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# Anthropization effects on freshwater fish communities in French Guiana : An environmental DNA approach

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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

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Présentée et soutenue par  
**Isabel CANTERA**

Le 20 décembre 2019

**Effet de l'anthropisation sur les communautés des poissons dans  
les cours d'eau Guyanais. Approche par ADN environnemental.**

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## *Chapter 1: General introduction*

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### **I) Biodiversity: a multifaceted entity threatened by human activities**

Biodiversity changes as a consequence of human activities, has been a key focus of political, economic, and scientific debates in the last decades. However, how are human activities inducing biodiversity changes and why is it critical for human societies?

Over the last centuries, human impact has deeply transformed the form and function of all ecosystems on earth. Before the Industrial Revolution, 50 % of the terrestrial biosphere was without human settlements or substantial land use and by the year 2000 only 25% remained wild (Ellis *et al.* 2010). This anthropogenic transition resulted from the widespread and growing presence of human populations and their economic development. Land transformation, mainly for agricultural intensification and infrastructure development, combined with the introduction of non-native species and the overexploitation of natural resources such as minerals, wood, water and animals are confronting ecosystems with unprecedented levels of disturbance (Dirzo and Raven 2003). Moreover, these activities are driving other environmental changes such as habitat loss, pollution, climate change and the alteration of biogeochemical cycles (Vitousek *et al.* 1997; Rockström *et al.* 2009). All of the impacts mentioned above are interacting and affecting directly (*e.g.* hunting and fishing) and indirectly (*e.g.* land use) the Earth's biodiversity.

Biodiversity is the variety of life and it can be described from local to global scales, and across different levels of organization, from the variation among genes to the diversity between species and their traits. Change is a natural feature of species and is the baseline of Evolution Theory as species emerge, adapt and/or become extinct without human actions. Naturally, extinction events are balanced by speciation events, but this balance has been disrupted. In fact, human alterations are accelerating the current rates of extinction as they are higher than expected from fossil records (Barnosky *et al.* 2011). In addition, Pimm *et al.* (1995), estimated that current extinction rates were 100 to 1000 times higher than pre-human rates. The global number of species is not the only diversity component that is altered by human impacts. Between 1970 and 2014, the population size of overall world's species declined by 60% (Living planet report 2018), with the Neotropics and freshwater ecosystems suffering the most dramatic

declines of population sizes (89% and 83% respectively), showing that the species from those ecosystems are highly threatened.

Globally, the number of species and their population sizes are declining and those estimates are the result of changes in biodiversity from local to regional scales. Locally, at the community level, species are responding to anthropogenic impacts by disappearing or shifting and/or adapting their distribution range, as well as changing their behavior and phenology. Newbold *et al.* (2015), using a global multi-taxa assessment, quantified that land use reduced, on average, 14 % of the species richness of local terrestrial communities. However, it has been illustrated that local communities facing human impacts do not always exhibit decreases of species richness but mostly changes on species composition (Sax and Gaines 2003; Thomas 2013). It is important to note that under severe disturbance levels, species richness always decreased. The alteration of the environment might result on local extirpations of sensitive species, but tolerant species can resist (McKinney and Lockwood 1999). Moreover, tolerant widespread species may colonize the disturbed community and benefit from the new environment thereby adding species to the community. Consequently, this species exchange among communities will lead to a homogenization of the species composition of communities and thus increase the similarity among communities within a region. For instance, marine fish communities under climate change showed important changes on species composition through time, with species from warmer southern localities colonizing northern localities, without systematic changes in species richness (Dornelas 2015). Similarly, land use led to taxonomic homogenization in communities of terrestrial plants, vertebrates and invertebrates (Newbold *et al.* 2018).

Taxonomic homogenization due to human impact will often be mediated by a transition from communities dominated by specialist species to a dominance of tolerant and widespread generalist species (McKinney and Lockwood 1999). The replacement of specialist species with unique functional traits by generalists more adapted to disturbed environments may eventually lead to a functional homogenization of communities (Clavel *et al.* 2011). Indeed, functional homogenization of bird communities under urbanization (Devictor *et al.* 2007b), land use and landscape fragmentation (Devictor *et al.* 2007a) has been recorded. General patterns of traits that replace specialist traits are omnivory, rapid growth and dispersal, as well as breeding in ephemeral habitats (McKinney and Lockwood 1999). Nevertheless, functional homogenization without associated taxonomic homogenization was found for North Sea fish communities after three decades of warming (McLean *et al.* 2019b). While diverging in species composition,

Southern and Northern North Sea communities converged towards similar traits such as small pelagic fishes with fast life history strategies.

Human activities are altering biodiversity across different geographical scales. From a local reorganization of species, through regional homogenization of taxonomic and functional diversity, to the global erosion of species. Importantly, ecosystem functions and services depend on local species and the traits they exhibit (Cardinale *et al.* 2012). Specifically, the loss of a species in an ecosystem can also lead to habitat loss, as well as the alteration of biogeochemical cycles and ecosystem productivity. Experimental studies illustrated that ecosystem functions, such as biomass production and nutrient cycling, were strongly influenced by changes in local diversity (Naeem *et al.* 1994; Tilman *et al.* 1996). Moreover, high local diversity was reported to increase ecosystem function, resistance and stability to environmental changes (Tilman *et al.* 2006). Accordingly, during drastic climate events, the productivity of low-diversity plant communities decreased by 50%, whereas that of high-diversity communities decreased by 25% (Isbell *et al.* 2015). The disruption of ecosystem functions results from the limited range of species-specific responses available after human impacts. This may be a consequence of the loss of specialist species or to the synchronized biological responses due to the biotic homogenization of communities. Furthermore, functional simplification may have a great impact on ecosystems processes given the strong links between organismal traits and ecosystem functioning (Cardinale *et al.* 2012). For example, experimental evidence pointed that functional composition and functional diversity were the main factors explaining ecosystem processes such as plant productivity, nutrient cycling, and light penetration (Tilman 1997).

Besides the ethical and aesthetical value of species, local diversity is essential to maintain the functioning of ecosystems and their benefits to societies. Biodiversity changes at local scales are more complicated to understand because they depend on the level and type of disturbance, the ecosystem and the studied taxa (Sax and Gaines 2003). Consequently, it is necessary to develop efficient sampling methods and frameworks to assess the effects of anthropization on local biodiversity in different regions around the world.

## **II) Tropical ecosystems: highly diverse and strongly vulnerable**

The type and intensity of human activities widely differ across ecosystems and regions. At global scale, land transformation has been highlighted as the hardest driver of changes in biodiversity, mainly by local extinction of associated species (Sala 2000). This impact is particularly accentuated in tropical rainforests, which are among the most threatened ecosystems on the world (Sala 2000; Morris 2010). In fact, agro-industrial and logging activities are removing thousands of hectares of tropical forest every year (Hansen *et al.* 2010).

Diversity patterns also differ across regions and ecosystems. This fact should be accounted to deeply understand the impact of human activities on biodiversity because the vulnerability of species may differ among ecosystems. Tropical regions host huge amounts of diversity (Barlow *et al.* 2018) with high species turnover between localities (Kraft *et al.* 2011). For instance, higher species richness were found in these ecosystems comparing with temperate ecosystems for freshwater fishes (Toussaint *et al.* 2016), mammals (Safi *et al.* 2011) and birds (Jetz and Rahbek 2002). Many hypotheses have been proposed to explain this high diversity: higher diversification rates (Rolland *et al.* 2014), higher available energy, diversity of habitats and/or decreased abiotic harshness (Cilleros *et al.* 2016). In view of its high diversity, tropical ecosystems are expected to be particularly resilient to human disturbances, as species loss may be compensated by the remaining species that perform similar functions. Indeed, the strength of human impacts on biological communities will depend on the levels of functional redundancy, which reflects how traits are ensured in terms of number of species and individuals (Naeem and Li 1997). While taxonomic diversity will count species, functional diversity will count functions, and species with the same functions will increase the functional redundancy in the community. This will increase the resilience of ecosystem processes under human disturbances, as the loss of some species will be compensated by the remaining functionally similar species. Accordingly, marine fish communities exhibiting high redundancy levels were less sensitive to global warming in the Seychelles islands (McLean *et al.* 2019a).

Functional diversity was also found to be concentrated in tropical ecosystems for freshwater fishes and mammals (Safi *et al.* 2011; Toussaint *et al.* 2016). Additionally, this rich functional diversity exhibited high levels of functional redundancy in mammals and freshwater fishes from the Afrotropical region. In contrast, low levels of redundancy were found for freshwater fishes in the Neotropical region. Specifically, the high functional diversity found in the Neotropical region was suggested to be due to a few orders with high levels of functional

uniqueness. Moreover, D'agata *et al.* (2016) found that in tropical coral reef fish communities, only 40% of trait combinations were redundant among species, leaving the other 60% highly vulnerable to fishing pressures. This trend to disproportionately pack into a few trait combinations was also found in a global study of the functional diversity of coral reef fish (Mouillot *et al.* 2014). Similarly, Leitão *et al.* (2016) illustrated that rare species had unique attributes and contribute disproportionately to the functional diversity of Australian birds and Amazonian fishes and plants. Tropical ecosystems have a high proportion of rare species, which are sensitive to local extinction induced by human impacts due to their low representativeness, narrow geographical size and habitat breadth (Leitão *et al.* 2016). Therefore, the low functional redundancy and the important contribution of sensitive rare species to trait diversity highlight the vulnerability of the functions supported by communities inhabiting tropical ecosystems.

### **III) Values and threats of Amazon freshwater ecosystems**

Among all tropical ecosystems, the Amazon forest hosts the highest levels of local diversity (Hubbell *et al.* 2008; Peres *et al.* 2010) and the most extensive tropical forest on the planet. For instance, this region hosts the most diverse freshwater fish fauna on earth, corresponding roughly to 20% of global fish species diversity (Lévêque *et al.* 2008). Additionally, this ecosystem provides significant goods and services for their inhabitants but also around the world, such as wood, timber and agricultural products. Importantly, many local populations still rely on Amazonian rivers and streams to transport use, water use and food acquisition. In spite, of its high value, little attention has been addressed to the management of freshwater ecosystems comparing to terrestrial ecosystems in the Amazonian region (Castello *et al.* 2013; Castello and Macedo 2016). Indeed, developing countries share the Amazonian forest, where the economic growth is favored over biodiversity conservation, which results in limited conservation policies, monitoring and data for empiric studies.

