highest explanatory power was the model considering deforestation over a 30 km upstream-downstream circular. This model explained 31.6% of the total deviance (Table S10). The anthropic variable was the most important, with a contribution of 16.4% to the total deviance. The geographic predictor was the second most important with 11.8% of the deviance explained while the most important environmental predictor explained only 1.1% of the deviance (Figure 3d). The functional turnover exhibited the same patterns of variation as functional β -diversity along increasing geographic distances and the deforestation gradient. The pattern of variations along the water temperature gradient was however different, with a stabilization of functional turnover from water temperature ranging from 27.5 to 35°C (Figure 4m, n, o).

3. Predicting taxonomic and functional fish diversity

The spatialized predictions of taxonomic and functional β -diversity and their turnover component based on geographical, environmental and anthropic variables show how β -diversity varies in space within and between basins in response to each factor (Figure 5).

Spatial predictions of taxonomic and functional β -diversity and turnover based on geography exhibited an east-west gradient (Figure 5a, d, g, j).

Spatial predictions of taxonomic β -diversity in response to environmental variables (elevation range and water temperature) showed that the main course of the Maroni and the Oyapock rivers as well as the Petit Saut Lake contained the fish communities with the highest rate of β -diversity (values approximately ranging from 0.42 to 0.84 GDM-unit, Figure 5b). When only considering taxonomic turnover predictions, similar communities between sections

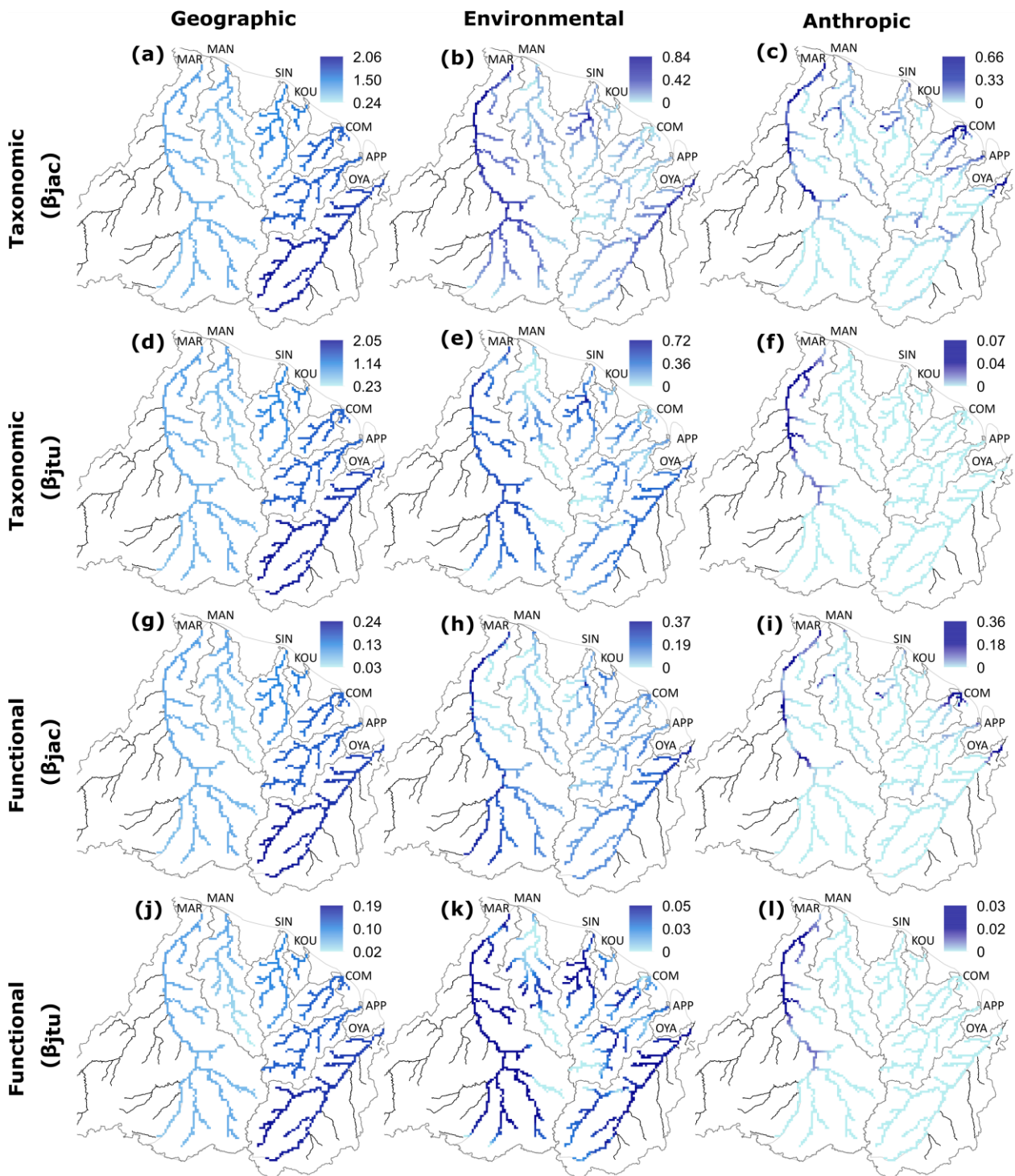


Figure 5. Colour maps resulting from the transformed layers of biological relevance presenting the predictions of both overall community dissimilarity (β_{jac}) and its turnover component (β_{jtu}) for taxonomic and functional diversity. Predictions were computed from the best GDMs considering the effect of each factor separately (Table S10). Similar colours depict similarities in assemblages. The colour gradient displays the variation of β -diversity along the geographic, environmental and anthropic gradients. Maroni (MAR), Mana (MAN), Sinnamary (SIN), Kourou (KOU), Comté (COM), Approuague (APP), Oyapock (OYA).

of the Mana, the Kourou, the Comté and the Approuague rivers and the Maroni, Oyapock and Sinnamary rivers are expected (Figure 5e).

Functional β -diversity predictions based on environmental variables (upstream precipitation seasonality, elevation range and water temperature) highlighted similarities between communities of the Oyapock and the Maroni courses but also between the inland rivers (Figure 5h). However, when only accounting for functional turnover, almost the entire Maroni, Sinnamary and Oyapock drainages as well as middle sections of the Mana, the Comté and the Approuague rivers exhibited similar communities (Figure 5k).

Predictions of taxonomic β -diversity in response to anthropic activities revealed that the downstream part of the Maroni as well as estuaries of the Comté and the Oyapock rivers

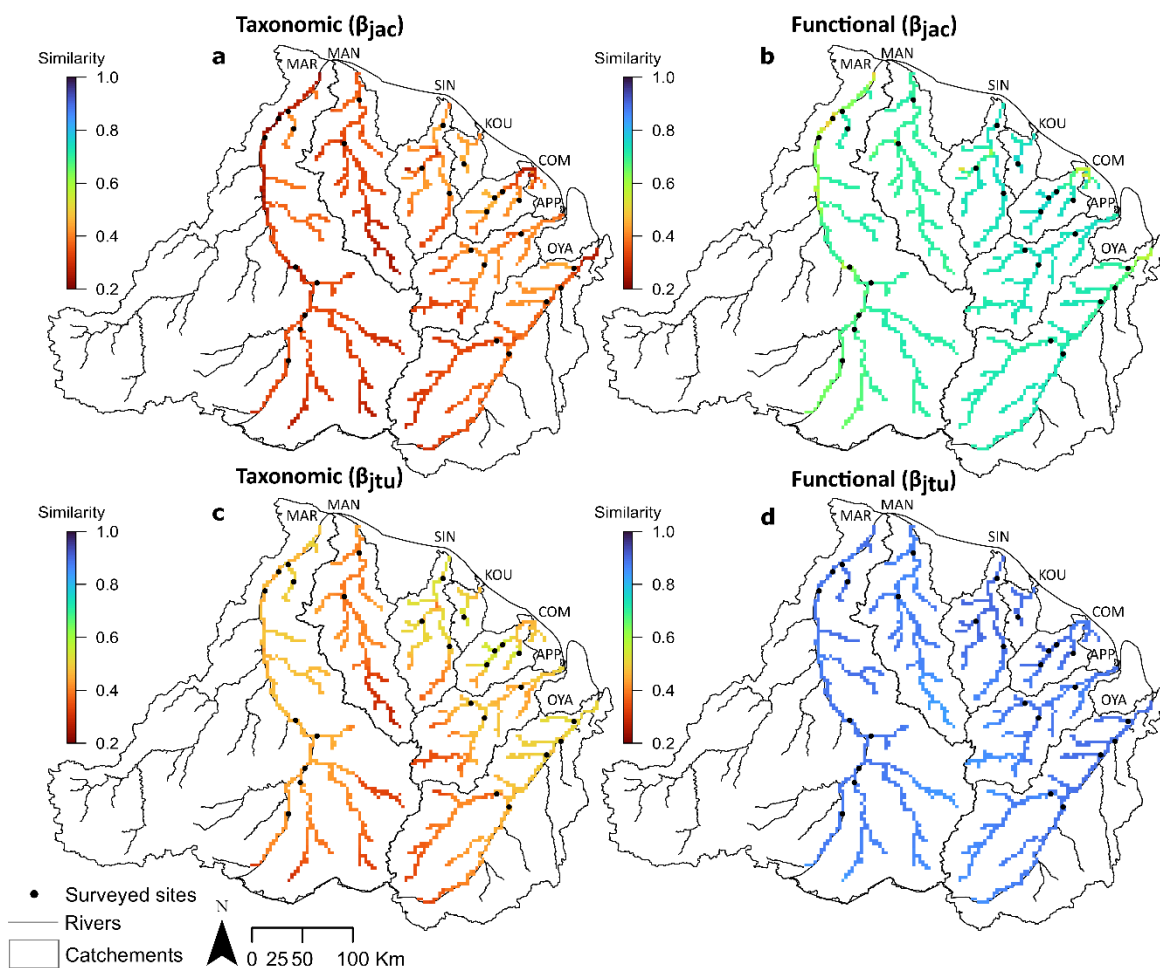


Figure 6. GDM predictions of β -diversity to assess the representativeness of survey locations. The similarity gradient indicates the mean predicted community similarity of each location (each 1km-pixel of the hydrographic network) to the 27 survey sites of the EU Water Framework Directive monitoring program (see methods). Maroni (MAR), Mana (MAN), Sinnamary (SIN), Kourou (Kou), Comté (COM), Approuague (APP), Oyapock (OYA).

displayed the highest rate of β -diversity (summed transformed predictor values were around 0.66, Figure 5c). In comparison, when considering taxonomic turnover predictions, only the middle and downstream course of the Maroni river exhibited the highest rate of β -diversity,

(summed transformed predictor values around 0.07, Figure 5f). Spatial predictions of functional β -diversity and turnover were similar to that of taxonomic β -diversity and turnover, respectively (Figure 5c, f, i, l). See Figure S3 for overall predictions of taxonomic and functional β -diversity and its turnover component in response to geographical, environmental and anthropic variables together.

Comparing the similarity between predicted fish communities and WFD survey locations indicates how these survey sites are representative of the regional fish fauna. Overall, the WFD survey sites had a better representation of the fish regional functional diversity (similarity values ranging from 0.48 to 0.76) than taxonomic diversity (similarity values ranging from 0.20 to 0.46) (Figure 6a, b). With fish community predictions based on taxonomic β -diversity, similarity to the WFD survey sites presented variations over the entire hydrographic network with the Kourou, sections of the Sinnamary and the Approuague and the downstream part of the Comté river exhibiting the highest similarities (0.40 – 0.45). Conversely, upstream sections of the Maroni and the Mana as well as estuaries of the Comté and the Oyapock rivers were the less well represented by the WFD survey sites (similarity values ranging between 0.20-0.25, Figure 6a). When only considering taxonomic predictions of fish community based on turnover, the overall similarity between regional taxonomic fish diversity and WFD survey sites increased (similarity values ranging from 0.27 to 0.55). This was particularly noticeable on the downstream section of the Maroni and on the estuaries of the Oyapock and the Comté rivers (Figure 6c). With fish community predictions based on functional β -diversity predictions, similarity between regional taxonomic fish diversity and WFD survey sites were high, with similarities to the survey sites ranging between 0.70 and 0.76 for most of the hydrographic networks. Only small sections of the Maroni, the Approuague, the Comté and the Oyapock had a low similarity to the WFD survey sites not exceeding 0.6 (range 0.48-0.60) (Figure 6b). When only considering fish community predictions based on functional turnover, the similarity to WFD survey sites increased for the entire river network and exhibited little spatial variations (similarity values ranging from 0.84 to 0.90, Figure 6d).

The comparison of similarity between each location and the entire region revealed unique fish assemblages. With fish community predictions based on taxonomic β -diversity, fish assemblages located downstream in the Maroni and in the Comté and Oyapock, estuaries were the less similar to the regional taxonomic diversity (similarity values ranging between 0.17-0.27, Figure 7a). Contrastingly, considering taxonomic fish community predictions based on turnover, the most unique assemblages were located at the upstream section of the Mana, and on upstream Maroni, Mana, Oyapock and Approuague tributaries (similarity values ranging

from 0.31 to 0.37, Figure 7c). Fish community pattern of uniqueness based on functional β -diversity was similar to that of taxonomic β -diversity while functional turnover exhibited no spatial variation of community uniqueness (Figure 7b, d). The index of uniqueness based on functional diversity nevertheless exhibited a narrower range than that based on taxonomic diversity (similarity values ranging from 0.48 to 0.75 for functional β -diversity and from 0.84 to 0.89 for functional turnover, Figure 7b, d).

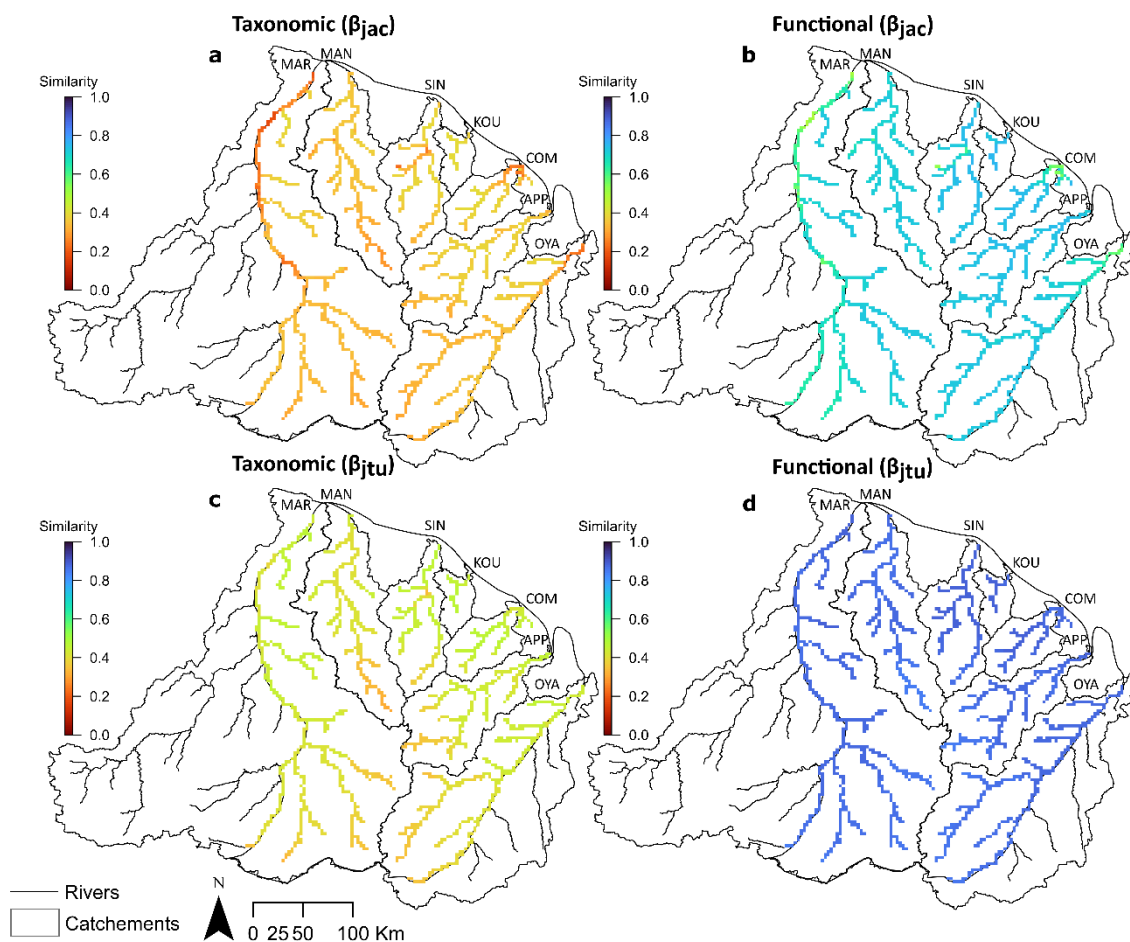


Figure 7. GDM predictions of β -diversity to assess the uniqueness of each location. The similarity gradient indicates the mean predicted similarity of each location (each 1km-pixel of the hydrographic network) in relation to the entire region. Maroni (MAR), Mana (MAN), Sinnamary (SIN), Kourou (Kou), Comté (COM), Approuague (APP), Oyapock (OYA).

Discussion

As freshwater ecosystems are organised in network structures following hydrologic pathways (Pringle, 2001), the river connectivity is an important determinant of biodiversity patterns. Thus, management strategies in those ecosystems need to be designed at the catchment scale (IPBES, 2019). However, conservation strategies are often insufficient because it requires knowledge of the biodiversity distribution, as well as of their determinants and processes

(assembly rules) at the scale of entire basins. This is particularly challenging in Neotropical freshwaters regions. These areas concentrate a vast number of species, many of which are rare, and present limited information on species spatial distributions (Mokany et al., 2014). This restricts the suitability of common species-level modelling approaches in depicting information on diversity distribution and its underlying mechanisms (Mokany et al., 2014; Pollock et al., 2020). Here we used community-level modelling, as an efficient alternative to classical species centered approaches (Pollock et al., 2020) combined with a multifaceted biodiversity approach.