Besides facing the same threats than other freshwater ecosystems, such as deforestation and pollution due to human settlement, damming, overharvesting, as well as intensified agriculture and livestock (Vörösmarty *et al.* 2010; Carpenter *et al.* 2011), Amazonian streams and rivers are highly threatened by unprecedented levels of mining, logging, oil and gas extraction. Those activities are polluting freshwater systems and altering their hydrology and physico-chemical conditions (Castello *et al.* 2013). Furthermore, those activities expanded from artisanal exploitation, to domestic and international markets. This results in an intensification of resource

exploitation and an increase of infrastructure constructions, which ultimately leads to large-scale degradation and disruption of the hydrological connectivity of Amazon freshwater ecosystems (Castello and Macedo 2016). Thus, assessing the integrity of freshwater systems becomes highly urgent due to the vulnerability of tropical biodiversity mentioned in section II. In particular, the Amazonian diversity was structured in a stable environment in terms of climate and landscape changes (Peres *et al.* 2010). Considering that historical stability promotes diversification rates (Ricklefs 2006) and thus higher levels of functional specialization and originality (Rodrigues-Filho *et al.* 2018), Amazonian biodiversity may be specially sensitive to the variation of environmental conditions induced by the current growing human perturbations.

#### **IV) Measuring human impacts on Amazonian fish communities: an overview**

Most of the studies assessing the impacts of mining and land use on Amazonian freshwater ecosystems concluded that they alter stream physical habitat and water chemistry (Mol and Ouboter 2004; Dias *et al.* 2010; Prudente *et al.* 2017). Contrastingly, the consequences on fish diversity are more contrasted. The commonly used diversity descriptors, the number of species and their abundance, were found not sensitive to human pressures or lacked of consistency. While Mol & Ouboter (2004) found an erosion of fish species richness due to small scale gold-mining, the majority of studies failed to detect changes on this variable (Bojsen and Barriga 2002; Brosse *et al.* 2011; Allard *et al.* 2016; Prudente *et al.* 2017). Surprisingly, Bojsen & Barriga (2002) found that total fish density increased with deforestation. Nonetheless, all the above-cited studies, found that species composition consistently changed under disturbance.

More recently, studies focused on the functional aspects of communities and this diversity facet appeared to be more sensitive to the effects of human activities. Indeed, low forest cover was found to induce trends towards functional homogenization in Brazilian streams (Bordignon *et al.* 2015; Arantes *et al.* 2018; Leitão *et al.* 2018), with functionally specialized species sensitive to forest loss. However, the identity of the shifts displayed some discrepancies among studies. Community shifts towards a dominance of periphyton-feeders under deforestation were observed in Ecuadorian (Bojsen and Barriga 2002) and Brazilian streams (Leitão *et al.* 2018). In opposition, in logged streams and rivers in French Guiana, phytophagous fish were unrepresented (Allard *et al.* 2016). Finally, planktivorous species were favored in areas with low forest cover in the Amazon river floodplain (Arantes *et al.* 2018).

The effects of anthropogenic activities on Amazonian freshwater ecosystems depend on the type and intensity of activities (Allard *et al.* 2016; Brejão *et al.* 2018). Even though the majority of studies failed to report a decrease in species richness, low levels of deforestation (<20% of deforested watershed) caused abrupt responses of Amazonian fishes (Brejão *et al.* 2018). Therefore, fish communities inhabiting Amazonian streams appear to be vulnerable to human activities. Species composition and functional diversity consistently responded to disturbances suggesting that the severity of human impacts should be assessed in a community ecology approach, evaluating both diversity patterns and processes (see below). Furthermore, all but one of the mentioned studies were performed in upstream streams, where the fauna and environment differ considerably from those in downstream rivers (Allard *et al.* 2016; Cilleros *et al.* 2017). Additionally, human impacts affect the two ecosystems differently: upstream streams are directly impacted whereas rivers are directly impacted but also may receive cumulative effects from upstream disturbances (Lindberg *et al.* 2011; McCluney *et al.* 2014). Thus, the effects of human activities on riverine communities need to be studied to have a more complete picture of the severity of biodiversity degradation in the Amazonian region.

#### **V) How are species assembled into communities?**

To assess the severity of human impact on local communities, it is important to define how species are assembled into communities. The answer roots on several ecological theories, encompassing different mechanisms and processes acting at different spatial and temporal scales. Local communities are the result of a hierarchical filter in which species are progressively filtered from a regional pool. The regional pool represents the global diversity of a region and is constrained by historical and evolutionary events (Chase 2003). For instance, the Neotropical region is characterized by a high species richness, which has been explained by the complex history of this region and the high diversification rates (Rolland *et al.* 2014). From the regional pool, species will be filtered by assembly rules (Gleason 1926; Keddy 1992) and/or neutral processes (Connor and Simberloff 1979). Assembly rules are deterministic processes constraining the co-occurrences of species in local communities, whereas neutral processes refer to stochastic events independent to the species traits or abiotic interactions (Hubbel 2001). These processes may act simultaneously and their relative importance depends on the considered spatial scale (Weiher and Keddy 1999; Webb *et al.* 2002). At large scales, the size of the local and regional species pools, as well as random colonization events and differences

in species dispersal abilities will determine the species capacity to arrive to a community (Hubbel 2001; Fraaije *et al.* 2015). At local scales, two main processes, based on the niche concept (Hutchinson 1957), will structure communities. First, local environmental conditions act as filters and select species able to persist in a given community according to their traits. This process is called environmental filtering (Keddy 1992). Then, limiting similarity will also shape local communities (MacArthur and Levins 1967), this process represents the competitive exclusion from a suitable environment by species having similar ecological strategies.

The ecological processes structuring local communities shape diversity patterns and the comparison between diversity facets across different spatial scales allow to disentangle their relative importance (Keddy 1992; Götzenberger *et al.* 2012). Lower functional dissimilarity compared to taxonomic dissimilarity among communities within a region may suggest that environmental conditions select particular species traits and strongly structures community assembly. For instance, this pattern was found for temperate fish communities in France, suggesting that they are mainly structured by environmental filtering (Cilleros *et al.* 2016). Contrastingly, fish communities inhabiting tropical streams in French Guiana exhibited higher functional dissimilarity among communities than taxonomic dissimilarity, suggesting that they are mainly structured by dispersal limitation (Cilleros *et al.* 2016). Furthermore, at local scale, communities mainly ruled by limiting similarity should harbour species with different ecological strategies than expected randomly (Weiher and Keddy 1999). In contrast, under predominant environmental filtering, communities are expected to have mostly similar species sharing traits that allow them to tolerate specific abiotic conditions.

As explained before, human activities are reorganizing local diversity patterns mediated by biotic homogenization and species loss. Moreover, considerable losses of functional diversity were recorded for coral reef fish communities due to increasing human population density (D'agata *et al.* 2014) and for amphibians, birds and mammals under land use (Ernst *et al.* 2006; Flynn *et al.* 2009). Thus, ecological processes shaping communities may be also influenced by anthropization. Accordingly, functional diversity was found to decrease faster than taxonomic diversity under global change (Kuczynski and Grenouillet 2018) and land use (Gutiérrez-Cánovas *et al.* 2015) in temperate freshwater ecosystems. This suggests that disturbed communities are mainly structured by environmental filtering, excluding functions not adapted to those altered environments (See Figure 1A for an illustration). Therefore, assessing how structuring processes interact with disturbance may provide a deeper understanding of the effect of anthropogenic activities on Amazonian biodiversity.

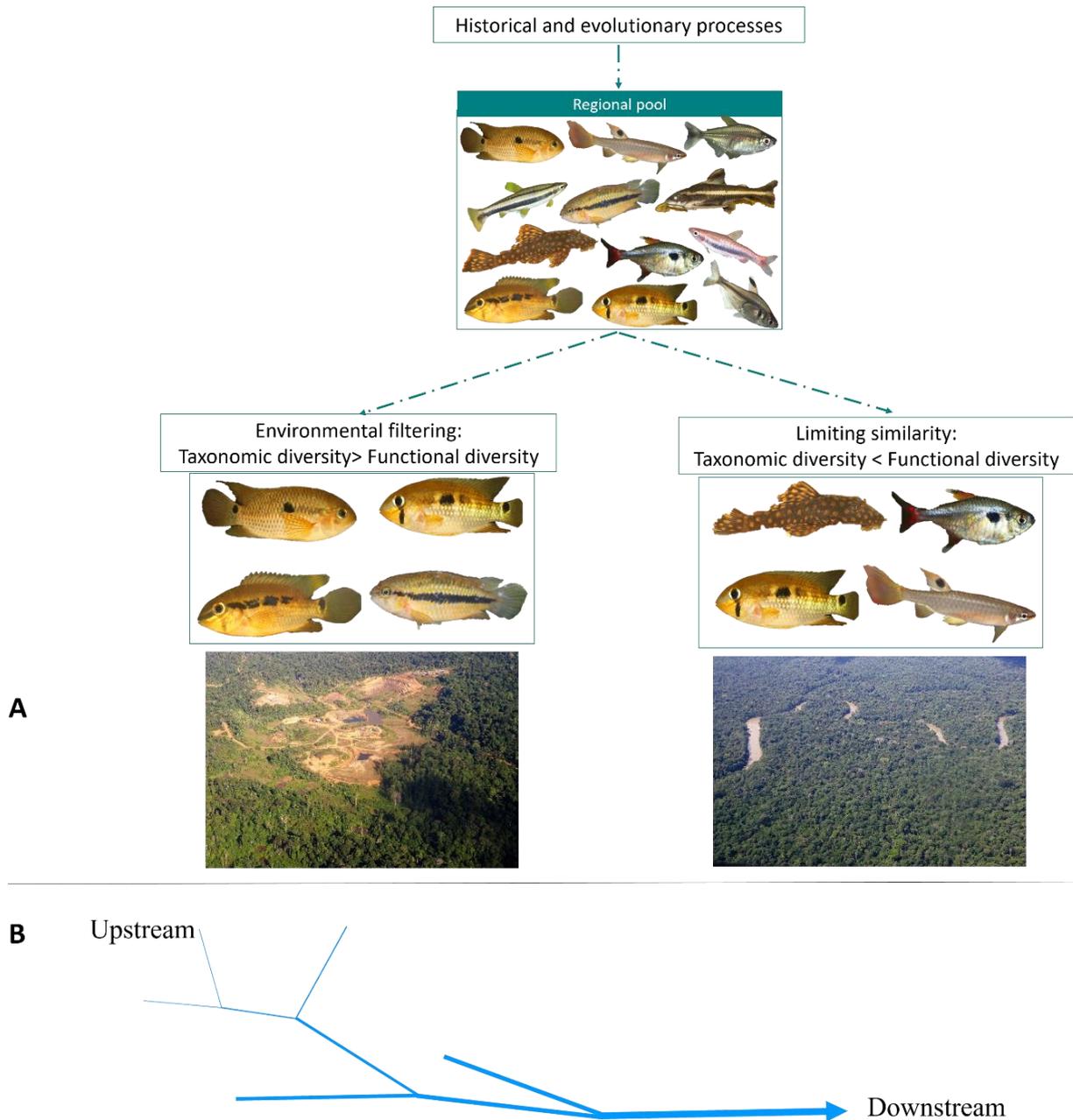


Figure 1: Illustration of the diversity patterns resulting from ecological process acting at local scale. Expectations under disturbance (A) and along the upstream-downstream gradient (B).

## VI) Assessing human impacts in a directionally connected network

Freshwater ecosystems are also among the most threatened ecosystems in the world (Sala 2000; Carpenter *et al.* 2011) and assessing the severity of human impacts on freshwater ecosystems needs to take into account their dendritic network structure. Indeed, an important feature of freshwater ecosystems is the longitudinal directional connectivity due to the movement of water

from the headwaters to the ocean (McCluney *et al.* 2014; Moore 2015). Therefore, two considerations are necessary to measure human impacts on diversity patterns and processes.

First, given the longitudinal connectivity of freshwater ecosystems, upstream disturbances can have consequences downstream. Indeed, effects of mountaintop mining have been documented for the water quality and biodiversity downstream from the mining sites (Palmer *et al.* 2010). Furthermore, the combined effect of multiple upstream perturbations can lead to cumulative downstream impacts (Lindberg *et al.* 2011) (Figure 2A). Accordingly, rivers are suggested to integrate and redistribute disturbance effects from upstream to downstream (Vörösmarty *et al.* 2010). Nonetheless, rivers can also promote resilience and resistance to human disturbances (Figure 2B). Specifically, river systems integrate processes across multiple spatial scales and broad distances over time resulting in temporal asynchrony and habitat heterogeneity across connected patches (McCluney *et al.* 2014). Individuals can move throughout the river system and recolonize new patches to avoid locally unsuitable conditions. Moreover, tributaries that are less or not impacted can vehicle undisturbed water inputs and therefore dilute disturbance effects (Vörösmarty *et al.* 2010). Thus, it is important to determine the spatial extent of upstream impacts to better assess the effects of human activities on freshwater communities.

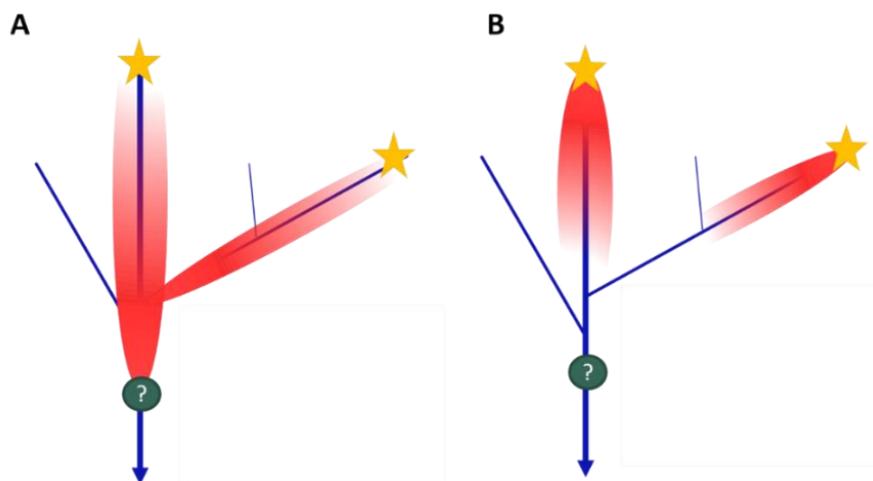


Figure 2: Possible downstream ecological responses to upstream perturbations.  
(A) Downstream sensitivity and cumulative effects.  
(B) Downstream resilience and resistance.

Second, environmental conditions and diversity patterns vary along the upstream-downstream gradient (Vannote *et al.* 1980; Ibanez *et al.* 2007). Consequently, network position may influence community assembly processes, as the relative importance of assembly processes may vary along natural environmental gradients. For instance, upstream streams are

strongly influenced by local environmental conditions and exhibit high environmental variability (Poff 1997), this leaves few species with particular traits able to tolerate these conditions. In opposition, environmental stability, habitat size and complexity increase downstream stability promoting high species richness in downstream large rivers. Therefore, in headwater streams, environmental filtering may be of greater importance, whereas limiting similarity may be predominant in downstream habitats (Figure 1B).

## **VII) The study area: French Guiana**

### *1) Description of the territory*

French Guiana is located in the Northern East of the Amazonian region (*sensu lato*, including the Guiana shield and the Amazon river drainage, see Figure 3). Almost the entire territory (*c.a.* 96%) is covered by a dense primary Amazonian rainforest, representing the largest area of unfragmented rainforest in the world (*c.a.* 80 000 km<sup>2</sup>). This territory is part of a unique geological unit, the Guiana Shield (Figure 3). The forests of the Guiana Shield cover around 30% of the Amazonian forest (*c.a.* 1.6 million km<sup>2</sup>).

A dense river network composed of seven large river basins covers French Guiana. Small streams (water depth <1 m; stream width <10 m) represent 70% of all running waters in the territory and have been found to display environmental conditions and diversity patterns contrasted with large rivers (Dedieu *et al.* 2015; Allard *et al.* 2016; Cilleros *et al.* 2017). This river network shelter typical Amazonian freshwater fauna with more than 400 described fish species that exhibit a high diversity of forms (See Figure 1 for some illustrations). In addition, the rivers basins share 50% of the species and the other half is represented by species endemic to the different basins (Le Bail *et al.* 2012). These distribution patterns result from a mixture of different species pools arising from the complex biogeographical history of the Neotropical region (Cilleros *et al.* 2016) and Guineans basins. In fact, most of the Amazonian basins dried up during the last Quaternary glaciation with the exception of the Maroni and the Eastern Amazon, which acted as fish refuges. Thus, post-glacial recolonization resulted from those river basins (de Mérona *et al.* 2012).

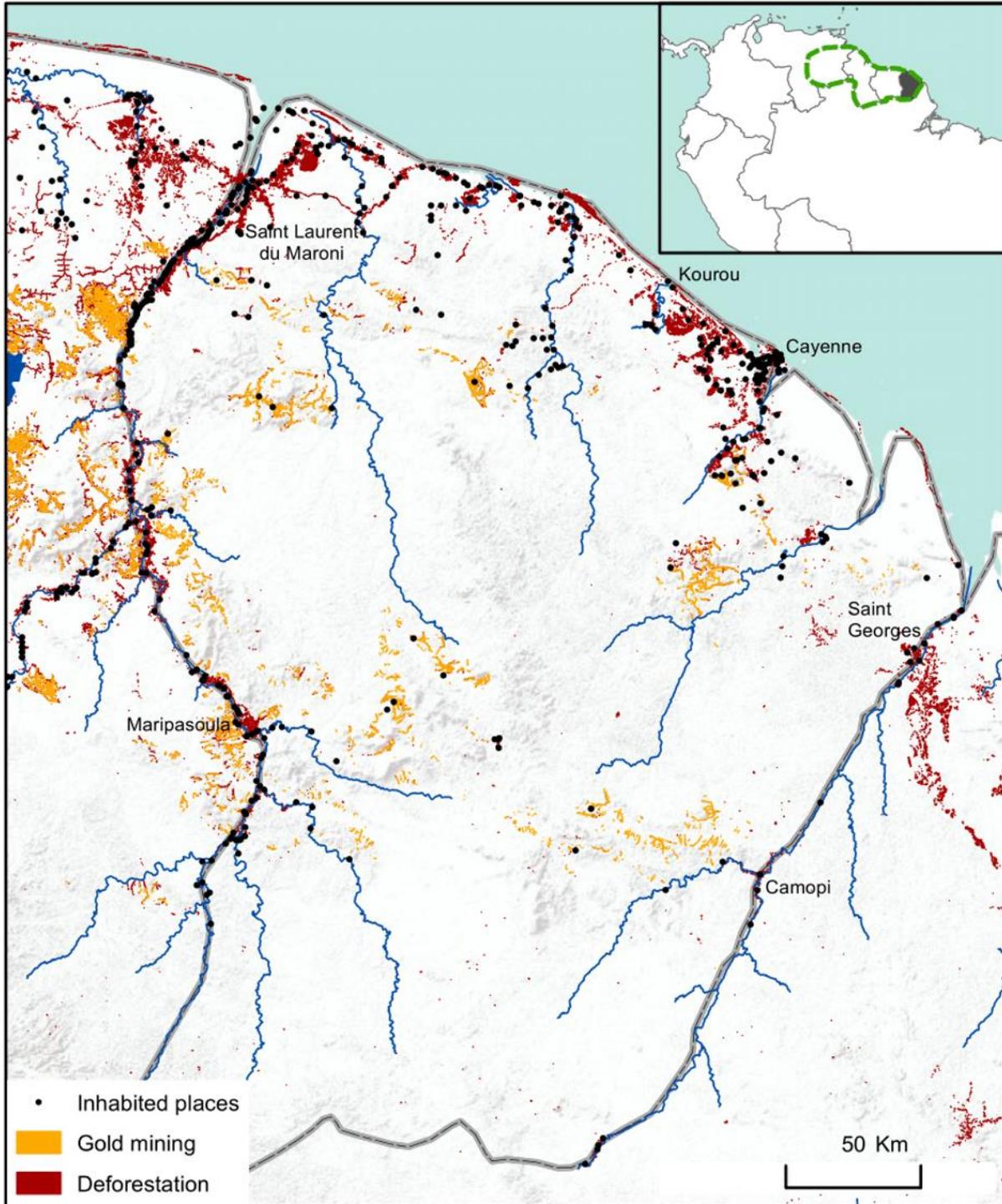


Figure 3: Map of the study area indicating the main human impacts threatening freshwater biodiversity in French Guiana. Deforestation and Gold-mined surfaces were extracted from landsat images (Hansen *et al.* 2010; WWF 2016; Rham *et al.* 2017). Inhabited places were obtained from Geonames website. The inset map on the right indicates the location of the study area in South America. The Guiana Shield is delimited with dashed green lines.

Despite representing the largest area of un-fragmented rainforest in the world, French Guiana is facing an unprecedented rise of human threats (Figure 3) due to deforestation for agriculture, gold-mining and urbanization, like the rest of the Guiana shield (Rham *et al.* 2017).