1. Fish assembly rules between and within basins

Multifaceted approaches of biodiversity provide complementary views on the processes that give rise to ecological patterns at different spatial scales (Loiseau et al., 2017). Here, taxonomic and functional β -diversity bring light onto the assembly rules driving Neotropical fish communities at both regional and river basin scales. Between basins, our findings revealed mismatching patterns between both facets of fish diversity. The higher taxonomic β -diversity than functional β -diversity indicates that dispersal limitation acts at a higher degree than environmental filtering in shaping fish community between basins (Benone et al., 2020; Carvajal-Quintero et al., 2019). This is also supported by the taxonomic β -diversity mostly explained by turnover between basins, as species replacement is expected to be higher between localities if species dispersal is limited (García-Navas et al., 2022; Peláez & Pavanelli, 2019). In addition, the high contribution of geographic distance to taxonomic β -diversity and turnover highlighted in the GDMs as well as the increase of the explanatory power of the models when accounting for historical connectivity between basins indicate that dispersal limitation is the major driver of taxonomic fish distribution at the regional scale. It is primarily due to the isolation between river basins by land and seawater, strongly limiting inter-basin dispersal (Dias et al., 2014). Such fish species turnover between drainages gave rise to an East-West similarity gradient of the river drainages, reflecting the historical fluvial connections of the region and the related species dispersal limitation.

Surprisingly, this pattern still holds at intra-basin level, although the mismatch between the taxonomic and functional β -diversity was less marked. Thus, dispersal limitation still occurs within basins. This process was already reported for fish faunas in small streams, where the main river stem and major tributaries act as barriers against dispersal of stream species (Cilleros et al., 2016), supporting the general prediction that upstream areas are isolated from each other (Brown & Swan, 2010; Schmera et al., 2018). Here, we show that dispersal limitation still holds

for faunas inhabiting large rivers, which contradicts the general theory specifying that large rivers are largely influenced by mass effects (the movement of animals through dispersal), and are therefore not constrained by dispersal limitation (Brown & Swan, 2010; Schmera et al., 2018). Such main stream dispersal limitation may be due to the natural discontinuities of the fluvial networks (Herrera-Pérez et al., 2019). In the particular case of Guianese fishes, the alternation of slow flowing areas with rapids and waterfalls, probably explains the observed dispersal limitation within the upstream-downstream course of the rivers.

This has a particular significance for conservation, as faunistic recovery from anthropogenic disturbances may be hampered by dispersal limitation. For instance, dispersal played an important role during the recovery of impacted zooplankton communities (Gray & Arnott, 2011). Naturally-discontinued river systems may be even more sensitive to anthropogenic fragmentation as it can result in a cumulative effect increasing the limitation of species movements along the hydrographic network (Gauthier et al., 2021). It particularly concerns freshwaters ecosystems in the Amazonian region, where the continuous development of dams (Castello & Macedo, 2016) acts as dispersal barriers, disconnecting populations and limiting re-colonization processes (Winemiller et al., 2016), and therefore reducing faunistic recovery in disturbed areas.

2. Anthropic disturbances shape fish diversity

While anthropic activities in this region was shown to drive drastic paired declines of fish taxonomic and functional diversity at the local scale (Cantera, Coutant, et al., 2022), we predicted at the regional scale that functional β -diversity would be more imprinted by anthropic activities than taxonomic β -diversity. We demonstrated that anthropic activities had a weaker contribution than historical and environmental determinants to the taxonomic distribution of fish at the regional scale. Nevertheless, anthropisation was here a major determinant of functional β -diversity, resulting in species trait turnover between sites. This mismatch pattern justifies the importance of multifaceted approaches to assess the impact of anthropic activities on biodiversity (Cilleros et al., 2017; Devictor et al., 2010; Jiang et al., 2021; Loiseau et al., 2017). Interestingly, we report a marked shared effect between environmental and anthropic factors in explaining functional β -diversity. Anthropic activities may thus reinforce environmental sorting by modifying environmental parameters through land exploitation and transformation (Roa-Fuentes et al., 2019; Villéger et al., 2010). Although deforestation was used as a proxy of anthropogenic disturbances including gold mining, logging, agriculture, and

human settlements, gold mining remains the main cause of deforestation in the Northern Amazonian region (Dezécache et al., 2017). Within the studied area, goldmining accounts for more than 40% of the deforested surfaces (Cantera, Coutant, et al., 2022). Goldmining generates both physical and ecotoxicological impacts by releasing pollutants in the water (mercury or cyanide), degrading the riverbed and the banks, and altering the water physico-chemistry (Gallay et al., 2018). We already reported that persistent impacts of goldmining throughout the study area drive fish assemblages towards the same local decline in functions, with a loss in small detritivores, invertivores and algae browser fish species (Cantera, Coutant, et al., 2022). Here, the congruent patterns of fish taxonomic and functional β -diversity but also between taxonomic and functional turnover in response to anthropisation reflects a functional homogenisation at the anthropised localities. At these localities, the compositional changes within communities result in an increase of similarity between anthropised sites and in a decrease in similarity between disturbed and undisturbed sites. Generally, functional homogenisation is the result of the replacement of species with unique functional attributes by functionally redundant species. The loss of functional strategies reflects the vulnerability of the studied ecosystems, altering functional diversity and ecosystem functioning (Clavel et al., 2011).

3. Regional predictions of French-Guianese fish diversity

In this study, using a community-level modelling approach, we illustrated how we can predict taxonomic and functional biodiversity distribution in species-rich environments and unexplored areas. The spatial GDM predictions provided a regional view of the spatial variation of fish assemblages based on historical, environmental and anthropic factors. For instance, we predicted the highest rate of β -diversity in response to anthropisation in assemblages located in the downstream section of the Maroni River and on the Oyapock and Comté estuaries as well as on some tributaries of the inland rivers. Predictions based on environmental parameters exhibited contrasting patterns. Taxonomic assemblages of the Maroni and Oyapock rivers were similar but distinct from those of the inland basins. This is possibly due to the length of the two rivers, crossing the entire territory and therefore facing a wider and similar range of environmental variations, notably elevation and water temperature. The assemblage similarity noticeably increased between all basins when only considering functional turnover. This suggests that the inland basins may present differences in the number of functional strategies compared to the Maroni and Oyapock.

Reliable predictions of β -diversity, conditioned upon models' explanatory power and quality can constitute a basis for biodiversity conservation studies. Mokany et al., (2022) provide a summary of relevant analyses that can be implemented from GDM predictions of β -diversity within the framework of biodiversity conservation studies. For instance, combining the assessment of regional community similarity to local communities and determining the biodiversity uniqueness of each locality can help identify whether sites surveyed as part of the European Water Framework Directive (WFD) monitoring program well represent regional biodiversity.

In general, the predictions demonstrated that functional β -diversity was better represented than taxonomic β -diversity in the WFD survey sites. Interestingly, the communities that were the most poorly represented in terms of functional diversity corresponded to communities exhibiting the highest β -diversity in response to anthropogenic disturbances. These communities were also predicted to be the most unique in terms of functional diversity. Regional functional turnover was well captured in the WFD sites compared to regional taxonomic turnover where the most poorly represented communities were expected to present the most unique taxonomic composition. Our results demonstrate that anthropic disturbances generate assemblages with atypical functional diversity that is poorly captured by the WFD survey sites. These results also indicate that achieving a meaningful taxonomic assessment of the diversity requires to survey sites distributed all along the hydrographic network. The representativeness of the regional fish diversity in the WFD survey sites could therefore be improved by adding additional localities hosting the most unique assemblages identified here, differing depending on the biodiversity facet.

Conclusion

Establishing appropriate conservation strategies to protect freshwater ecosystems at the catchment scale first necessitates disentangling the assembly rules of freshwater communities. This is challenging in species-rich environment because of the lack of knowledge on species distribution. The recent advances of eDNA metabarcoding coupled with adequate biodiversity modelling tools allows implementing biodiversity distribution models with high explanatory power that can further be used to project biodiversity at a regional scale. Our study revealed a mismatch between the taxonomic and the functional facet of fish assemblages. While French-Guianese fish taxonomic diversity was mainly structured by dispersal limitation at both intra and inter-basin scales, functional diversity was rather influenced by environmental and

anthropic determinants. Importantly, regional predictions of fish biodiversity indicated that survey sites of the EU Water Framework Directive monitoring program failed to fully capture regional taxonomic diversity but also assemblages presenting unique functional diversity in response to human disturbances. This study presents a promising approach to map spatial biodiversity, design efficient networks of survey sites, and to quantify the anthropogenic impact on species rich environments.

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Author contribution

O.C., C.J., K.M., S.B. and J.M. conceived the ideas and designed methodology; O.C., S.B., J.M., collected the data; A.V. and T.D. supervised the laboratory work and conducted the bioinformatic analyses; R.C. provided part of genetic material; O.C. analysed the data; O.C., C.J., K.M., S.B., J.M., I.C. led the writing of the manuscript. All authors contributed critically to the manuscript.

Supplementary Materials

See the online version of the article

Chapter III. Human responses to biodiversity modifications

1. Part 1

Confronting human's perceptions and eDNA measures of biodiversity

Manuscript in preparation

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This chapter is exploratory as we sought to initiate a multidisciplinary approach between social and ecological sciences. Fieldwork was initially scheduled in November 2020, but due to COVID19 pandemic, it was postponed several times and was finally achieved in April 2022. Our initial plan was to focus our study on the course of the Camopi River, characterized by a strong anthropogenic gradient, with the downstream course strongly impacted by human activities and the upstream area free from human disturbances. The Camopi river is a tributary of the Oyapock river and connects the main linear at the Camopi village (Figure 1). It is the territory of the Teko community, the only Amerindian community presents only in French Guiana. They mainly occupy the village of Camopi and upstream areas. Illegal gold mining is also developed in the downstream part of the Camopi River but the tributary is free from anthropogenic pressures from the confluence with the “Crique Inipi”. While all the necessary permits had been obtained, we were eventually not authorized to navigate on the Camopi River by the village chef once we arrived at the Camopi village. This was unexpected as our field trip was organized in collaboration with an ethnologist who has been working with the Teko for several years. We therefore changed the locality of our survey campaign and relocated our sampling sites along the upstream part of the Oyapock river, starting from downstream Camopi village. The anthropisation gradient in this river section is different from that of Camopi. The river section is delimited by two inhabited sectors and travels between them are regular although not intense (2 to 3 boats per day maximum). This last minute change rescued our project, although the anthropisation gradient was less clear on the Oyapock than on the Camopi river. On the other hand, it offered the opportunity to work with the Wayãpi community who benefits from more ethnologic knowledge than the Teko community.

Introduction

The loss of biological diversity is a critical global environmental issue. While biodiversity is being dramatically eroded, associated ecosystem services are also affected, putting at risk human societies (Ceballos et al., 2017). Protecting nature for humans' sake (instrumental values) or for nature's intrinsic values has been extensively debated (Chan et al., 2016). On the one side, considering the instrumental values of nature involves preserving human resources and ecosystem services while considering nature intrinsic values seeks to protect nature more holistically, regardless of humans' needs (Chan et al., 2016; Ernoul et al., 2018). Historically, natural sciences have been the primary source of information to guide environmental policies, centered on the intrinsic values of nature (Bennett et al., 2017).

Progressively, conservation biology recognised humans as external drivers of biodiversity changes and as beneficiaries of ecosystem services. These complex interactions led to reconceptualise the human-nature relationship evidenced today with well-known concepts such as sustainable development or socio-ecological systems (Teel et al., 2018). In 1985, the influential article of Soulé, (1985) introduced social sciences as a discipline of conservation biology. In such way, the debate over the protection of instrumental or intrinsic values of nature evolved toward the integration of both values, giving rise to a so-called “relational values” (Chan et al., 2016). This third class of values is not represented by material values, but rather by the relationships and responsibilities to them. It can be translated into values such as justice, reciprocity or care (Chan et al., 2016). There is now a consensus on the need to integrate multidisciplinary approaches, such as combining social and ecological sciences in conservation biology (Bennett et al., 2017). While several studies have aimed to include human perceptions and uses of biodiversity, integrative approaches have not yet become a standard (Bennett et al., 2017). This lack of multidisciplinary studies is problematic because poor assessments of ecosystem and biodiversity social values impede the efficiency of conservation strategies (Ernoul et al., 2018).

Conservation social sciences, defined as “the subset of the classic and applied social science disciplines that focus particularly on conservation or environmental management” provide a vision on the human dimension of conservation (Bennett et al., 2017). Different approaches have been developed in conservation multidisciplinary studies, such as comparative analyses. In particular, socio-ecological comparisons of biodiversity have been used to explore different questions. Several studies use Local Ecological Knowledge (LEK), the environmental knowledge and/or perception of people living in close contact to nature (Braga-Pereira et al., 2022), as a biodiversity monitoring tool (Braga-Pereira et al., 2022; Camino et al., 2020; Madsen et al., 2020; Shaw et al., 2016; Zayonc & Coomes, 2022). In this way, comparing LEK-based biodiversity inventories to ecological-based inventories can help estimate the biodiversity representativeness of social knowledge and/or perception. For instance, Camino et al., (2020) evaluated the performance of LEK-based inventories by comparing it with that of standard-methods to monitor terrestrial mammals. They showed that LEK is efficient to obtain information on large mammals in remote areas. Similarly, Zayonc & Coomes, (2022) demonstrated that LEK household surveys were as accurate or even outperformed conventional surveys (line-transects and camera trapping) for certain aquatic, ephemeral, and cryptic terrestrial species. Among the same lines, Braga-Pereira et al., (2022) demonstrated that LEK-based surveys provided reliable estimation of abundance for a wide diversity of taxa by

comparison to traditional line-transect inventories. They found concordance in population abundance of diurnal and game species between line-transects and LEK-based surveys, while they retrieved no significant correlation for nocturnal and non-game species. In all these studies, the multidisciplinary approach with social sciences aims to improve conservation management practices by including different stakeholders that can provide in certain cases more cost-efficient and rapid assessment of biodiversity than natural sciences (Bennett et al., 2017). Another approach is the collection of biodiversity LEK through free lists. With this approach, non-oriented questions can help describe the nature instrumental values of local populations. It allows identifying the key resources but can also highlight the threats to the resource and environment, as perceived by local populations (Longin et al., 2021). In such a way, local population needs and nature needs may be better identified and conciliated into conservation management policies. It can then result in more socially equitable conservation strategies and enables planning conservation initiatives that match different social, economic, cultural and governance contexts (Bennett et al., 2017).

This last approach is particularly relevant when the use of natural resources is conflictual between different natural beneficiaries. This context is encountered in French Guiana. French Guiana is located in the Guiana shield and presents well-preserved and poorly fragmented rainforests and freshwaters but face increasing threats due to the illegal exploitation of gold and the rapid demographic increase (Cantera, Coutant, et al., 2022; Su et al., 2021). This territory is home to different ethnic groups that drastically differs in their use of natural resources but also in their relationship with nature (Tritsch, 2013; Tritsch et al., 2011, 2015). The littoral presents the highest population density with an occidental lifestyle and economy but is also inhabited by native Amerindian ethnic groups, namely the Palikour or Paykweneh, the Lokono or Arawak and the Kali'na. Two ethnic groups inhabit the inland: the native Amerindians and the afro-descendant Bushinengués. These ethnic groups, and particularly Amerindians populations, remain strongly anchored to a subsistence economy. As Amerindians do not practice animal farming, most protein resources come from fishing and hunting activities. Rivers and the surrounding forest thus represent a pantry for Amerindians, who maintain strong cultural and historic ties to these ecosystems (Tritsch, 2013). Yet, the impacts of gold mining, mainly the alteration and contamination of water and by extent, biodiversity, dramatically affect the Amerindian livelihoods (Tritsch, 2013).

In this study, we led a comparative analysis of social perceptions and scientific measures of biodiversity along the Oyapock river, representing the border between French Guiana and Brazil. We collected biodiversity perceptions of Wayãpi and Teko Amerindians using free list

surveys at different sites along the river, which faces different levels of anthropogenic pressure. In parallel, environmental DNA (eDNA) was also collected in the sites. In this study, our first objective was to determine whether human perceptions of biodiversity bring concordant information on species distribution with that obtained from scientific measures. Second, by identifying the similarities and dissimilarities with scientific measures of biodiversity, we aimed at describing the nature instrumental values of Wayãpi and Teko Amerindians. A strong congruence between scientific measures and local people perceptions would indicate that local knowledge can be used as measure of biodiversity conservation status, enabling therefore rapid and efficient assessments of biodiversity through local knowledge. Instead, a mismatch between scientific biodiversity measures and local perceptions will indicate that both visions are distinct and complementary facets of biodiversity. In this way, both visions would deserve to be considered for biodiversity management, paving therefore the way for a better integration of indigenous nature instrumental values into conservation policies.

Material and Methods

1. Study area

The main channel of the Oyapock river is the border between Brazil and French Guiana. It is 404 km long and shelters 195 described fish species. We conducted our exploratory approach on the upper section of the Oyapock River, departing a little downstream from Camopi village (Figure 1). This section is less heavily affected by human activities than the downstream part of the river. The upstream section of the Oyapock drainage, from Camopi to the source, is indeed under protection on both Brazilian (Tumucumaque National Park) and French (Guiana Amazonian Park) sides, with only subsistence fishing and hunting allowed. Nevertheless, illegal gold mining is also operating in this area, mainly near Camopi village along the downstream section of the Camopi river (Figure 1). Amerindians inhabiting the upper Oyapock section belong to the Teko and Wayãpi ethnic groups, accounting together for approximately 2000 people (1032 in Camopi and 560 in Trois-Sauts) practising subsistence slash-and-burn agriculture that provides carbohydrates from cassava roots, and hunting and fishing providing proteins.