In the last decades, gold-mining activities have increased with the rise in the gold price (Hammond *et al.* 2007). Even though deforestation induced by gold-mining still represents a low proportion compared to other regions (Rham *et al.* 2017), its rapid expansion is alarming. Deforestation induced by mining activities increased from more than 40 km<sup>2</sup> of forest in 2001, to nearly 115 km<sup>2</sup> in 2006 (Hammond *et al.* 2007). For instance, in the sampling sites used for this work (see below), the percentage of deforested surfaces upstream of the sites for gold-mining increased considerably (Figure 4) between 2000 and 2015. Mining activities are developed through legal enterprises or illegal small-scale mining. Besides the impact of deforestation for roads or infrastructure on the surrounding vegetation, this activity has a detrimental effect on the benthic habitat and turbidity levels (Mol and Ouboter 2004; Dedieu *et al.* 2014). These consequences deeply affect community structure even after the cessation of the mining activity (Brosse *et al.* 2011; Tudesque *et al.* 2012). Furthermore, the mercury used to amalgamate the gold is accumulated downstream and bio-amplifies through trophic transfer (Hammond *et al.* 2007).

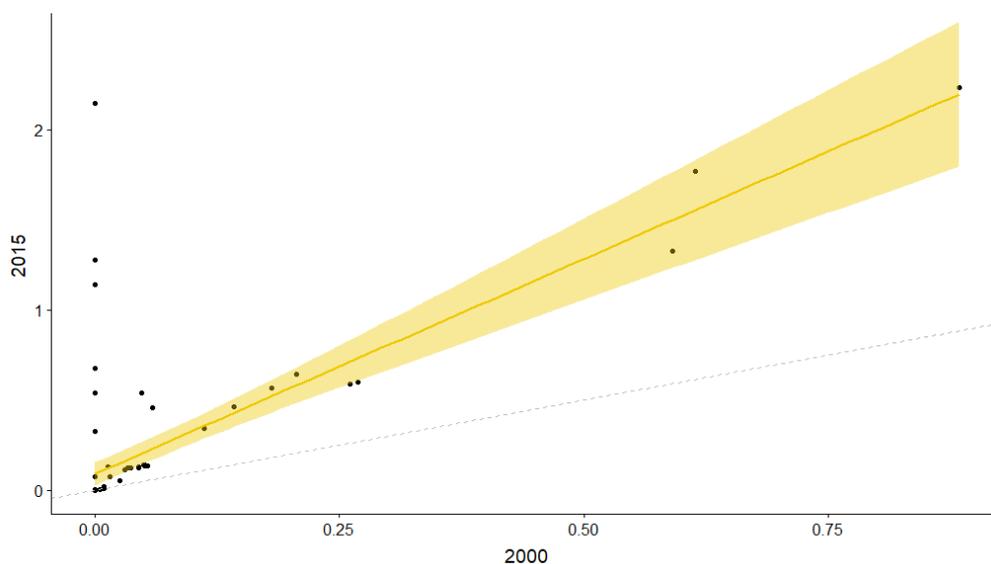


Figure 4: Comparison of the percentage of surfaces deforested for gold-mining between 2000 and 2015 across French Guiana. The percentage of deforested surfaces was calculated within the sub-basin area upstream of our sampling sites (see Figure 6).

## 2) *Sampling issues*

Unfortunately, there is a technical issue in French Guiana for sampling freshwater fish communities. Traditional sampling methods are destructive (Hubert *et al.* 2012) or inefficient and vary between streams and rivers (Allard *et al.* 2014; Cilleros *et al.* 2018). Small streams have been usually sampled using rotenone or electrofishing. However, rotenone is banned in

the territory because it is destructive and the low conductivity of the Guianense water makes electro-fishing inefficient. For rivers, the current fish sampling method is gillnet sampling. This method corresponds to passive captures based on the movement of fishes and is thus species selective (Murphy and Willis 1996; Cilleros *et al.* 2018). In fact, the probability of catching fishes with this method will vary depending on species morphology (small species are not caught and species with high bodies and prickly teeth or fins have a higher probability of catching than elongated species) and behavior (gregarious and mobile species have a higher probability of capture than solitary and less mobile species). Moreover, gill nets are also habitat selective since they can be only installed in deep and stagnant waters. Finally, both types of sampling methods collect fishes in a limited range of habitats, giving partial inventories of the fauna (Cilleros *et al.* 2018).

The environmental DNA (eDNA) metabarcoding approach has been claimed as a promising tool for measuring biodiversity (Taberlet *et al.* 2012). In aquatic systems, the method involves capturing DNA molecules that flow in the water. The obtained DNA is extracted, amplified, sequenced and assigned to species by comparing the DNA sequences to a reference molecular database (see Figure 5). Ultimately, species inventories can be built according to the detected species.

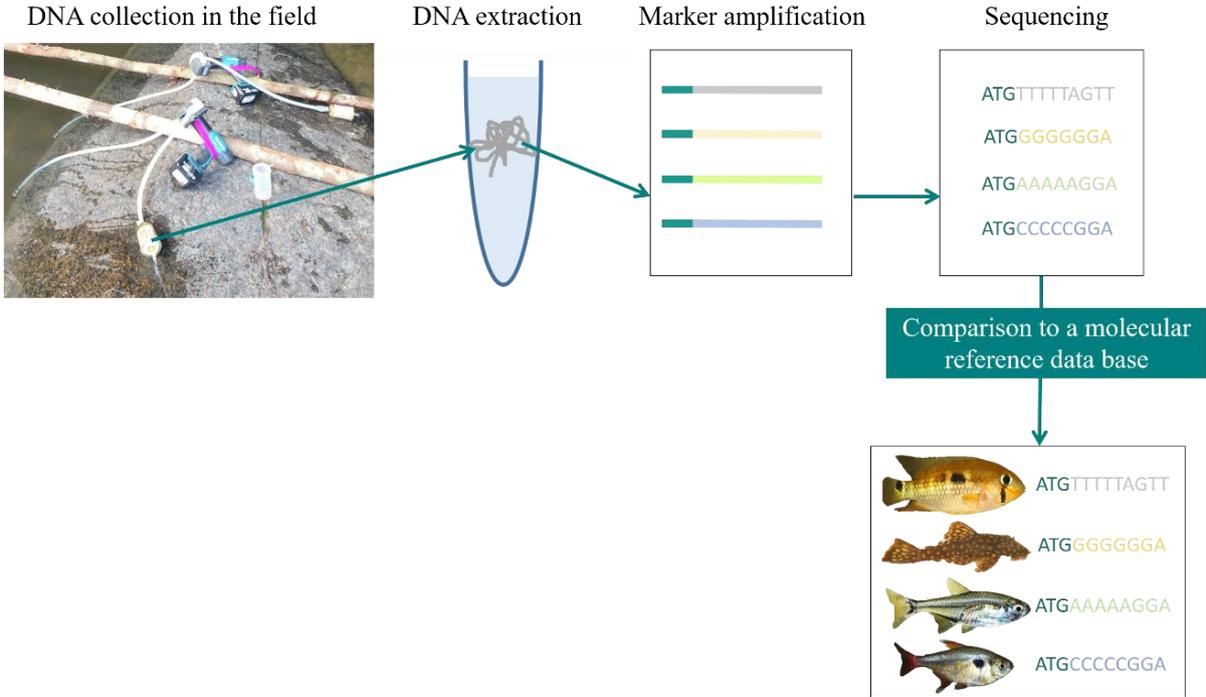


Figure 5: Illustration of the main steps of the eDNA procedure. Water is filtrated to collect the DNA released by organisms. The DNA is extracted, amplified using taxa specific primers and sequenced. The obtained reads are filtered using bio-informatic analyses and assigned to species present in a reference database according to a similarity threshold.

In spite of a wide use in temperate rivers and streams (Civade *et al.* 2016; Valentini *et al.* 2016; Pont *et al.* 2018), the method is still under development in other ecosystems, such as tropical ecosystems. In Guianese streams and rivers, the method has proved to be efficient (Cilleros *et al.* 2018; Jerde *et al.* 2019). Preliminary tests in French Guiana were performed, in which 39 freshwater fish communities were sampled using the protocol designed by Valentini *et al.* (2016) for temperate rivers. These tests showed that one water sample permitted to detect a substantial part of the fauna without erroneous detections (*i.e.* species not expected to occur in the detected sites according to their known habitat preferences and watershed occurrence). However, this standard protocol did not permit to detect the whole fish fauna of the studied sites (Cilleros *et al.* 2018) compared to traditional methods. We hence hypothesized that increasing the sampling effort will enhance detection rates. Indeed, some aspects of the method remain poorly evaluated even in temperate ecosystems. The growing interest in this method resulted in the development of a plethora protocols for each step of the eDNA procedure and the protocol choice may influence the detection of aquatic species (Goldberg *et al.* 2016). Despite an extended literature on optimizing the analyses of eDNA samples to improve detection performance (marker choice, extraction, sequencing and bioinformatics protocols), the sampling effort (*i.e.* the volume of sampled water) has benefitted from much less attention. This results in a high variability in sampling efforts across studies (ranging from few centiliters to tens of liters), making comparisons between studies difficult and raising uncertainties about the completeness of such inventories. Thus, this method needs to be optimized to be used for the assessment of human impact on tropical rivers and streams.

## VIII) Objectives

The main objective of this work is to better understand how deeply anthropogenic disturbances are affecting fish communities in Amazonian streams and rivers. A community ecology approach is proposed to define how anthropogenic activities are affecting taxonomic and functional diversity patterns, as well as ecological processes, in which the longitudinal connectivity of freshwater ecosystems is considered. This work was performed using a non-invasive sampling method that allows to equally sampling streams and rivers across French Guiana.

This work is divided in 4 parts:

1) *Optimization of the eDNA method for sampling species-rich communities in tropical rivers*

The aim of this part is to determine the sampling effort (filtered water volume) needed to get optimal inventories of fish assemblages in species-rich tropical streams and rivers using eDNA. Ten eDNA replicates were collected in six sites (Figure 6) following the protocol developed by Valentini *et al.* (2016) for temperate rivers. Each replicate was collected by filtering water for 30 minutes, corresponding to 34 liters of filtered water. Specifically, we sought to define the optimal sampling effort to describe communities through three diversity descriptors: species richness, dissimilarity of species composition and community structure patterns among sites. Additionally, we compared eDNA inventories with capture-based inventories collected in the same sites. The sampling, laboratory and bio-informatic protocols validated during this study achieved in 2016, were then used with the optimal sampling effort for the rest of the 81 other study sites sampled in 2017.

2) *Definition of the spatial extent and strength of anthropogenic impacts on fish biodiversity in rivers*

The main goal of this part was to measure the strength of anthropogenic impacts on fish taxonomic diversity and functional diversity in riverine habitats. We also investigated the optimal spatial extent to measure upstream anthropogenic effects on local fish fauna. The eDNA technique validated in Chapter 2 was used to collect data from 50 river sites (see Figure 6). The intensity of anthropogenic impacts was calculated by summing deforested surfaces due to gold-mining, agriculture and urbanization extracted from GIS data. Therefore, we used a global deforestation variable that accounts for the effects of those three types of human impacts.

Upstream deforestation intensity at each site was then calculated at different spatial extents by widening the spatial extent in which deforestation surfaces were calculated, from the immediate vicinity of the site (0.5 km upstream) to 150 km upstream. From this Chapter the molecular reference database developed by (Cilleros *et al.* 2018) was actualized. In 2016, I collected tissue from 264 fish individuals across French Guiana. This allowed to add 158 individuals and 24 species to the reference database, which includes now 255 species and 661 individuals.

3) *How anthropogenic impacts modify diversity patterns and ecological processes?*

The aim of this part was to compare the effects of two environmental gradients, a deforestation gradient and the upstream-downstream gradient, on fish diversity patterns but also on the ecological processes shaping fish communities. We used 50 river sites and 37 stream sites (Figure 6) sampled with the eDNA protocol validated in Chapter 2. The deforestation intensity upstream from our fish sampling sites was calculated using the method developed in the Chapter 3. Ecological processes were assessed at local scale by analysing the relationships between taxonomic and functional richness. These relationships were confronted to null models simulating random species assembly, which permitted to test the hypothesis that deforestation constitutes a strong environmental filter and therefore drive assemblages toward non-random functional and ecological clustering (see Chapter 1, part V). Rivers and streams sites were considered separately given that stream and river fauna and environment significantly differ.

4) *How anthropogenic impacts modify the functional structure of fish communities?*

In this part, the multifaceted effects of anthropogenic impacts on the functional structure of fish communities were described. Thus, we deepened the results of Chapter 4 by not only considering the effect of anthropogenic disturbances on functional richness, but also on the overall functional structure of assemblages including different facets such as functional richness, divergence, evenness and identity as proposed by Villéger *et al.* (2008) and Mouillot *et al.* (2013). As in Chapter 3, we used 50 river sites and 37 stream sites (Figure 6) sampled using the eDNA protocol validated in Chapter 2 and we analyzed separately stream and river communities. Deforestation intensity upstream from our fish sampling sites was calculated using the method developed in the Chapter 3.

## IX) Data description

### 1) Sampling sites

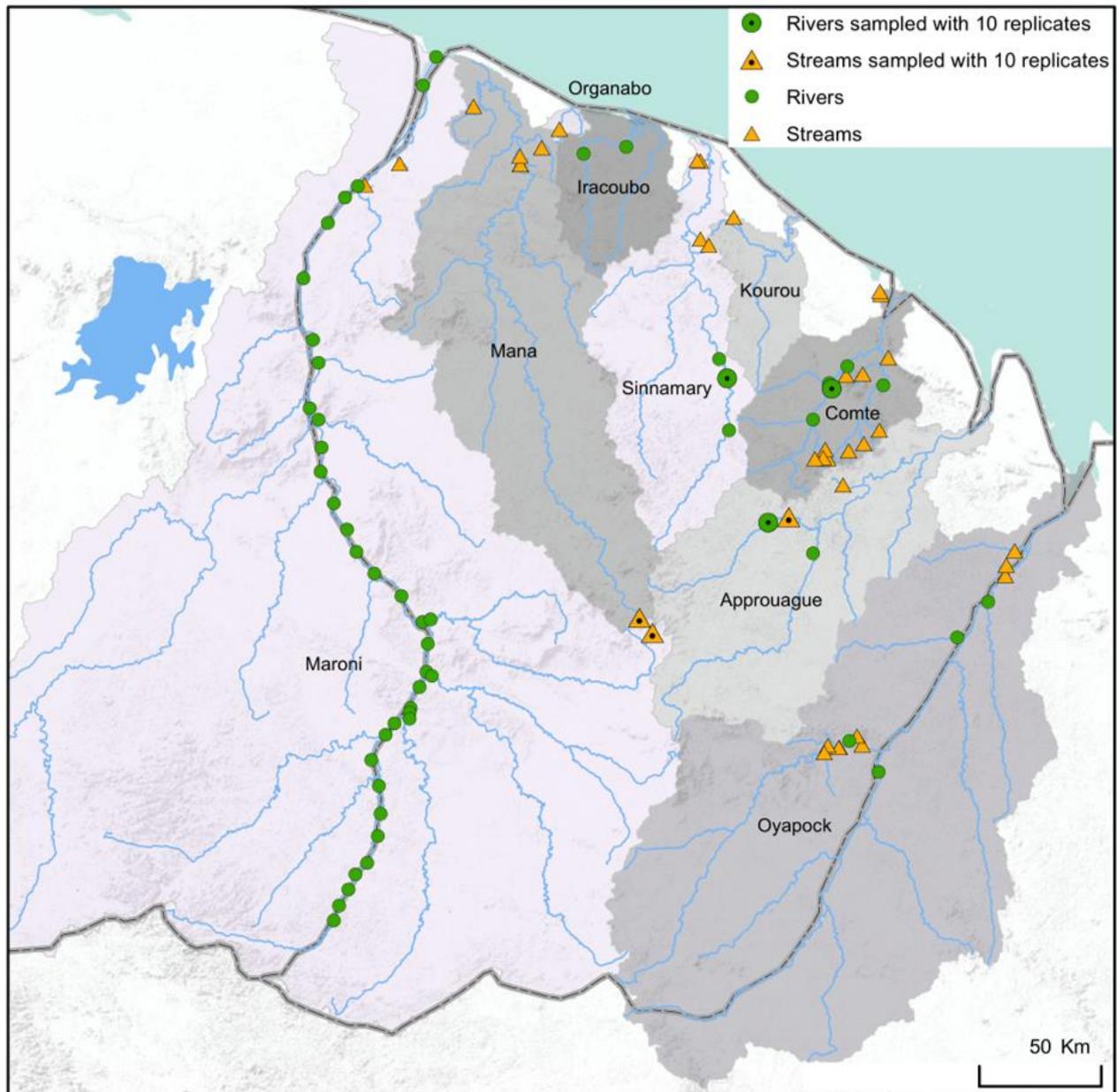


Figure 6: Map of the study area indicating the 86 fish sampling sites used for this work. Orange triangles correspond to stream (N= 37) sites and green circles correspond to river sites (N= 50) sampled with one eDNA replicate. Black dots in the center of the symbols indicated the sites used in the Chapter 2, which were sampled with 10 replicates. For the Chapter 3 only river sites were used. For the Chapters 4 and 5 all sites were used. The 9 main river basins were indicated.

For this work, 87 sites were sampled during the dry season (September-November) across nine river basins of French Guiana from 2016 to 2017 (Figure 6). We sampled 50 river sites and 37 stream sites. The stream sites were less than 10 meters wide and 1 meter depth, while river sites were wider than 30 meters and deeper than 1 meter. This distinction between streams and rivers is frequently used to distinguish these two distinct environments where the

fauna and environmental conditions significantly (Dedieu *et al.* 2015; Allard *et al.* 2016). Moreover, sampling sites were selected to take into account undisturbed sites but also sites subject to human disturbances such as urbanization, agriculture and gold-mining. The sampling was funded by the TULIP and CEBA Labex, the DEAL Guyane, Office de l'Eau Guyane (Aquatic Metabarcoding project), SPYGEN (for more details see Appendix). The Parc Amazonien de Guyane and Hydreco provided logistical facilities to access to some of the sites. Of the 87 sites, I contributed to the sampling of 50 sites and the remaining 37 sites were sampled during a VigiLife project along the Maroni River.

## 2) Biodiversity measures

For each site, the number of detected species in the eDNA samples was used to measure taxonomic diversity. To describe the functional diversity of the sampled communities, morphological and ecological traits were attributed to the detected species using information from Fishbase (www.fishbase.org), the Atlas of fish species from French Guiana (Planquette *et al.* 1996; Le Bail *et al.* 2000) and fish pictures. We used two types of traits as they are complementary to measure the functional diversity of freshwater fish (Kuczynski *et al.* 2018a).

<i>Functional trait</i>	<i>Measure (ratio or categories)</i>	<i>Function</i>	<i>Type</i>	<i>References</i>
Maximum body length	Fish base (cm)	Synthetic: metabolism, trophic impacts, locomotion nutrient cycling		Toussaint et al. (52) and Blanchet et al. (36)
Body elongation	Bl/Bd			Reecht Yves (53)
Eye vertical position	Eh/Bd			Winemiller (54)
Body lateral shape	Hd/Bd			Toussaint et al. (52)
Pectoral fin vertical position	PFi/Bd	Locomotion	Morphological	Dumay et al. (55)
Pectoral fin size	PFl/Bl			Fulton et al. (56)
Caudal peduncle throttling	CFd/CPd			Webb (57)
Relative eye size	Ed/Hd			Boyle & Horn (58)
Oral gape position	Mo/Bd			Dumay et al. (55)
Relative maxillary length	Jl/Hd	Food acquisition		Toussaint et al. (52)
Relative barbell length	Bbl/Bl			Villéger et al. (59)
Territoriality	Yes, no			
Motility	Mobile, sedentary	Behavior		
Gregariousness	Gregarious, solitary		Ecological	Villéger et al. (59)
Position in the water column	Benthic, benthopelagic, pelagic	Habitat preference		
Preferred substrate	Hard, soft, none			

Table 1: Morphological and ecological traits and measures used to describe functional diversity for Chapter 2, 3 and 4. Their corresponding functions are indicated. See Su *et al.* (2019), Toussaint *et al.* (2016) and Villéger *et al.* (2010) for details on morphological measures.

For the morphological traits, 12 measurements (Figure 7) were achieved on side view pictures gathered during the last 10 years to compute 10 unit-less ratios reflecting two key

functions: locomotion and food acquisition (see Table 1 for details). Locomotion is related to habitat use, vertical position in the water column, hydro-dynamism, as well as fin use for manoeuvrability, propulsion and acceleration efficiency. Food acquisition considers the functional traits related to the size of food items, feeding method in the water column, filtering ability, prey detection and trophic position. We measured morphological traits on as much individuals as possible (1 to 20, according to the species) and used the average value of all measures per species. We did not consider intraspecific variability in morphological traits, because it has been found to be negligible in a recent study conducted using the same dataset (Toussaint *et al.* 2018). Additionally, the maximum body length of species obtained from Fishbase ([www.fishbase.org](http://www.fishbase.org)) was used to represent species maximal body size, which is considered as a synthetic functional trait (Blanchet *et al.* 2010). Thus, we used 11 continuous variables to characterize fish morphological diversity.