2. Environmental DNA

2.a. *Water sampling*

The linear from our two farthest sampling sites measures 160 km. Along this linear, we collected environmental DNA at 20 different sites. We conducted the survey during the wet season (April 2022). This was necessary to access some locations that are protected by rocky barriers hardly crossable during the dry season. In each site, we implemented the protocol developed in Cantera et al., (2019). At all rivers, the sites were sequentially sampled from downstream to upstream. We collected the eDNA by filtering two replicates during 30 minutes. A peristaltic pump (Vampire Sampler; Buerkle GmbH, Bad Bellingen, Germany) and single-use tubing were used to pump the water into a single-use filtration capsule (VigiDNA, 0.45 μm ; SPYGEN, Bourget-du-Lac, France). The tubing input was placed a few centimetres below the water surface in zones with high water flow as recommended by Cilleros et al., (2019). Sampling was performed in turbulent areas to ensure optimal eDNA homogeneity throughout the water column. To avoid eDNA cross-contamination among sites, the operator remained on emerging rocks downstream from the filtration area. At the end of filtration, the capsule was emptied, filled with 80 mL CL1 conservation buffer (SPYGEN), and stored in the dark up to one month before the DNA extraction.

2.b. *eDNA laboratory and bioinformatics*

For the DNA extraction, each filtration capsule was agitated on an S50 shaker (Ingenieurbüro CAT M. Zipperer GmbH, Ballrechten-Dottingen, Germany) at 800 rpm for 15 min, decanted into a 50 mL tube, and centrifuged at $15,000 \times g$ and 6°C for 15 min. The supernatant was removed with a sterile pipette, leaving 15 mL of liquid at the bottom of the tube. Subsequently, 33 mL of ethanol and 1.5 mL of 3 M sodium acetate were added to each 50 mL tube, and the mixtures were stored at -20°C for at least one night. The tubes were then centrifuged at $15,000 \times g$ and 6°C for 15 min, and the supernatants were discarded. Then, 720 μL of ATL buffer from a DNeasy Blood & Tissue Extraction Kit (Qiagen, Hilden, Germany) was added. The tubes were vortexed and the supernatants were transferred to 2 mL tubes containing 20 μL Proteinase K (Macherey-Nagel GmbH, Düren, Germany). The tubes were then incubated at 56°C for 2 h. DNA extraction was performed using a NucleoSpin Soil kit (Macherey-Nagel GmbH, Düren, Germany) starting from step six of the manufacturer's instructions. Elution was performed by adding 100 μL of SE buffer twice. After the DNA

extraction, the samples were tested for inhibition by qPCR (Biggs et al., 2015). Briefly, quantitative PCRs were performed in duplicate for each sample. If at least one of the replicates showed a different Ct (Cycle threshold) than expected (at least 2 Cts), the sample was considered inhibited and diluted 5-fold before the amplification.

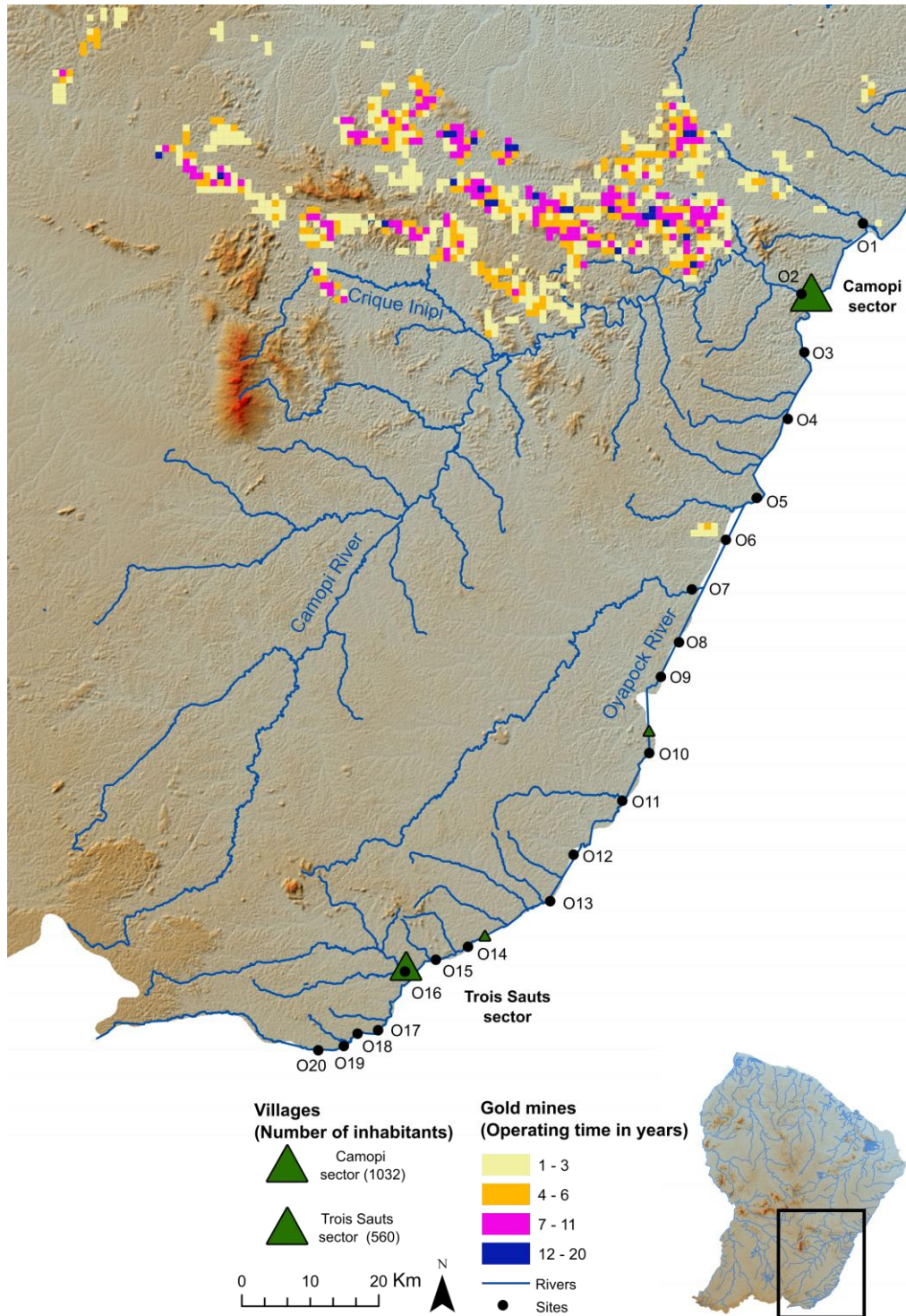


Figure 1. Study area and sampling sites. Each 1 km pixel contains a gold mine abandoned or still operating. Gold mine operating time (years) was shared by the Parc Amazonien de Guyane (PAG) and the Observatoire de l'Activité Minière (OAM). The number of habitants was shared by the PAG.

For fishes, we used the “teleo” primer (Valentini et al., 2016) (forward: 3'-ACACCGCCCGTCACTCT-5'; reverse: 3'-CTTCCGGTACTTACCATG-5'), which has been shown to provide efficient discrimination of the local fish fauna (Cantera et al., 2019; Cilleros et al., 2019). For all the other taxonomic groups, the 12S-V5 vertebrate marker (Riaz et al., 2011) (forward: 3'-TAGAACAGGCTCCTCTAG-5'; reverse: 3'-TTAGATACCCCACTATGC-5') was used. This primer set also effectively distinguishes local mammal species (Coutant et al., 2021; Kocher, Thoisy, Catzeflis, Huguin, et al., 2017). The DNA amplifications were performed in a final volume of 25 μ L containing 1 U AmpliTaq Gold DNA Polymerase (Applied Biosystems, Foster City, CA, USA), 0.2 μ M of each primer, 10 mM Tris-HCl, 50 mM KCl, 2.5 mM MgCl₂, 0.2 mM of each dNTP, and 3 μ L DNA template. Human blocking primer was added to the mixture for the “teleo” primers (Valentini et al., 2016) (5'-ACCCTCCTCAAGTATACTTCAAAGGAC-C3-3') and for the “12S-V5” primers (De Barba et al., 2014) (5'-CTATGCTTAGCCCTAAACCTCAACAGTTAAATCAACAAAAGTCT-C3-3') at final concentrations of 4 μ M and 0.2 μ g/ μ L bovine serum albumin (BSA; Roche Diagnostics, Basel, Switzerland). Twelve PCR replicates were performed per field sample (85 sites x 2 field replicates x 12 PCR replicates, 2040 PCR replicates in total). The forward and reverse primer tags were identical within each PCR replicate. The PCR mixture was denatured at 95 °C for 10 min, followed by 50 cycles of 30 s at 95 °C, 30 s at 55 °C and 1 min at 72 °C, and a final elongation step at 72 °C for 7 min. This step was conducted in a dedicated room for DNA amplification that is kept under negative air pressure. The success of the amplification was verified using capillary electrophoresis (QIAxcel; Qiagen GmbH) and the samples were purified using a MinElute PCR purification kit (Qiagen GmbH). Before sequencing, purified PCR products were quantified using capillary electrophoresis and then pooled in equal volumes to achieve an expected sequencing depth of 500,000 reads per sample before DNA library preparation.

For fish and game species analyses, six and two libraries were prepared, respectively. The library preparations were realised following the Illumina TruSeq DNA PCR-Free library prep protocol and the sequencing was performed by the DNAGensee laboratory (Le Bourget du Lac), using the MiSeq sequencing platform. To monitor for contaminants, five negative extraction controls were performed for each of the primers (“teleo” and “12S-V5”). All of them were amplified and sequenced by the same methods as the samples and in parallel to them. In addition, Fourteen negative PCR controls (ultrapure water; 12 replicates) were amplified and sequenced in parallel to the samples. Three were amplified with the “teleo” primers and one was amplified with the “12S-V05” primers.

The sequence reads were analysed with the OBITools (Boyer et al., 2016) according to the protocol described by Valentini et al., (2016). Briefly, the forward and reverse reads were assembled with the `illumina-paired-end` programme. The `ngsfilter` programme was then used to assign the sequences to each sample. A separate dataset was created for each sample by splitting the original dataset into several files with `obisplit`. Sequences shorter than 20 bp or occurring less than 10 times per sample were discarded. Sequences labelled by the `obiclean` programme as “internal” and probably corresponding to PCR errors were also discarded. The `ecotag` programme was used for taxonomic assignment of molecular operational taxonomic units (MOTUs). An updated version of the reference database from Cilleros et al., (2019) was used. There were 362 Guianese species for the fish analyses. The GenBank nucleotide database was consulted, but it contained little information on the Guianese fish species. Most of the sequences were derived from Cilleros et al., (2019). Species-level assignments were validated only for $\geq 98\%$ sequence identity with the reference database. Sequences below this threshold were discarded. We discarded all MOTUs with a frequency of occurrence below 0.001 per library in each sample, considered as tag-jumps (Schnell et al., 2015). These thresholds were empirically determined to clear all reads from the extraction and PCR negative controls included in our global data production procedure as suggested by De Barba et al., (2014); Taberlet et al., (2018). These bioinformatic analyses provided a species by site matrix with read number per species (Table S1).

3. Social surveys

At each sampling sites, we conducted semi-orientated free list surveys with Amerindians from the Wayãpi and Teko ethnic groups. Nine Amerindians participated in the social survey in total. At each site, one participant answered the survey conducted by one interviewer (Damien Davy or Opale Coutant), directly on site. The participants were all voluntary and sensitized to the interest of the study. Participants were anglers and hunters from Camopi and Trois-Sauts sectors and thus possessed a good knowledge of the upstream section of the Oyapock River. They are recognised for their biodiversity knowledge, their fishing and hunting skills and their ethnic group knowledge. The surveys were semi-orientated as we asked open-ended questions in semi-structured interviews, with predefined themes. The themes were separated into fish diversity, game-species diversity (including bird, mammals, and reptiles), gold mining threats and slash-and-burn agriculture (Supplementary Table 1). For the fish diversity and game-species themes, the participants were asked several questions about the

general quality of the site for fishing and hunting and then, they were asked which species can be encountered at the site.

4. Data analyses

Species by site matrices were built for both approaches (ecological and social surveys). In certain cases, generic names of groups of species were given, such as toucans or peccaries. As these categories include several species, we built the same categories for eDNA-based inventoried and only accounted for the presence or absence of the species group when comparing both approaches. The comparisons were only conducted based on the species cited in the social surveys. We categorized sites based on the classification determined by the participants for fish diversity and for games species diversity. For fish, the sites were either described as good sites for fishing harbouring a lot of fish (referred to as “good” sites for fish), or as medium-quality sites sheltering less fish than good sites (referred to as “medium” sites for fish). For games species, sites were described as good sites with many game species (referred to as “good” for game species) or as poor sites, therefore with fewer animals (referred to as “poor” in game species). Another category for game species was defined, namely the site quality for hunting, with good-quality sites or poor-quality sites for hunting. The two classifications are different because a site with many game species and considered as “good” is not necessarily a “good” hunting site, as the selection of hunting areas may depend upon other factors.

We compared diverse attributes of the biodiversity inventories built from both methods, namely (i) the site species richness, (ii) the species occurrence percentages and (iii) the species composition of sites. (i) For site species richness, we compared the number of species per site between both methods but also between site categories determined from social surveys, within each approach. (ii) For species occurrence percentages (the percentage of sites in which a species was cited for social surveys or detected for eDNA), we compared within each approach the species occurrence percentages between the two site categories (good vs medium sites for fish and good vs poor sites for game species). We also compared the species occurrences between both methods for each site category. Pairwise comparisons were realised with Mann–Whitney U-tests for non-parametric and unpaired samples. (iii) For the comparison of site species composition, we built distance matrices. As the β -diversity and the α -diversity are interrelated, classical metrics of dissimilarity are influenced by the number of species present in each pair of samples. To address this issue, we built null models using the Raup crick metric

(β_{rc}) to determine whether pairs of sites were more or less similar than expected under stochastic effects (Chase et al., 2011). This metric expresses the dissimilarity between two communities relative to the null expectation of randomly assembled communities by estimating the probability that two randomly drawn communities have the same number or more species in common than the two observed communities. The resulting value represents a β -diversity metric ranging from -1 to $+1$. A negative β_{rc} value stands for communities more similar than expected by chance, while a positive value refers to communities less similar than expected by chance. A null value indicates that community assembly is highly stochastic. The dissimilarity matrices were then implemented into Non-Metric Multidimensional Scaling (NMDS) to visualise how the species composition of the sites is discriminated according to the site classification defined by social surveys and between both approaches. Procrustean analyses using the *procruste* and the *protest* functions of the *vegan* package (Oksanen et al., 2017) were then used to test for a correlation between ecological and perceptions of site species composition. NMDS were computed separately for fish and game species and for eDNA and social surveys. Finally, we fitted environmental variables onto the NMDS using the *envfit* function from the *vegan* package to identify any correlations between the variables and the ordination axes (Supplementary Table 2). The determination coefficients R^2 were calculated to assess the strengths of the correlations between the axes and the variables. Variables with high R^2 were strong ordination predictors. P-values were computed by comparing the observed and simulated R^2 based on 9999 random data permutations. To quantify the variable contributions, the continuous variables were transformed onto vectors directed according to their correlation with the axes. Their lengths were proportional to the strengths of the correlations between the ordinations and the traits (R^2). For the categorical variables, the average ordination scores were computed for the scores of all species belonging to each factor level to locate categories in the functional spaces. The analyses were conducted separately for fish and game species.

Results

1. General results

In total, 23 fish species and 18 game species including mammals, birds, and reptiles were cited in the social surveys over the 20 sampling sites. In comparison, 118 fish species and 115 species other than fish were recorded with eDNA at the same sites. One fish species (*Herootes efasciatus*) and two game species (the caimans of the genus *Paleosuchus spp.* and the green

iguana, *Iguana iguana*) were indicated as occurring at the sites but were not detected with eDNA. Fifteen sites were classified as good areas and five sites were considered as medium-quality areas for fish. For game species, 13 sites were cited as good sites for game species, while seven sites were considered as poor sites for game species.

2. Site species richness

For the surveys, the site species richness ranged from 2 to 14 (median = 6) for fish and from 2 to 11 (median = 8) for game species in the sites classified as “good” for fish and game species (Figure 2a and b). At the site classified as “medium” sites for fish, fish species richness ranged from 4 to 10 (median = 5) (Figure 2c). The species richness of game species ranged from 1 to 9 (median = 3) for sites considered as “poor” sites in game species (Figure 2d).

With eDNA, the site species richness (the richness in species detected using eDNA within the set of species cited by Amerindians during the survey) ranged from 10 to 19 (median = 16) for fish and from 7 to 15 (median = 13) for game species in the sites classified as “good” sites for fish and game species (Figure 2a and b). At the site classified as “medium” sites for fish, fish species richness ranged from 13 to 19 (median = 16) (Figure 2c). The species richness of game species ranged from 10 to 15 (median = 13) for sites considered as “poor” sites in game species (Figure 2d).

For the surveys, there was no significant difference in fish species richness between the sites classified as “good” and “medium” for fish. In comparison, the species richness of the game species recorded for the “good” sites was significantly higher than that of the “poor” sites (2 times higher in average) (Table 1).

With eDNA, there was no significant difference between the fish species richness of the “good” and “medium” sites. Similarly, no significant difference was retrieved for the species richness of game species between the “good” and the “poor” sites (Table 1).

Comparing species richness retrieved with eDNA and social surveys for each site category showed that eDNA species richness was systematically greater than survey species richness (Table 1). At the “good” sites, the eDNA species richness of fish and game species were in average 2.79 times and 1.69 times greater than that obtained with surveys, respectively (Figure 2a, b & Table 1). Similarly, the eDNA species richness of fish and game species at the “medium” and “poor” sites were 2.55 times and 3.64 times higher than that recorded with surveys, respectively (Figure 2c, d & Table 1).