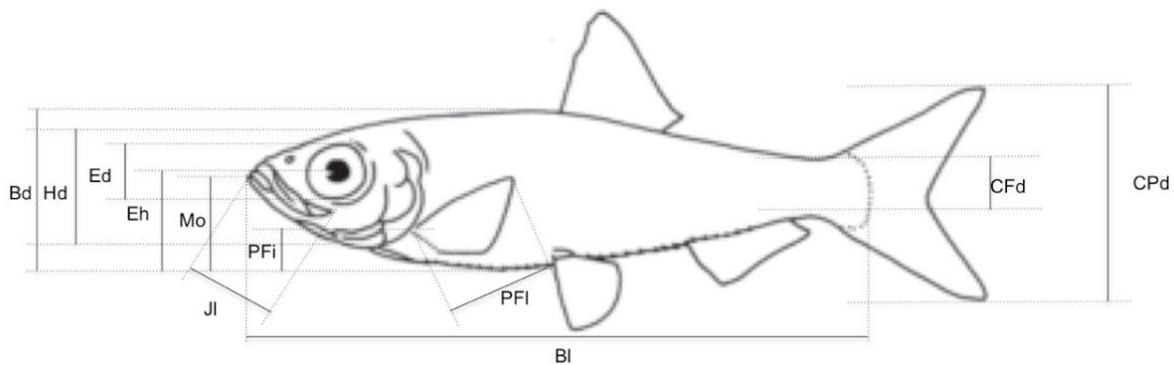
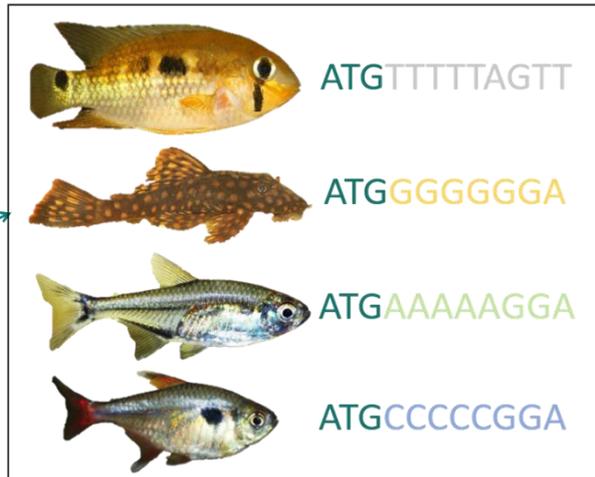


Figure 7: Morphological measures (from Toussaint *et al.* 2016).

For ecological traits, I compiled a database of ecological traits using Fishbase ([www.fishbase.org](http://www.fishbase.org)) and the Atlas of fish species from French Guiana (Planquette *et al.* 1996; Le Bail *et al.* 2000). The ecological database includes five qualitative traits related to trophy, behavior and habitat (see Table 1 for details) preference of 390 Guianese species.

The ecological and morphological traits were combined to build functional spaces and assess functional diversity through different facets according to the Chapter. Trait distances between all the species detected in each part of the study were calculated using Gower's distance, which considers different types of traits (here categorical and continuous) while standardizing them and handling missing data. The distance matrix was ordered into a multidimensional space using Principal Coordinates Analysis (PCoA). Then, a global functional space was built with the five retained axes of the PCoA, which accounted for 45% of total variance. The number of dimensions was chosen as the optimal number of axes determined according to (Maire *et al.* 2015).

## Chapter 2: Optimization of the eDNA method for sampling species-rich communities



### Optimizing environmental DNA sampling effort for fish inventories in tropical streams and rivers.

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## Abstract

Environmental DNA (eDNA) metabarcoding is a promising tool to estimate aquatic biodiversity. It is based on the capture of DNA from a water sample. The sampled water volume, a crucial aspect for efficient species detection, has been empirically variable (ranging from few centiliters to tens of liters). This results in a high variability of sampling effort across studies, making comparisons difficult and raising uncertainties about the completeness of eDNA inventories.

Our aim was to determine the sampling effort (filtered water volume) needed to get optimal inventories of fish assemblages in species-rich tropical streams and rivers using eDNA. Ten DNA replicates were collected in six Guianese sites (3 streams and 3 rivers), resulting in sampling efforts ranging from 17 to 340 liters of water.

We show that sampling 34 liters of water detected more than 64% of the expected fish fauna and permitted to distinguish the fauna between sites and between ecosystem types (stream *versus* rivers). Above 68 liters, the number of detected species per site increased slightly, with a detection rate higher than 71%. Increasing sampling effort up to 340 liters provided little additional information, testifying that filtering 34 to 68 liters is sufficient to inventory most of the fauna in highly diverse tropical aquatic ecosystems.

## Introduction

In recent years, environmental DNA (eDNA) metabarcoding has been claimed as a promising tool to estimate biodiversity and its change through time (Taberlet *et al.* 2012; Thomsen and Willerslev 2015; Keck *et al.* 2017). In particular, this technique is now employed to identify the free DNA released by organisms in their environment (Taberlet *et al.* 2012). In aquatic ecosystems, the use of eDNA has been widely developed during the last years and has turned from the detection of specific species of amphibians, fish, mammals, insects and crustaceans (Thomsen *et al.* 2012) to the detection of whole communities (Evans *et al.* 2017; Lopes *et al.* 2017; Civade *et al.* 2016; Hänfling *et al.* 2016; Olds *et al.* 2016; Valentini *et al.* 2016). The latter studies besides reconstructing entire aquatic communities of fishes and amphibians, compared the detection performance between eDNA metabarcoding and capture-based sampling methods used to collect specimens in streams and rivers. Through this, they showed that both methods provided similar or more complete species inventories, hence opening avenues to use this method for ecological and conservation studies.

Obtaining biodiversity inventories with eDNA metabarcoding requires several subsequent steps including: DNA sampling and collection, laboratory protocols (DNA purification, marker targeting and sequencing) bioinformatics analyses and taxonomic assignment of sequences. The growing interest in this method resulted in the development of a considerable variety of protocols for each step of the eDNA procedure (Goldberg *et al.* 2016). This makes comparisons between studies challenging considering that it has been illustrated that the choice of markers (Hänfling *et al.* 2016; Evans *et al.* 2017), DNA collection methods (Deiner *et al.* 2015; Eichmiller *et al.* 2016) and laboratory protocols (Deiner *et al.* 2015; Eichmiller *et al.* 2016; Evans *et al.* 2017) may influence the detection of aquatic species. Furthermore, the environmental conditions and the targeted taxon can also affect detection rate because eDNA release varies among taxa (Deiner *et al.* 2015; Mächler *et al.* 2016) and water physiochemical factors may impact eDNA degradation (Barnes *et al.* 2014). Therefore, the performance of biodiversity detection in the water depends on a combination of protocols choice, as well as the environmental conditions and the targeted taxonomic group.

Despite an extended literature about the optimization of eDNA samples analysis to improve detection performance, less attention has been paid to how eDNA sampling design can be optimized. Consequently, there is a wide range of variation in the volume of sampled water among studies, ranging from a few centiliters to tens of liters (Mächler *et al.* 2016).

Nonetheless, sampling effort is a fundamental aspect for any ecological study or monitoring procedure (Gotelli and Colwell 2001) and might deeply affect results and interpretations. Some eDNA studies suggested that increasing the volume of sampled water improved the quality of the biodiversity assessment. For example, detection rates of anurans in tropical streams were higher when increasing sampling effort from 20 to 60 liters of water (Lopes *et al.* 2017). Moreover, Mächler *et al.* (2016) found a significant positive relationship between the sampled water volume and the detection rate for a macro-invertebrate species. In spite of this, due to financial and technical limitations, a threshold must be fixed in order to optimize eDNA inventories. This consists in determining the best compromise between sampling effort (and its associated financial and time costs) and accuracy of the biodiversity estimate.

Recently, the sampling effort needed to accurately estimate the fish species richness in temperate lakes has been assessed using spatial replicates and revealed that 5 to 20 liters of water were needed to detect the entire fish fauna (Hänfling *et al.* 2016; Evans *et al.* 2017). However, to date, the optimization of the eDNA sampling effort for the assessment of the whole community diversity in running waters (streams and rivers) has never been assessed. A better understanding of this effect will allow optimizing sampling efforts without reducing diversity estimates. For instance, Nascimento *et al.* (2018) found that the volumes of sampled sediments strongly impacted diversity assessments of benthic eukaryotic communities. The stakes of this understanding will be higher in tropical ecosystems, where large sampling efforts are often needed (Schneck and Melo 2010). Indeed, describing tropical communities can be challenging given the wide range of species diversity they host (Albert and Reis 2011), and the strong contribution of rare species to tropical biodiversity and ecosystem functioning (Mouillot *et al.* 2013, 2014).

The aim of this study was to determine the optimal sampling effort for fish inventories using eDNA metabarcoding in tropical streams and rivers. We built on preliminary tests in French Guiana in which 39 freshwater fish communities were sampled using the protocol designed by Valentini *et al.* (2016) for temperate rivers. Those tests showed that one water sample of *ca.* 50 liters permitted to detect a substantial part of the fauna without erroneous detections (Cilleros *et al.* 2018) (*i.e.* species not expected to occur in the sampled sites according to their known habitat preferences and watershed occurrence). Nevertheless, the standard protocol designed by Valentini *et al.* (2016), did not permitted to detect the whole fish fauna of the studied sites (Cilleros *et al.* 2018) comparing with traditional methods. We hence

hypothesized that increasing sampling effort will enhance detection rate. To test this, we filtered water in four highly diverse Guianese streams and rivers using the VigiDNA 0.45 µm; SPYGEN filtering system. In each site we took 10 replicates. Each replicate was collected by filtering for 30 minutes, corresponding to 34 liters of filtered water (standard protocol). We then analyzed how sampling effort (from 34 to 340 liters) affects the estimation of fish biodiversity. Specifically, we sought to define the optimal sampling effort to describe communities through three diversity descriptors: species richness, dissimilarity of species composition and community structure patterns between sites. In addition, two sites were sampled for half of the time (relaxed protocol) than the other four sites to test whether reducing the filtering volume to 17 liters per replicate will degrade the diversity estimates (due to a lower filtered volume), or will improve the results as increasing filtering time can increase the accumulation of PCR inhibitors in the filter (Matheson *et al.* 2014).

## **Materials and methods**

### *eDNA sampling*

This study was conducted in French Guiana in November 2016 (during the dry season). This territory is subjected to an equatorial climate, and is covered by a dense primary rainforest. Freshwater bodies in this country host nearby 405 fish species (Le Bail *et al.* 2012), making Guianese freshwater ecosystems and excellent place to optimize eDNA sampling effort in species-rich communities. Six sites corresponding to three small streams and three rivers, were sampled (See Figure 6 in Chapter 1-VIII). Stream sites (S1, S2, and S3) are less than 10 meters wide and 1 meter depth whereas river sites (R1, R2, and R3) are wider than 30 meters and deeper than 1 meter. Those sites belong to distinct watersheds (Mana (S1); Maroni (S2); Comté (R1); Sinnamary (R2); Approuague (S3, R3)). They are free from human settlements upstream and are therefore little affected by human activities (See supplementary Table S1 for more details on localities and their characteristics).

At each site, 10 filtrations were performed in the same place, resulting in 10 field replicates per site. Each filtration was done following Valentini *et al.* (2016) protocol for running waters. Per replicate, we filtered 34 liters of water during 30 minutes in four sites (S1, S2, R1 and R2). In two complementary sites (S3 and R3) we filtered 17 liters of water during 15 minutes. This resulted in two different treatments called “standard protocol” and “relaxed

protocol” respectively. This permitted to test if filtering volume can be optimized without decreasing detection performance. For each replicate, a peristaltic pump (Vampire sampler, Burlke, Germany) and a single-use tubing were used to pump the water into a single-use filtration capsule (VigiDNA 0.45  $\mu\text{m}$ ; SPYGEN, le Bourget du Lac, France). The input part of the tubing was placed few centimeters below the surface in zones with high water flow as recommended by Cilleros *et al.* (2018). Sampling was achieved in turbulent area (rapid hydromorphologic unit) to ensure an optimal homogenization of the DNA throughout the water column. To avoid DNA contamination among sites, the operator always remained downstream from the filtration area and stayed on the bank (for streams) or on emerging rocks (for rivers). At the end of the filtration, the filtration capsule was emptied of water, filled with 80 mL of CL1 conservation buffer (SPYGEN) and stored in individual sterile plastic bags kept in the dark. Samples were then stored at room temperature for less than one month before DNA extraction.

Code	Site name	Watershed	Width (m)	Latitude	Longitude	Distance from the source (km)	Sampling protocol
S1	Crique à l’est	Mana	3.4 - 3.97	3.66	-53.22	4.1	Standard
S2	Point chaud	Maroni	4.1 - 10	3.61	-53.17	8.3	Standard
S3	Crique Museum	Approuague	1.7 - 5.5	4.04	-52.68	4.7	Relaxed
R1	Lysis	Comté	45 - 55	4.51	-52.51	89.3	Standard
R2	Saut dalles	Sinnamary	30 - 40	4.55	-52.90	124.6	Standard
R3	Aratai	Approuague	30 - 40	4.03	-52.70	95.5	Relaxed

Table 1: Site characteristics: site local name, watershed membership, average width in meters, site position (WGS84) and distance from the source in kilometers. The standard sampling protocol consists in collecting eDNA from 34 liters of filtered water whereas the relaxed protocol consists in collecting eDNA from 17 liters of filtered water.

### *Laboratory and bioinformatics analyses of eDNA*

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 3M sodium acetate were added to each 50-mL tube and stored for at least one night at  $-20^{\circ}\text{C}$ . The tubes were centrifuged at 15 000  $\times g$  for 15 min at  $6^{\circ}\text{C}$ , and the supernatants were discarded. After this step, 720  $\mu\text{L}$  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  $\mu$ L 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  $\mu$ L of SE buffer twice. Four negative extraction controls were also performed. They were amplified and sequenced in the same way as and in parallel to the field replicates to monitor possible laboratory contaminants. After the DNA extraction, the samples were tested for inhibition by qPCR following the protocol in Biggs *et al.* (2015) If the sample was considered inhibited, it was diluted 5-fold before the amplification.

We performed DNA amplifications in a final volume of 25  $\mu$ 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 MgCl<sub>2</sub>, 0.2 mM of each dNTP, 0.2  $\mu$ M of “teleo” primers (Valentini *et al.* 2016) and 3  $\mu$ L of DNA template. We also added human blocking primer for the “teleo” primers with a final concentration of 4  $\mu$ M and 0.2  $\mu$ g/ $\mu$ L of bovine serum albumin (BSA, Roche Diagnostic, Basel, Switzerland) to the mixture. We performed 12 PCR replicates per field replicate. 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 done in a room dedicated to amplified DNA with negative air pressure and physical separation from the DNA extraction rooms (with positive air pressure). We also amplified the four negative extraction controls and three PCR negatives controls (with 12 replicates as well) and sequenced them in parallel with the 720 PCR replicates (6 sites, 10 field replicates per site and 12 PCR replicates per field replicate). We pooled the purified PCR products in equal volumes to achieve an expected sequencing depth of 500,000 reads per sample before the libraries 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 HiSeq 2500 (2x125 bp) (Illumina, San Diego, CA, USA) and the HiSeq SBS Kit v4 (Illumina, San Diego, CA, USA) following the manufacturer's instructions at Fasteris facilities (Geneva, Switzerland).

The sequence reads were analyzed using the programs in the OBITools package (<http://metabarcoding.org/obitools> (Boyer *et al.* 2016)) following the protocol described in

Valentini *et al.* (2016). The ecotag program was used for the taxonomic assignment of molecular operational taxonomic units (MOTUs) using a threshold of 98% of identity with the reference database available from Cilleros *et al.* (2018), that counts 130 Guianese fish species. The GenBank nucleotide database was checked but Guianese fishes being poorly informed (most of the sequences are from Cilleros *et al.* (2018)), it did not provided additional information in our case. We discarded all MOTUs with a frequency of occurrence below 0.0003 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) and Taberlet *et al.* (2018).

#### *Comparisons with traditional capture-based methods*

All the capture-based samplings were achieved during the dry season from 2008 to 2016 as part of research and biodiversity management programs supported by the French ministry of environment (DEAL), the French Guyana National park (PAG), and the French National Center for Scientific Research (CNRS). Stream fishes were sampled using rotenone, following the protocol described by Allard *et al.* (2016). Riverine fishes were sampled using a standardized gill-net protocol designed by Tejerina-Garro and De Mérona (2001). Since neither rotenone nor gill-net samples provide an exhaustive image of the fish fauna (Cilleros *et al.* 2018), we combined local inventories using gillnets and rotenone available in each site and eDNA results to estimate the overall fauna inhabiting each site.

Additionally, we compared the occurrence of species in the eDNA replicates with the commonness of the species. Since absolute commonness values are not available, the percentage of occurrence of each species in the watershed was used as a surrogate to species commonness (species occurring in more than 50% of the sampling occasions) or rarity (species occurring in less than 50% of sampling occasions) (Gaston, K. J 1994). More specifically, we compared in each site, the percentage of eDNA replicates in which a species was detected against the percentage of sites in which the species was captured though all the capture-based sampling campaigns ran since 2008 in the stream or river stretches of the considered watershed for stream and river eDNA sites, respectively. In streams, the captures were performed in 25, 50, and 34 sites in the Mana, Maroni and Approuague watersheds, to which sites S1, S2 and S3 belong, respectively. In rivers, the captures were performed in 31, 26, and 36 sites in the Comté,

Sinnamary and Approuague watershed, to which sites R1, R2 and R3 belong, respectively.

### *Statistical analyses*

The obtained sequences were used to build a presence/absence matrix per field replicate and per site, in which only taxa detected to the species level were incorporated. Species accumulation curves (Gotelli and Colwell 2001) with confidence intervals were drawn for each site using the *speccacum* function to examine the impact of replication on the number of species detected. Additionally, expected species richness and confidence intervals were calculated for each site using the Chao II estimator (Chao 1989). This allowed to estimate the detection rate (*i.e.* the percentage of detected fauna with the eDNA) according to sampling effort (from one to ten replicates per site). The dissimilarity in species composition among replicates was assessed by calculating pairwise Jaccard's distances with the *vegdist* function. Then, the dissimilarity values were ordinated using non-metric multidimensional scaling (NMDS) to visualize how replicated eDNA data discriminate sites and habitat (streams vs. rivers) patterns and to determine the sampling effort needed to identify community changes among sites. Differences in species compositions between sites and habitat types were statistically tested by permutational analysis of similarities (ANOSIM). This analysis tool allows to test the statistical significance of dissimilarity between groups comparing to the within groups dissimilarity using the rank of dissimilarity values (Clarke 1993). All the statistical analyses were performed in R (R Core Team 2016) using the vegan package version 2.4-4 (Oksanen *et al.* 2013).

## **Results**

### *Total biodiversity detected*

In total, 40,838,558 reads were obtained. After the bioinformatic filtering (see Materials and Methods) 22,488,969 reads were retained, corresponding to 55.1% of the total reads. We found reads in all of the 720 PCR replicates while no reads were found in the extraction and PCR controls. Among all the sites and replicates, we detected 106 species, seven genus (*Bryconops*, *Guianacara*, *Krobia*, *Laimosemion*, *Leporinus*, *Moenkhausia*, *Pimelodella*) and two families (Characidae, Hypopomidae). A total of 279 species occurrences were detected in the six sites. Among those occurrences, only 5 (1.8%) were not consistent with the known distribution of the

species per watershed and habitat preference. The total number of species detected per site, when summing across the 10 replicates, ranged from 21 to 60, which accounts for 57 to 83% (on average 71%) of the local fauna derived from fish surveys using both capture-based and eDNA samples (see Materials and Methods) in each site (Figure 1). A proportion of the undetected species using eDNA are not informed in the molecular reference database (on average 19% of the fauna), but some species were not detected although referenced in our reference database (on average 10% of the fauna). This explains why Chao II estimated a lower species richness than the combined eDNA and capture-based inventories. Nevertheless, Chao II estimations of species richness using eDNA samples remained consistent with the combined eDNA and capture-based inventories (Figure 1).