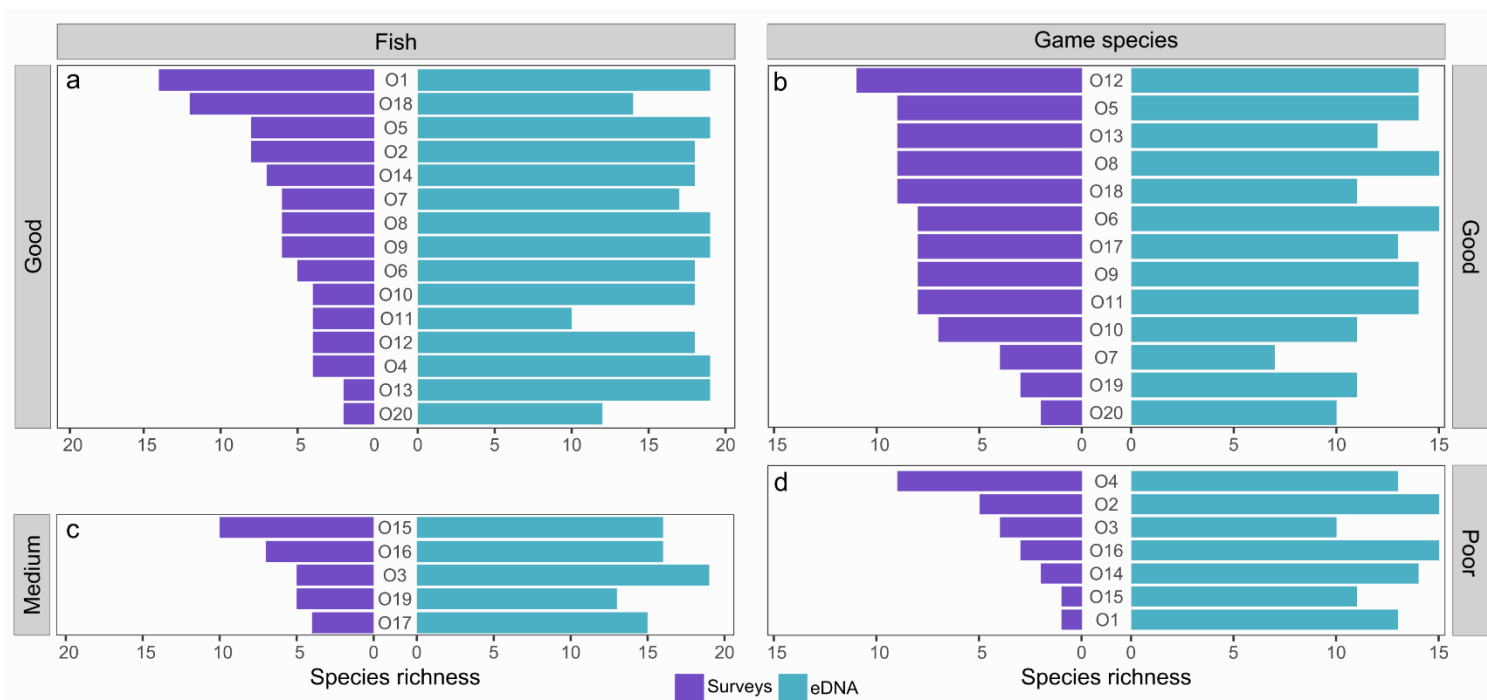


Figure 2. Site species richness obtained with social surveys and eDNA for each site category. Only the species cited in the social surveys were considered in the comparisons.

3. Species occurrence percentages

With the social surveys, the fish occurrence percentages ranged from 1.09 to 35.87 % (median = 6.52) while it ranged from 13.68 to 25.26 % (median = 20) for game species at the “good” sites (Figure 3a, b). At the “medium” and “poor” sites, the fish and game species occurrences ranged from 0 to 32.26 % (median = 6.45) and from 4 to 44 % (median = 16), respectively (Figure 3c, d). There was no significant difference in fish occurrence percentages obtained from social surveys between the sites classified as “good” and “medium” (Figure 3a, c & Table 1) and in game species occurrence percentages between the sites classified as “good” and “poor” (Figure 3b, d & Table 1).

With eDNA, the fish occurrences ranged from 0 to 25.68 % (median = 5.84) while it ranged from 15.53 to 27.33 % (median = 20.50) for game species at the “good” sites (Figure 3a, b). At the “medium” and “poor” sites, the fish and game species occurrences ranged from 0 to 24.05 % (median = 6.33) and from 15.38 to 25.27 % (median = 20.88), respectively (Figure 3c, d). There was no significant difference between fish occurrence percentages retrieved with eDNA between the sites categorised as “good” and “medium” (Figure 3a, c & Table 1) and in game species between the sites classified as “good” and “poor” (Figure 3b, d & Table 1).

Similar trends were reported for the occurrence percentage comparisons between surveys and eDNA. At the “good” and “medium” sites, fish occurrence percentages retrieved with eDNA did not significantly differ to that obtained with social surveys (Figure 3a, c & Table 1). Game species occurrence percentages retrieved with eDNA were not significantly different to that obtained with surveys at the “good” sites (Figure 3b & Table 1). The statistical comparison between eDNA and survey game species occurrence at the “poor” sites was not possible due to the small sample size (Table 1).

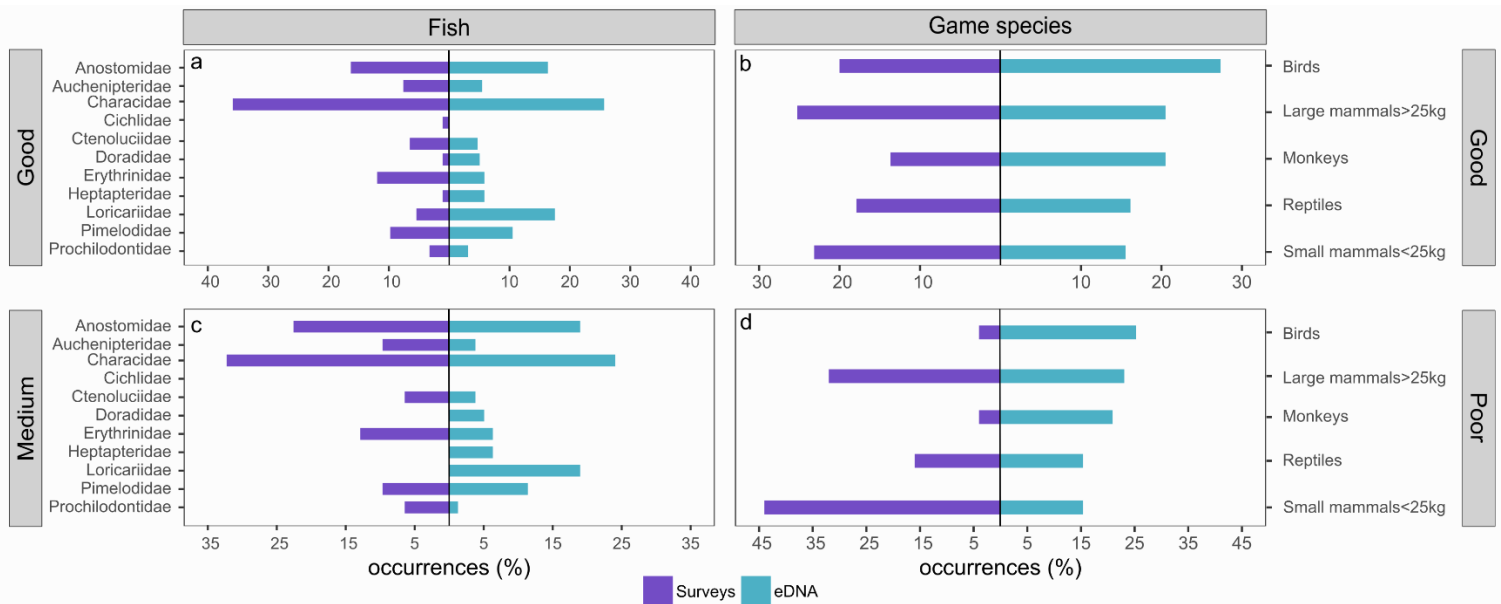


Figure 3. Percentage of species occurrences obtained with social surveys and eDNA for each site category. Only the species cited in the social surveys were considered in the comparison.

Table 1. Species richness and occurrence percentages comparisons between each site category and between social surveys and eDNA inventories. SR refers to species richness, O refers to occurrences, ns refers to not significant. Kruskal-Wallis statistical tests were used to compared species richness and occurrence percentages obtained with the same method between site categories. Mann Whitney-U tests were used to compare species richness and occurrence percentages obtained with eDNA and social surveys for each site category.

	eDNA	Surveys	eDNA - Surveys	
Site quality	Good - Medium	Good - Medium	Good	Medium
Fish	SR ($\chi^2=1.70$, df=1, p=ns) O ($\chi^2=0$, df=1, p=ns)	SR ($\chi^2=0.70$, df=1, p=ns) O ($\chi^2=0.24$ df=1, p=ns)	SR (W=221, n=15, p<0.001***) O (W=57, n=11, p=ns)	SR (W=25, n=5, p<0.05*) O (W=59, n=11, p=ns)
Site quality	Good - Poor	Good - Poor	Good	Poor
Game species	SR ($\chi^2=0.20$, df=1, p=ns) O ($\chi^2=0.01$ df=1, p=ns)	SR ($\chi^2=5.05$, df=1, p<0.05*) O ($\chi^2=0.1$ df=1, p=ns)	SR (W=157, n=1, p<0.001***) O (W=12, n=5, p=ns)	SR (W=49, n=1, p<0.01**) NA

The same tests were run without sorting species into categories. For both fish and species, there was no significant difference between occurrence percentages retrieved with

eDNA and surveys, but also between “good” and “poor” sites within each biodiversity approach.

4. Site species composition

For both fish and game species, the site composition in species retrieved by eDNA and by social surveys were significantly different and appeared as distinct clusters in the NMDS (Figure 4). However, within each biodiversity approach, the site species composition in fish and game species was not distinguishable between “good” and “medium” sites and between “good” and “poor” sites, respectively (Figure 4). Nevertheless, with both surveys and eDNA, “medium” and “poor” sites were nested into “good” sites. This resulted in overlapping clusters of “good” and “medium” sites (Figure 4a) and of “good” and “poor” sites (Figure 4b). It indicates that the fauna present at “medium” and “poor” is a subset of that of “good” sites, and it therefore less diversified.

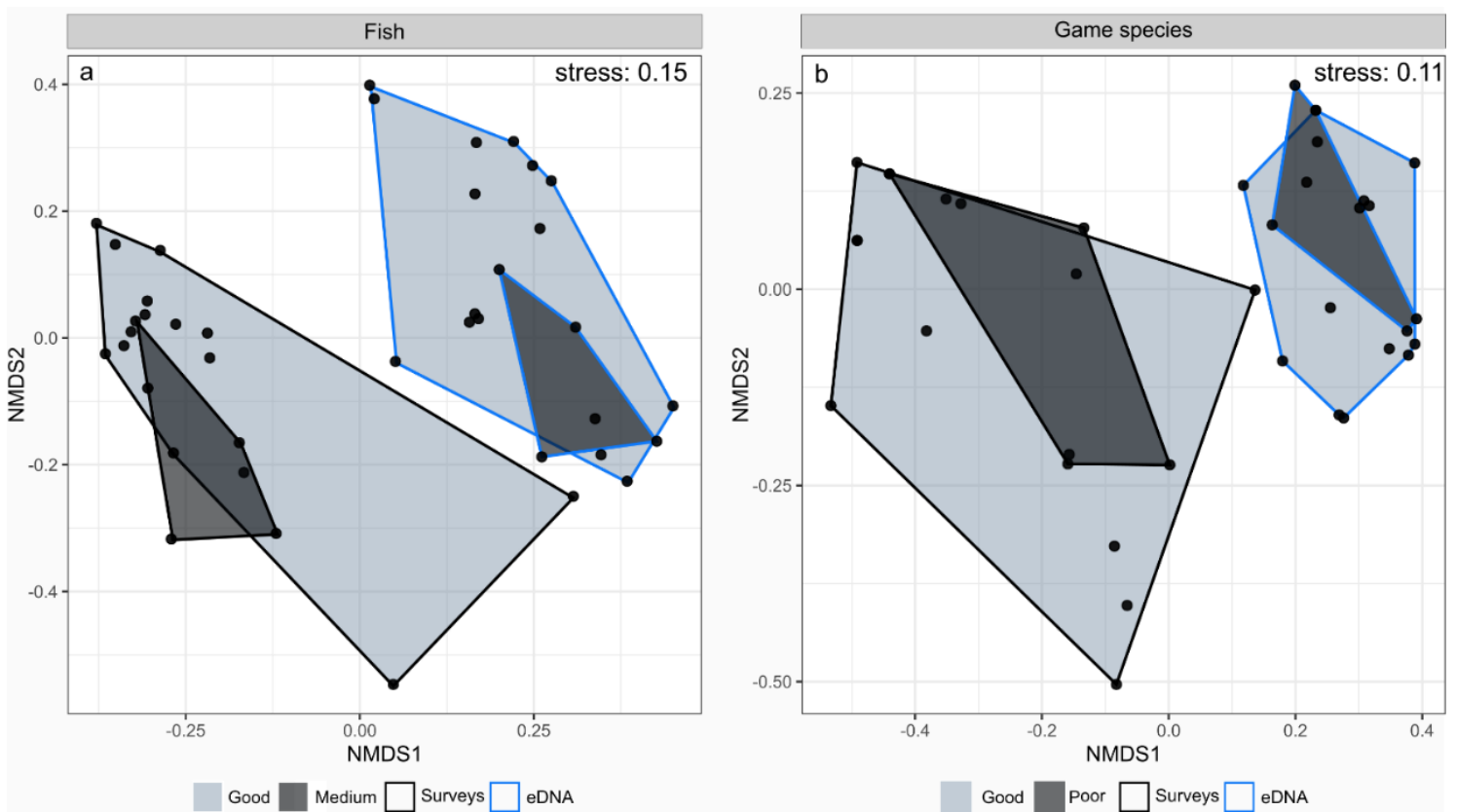


Figure 4. Non-metric multidimensional scaling (NMDS) based on the β_{rc} indice displaying site compositions in fish and game species. Only the species cited in the surveys were considered.

For fish, the ProTest analysis showed non-significant concordance in site species composition between eDNA and survey sites (NMDS axes 1–2, $m^2 = 0.91$, $p = 0.19$, Figure S1). Similarly, there was no significant concordance in site species composition between eDNA and survey sites for game species (NMDS axes 1–3, $m^2 = 0.86$, $p = 0.25$, Figure S1).

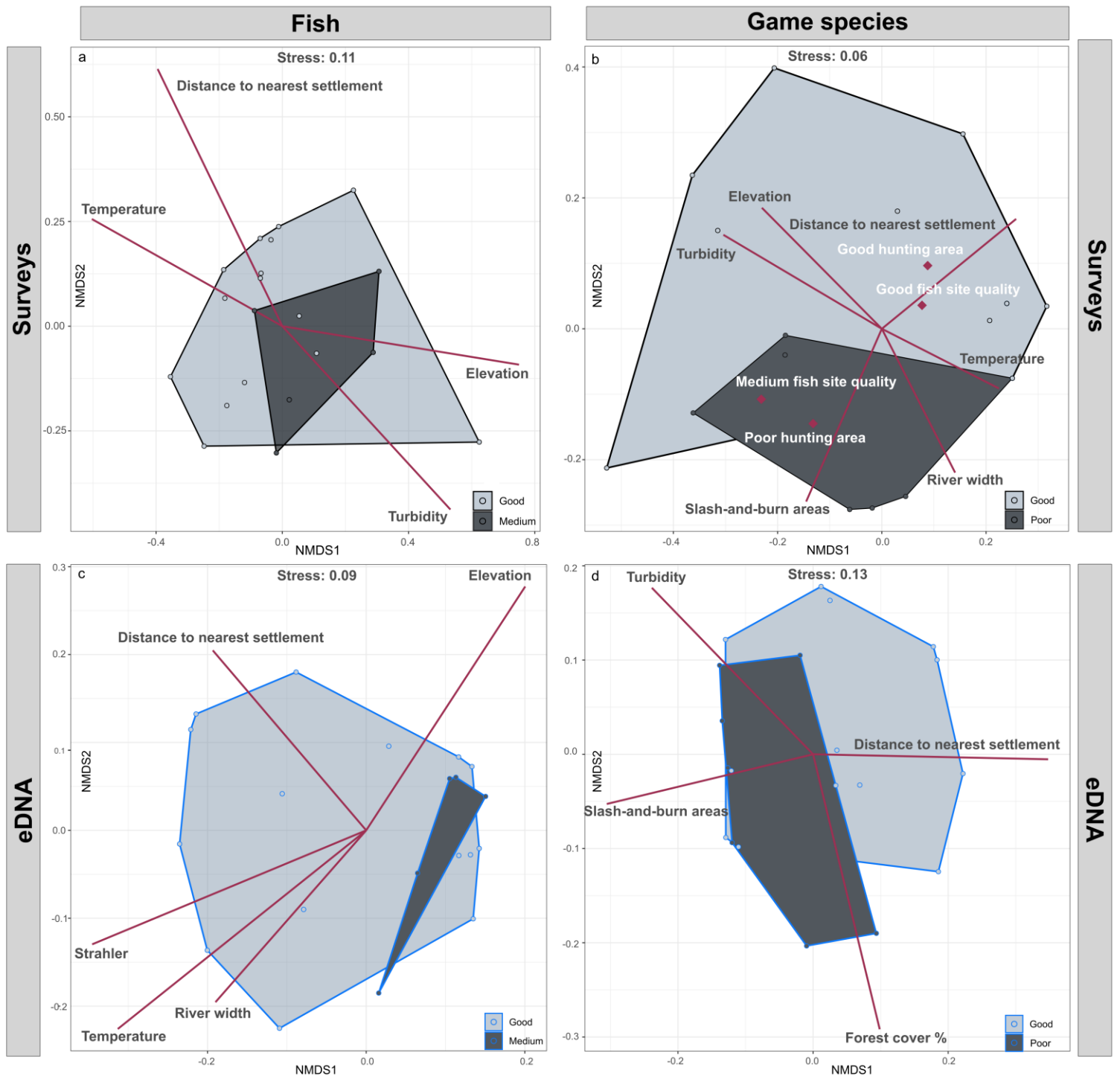


Figure 5. NMDS ordinations of fish and game species site composition based on the β_{rc} indice for eDNA and social surveys. Only the species cited in the surveys were considered and sites were clustered based on site quality for fish and for game species. Significant correlated continuous (red lines) and categorical (diamond symbols) environmental variables are displayed onto the ordinations.

For fish and for the social surveys, the elevation and the distance to the nearest settlement variables were the most strongly correlated to the ordination ($r^2 = 0.48$ and 0.45 respectively). The turbidity and the temperature were the least correlated to the ordination ($r^2 = 0.39$ and 0.36 respectively; Figure 5a & Supplementary Table 3). By contrast with eDNA, the two most strongly correlated variables were the temperature and the Strahler order ($r^2 = 0.65$ and 0.59 respectively). The Elevation variable showed a slightly weaker correlation ($r^2 = 0.51$). The distance to the nearest settlement and the river width had a similar correlation coefficient ($r^2 = 0.35$ and 0.32 respectively, Figure 5c & Supplementary Table 3). All these variables were associated to the cluster of “good” sites for fish on the eDNA ordination.

For game species and the social surveys, the turbidity, the distance to nearest settlement, the presence of slash-and-burn agriculture and the elevation correlated the strongest the ordination ($r^2 = 0.69, 0.58, 0.55$ and 0.53 , respectively). Beside the presence of slash-and-burn agriculture, all of these variables were correlated to the cluster of sites considered as “good” sites for game species. Interestingly, the categorical variables describing the site quality for fish and the site quality for hunting derived from the surveys also correlated the ordination ($r^2 = 0.23$ and 0.26 respectively). Within these categories, the medium sites for fish and the poor sites for hunting variables overlapped the cluster of sites considered as “poor” in game species. Likewise, the variables good sites for fish and good sites for hunting overlapped the cluster of sites considered as “good” sites for game species. Slash-and-burn agriculture ($r^2 = 0.54$) and river width variables ($r^2 = 0.41$) also correlated the cluster of sites considered as “good” sites for game species. Finally, the temperature correlated with both site category clusters ($r^2 = 0.35$, Figure 5b & Supplementary Table 3). Contrastingly with eDNA, the distance to the nearest settlement was the most correlated to the ordination ($r^2 = 0.43$) and was associated to the cluster of “good” sites for game species. The turbidity, the slash-and-burn agriculture and the percentage of forest cover had a similar correlation coefficient (r^2 ranging from 0.31 to 0.34) and were rather associated to the cluster of “poor” sites in game species (Figure 5d & Supplementary Table 3).

Discussion

Comparing social perceptions from surveys to scientific measures of biodiversity evidences similarities and dissimilarities in the way biodiversity is considered. On the one hand, similar perceptions to scientific measures of biodiversity indicate that Amerindians perceive biodiversity distribution regardless of their use of it. On the other hand, dissimilarities between

both approaches may suggest that Amerindians also perceive biodiversity based on their nature instrumental values and therefore, their use of it.

Considering only the species mentioned in the surveys, the site species richness was systematically higher for both fish and game species with eDNA. In addition, site composition in fish and game species between both approaches was also consistently significantly different. These dissimilarities in species richness and composition suggest that social perceptions of biodiversity does not reflect that measured with eDNA. Nevertheless, such discrepancies do not reflect a lack of knowledge from Amerindians, who rightly identified the distribution range of the species. For instance, Amerindians consider that *Tometes trilobatus*, *Myleus rhomboidalis* and *Boulangerella cuvieri* never occur upstream from the Mutaquere confluence, which is consistent with the scientific knowledge on the distribution of these species. Similarly, local people rightly distinguished between small stream and large river species even when the species belong to the same family. In addition, fish and game species did not significantly differ in occurrence percentages between both surveys and eDNA. These reveal that overall, fish and game species commonness and rarity are similarly estimated by Amerindians and eDNA over the entire study area. Nevertheless, results may change if species are grouped into relevant categories, more representative of the different values that Amerindians attribute to species according to their need.

Sites were classified as “good” or “medium” site for fish. However, there was no significant difference of site species richness between “good” and “medium” sites for surveys and for eDNA. The results were similar for game species, except for site species richness retrieved with surveys that was significantly higher in “good” than in “poor” sites. This lack of significant difference within this classification suggests that this site classification may not be defined only based on the quantity of species encountered in the sites. In addition, with the surveys, the NMDS showed that the sites classified as “medium” and “poor” presented a smaller variability in species composition and were inhabited by a subset of the species composing the “good” sites. Although eDNA site composition is different to that of surveys, a similar trend can be observed. In this way, species diversity may be an important criterion to site classification described by Amerindians. In fact, it may be influenced by other criteria, such as species abundance or species values for native population. For instance, studies reported that primates such as spider monkeys were of high values for Amerindians whereas it is rather an elusive species (de Thoisy et al., 2005; Richard-Hansen et al., 2019). In fact, social perception of biodiversity in a context of subsistence hunting may reflect foraging strategies. In this way, the species reported at sites may represent the most optimal choice of species to hunt or fish

considering the trade-off between different parameters such as species probability of encounter, costs of trips or protein uptake (Levi et al., 2011).

For fish, the significant contribution of elevation and water temperature variables to explain the site composition suggests that Amerindians perceive differences in fish assemblages with the variations of these variables. These latter variables are known to be important drivers of fish diversity distribution, correlating with the upstream-downstream increase in river size and discriminating stream and river fauna (González-Trujillo et al., 2021). Moreover, the significant correlation of the distance to the nearest settlement demonstrates that Amerindians also perceive a difference in fish assemblages depending on the distance to the villages. On the Maroni River, Amerindians recognised that the fishing pressure from subsistence hunting is detrimental to the fish resources, especially with the use of “western” fishing methods such as nets that are less selective than traditional fishing methods (Longin et al., 2021). The fishing pressure may be higher around villages because fishing trips far from villages require having a boat and involve different costs. This may explain why this variable is also an important driver of fish diversity with eDNA inventories. For instance, it was mentioned that the remote sites O3, O6, O8 and O13 are or were sites used for plant-based fish poisoning, a traditional fishing method that is practiced only occasionally, but that aims at collecting many fishes at the same time. It is usually practiced far from villages where the resources are more abundant (Longin et al., 2021). Finally, the significant influence of water turbidity on the fish assemblages indicates that Amerindians are well aware of how fish biodiversity is modified depending on water turbidity and associated mining activities. Nevertheless, this is no surprise and only an additional evidence that these populations have long been exposed to illegal gold mining activities and associated environmental alterations. These results were already reported in a sociological study on the Maroni river (the border between French Guiana and Suriname) where Amerindians fisher’s first three major concerns were “Gold mining”, “Water turbidity” and, “Fish health” (Longin et al., 2021).

For the site composition in fish obtained with eDNA, beside the distance to the nearest settlement, the other influential variables were mostly geographical and environmental. The temperature, the Strahler order, the elevation, and the river width are variables recognised to influence freshwater species distribution within a river basin (Carvajal-Quintero et al., 2019).

For game species with the social surveys, the “poor” sites were not totally nested in the “good” sites. Surprisingly, the water turbidity was the most strongly correlated variable to game species assemblages. It shows that Amerindians also perceive a change in game species with variations in water turbidity. However, it may also be because water turbidity indicates a

proximal activity of illegal gold miners that also involves deforestation and poaching (de Thoisy et al., 2016; Hammond et al., 2007). Other anthropic variables were associated to the ordination, such as the presence of slash-and-burn areas within a radius of 1 km. This could translate the impacts on site composition in game species because the vegetation is first cleared and then changed, but also because subsistence hunting occur in these areas (Richard-Hansen et al., 2019). The site classification based on fish correlated the ordination and matched the game species site classification. Sites considered as good for fish were “good” sites with many game species, and inversely. The same trend was also verified for the classification of sites for hunting. Overall, it suggests that sites described as harbouring many game species are also considered as good hunting areas but also good fishing areas. As for fish, the distance to the nearest settlement also influenced the perceived site composition in game species. Besides anthropic variables, environmental variables such as water temperature, elevation, and river width also correlated the ordination. Interestingly, this shows that for Amerindians, game species diversity is associated to fish species diversity, both influenced, in part, by aquatic environmental variables. This association could stem from hunting practices as hunting areas are often reached by boat and the quality of a site for hunting could be estimated by water quality and river morphology. Both can indicate the presence of illegal gold mines settled on small streams, altering water chemical parameters (Cantera, Coutant, et al., 2022; Hammond et al., 2007). Moreover, river morphology may also condition the forest access and thus the hunting frequency, which in turn affects the site composition in game species (Richard-Hansen et al., 2019).

Site composition in game species retrieved by eDNA was not correlated with aquatic variables except water turbidity. It varied instead with terrestrial and variables related to anthropisation such as the presence of slash-and-burn agriculture, the distance to the nearest settlement and the upstream percentage of forest cover. Contrary to fish, site composition in game species was therefore more influenced by anthropic activities. This is consistent with a recent study highlighting the effects of deforestation on fish and mammals. Both taxonomic groups are severely impacted, but mammals declines were in average higher than fish declines (Cantera, Coutant, et al., 2022).

Similar socioecological approaches were already conducted on the Maroni river to document fisher’s perception of resources (Longin et al., 2021) and over the entire territory to describe hunting practices and trends (Richard-Hansen et al., 2019). Nevertheless, combining social surveys with eDNA biodiversity inventories allowed integrating multiple taxa and depicting the complex interactions between them and the environment. This comparative

analysis showed that at the species level, native Amerindian populations have accurate knowledge about species distributions, as already evidenced in several studies (Braga-Pereira et al., 2022; Camino et al., 2020; Zayonc & Coomes, 2022). Nevertheless, the differences observed in site species richness and composition between both approaches demonstrates that native populations have a different perception of biodiversity distribution that reflects their subsistence economy and resources requirements. Importantly, the study also shows the awareness of Amerindians regarding human impacts on biodiversity. The correlation between anthropic variables and the perceived variations in fish and game species diversity well illustrates their expositions to anthropic activities such as illegal gold mining. It also shows their awareness about biodiversity modifications associated to subsistence living, generated by the adoption of western fishing and hunting practices (Longin et al., 2021; Tritsch et al., 2011).

These results are concerning because the Oyapock river considered in this study is much less affected by gold mining than the Maroni river, yet the impacts are already sufficient to influence the biodiversity perceptions of indigenous populations.

Conclusion

Integrating social science into conservation studies using comparative analyses provides a vision of the human dimension of conservation (Bennett et al., 2017). Here, Amerindians perceptions of biodiversity converged with scientific measures of biodiversity regarding anthropogenic disturbances. In addition, this comparative analysis showed how their perceptions of biodiversity is conditioned by their resource needs. Based on the results, conserving biodiversity for native populations requires the maintenance of species abundances and diversity, more than species richness. Moreover, conserving biodiversity also involves the preservation of aquatic ecosystems, as their perception of game species diversity is influenced by the fish diversity and the state of rivers. Considering these aspects into conservation policies would be beneficial to match different social contexts and would help contribute to more socially equitable conservation processes.

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Author contribution

O.C., D.D, S.B. and J.M. conceived the ideas and designed methodology; O.C., S.B., J.M. and D.D. collected the data; A.V. and T.D. supervised the laboratory work and conducted the bioinformatic analyses; O.C. analysed the data; O.C. led the writing of the manuscript. All authors contributed critically to the manuscript.

Supplementary Materials

Supplementary Table 1. Social surveys.

A - Description du site								
	Ecologique				Téko			
Unité paysagère								
Morphologie du fleuve								
Météo (pendant enquête)								
Profondeur								
Fruitaison (alentour)								
Critère bonne zone de pêche								

B - Pêche								
B.1 - Fréquence et méthode de pêche								
Nombre de pirogues	Beaucoup	Moyen	Peu	Aucune	Autre:			
Méthodes de pêche	Nivrée	Plongée	Epervier	Tramail	Hameçon	Trap de nuit	Ligne de fond	Autre:

B.2 - Espèces symboliques pêchées						
Nom Teko	Nom commun	Quantité				Usage
	Aimara	Beaucoup	Moyen	Peu	Aucun	
	Pacu	Beaucoup	Moyen	Peu	Aucun	
	Coumarou	Beaucoup	Moyen	Peu	Aucun	

B.3 - Quoi d'autre ici ?							
Classement des observations	Nom Teko	Nom commun	Quantité				Usage
			Beaucoup	Moyen	Peu	Aucun	
			Beaucoup	Moyen	Peu	Aucun	
			Beaucoup	Moyen	Peu	Aucun	
			Beaucoup	Moyen	Peu	Aucun	

Nb espèces bonnes a manger	Toutes	Certaines	Pas beaucoup	Rien	Autre:			
Nb espèces pas mangée	Toutes	Certaines	Pas beaucoup	Rien	Autre:			

B.4 - Quels poissons ont disparu ?					
Classement des observations	Nom Teko	Nom commun	Pourquoi ?		Usage

C - Chasse					
C.1 - Fréquence et méthode de chasse					
Bon coin de chasse	Oui	Non	Moyen	Autre:	
Nombre de pirogues	Beaucoup	Moyen	Peu	Aucune	Autre:
Méthode de chasse	Fleuve	Forêt	Affut	Fusils	Autre:
Quand	Jour	Nuit	ombée de la nu	Levée du jour	Autre:
Fréquence	Jour	Semaine	Mois	An	Autre:

C.2 - Espèces chassées							
Classement des observations	Nom Teko	Nom commun	Quantité				Usage
			Beaucoup	Moyen	Peu	Aucun	
		Caiman	Beaucoup	Moyen	Peu	Aucun	
		Iguane	Beaucoup	Moyen	Peu	Aucun	
		Tapir	Beaucoup	Moyen	Peu	Aucun	
		Biche	Beaucoup	Moyen	Peu	Aucun	

C.3 - Qu'est-ce qui a disparu?					
Classement des observations	Nom Teko	Nom commun	Pourquoi		Usage

C.4 - Qu'est ce qu'on peut voir ? (chien bois/félin)					
Classement des observations	Nom Teko	Nom commun	Pourquoi		Usage

D - Orpillage					
Nombre de pirogues	Beaucoup	Moyen	Peu	Aucune	Autre:
Intensité	Beaucoup	Moyen	Peu	Aucune	Autre:
Fréquence	Jour	Semaine	Mois	An	Autre:
Qualité de l'eau	Très sale	Sale	Pas buvable	On peut boire	Autre:
Orpillage depuis quand	Longtemps	Pas longtemps	Maintenant	Autre:	
Tendance	Augmentation	Diminution	Stable	Autre:	

E - Abbatis	
Nombre	
Distance au dernier abbati	
A qui appartient l'abbati	

Supplementary Table 2. Description of the environmental variables fitted onto the eDNA and survey site ordination for fish and game species

Variable	Description
Tributary or main channel	Affluent or main channel
Site disturbance	Either classified as gold mining, ancient gold mining or reference (without disturbances)
Elevation	Site elevation in meters
Conductivity	Site conductivity in μS (field measure)
Temperature	Site temperature in $^{\circ}\text{C}$ (field measure)
pH	Site pH (field measure)
Turbidity	Site turbidity (field measure)
O ² Saturation	Site water saturation in O ² in % (field measure)
O ²	Site concentration of dissolved O ² in mg/L (field measure)
Strahler	Strahler order ranging from 1 to 8
Distance to mouth	Site distance to the estuary
River width	Site river width (between banks) in meters
Landscape type	Between upstream rapids, downstream rapids, upstream affluent and without rapid
Fish site quality	Classification derived from the surveys, either good or medium site for fishing
Game species site quality	Classification derived from the surveys, either good or poor site for game species
Hunting site quality	Classification derived from the surveys, either good or poor site for hunting
Water quality	Classification derived from the surveys, either drinkable, clear but not drinkable, not drinkable
Slash-and-burn areas	Presence of slash-and-burn areas near the sites (< 1km away), either 1 for presence, 0 for absence
Forest cover %	Upstream site forest cover in % (over a surface of 30 km from sampling sites, see Cantera et al. (2022))
Gold mining %	Upstream deforestation due to gold mining in % (over a surface of 30 km from sampling sites, see Cantera et al. (2022))
Deforestation %	Upstream deforestation in % (over a surface of 30 km from sampling sites, see Cantera et al. (2022))
Distance to nearest settlement	Distance to the nearest settlement in km calculated along the hydrographic network (either Camopi or Trois-Sauts)

Supplementary Table 3. NMDS scores and correlations (r^2) of environmental variables with the NMDS ordinations of surveys and eDNA sites and the significance of the correlation based on envfit function (9999 permutations).

Variables	NMDS1	NMDS2	R ²	p.values
<i>Fish social surveys</i>				
Elevation	0.99259	-0.12155	0.4782	0.0034**
Temperature	-0.92072	0.39022	0.3599	0.0240*
Turbidity	0.77246	-0.63506	0.3981	0.0151*
Distance to nearest settlement	-0.54103	0.84100	0.4467	0.0057**
<i>Game species social surveys</i>				
Elevation	-0.77757	0.62880	0.5269	0.0020**
Temperature	0.92999	-0.36759	0.3516	0.0245*
Turbidity	-0.89981	0.43629	0.6869	0.0001***
River width	0.53487	-0.84493	0.4101	0.0109*
Slash-and-burn areas	-0.50442	-0.86346	0.5465	0.0018**
Distance to nearest settlement	0.85375	0.52068	0.5816	0.0010*
Good fish site quality	0.0764	0.0366	0.2259	0.0078*
Medium fish site quality	-0.2291	-0.1097	0.2259	0.0078*
Good hunting area	0.0880	0.0954	0.2655	0.0054*
Poor hunting area	-0.1320	-0.1432	0.2655	0.0054*
<i>Fish eDNA</i>				
Elevation	0.58574	0.81050	0.5116	0.0032**
Temperature	-0.81097	-0.58509	0.6513	0.0002 ***
Strahler	-0.93625	-0.35133	0.5946	0.0004***
River width	-0.69713	-0.71694	0.3245	0.0330*
Distance to nearest settlement	-0.68669	0.72695	0.3464	0.0270*
<i>Game species eDNA</i>				
Turbidity	-0.80376	0.59495	0.3142	0.0395*
Slash-and-burn areas	-0.98541	-0.17017	0.3422	0.0332*
Distance to nearest settlement	0.99988	-0.01530	0.4316	0.0101*
Foret cover %	0.32031	-0.94731	0.3381	0.0333*

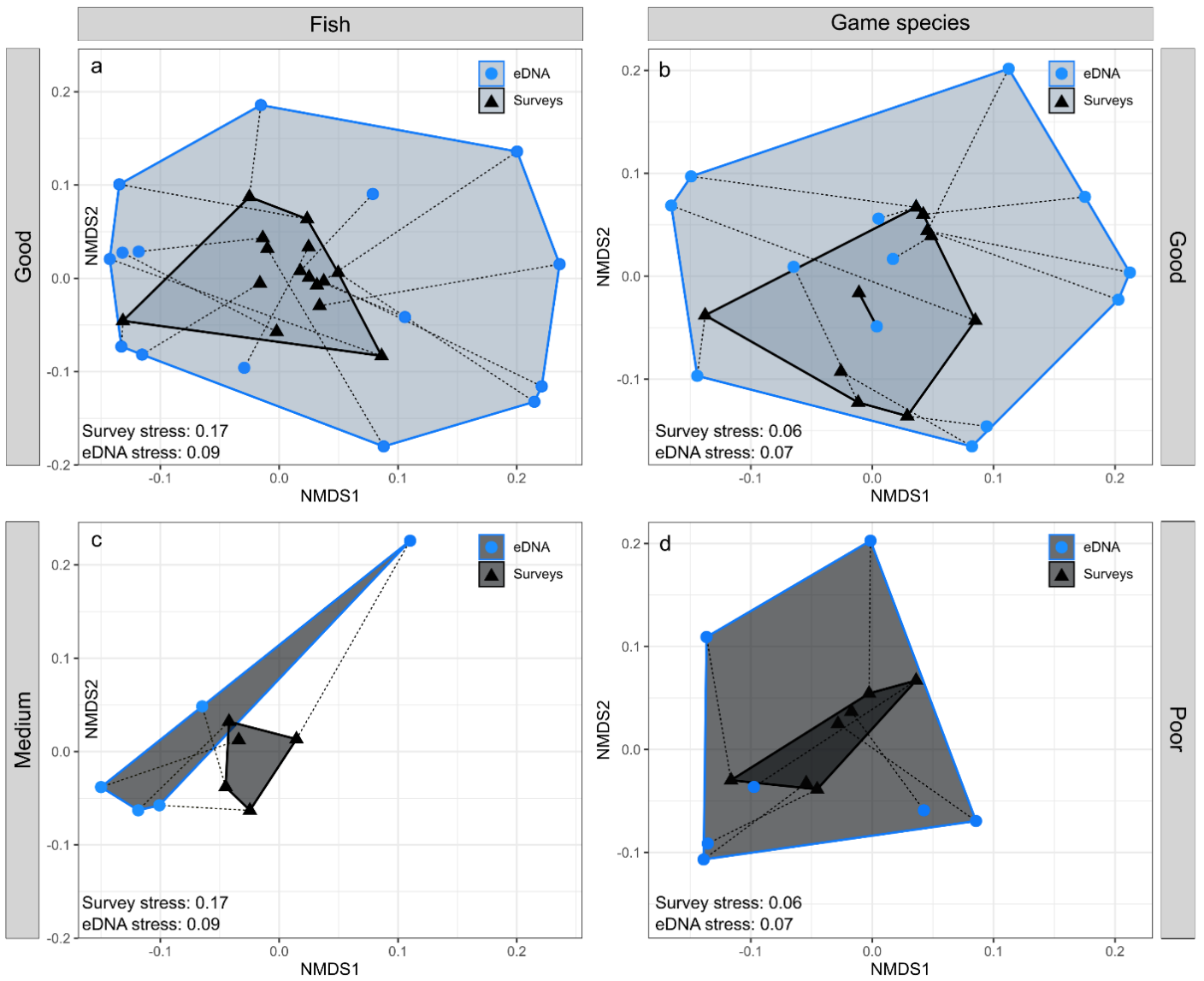


Figure S1. Procrustes analyses of eDNA and survey ordinations based on fish and game species site composition. The sites were categorised as “good” or “medium” site for fish and “good” or “poor” sites for game species. For visual clarity, “good” and “medium/poor” ordinations are displayed in different quadrants.

General Discussion

Freshwaters are being modified by anthropogenic activities at a critical pace, threatening the unique biodiversity they host, especially in tropical regions (Albert et al., 2021; Albert & Reis, 2011; Dudgeon, 2019; Reid et al., 2019). In this PhD, we attempted to highlight the mechanisms by which anthropic pressures alter ecosystems, but also to quantify the resulting biodiversity changes at both local and regional scales in French Guianese rivers. We also introduce a novel approach in which humans' perceptions of biodiversity are also integrated in our assessment of biodiversity alterations.

In this general discussion, I discuss the relevance of our methodological advancements with eDNA metabarcoding to unlock regional and cross-taxa biodiversity assessments and address some of the remaining limits of the method. I also discuss our ecological findings, providing a complete picture of the assembly rules shaping fish biodiversity and how anthropogenic pressures modify these assembly rules.

1. Optimising and extending the application of environmental DNA

Environmental DNA coupled to metabarcoding approach is increasingly used and have been applied to inventory all branches of life, from a variety of matrices (water, soil, air). The collection of eDNA from water is the most widespread application of the method, and fish is the most investigated taxon (Tsuji et al., 2019). The success of the method in water ecosystems probably stems from the limitations of conventional methods used for fish monitoring. Capture- and observation-based methods such as electrofishing, nets and traps or underwater visual and video surveys provide relevant information about fish repartition, but they are labour-intensive, species-selective, scale-constrained and can be destructive to the fauna and habitats (Yao et al., 2022). In aquatic ecosystems, aquatic eDNA metabarcoding have showed higher detection sensitivity, finer spatial resolution and superior cost-efficiency advantages than capture or observation-based methods (Fediajevaite et al., 2021; Rees et al., 2014; Taberlet et al., 2018; Yao et al., 2022). eDNA presents, therefore, a great potential to access detailed information about fish distribution. In French Guiana, the development of eDNA-based monitoring is unlocking fish biodiversity studies that were up to now limited by the biases and constraints of capture and observational methods. For fish, it is particularly relevant because the only reliable conventional method is net sampling, the low water conductivity hampering the use of electrofishing.

The rapid methodological advances in aquatic eDNA metabarcoding result in a continuous improvement and optimisation of the protocols. Protocol comparisons are therefore necessary to avoid the obsolescence of datasets. Many studies compare eDNA protocols in the literature (Deiner et al., 2018; Majaneva et al., 2018). Nevertheless, they do not compare the ecological interpretation of eDNA data, but rather synthetic estimates of raw data such as eDNA concentrations or read number. In this case, they do not aim at comparing protocols to keep valorise old datasets but rather to evaluate protocol performances. The first part of chapter I justifies the need to investigate the influence of protocol differences on ecological results. Both evaluations are necessary because ecological outputs can be decoupled from synthetic estimates of raw data. For instance, one protocol may be more sensitive and provides a greater DNA concentration or read numbers compared to another, but the number of detected species may remain the same. This first chapter also demonstrates the importance of the material used to collect aquatic eDNA. In the literature, filter clogging is often reported as a constraint to the filtration of large volumes of water (Hunter et al., 2019; Spens et al., 2017; Takahara et al., 2012; Thomas et al., 2018; Turner et al., 2014). In all these cases, researchers used disk membranes or Sterivex filters possessing a small surface of filtration and allowing the filtration of 50 mL to 12 L. Clogging issues are problematic because they may critically limit the volume of filtration and lead to incomplete inventories. For instance, in our study system, reaching the optimal sampling effort requires the filtration of 64L of water (Cantera et al., 2019). In our case, clogging issues occur but do not represent a constraining factor and this is possibly due to the large surface of filtration that possess our filters (500 cm² to 1300 cm²). In species-rich environment, we therefore recommend the use of filters with large membranes. Overall, the first part of Chapter I evidences the robustness and the replicability of our aquatic eDNA metabarcoding method and therefore ensure the reliability of our inventories, which is crucial to initiate ecological studies on fish and long-term temporal follow-ups of biodiversity.

Besides being intensively used to monitor fishes, aquatic eDNA collects information on various taxa. Proofs of concept to detect non-aquatic species from aquatic eDNA has been multiplied these past three years (Broadhurst et al., 2021; Lozano Mojica & Caballero, 2021; Lyet et al., 2021; Macher et al., 2021; Polanco F. et al., 2021; Saenz-Agudelo et al., 2022; Sales, Kaizer, et al., 2020; Sales, McKenzie, et al., 2020). These researches demonstrate that eDNA from non-aquatic vertebrates can be efficiently collected from water in rivers, coastal wetlands or estuaries. However, there is a consensus on the lack of replicability and robustness of this application that could be solved by adapting protocols, particularly the sampling efforts

(Macher et al., 2021) and the type and number of primers used. Nevertheless, the congruence between eDNA detections of non-aquatic vertebrates and traditional methods indicate that it can offer sufficient information to solve many conservation problems (Coutant et al., 2021; Lyet et al., 2021; Polanco F. et al., 2021; Sales, Kaizer, et al., 2020). The second part of Chapter I contributes to this recent literature and highlights the potential of aquatic eDNA metabarcoding to collect relevant information of species distribution for different taxa, simultaneously. Although some species remain consistently better detected by traditional line-transect observations, it provided information on mammals in areas that are difficult to access by means of rapid water filtrations (30 minutes per site). In comparison, conducting line-transects in remote areas requires the creation of trails and several days of surveys with experts in the taxonomic groups of interest. In our study system, this study opened new avenues to lead multi-taxa ecological studies such as the study presented in the first part of Chapter 2.

One particular aspect that conditions the detectability of species and therefore the relevance of eDNA-based inventories, is the “ecology” of eDNA, including eDNA production, eDNA transport and eDNA degradation (Pawlowski et al., 2020). eDNA production is the shedding of DNA into the environment and is largely dependent on the taxonomic groups, species abundances and densities and, their biological and physiological features (Pawlowski et al., 2020). In consequence, some taxa are known to release larger quantities of DNA to the environment than others, making quantitative interpretation of eDNA data complicated (Pawlowski et al., 2020). In this PhD, we showed that mammals, be they in contact or not with water, also release large quantities of DNA into freshwater environments. In another hand, the application of aquatic eDNA in lotic environment such as rivers is the most widespread (Yao et al., 2022), but the transportation and the degradation of eDNA in the water are pivotal parameters that can bias ecological interpretations. DNA transport mainly results from hydraulic properties of the running waterbodies such as water discharge and topography (Jane et al., 2015; Pawlowski et al., 2020). In addition to eDNA transport, the travelling distance of DNA also depends on its degradation rate conditioned by local environmental parameters such as pH, temperature or solar radiation (Barnes & Turner, 2016). Both eDNA transportation and degradation determined the spatial scale at which biodiversity information is integrated. A wide range of eDNA distances of detection is reported in the literature (from less than 50 m up to 130 km) (Civade et al., 2016; Deiner et al., 2016; Laramie et al., 2015; Pont et al., 2018). For instance, Van Driessche et al., (2022) evaluated the effects of river discharge and source biomass effects on the downstream dispersal of DNA, using a longitudinal cage study in two

temperate river sections. They evidenced a reduction of eDNA concentrations 2 km downstream from caged fish, strengthened by a dilution effect. With a higher discharge rate, however, they found higher species detection probabilities at increasing distance from the cages. This study illustrates the complex interactions between eDNA sources and water-body specific characteristic, such as source biomass and water discharge, that condition eDNA dispersal. In our study system, using a community-level approach and, comparing fish community distance decay of similarity between eDNA and capture-based inventories, Cantera, Decotte, et al., (2022) demonstrated that the travelling distance of eDNA in the water is short enough to provide local information about fish distribution.

In conclusion, eDNA metabarcoding is a tool with a great potential as it offers the opportunity to realised cross-taxa and regional monitorings of biodiversity, while being less labour-intensive and time-consuming than traditional inventory methods. These features are particularly interesting in species-rich ecosystems and remote areas that are difficult to access. Nevertheless, the relevance of the inventories is conditioned by many parameters, some of them resulting from the “ecology” of eDNA, that need to be investigated to avoid biased ecological interpretations of eDNA data. Because these parameters vary depending on the studied systems but also with technological advancements, a wide variety of protocols have been developed throughout the scientific communities. In consequence, comparing the trends of biodiversity worldwide necessitates the implementation of studies that compare raw eDNA data but also the ecological interpretations of the eDNA data.

2. Investigating biodiversity distribution patterns at local and regional scales to assess anthropogenic impacts on biodiversity

Besides presenting a good state of preservation and therefore a high conservation value, French Guiana is also an excellent natural laboratory for investigating the impacts of human activities on biodiversity. It is small enough to have regional knowledge of biodiversity distribution, but at the same time, faces a similar context of anthropisation than the rest of the Amazonian region, although at a much weaker level. The contribution of aquatic eDNA allowed, for the first time, a thorough investigation of human impacts estimated by deforestation, on multiple taxa and at both local and regional scales in this territory.

In the first part of Chapter II, we revealed the high vulnerability of freshwater ecosystems and the biodiversity it shelters. We show how low levels of anthropisation are responsible for drastic declines of fish and mammal biodiversity. Deforestation generates a

variety of direct but also indirect impacts. Among the indirect impacts, it has been documented that in deforested areas, the deposition of large woody debris during the rainy season decreases. This causes a loss of spatial heterogeneity and habitats and ultimately, a loss of species abundances and richness (Sweeney et al., 2004; Wright & Flecker, 2004). Deforestation can also decrease the flux of organic matters such as dissolved organic carbon from terrestrial land to water, affecting detritivorous species and consequently the entire trophic network (Sweeney et al., 2004). In addition, deforestation can generate opposite impacts, but at different temporal and spatial scales. Over a short timescale, deforestation and associated land uses, such as gold mining, reduce the amount of light penetrating the water through a temporary increase of water turbidity, particularly downstream from the deforested areas. This affects photosynthetic activities and, therefore, the production of dissolved oxygen. On the other hand, it also affects species fitness as the visibility of aquatic species essential for foraging, reproduction, eggs and larvae developments decreases in turbid waters (Boyd, 2015; Dedieu et al., 2015; Sweeney et al., 2004). Over a longer timescale, the remnant impacts of deforestation are characterised by an increase in the amount of light penetrating the water with the removal of the riparian vegetation. This will particularly occur at the deforested localities and favour photosynthetic activities and primary productivity, which can, in turn, give rise to water eutrophication and therefore generates a risk of anoxia for freshwater species (Larson et al., 2019; Sweeney et al., 2004). These impacts vary spatially, as the river course of the deforested localities will receive more light than downstream areas not deforested. Be they direct or indirect, these impacts reverberate far from the deforested localities via the downstream transfer of anthropic disturbances, mediated by the river hydrologic connectivity (Anderson et al., 2018; Leal et al., 2016; Leitão et al., 2018; Lindberg et al., 2011; McCluney et al., 2014; McManamay et al., 2017; Pringle, 2001). This spread of human disturbances is even recognized as an important driver of biogeochemical cycles and aquatic biodiversity modifications at local but also global scales (Lawrence & Vandecar, 2015; McCluney et al., 2014). In addition, we demonstrated that declines of local terrestrial biodiversity were also associated to remote deforestation impacts, evidencing the lateral connectivity between freshwater and terrestrial ecosystems (Cantera, Coutant, et al., 2022; Leal et al., 2020; Pringle, 2001). This underlines the multifaceted link between forest and freshwater ecosystems, often less considered in the literature when investigating anthropic impacts (but see (Leal et al., 2020)). Terrestrial and freshwater ecosystems exchange flows of water, energy, organic and inorganic materials (Junk et al., 1989; Vannote et al., 1980) but the extent and the quantification of their relationship remained poorly understood in the tropics (Lo et al., 2021). Importantly, we also demonstrated that with the river

hydrologic connectivity, defining the spatial scale at which human disturbances operates is crucial. In our study system, the deforestation impact on freshwater and terrestrial biodiversity was under-estimated when only considering spatial extent under 30 km. These results thus encourage the determination of the most relevant spatial scale at which considering anthropogenic pressures and invite reconsidering studies that only account for the proximal and direct effects of deforestation on biodiversity, in the light of these findings.

Moving from a local (α -diversity) to a regional (β -diversity) analysis of biodiversity brought novel information about drivers of fish distribution and the underlying processes in French Guiana. We demonstrated that a high fish dispersal limitation resulting from a long isolation between basins (+20 000 years) had a major impact on fish taxonomic assemblages between the three largest rivers of the territory. Unexpectedly, we also found a dominant effect of dispersal limitation over environmental filtering within basins. Although dispersal limitation was already documented for small stream fish assemblages (Cilleros et al., 2016), river fish assemblages are usually expected to be shaped by mass effect dispersal, where high dispersal, in addition to environmental factors, alters species composition (Leboucher et al., 2020). In fact, the river spatial configuration in dendritic structure complicates the identification of the spatial processes shaping diversity. Contrary to two-dimensional landscapes such as forest or grassland, local dispersal is not random, and the distance and the direction of dispersal is dictated by the landscape structures and by physical flows (Altermatt, 2013). It thus affects the balance of dispersal-driven regional effects and local environmental factors, depending on the position in the riverine network. Due to the dispersal pathways, more organisms disperse from the headwaters to main channels, while relatively few organisms move toward the headwaters. Exchange among localities in headwaters is therefore low, but high in main channels. These differences in dispersal suggest that communities from headwaters are more shaped by environmental filtering while those of main channels are structured by mass effect (Altermatt, 2013; Brown & Swan, 2010). These predictions have been synthesized in the network position hypothesis (Schmera et al., 2018). In our study system, one possible explanation for the dominance of dispersal limitation in shaping assemblages within basins may be the regular presence of rapids that represent natural discontinuities and possibly dispersal barriers. These results are of particular importance because fragmentation in dendritic landscapes can be more deleterious to biodiversity than in two-dimensional networks (Fagan, 2002). In these latter landscapes, multiple paths of movement are possible between patches, whereas fragmentation in dendritic landscapes can totally isolate patches (Fagan, 2002). In this way, organisms'

emigration and immigration are hindered, as well as gene flow. Population composed of small fragmented patches can thus be particularly at risk. Ecological systems with naturally reduced connectivity may be, therefore, more sensitive to anthropic fragmentation because of the accumulation of both natural and anthropic fragmentation effects (Gauthier et al., 2021). Moreover, as rapids are more or less immersed depending on the season, dispersal limitation may present a seasonal dynamic inducing a temporal variability of metacommunity processes. This was evidenced in Gauthier et al., (2021), who demonstrated that dispersal-based metacommunity processes can vary with the wet-dry seasons. In their study system, the dry season was characterized by drying events responsible for temporary dispersal limitation. This could have important conservation implications for ecosystem assessment and management, as certain hydrologic phases could be more relevant to monitor than others (Gauthier et al., 2021). In a context of global changes, a decrease trend of water flow during the wet season could represent a persistent dispersal barrier isolating populations permanently, while an increase in the frequency of extreme climatic events could temporarily erase certain natural dispersal barriers.

In this chapter, we also showed that deforestation is an important driver shaping fish functional assemblage at the territory scale. This is concerning because French Guiana is the territory harbouring the highest forest cover percentage (98.6%) in the world (Food and Agriculture Organization of the United Nations, 2015). Although variations in both taxonomic and functional fish assemblages are similar, we showed that deforestation explained a highest percentage of functional variation in the assemblages. This suggests that deforestation modifies fish assemblages based on species attributes, favouring specific sets of traits and that these functional changes induces paired taxonomic changes (Jarzyna & Jetz, 2018; Villéger et al., 2010). In addition, by decomposing overall β -diversity, we showed that the turnover component of β -diversity in fish assemblages was the most strongly correlated to deforestation. It shows that the nestedness component of β -diversity blur the effects of deforestation on fish biodiversity. Deforestation, therefore, majoritarily modifies functional assemblages by selecting different sets of species than that of non-deforested areas. These results are convergent with that of the first part of this chapter, indicating paired losses of fish taxonomic and functional assemblages (Cantera, Coutant, et al., 2022). In both cases, it highlights functional and taxonomic homogenization at the deforested localities, a widely documented pattern of biodiversity modification at a regional scale (Olden et al., 2004). The findings of this chapter contrast that of Toussaint et al., (2016), suggesting that Neotropical freshwater fishes exhibit the highest functional diversity worldwide and therefore present low vulnerability given high

levels of functional redundancy. Nevertheless, in French Guiana, the species that are functionally redundant only represent a weak portion of the regional functional diversity, while the majority of the functional diversity is supported by few species (90% of the fish regional functional space is supported by 5% of species) (Su et al., 2019). For this reason, the loss of only a few species can critically decrease the functional diversity of assemblages, even if they present some level of functional redundancy. In addition, the loss of functionally unique species may affect other species as it can impair important species interactions and ecosystem processes (Vitule et al., 2017).

In conclusion, this chapter provides a comprehensive summary of the mechanisms and the drivers structuring freshwater biodiversity in French Guiana. It emphasizes the multidimensionality of biodiversity and the complexity of the mechanisms structuring biodiversity. Our results justify the necessity of a multifaceted approach of biodiversity, considering the different biodiversity facets and components, to properly identify the impacts of anthropisation on biodiversity. Importantly, it shows how low levels of anthropisation are modifying biodiversity distribution at local and regional scales.

3. Human responses to biodiversity modifications

Biological sciences provide the foundations for understanding species distribution and for identifying the most vulnerable species and ecosystems. Yet, there is still a disconnection between our biological knowledge and the success of conservation measures. Part of the reason is that conservation is about people as much as it is about species and ecosystems (Mascia et al., 2003). Social science knowledge brings information about the required use of nature by humans, whereas biology indicates the limits to a sustainable exploitation of nature (Mascia et al., 2003; Sandbrook et al., 2013). Despite extensive effort to join natural and social sciences around the question of conservation, the place of social research in conservation remains a source of misunderstanding, miscommunication and contention among researchers (Sandbrook et al., 2013). The important roles of indigenous and local populations have been increasingly recognised to guide conservations policies. It rooted in the observation that areas of high values for biodiversity conservation also exhibited a high cultural diversity (Fernández-Llamazares & Cabeza, 2018; Kohler & Brondizio, 2017). These efforts translated into different ways from the increase of land prospections for conservation, a boom in the establishment of protected areas, to the inclusion of local and indigenous knowledge in biodiversity assessments (Fernández-Llamazares & Cabeza, 2018; Kohler & Brondizio, 2017). An increasing body of the literature

is valuing Local Ecological Knowledge to improve biodiversity assessments, providing important information about species distributions (Braga-Pereira et al., 2022; Camino et al., 2020; Madsen et al., 2020; Vierros et al., 2020; Zayonc & Coomes, 2022). However, Local Ecological Knowledge can also reflect the instrumental use of nature by local populations (Longin et al., 2021; Richard-Hansen et al., 2019). In comparison to scientific measures of biodiversity, discrepancies between both approaches can indicate how local populations exploit the resources, but also inform the temporal and spatial dynamic of the resource exploitation in a context of global changes. This is of paramount importance to take into account the realities that face local populations, living between traditional cultures and modernizing world, and to better understand and integrate the concrete needs of indigenous populations into conservation policies (Kohler & Brondizio, 2017).

In this third chapter, following previous works conducted on the Maroni river (Longin et al., 2021) and all over the territory (Richard-Hansen et al., 2019), we sought to compare scientific measures of biodiversity retrieved by eDNA and Amerindian perceptions of biodiversity. Our initial planned study changed because we were not authorized to navigate on the Camopi river and this led to a major change in the study system. Our study was relocated on a section of the Oyapock river that is delimited by two inhabited sectors. Consequently, the river section is characterized by a less strong anthropogenic gradient than on the Camopi river because inhabitants regularly travel from one sector to another, hunting and fishing on the way. Still, we obtained informative results from the social surveys and the comparison with eDNA biodiversity inventories helped identify factors influencing biodiversity perceptions of Amerindians. Restricting the analysis to the species mentioned in the survey that are all species of interest to Amerindians (Richard-Hansen et al., 2019), we showed that species richness and species composition of the sites were systematically different from that of eDNA inventories. This suggests that when asked to describe biodiversity through free-listing surveys, Amerindians spontaneously provide information about the species that are the most worth to be fished or hunted at the sites and do not seek to perform complete inventories of the fauna. Yet, when asked distribution information about specific species, they shared accurate knowledge supporting that of eDNA. Unsurprisingly, this emphasizes the extent to which these indigenous populations rely on natural resources but also demonstrates that several factors influence their biodiversity perceptions, varying depending on the sites and the taxonomic groups. On the one hand, environmental factors such as water temperature, elevation and river width known to significantly determine species distribution, explained the variation in species composition mentioned at the different sites (Carvajal-Quintero et al., 2019). On the other hand, the influence

of human-related factors underlined how Amerindian are facing a context of resource depletion. The influence of water turbidity demonstrates that their resources are affected by gold mining and deforestation, while the influence of the distance to the nearest settlement and of slash-and-burn agriculture reveal that their subsistence economy contributes to affect the resource availability. This has already been evidenced in Longin et al., (2021) on the Maroni river, where the main concerns of indigenous fishers were illegal gold mining and the French westernisation of lifestyle, changing the fishing and hunting practices, such as the use of fishing nets (Longin et al., 2021). This is concerning because gold mining is much less developed in the Oyapock river than in the Maroni river (Cantera, Coutant, et al., 2022), yet it is already sufficient to severely impair biodiversity and by extent Amerindian's subsistence economy. The westernisation of lifestyle taking shape in the mid-1950s has also dramatically changed the mobility of Wayãpi and Teko Amerindians. They became more sedentary, leading to small processes of urbanisation and occasioning a gradient of pressure on resources around villages, while mobility had always played a central role in their natural resources management strategies in the past (Longin et al., 2021; Tritsch et al., 2015).

This work aimed at contributing to a better conciliation of biodiversity and indigenous population needs into conservation policies. It remains exploratory, and the methodology needs several improvements. First, the number of participants should be increased to improve the representativity of the biodiversity perceptions. In addition, we initially planned to collect semi-quantitative data on species abundances, but it did not work as expected, as participants would either answer using the terms “little” or “many”. One method to collect semi-quantitative data would be to use visual illustrations representing different quantities and ask the most appropriate quantity for each species, as experimented in Braga-Pereira et al., (2022).

4. Perspectives

1.a. Enhancing eDNA-based methods to foster ecological studies

One of the main limitations of our eDNA methodology to inventory biodiversity is the access to qualitative data (presence/absence) and semi-quantitative data (occurrences) only, and the lack of quantitative information (abundances) about species. Qualitative and quantitative data provide complementary information, with species presence/absence reflecting species distribution and species abundance revealing trend in population size (Howard et al., 2014). However, due to the highest economic cost and the labour-intensive work to collect abundance

data, often not representative of the true species abundances, presence/absence data or occurrences (the proportion of monitoring sites within a region where a species is present) are often used as a surrogate for population size or species abundance (Joseph et al., 2006; Mackenzie, 2005).

Despite the sequencing costs and the limits due to the completeness of reference database, eDNA has improved biodiversity monitoring worldwide thanks to simple field protocols requiring only little equipment and workforce. While the most reliable information on biodiversity it provides so far is species detection/non-detection extrapolated to species presence/absence, methodological improvements to derive quantitative data would not necessarily complicate field protocols. The stakes of obtaining quantitative data from eDNA biodiversity inventories are therefore high. The limitations first stems from the “ecology” of eDNA and the disconnection between species and the amount of eDNA they shed in the environment (Lamb et al., 2019; Pawlowski et al., 2020). In fact, the quantities of eDNA released vary depending on species morphological, physiological and behavioural characteristics (Thalinger, Rieder, et al., 2021). Second, the PCR procedure does not amplify the DNA proportionally to the initial DNA concentrations, and it will notably vary depending on primer affinity to target DNA fragments (Nichols et al., 2018).

At the level of the community, the relation between species abundances and quantities of DNA is not always straightforward (Lamb et al., 2019), despite many studies establishing this relation, where the relative read abundances is interpreted as an estimator of relative species biomass (Di Muri et al., 2020; Lacoursière-Roussel, Côté, et al., 2016; Takahara et al., 2012). However, identifying a phylogenetic or functional signal in the shedding rate of DNA could help to avoid the under or overestimation of certain species abundances or biomass (Thalinger, Rieder, et al., 2021). For instance, Van Driessche et al., (2022) have evidenced a differential DNA shedding rate between several fish species, with the larger roach (*Rutilus. rutilus*) releasing smaller amounts of DNA per gram body mass than the round gobies (*Neogobius. melanostomus*), four time smaller on average. On the other and, overcoming the limits related to DNA amplification could at least provide relative abundances of species (in contrast to absolute abundances) and therefore, a hierarchical abundance order between species (Pont et al., 2022). The relative number of reads could therefore be a good estimate of the relative abundance of species if the amplification efficiency is comparable for the different species (Pont et al., 2022). This can be achieved using quantitative PCR (qPCR) (Pont et al., 2022) but also by replacing the PCR amplifications by other molecular methods such as hybridization capture as it has been experimentally tested by Mariac et al., (2018).

At the level of the species, these limits are less problematic, and an abundant literature already derives quantitative information from eDNA (Pont et al., 2018; Wilcox et al., 2016; Yates et al., 2019), although its linear relation with species abundance is not systematic (Fonseca, 2018; Nichols et al., 2018). In fact, in contrast to communities, relating a species abundance to the quantity of DNA it releases may be more easily achieved with empirical studies. In addition, with species-specific primers, there are molecular methods that can allow a quantification of DNA such as quantitative qPCR or the more sensitive Digital PCR (dPCR) (Doi et al., 2015). One of the ongoing projects of our research team is to estimate abundances of *Hartiella* species using dPCR to quantify DNA abundance. This genus possesses very limited dispersal abilities and comprises species endemic to certain waterfalls. In consequence, they are highly threatened and classified as critically endangered by the IUCN red list.

Regarding the monitoring of mammal biodiversity with aquatic eDNA, adapting the sampling effort to the quantity of eDNA released by these organisms would increase the robustness and the representativity of the inventories. This could be achieved using the same methodology as developed in Cantera et al., (2019). Recently, Macher et al., (2021) demonstrated that increasing the number of water sample replicates (1 L per sample) to 18, increased the mammal detection probability by 68.9%. In addition, one other interesting pilot study to conduct would be to analyse the eDNA content of small puddles laying under the forest cover that are formed by pluvial water run-off. Because DNA from non-aquatic organisms is probably present in highest quantities on the forest floor than in rivers, water run-offs could concentrate eDNA in these puddles. It may also integrate biodiversity information of species that avoid rivers and stream or species that release very small amount of DNA in the environment, and therefore not detectable in rivers.

1.b. Filling ecological knowledge gaps of the Neotropical biodiversity

Whereas French Guianese fish taxonomy is probably the best known in South America (Le Bail et al., 2012), many knowledge gaps remain regarding species ecology. For instance, there is little documentation about the seasonal dynamics of species. Yet, the water discharge drastically differs between the wet and the dry seasons, probably affecting the dispersal of species. There are several cases of species with restricted spatial distribution, remaining unexplained. For instance, on the Oyapock river, the electric eel (*Electrophorus electricus*) and the stingrays (*Potamotrygon spp.*) only occur downstream the Maripa rapids located 60 km upstream the estuary. The reason of their absence in the upstream Oyapock remains unexplained, but it is suggested so far that the rapids represent a natural barrier to their

dispersion, although the rapids can be almost immersed during the rainy season. Yet, on the Maroni river, both species can be encountered upstream large rapids. Similarly, the confluence between the Oyapock and the Rio Mutaquere (upstream Oypapock) represents the last area where the species *Tometes trilobatus*, *Myleus rhomboidalis* and *Boulangerella cuvieri* can be encountered whereas no apparent barrier is identifiable in this area. A last example relates to the coulimata (*Prochilodus rubrotaeniatus*), which is known by the Wayãpi Amerindians to be a migratory species, heading to the upstream Oyapock near Trois-Saut sector in April for the laying period. Those examples illustrate the need to improve our knowledge on fish species ecology and distribution, and the development of eDNA-based methods could help to set systematic biodiversity surveys on selected sites.

Seasonal studies of French Guianese fish communities could bring interesting insights about fish dispersal and population dynamics. For this purpose, eDNA offers great opportunities thanks to its easy on-field implementation. Yet, eDNA transportation and persistence in the water is influenced by multiple parameters from hydrological conditions, topographical parameters, to environmental factors and water chemistry (Barnes et al., 2014; Harrison et al., 2019; Pont et al., 2018; Troth et al., 2021). These factors exhibit seasonal changes. For instance, the alternations of the wet and dry seasons in French Guiana are characterized by consequent variations in water flow. Therefore, eDNA may also present seasonal variation patterns that need to be examined to understand if it reflects organism seasonal population dynamics. Several authors have studied the dynamics of eDNA and have showed the ability of eDNA to provide information on spatial dynamics of fish communities (Civade et al., 2016; Jane et al., 2015; Pont et al., 2018). In contrast, some studies also showed that eDNA collection can be affected by water flow, the DNA being more diluted with higher level of water (Jane et al., 2015; Thalinger, Kirschner, et al., 2021). To understand the seasonal dynamics of riverine fish communities, it is therefore necessary to analyse the seasonal variations of eDNA and this will be one of the next research questions of our research group. To investigate this question, we inventoried the fish and mammal faunas of 20 sites in the upstream Oyapock during the dry (November) and wet (April) seasons.

Investigating the seasonality of eDNA in the water will probably also provide useful knowledge about monthly and annual variations of eDNA. Indeed, the ecological questions of this PhD were addressed only with a spatial dimension but did not include any temporal dimension. Yet, investigating the modifications of biodiversity throughout time is crucial to assess the growing impacts of anthropisation on ecosystems and, more generally, the impacts of global changes. Coupled to methodological advancements providing quantitative data,

eDNA could represent a robust and reliable tool to monitor these biodiversity changes and guides conservation policies. This has already been initiated by our research group with the NAMCO project (Neotropical Aquatic eDNA Monitoring Consortium). The fish communities of six river localities are being monitored monthly in French Guiana but also in other South American countries, namely Colombia, Brazil and, Bolivia. In addition, the Vigilife observatory aims at deploying and consolidating long-term ecosystem monitorings on a global scale, using standardized eDNA methods. 30 sentinel rivers comprising the Maroni river were chosen to be monitored at different time steps (<https://www.vigilife.org/nos-programmes/>).

Extending the spatial and temporal monitoring of biodiversity outside French Guiana will require the extension of reference databases. The neotropics present the highest fish diversity on the planet (Toussaint et al., 2016) and the establishment of complete reference databases is laborious as more than 4,000 species inhabit neotropical freshwaters (Toussaint et al., 2016), among which, many species are rare (occur at low densities, or have restricted spatial distributions) (Albert & Reis, 2011). In the case of incomplete reference databases, the analysis of marker-gene data may be more reliable using error-based models such as resolving amplicon sequence variants (ASVs) than clustering methods such as molecular operation taxonomic units (OTUs) (Callahan et al., 2017). The OTU approach consists in the clustering of reads into molecular operational taxonomic units (MOTUs) by means of an arbitrary similarity threshold (usually 97%) (Joos et al., 2020). The ASV approach, by contrast, partitions the reads based on error models to correct sequencing errors (Callahan et al., 2017). Unlike OTU approaches, ASV approaches are reusable across studies and are not limited by incomplete reference databases because they are discriminated from sequencing errors and therefore represent a biological reality outside the datasets. Contrastingly, OTU clusters are shaped based on the sequence diversity of the datasets and the biological variation that is not represented in the reference database is lost during taxonomic assignment (Callahan et al., 2017; Joos et al., 2020). In this case, with incomplete reference databases, ASVs may be only identified at higher taxonomic levels than the species levels, but it still allows estimations of ecological diversity, comparable across localities (Callahan et al., 2017; Joos et al., 2020). It remains, nevertheless, pivotal to question the influence of incomplete reference databases on the ecological outputs, such as functional or phylogenetic diversity. A similar question was investigated by Calderón-Sanou et al., (2020), who explored the influence of methodological choices in eDNA-based methods, on taxonomic ecological patterns. They showed that OTUs clustering, removal of PCR errors and cross-sample contaminations have major impacts on taxonomic ecological outputs.

Conclusion

A retrospective summary of eDNA metabarcoding-based ecological studies in French Guiana

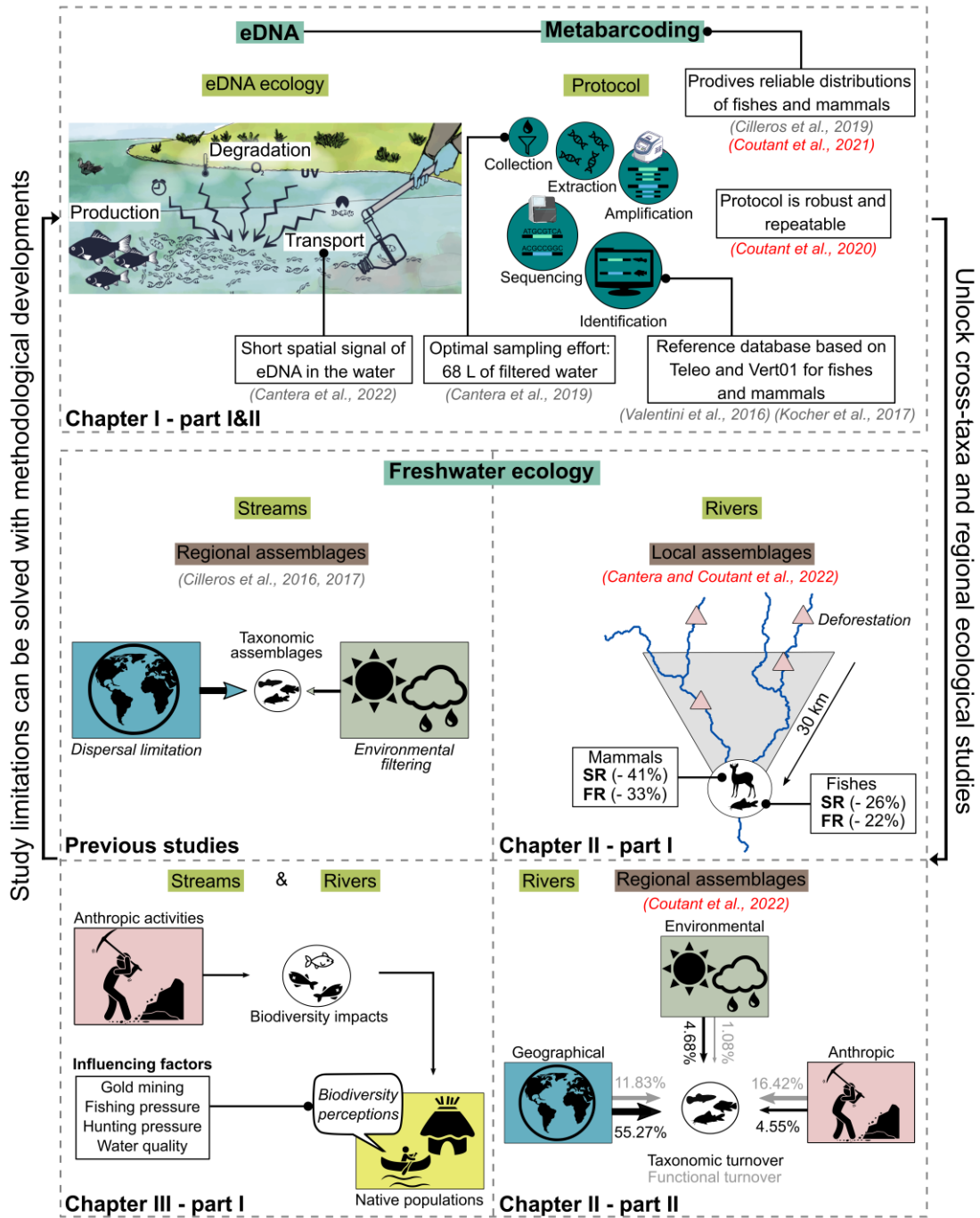
Since Cilleros et al., (2019) who first implemented eDNA metabarcoding in French Guianese rivers in 2014, our method has been greatly improved. For fish communities, we have been building a local database of French Guianese fish, which is now almost complete for strict freshwater fish, comprising 358 out of the 391 species described in the territory. We established that the filtration of 68L of water was the optimal sampling effort and showed that our inventories were more complete than inventories obtained with traditional fishing methods (Cantera et al., 2019). Then, we shifted to an optimised version of our ancient protocol (Pont et al., 2018) and compared it to the old protocol (Valentini et al., 2016), presenting differences in the collection, extraction and detection steps. This comparison demonstrated the robustness of the method, as the ecological results remained not influenced by the variations in the protocols (Coutant et al., 2020). Following this work, Cantera, Decotte, et al., (2022) addressed the question of the spatial signal of eDNA in the water. Using a community ecology approach, they showed that eDNA-based inventory exhibited a similar and even steeper distance decay of similarity than inventories based on traditional methods. This testified that eDNA integrates biodiversity information at a similar spatial scale than traditional methods, describing fish fauna over a few hundred metres. We then extended the application of our eDNA metabarcoding approach to inventory other taxonomic groups than fish. Kocher, Thoisy, Catzeflis, Huguin, et al., (2017) established a nearly exhaustive reference library for mammals species found in French Guiana. They show that the 12S marker allowed accurate taxonomic assignments despite its very short size, and that primer-binding sites were highly conserved, which is crucial to avoid PCR amplification biases potentially leading to detection failure. For the first time, this work allowed the simultaneous inventory of fish and mammals communities at the sampling sites. We showed that eDNA-based inventories of emblematic Amazonian mammals correctly reflected their spatial distribution and provided new occurrences of species. Nevertheless, the study also revealed that the sampling effort should be adapted to obtain more complete inventories including the rarest species releasing less eDNA in the water (Coutant et al., 2021).

French Guianese fish taxonomy is one of the best known of South America, probably because despite being very diversified, the number of fish species remains far inferior to that of the Amazon basins. Cilleros et al., (2016) initiated community ecology studies on freshwater fish before the implementation of eDNA metabarcoding. They first investigated assembly rules

of fish communities inhabiting small streams and revealed that dispersal limitation was the predominant process structuring Guianese stream fish, causing strong species replacement. Later on, they disentangled the strength of spatial (longitudinal and environmental) drivers and scale-specific (drainage basin, reach and local scale) determinants of species richness and composition of freshwater fish assemblages from small streams. They evidenced a zonation pattern of species richness, increasing from upstream to downstream but also with local habitat structural diversity, independent of the stream position in the upstream–downstream gradient (Cilleros et al., 2017). While these works focused so far on small streams and at local scales, the continuous improvements of eDNA metabarcoding in French Guianese rivers unlocked the ecological study of fish and mammal fauna at a regional scale. More importantly, it offers the opportunity to evaluate the growing impacts of anthropogenic pressure on biodiversity. At a local scale, we underscored the vulnerability of Amazonian biodiversity even to low anthropisation levels. Our findings suggested that anthropisation had a spatially extended impact on both fish and mammal fauna. We showed that forest cover losses of <11% in areas up to 30 km upstream from the biodiversity sampling sites are linked to reductions of >22% in taxonomic and functional richness of both terrestrial and aquatic fauna. The paired taxonomic and functional declines indicated a loss of species with unique traits belonging to the detritivore and invertivore guild for fish and to the top predators for mammals (Cantera, Coutant, et al., 2022). At a regional scale, we disentangled the different drivers structuring the distribution of freshwater fish inhabiting large rivers (Coutant et al., 2022). We revealed that taxonomic assemblages were mainly shaped by dispersal limitation. Interestingly, this was true for both inter and intra-basin diversity. These findings are important because it is traditionally expected that river fish assemblages are structured by a mass effect dispersal. Once again, this underlined the vulnerability of freshwater fish biodiversity, as they will be probably more vulnerable to anthropic fragmentations if they are naturally limited in their dispersal capacities. In addition, we demonstrated that functional assemblages and particularly species replacement was strongly influenced by deforestation. Variations in the functional assemblages induced similar taxonomic variations at the regional scale, and this confirmed our previous findings supporting the loss of functionally unique species, generating a biotic homogenization at the anthropised localities (Coutant et al., 2022). Finally, we explored the impacts of a modified biodiversity on humans by investigating the biodiversity perceptions of local Amerindian populations. We found that Amerindians' perception of biodiversity was different from that measured with eDNA. Their biodiversity perceptions were primarily influenced by anthropic factors, namely gold mining and subsistence fishing and hunting, but also by environmental parameters known

to be determinant drivers of fish and mammal distributions. This emphasized how local populations are being affected by the human alteration of biodiversity and calls for a more thorough investigation of these impacts on their resources.

While these researches bring a better comprehension of the biodiversity distribution of freshwater and terrestrial species and the impacts of anthropisation in French Guiana, it also has a more global scope, providing useful insights for the development of robust eDNA metabarcoding methods in tropical ecosystems and species-rich environments. Understanding both biodiversity modifications and the impacts on local human populations will also be crucial to set effective conservation policies and management strategies in one of the most preserved territory on the planet.



A pictorial summary of the methodological and ecological studies of our research team in French Guiana. The contributions of this PhD are highlighted in red. The image from the first left panel comes from Pawloski et al. (2020), eawag.ch. The side arrows indicate the relationships between the inventory method and the ecological studies. In French Guiana, our research team used eDNA metabarcoding as a fish inventory method for the first time in 2014. Since then, several studies have been conducted to improve the reliability and the robustness of the method. By providing information on fish and mammal distributions at the scale of the territory, it unlocked the development of cross-taxa and regional ecological studies, such as revealing the spatial structuration of biodiversity and the major drivers shaping species distributions or assessing the impacts of anthropogenic disturbances on biodiversity. In addition, ecological study limitations, notably associated to incomplete information on biodiversity, help identify new relevant methodological developments regarding the inventory method. For instance, the lack of reliable quantitative data such as species abundances calls for new methodological developments of eDNA-based inventory methods. SR (species richness), FR (functional richness).

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Appendices

Table A1. Morphological and ecological traits and measures and associated descriptions, used to describe functional diversity in chapter II. See Su et al., (2019), Toussaint et al., (2016) and Villéger et al., (2010) for details on morphological measures.

Codes	Mesures	Descriptions
Bl	Body length	Standard length (snout to caudal fin basis)
Bd	Body depth	Maximum body depth
Bd2	Maximum body depth	Vertical distance between the dorsal and ventral faces
Hd	Body depth at the vertical of the eye	Body depth at the vertical of the eye
CPd	Caudal peduncle depth	Minimum depth
CFd	Caudal fin depth	Maximum depth
Ed	Eye diameter	-
Eh	Eye position	Vertical distance between the center of the eye and the bottom of the head
Eh2	Eye position	Vertical distance between the center of the eye and the ventral face
Eh3	Eye position	Vertical distance between the bottom of the eye and the bottom of the head
Mo	Mouth position	Vertical distance from the top of the mouth to the bottom of the head along the head depth axis
Mo2	Mouth position	Vertical distance between the top of snout to the ventral face
Jl	Maxillary jaw length	Snout to the corners of the mouth
Bbl	Barbel maximum length	Length of the longest barbel
PFl	Pectoral fin length	Maximum length (horizontal)
PFd	Pectoral fin depth	Maximum depth (vertical)
Pp	Pectoral fin position Body depth	Maximum body depth (often at the vertical of pectoral fin insertion)
PFi	Pectoral fin position	Vertical distance between the insertion of the pectoral fin to the bottom of the body
PFi2	Pectoral fin position	Vertical distance between the top insertion of the pectoral fin to the ventral face
CFs	Caudal fin surface	Total fin surface
PFs	Pectoral fin surface	Total fin surface

Figure A1. Morphological measures from Toussaint et al., (2016).

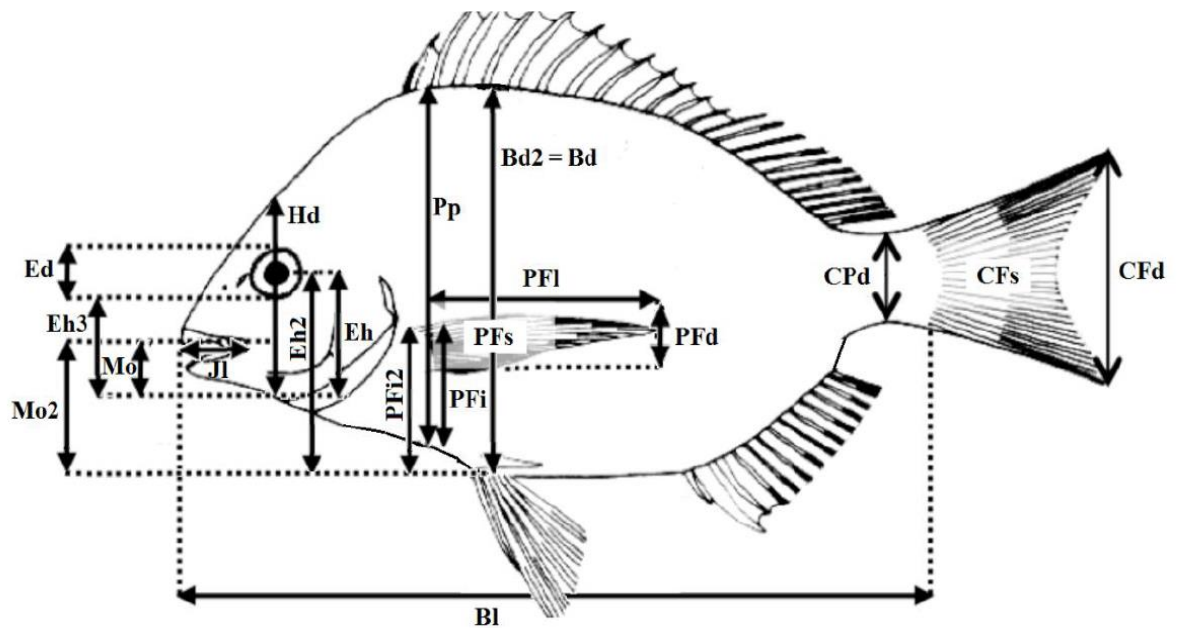


Table A2. Morphological and ecological traits and measures used to describe functional diversity for chapter II and their corresponding functions. See Su et al., (2019), Toussaint et al., (2016) and Villéger et al., (2010) for details on morphological measures.

Functional trait	Measures (ratio or categories)	Function	Type
Maximum body length	Fish base (cm)	Synthetic: metabolism, trophic impacts, locomotion nutrient cycling	Morphological
Body elongation	Bl/Bd	Locomotion	
Eye vertical position	Eh/Bd		
Body lateral shape	Hd/Bd		
Pectoral fin vertical position	PFi/Bd		
Pectoral fin size	PFI/Bl		
Caudal peduncle throttling	CFd/CPd	Food acquisition	
Relative eye size	Ed/Hd		
Oral gape position	Mo/Bd		
Relative maxillary length	Jl/Hd		
Relative barbell length	Bbl/Bl	Behavior	
Territoriality	Yes, no		
Motility	Mobile, sedentary		
Gregariousness	Gregarious, solitary	Habitat preference	
Position in the water column	Benthic, benthopelagic, pelagic		
Preferred substrate	Hard, soft, none	Ecological	

Summary

Anthropisation and biodiversity: Human disturbances and anthropic responses to the decline of vertebrate's biodiversity in Guianese rivers

In a context of global changes and biodiversity erosion, quantifying biodiversity alterations constitutes a fundamental stake. Yet, these quantifications remain incomplete in species-rich environments such as tropical regions. These alterations affect, in turn, human populations that thus need to adjust their exploitation of resources. In French Guiana, the rapid population growth increases the direct and indirect anthropogenic pressures on ecosystems. This PhD aims at identifying and quantifying the biodiversity modifications generated by anthropogenic activities and at characterising in turn, the repercussions of a modified biodiversity on local indigenous populations. These questions are studied thanks to the development of aquatic environmental DNA (eDNA) metabarcoding, used to inventory fish diversity. A first methodological chapter validates the robustness of eDNA-based inventories and broadens its application to the survey of mammals. Thanks to these methodological studies, in a second chapter, modelling approaches allow the identification of the spatial extent and the intensity at which anthropogenic pressures affect freshwater and terrestrial biodiversity. These advancements also allow determining the relative contribution of environmental, biogeographical and anthropic factors to the spatial structuration of biodiversity. Finally, in a last chapter, a multidisciplinary approach comparing social and ecological assessments of biodiversity allows characterising the factors that influence the biodiversity perception of *Wayãpi and Teko* indigenous population.

Anthropisation et biodiversité : Impacts humains et réponses anthropiques au déclin de biodiversité des vertébrés des cours d'eau de Guyane

Dans un contexte de changements globaux et d'érosion de la biodiversité, quantifier les altérations de la biodiversité constituent un enjeu fondamental. Pourtant, ces quantifications demeurent incomplètes dans les zones tropicales à forte biodiversité. Ces altérations affectent en retour les populations humaines qui doivent alors ajuster leur exploitation de l'environnement. Ainsi, l'essor démographique en Guyane accroît les pressions directes/indirectes sur les écosystèmes. Ma thèse vise à identifier et quantifier les modifications de biodiversité générées par l'anthropisation et à caractériser en retour les répercussions d'une biodiversité modifiée sur les populations humaines locales. Ces questions sont étudiées grâce au développement de l'ADN environnemental (ADNe) aquatique, utilisé pour inventorier la faune piscicole. Un premier axe de recherche méthodologique a validé la robustesse des inventaires réalisés avec l'ADNe et étendu son champ d'application à l'inventaire de mammifères. Grâce à celui-ci, dans un deuxième axe de recherche, des approches de modélisation ont permis d'identifier à quelle distance et avec quelle intensité les pressions humaines affectent la biodiversité des cours d'eau et des écosystèmes forestiers à proximité. Ces travaux ont également permis de déterminer les rôles relatifs de l'environnement, de la géographie et de l'homme sur la structuration spatiale de la biodiversité. Enfin, dans un dernier axe, une approche exploratoire multidisciplinaire comparant des évaluations sociologiques et écologiques de la biodiversité a permis de caractériser les facteurs influençant la perception de la biodiversité des populations Amérindiennes *Wayãpi and Teko*.