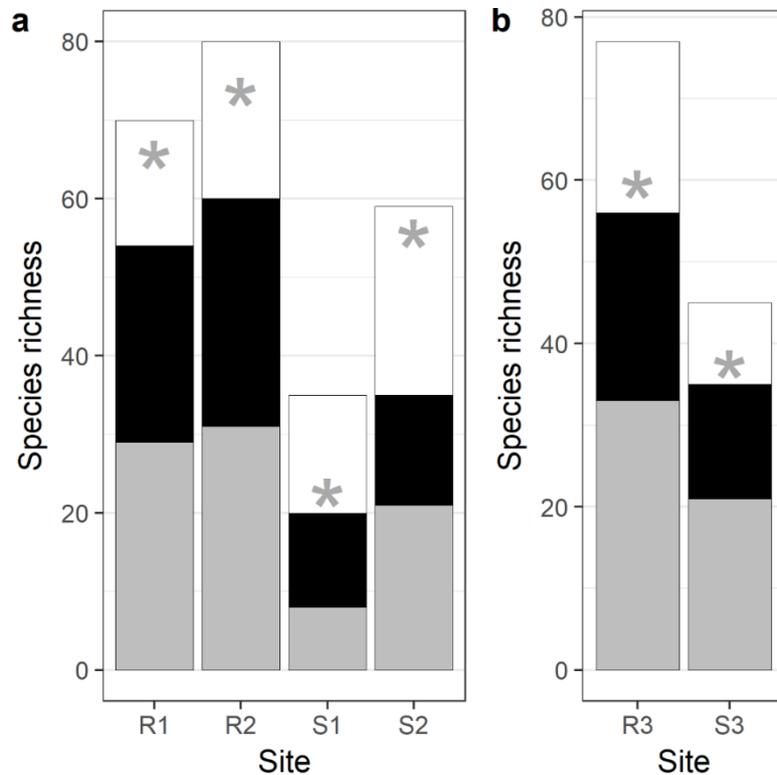


Figure 1: Species richness per site detected with traditional capture-based and eDNA metabarcoding methods with the standard (a) and relaxed (b) protocols. The species caught only with traditional methods are indicated with white, those detected only with eDNA are indicated with grey, and those detected by both eDNA and traditional methods are indicated with black. The Chao II estimation of species richness using eDNA samples is indicated with grey asterisk. R1, R2 and R3 are river sites and S1, S2 and S3 are stream sites.

#### *Replication effects on detected species richness*

Under the standard protocol, replicates provided consistent numbers of detected species, as shown by the narrow interquartile ranges in Figure 2a. This repeatability was particularly marked in stream sites where species richness differed by fewer than three species between replicates. For river sites, species richness varied by up to 10 species between replicates. The number of species found in the sites sampled under the relaxed protocol was less consistent among replicates, with a variation of up to 15 species between replicates for the stream site and up to 19 species between replicates for the river site (Figure 2b).

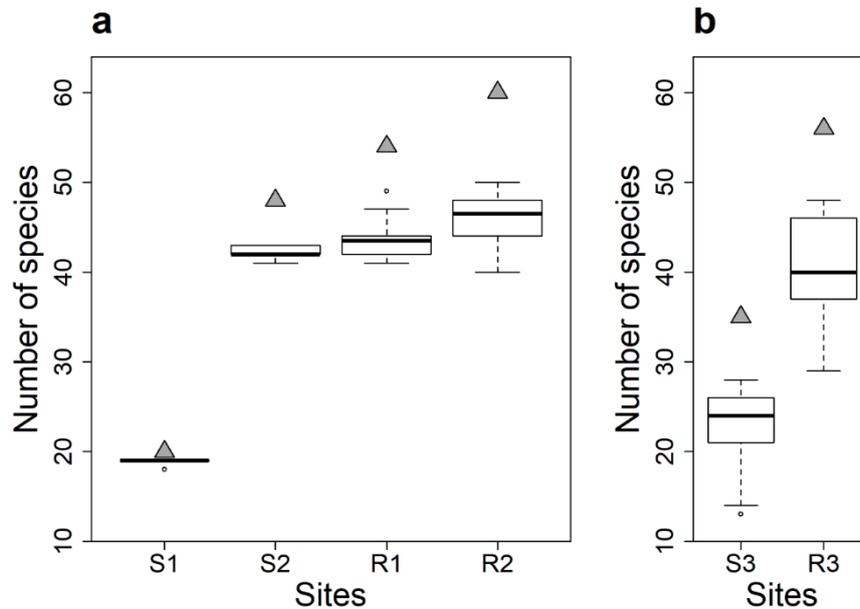


Figure 2: Number of detected species among the ten replicates for each site. Boxplots indicate the number of detected species per replicate. Triangles indicate the total number of species detected in each site (combining the 10 replicates). (a) Sites sampled under the standard protocol. (b) Sites sampled under the relaxed protocol. R1, R2 and R3 are river sites and S1, S2 and S3 are stream sites.

With one replicate, detection rate represented 64-95 % of the Chao II estimation of expected species richness (Figure 3). Using the standard protocol, a single replicate detected, on average, 67% of the expected richness in rivers and 87% of the expected richness in streams. Using the relaxed protocol, detection rate was lower in the stream site (*i.e.* 79%), but remained similar to that obtained with the standard protocol in the river site (*i.e.* 69%). Adding a second replicate slightly increased detection rate in sites sampled under the standard protocol, with a gain of less than 4% and 7% in species richness for stream and river sites, respectively (Figure 3a-d). In contrast, under the relaxed protocol, adding a second replicate increased detection rate by more than 10% (Figure 3e-f). Finally, increasing sampling effort from three to 10 replicates marginally affected the estimates of species richness using the standard protocol, whereas a substantial gain of species was still observed when increasing the sampling effort with the relaxed protocol. In the latter case, species accumulation curves did not saturate from one to 10 replicates (Figure 3e-f), while a species saturation was obtained until the second replicate using the standard protocol (Figure 3a-d). In addition, confidence intervals of the estimated species richness with the relaxed protocol were larger than those obtained using the standard protocol. This indicates that the standard protocol consistently detected similar species richness in the 10

replicates whereas substantial variations among replicates were observed using the relaxed protocol.

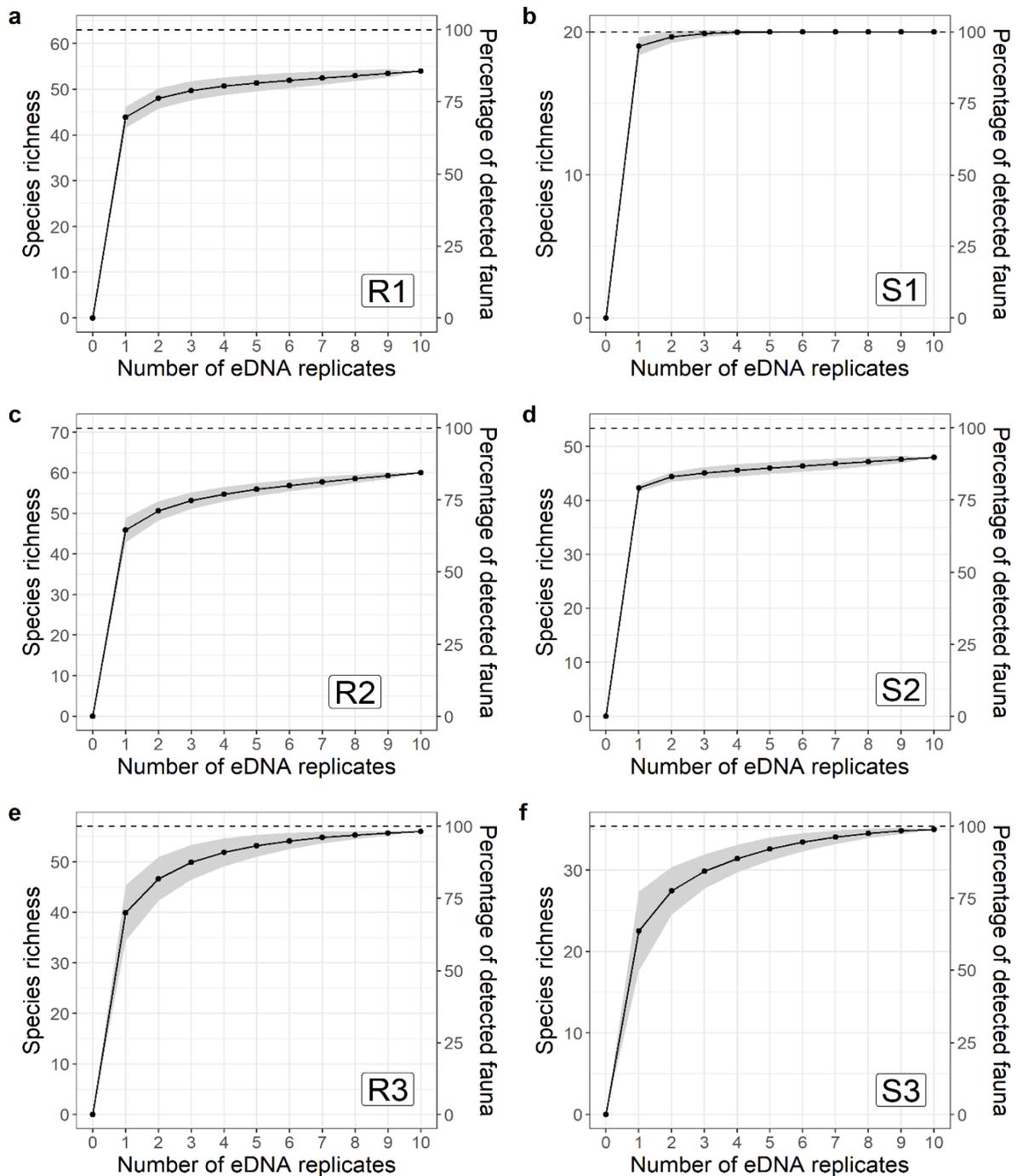


Figure 3: Species accumulation curves (solid lines) with increasing number of replicates for sites sampled under the standard protocol (a-d) and the relaxed protocol (e-f). River sites are on the left and stream sites on the right. Confidence intervals are represented by the shaded area. Estimated species richness with the Chao estimator are indicated with a dashed line. The percentage of detected fauna per replicate according to the Chao estimator is represented on the right axis.

### *Species composition among replicates*

The differences in species composition between replicates were low for the sites sampled under the standard protocol (Figure 4a). Pairwise Jaccard's dissimilarity indices ranged from 0.07 to 0.32 for rivers (mean= 0.22) and from 0 to 0.19 (mean= 0.17) for streams, with significantly higher dissimilarity values between replicates in rivers than in streams (Kruskal-Wallis rank sum test:  $\chi^2 = 27.2$ ;  $p < 2.2e-16$ ). On average, river faunas differed by 22% between replicates, whereas stream faunas differed by less than 17%. These results contrasted with those obtained using the relaxed protocol (Figure 4b), which showed a mean species dissimilarity between replicates higher than 30% for both stream and river sites. Accordingly, species dissimilarity between replicates was significantly higher with the relaxed protocol than with the standard protocol (Kruskal-Wallis rank sum test:  $\chi^2 = 149.76$ ;  $p < 1.8e-07$ ).

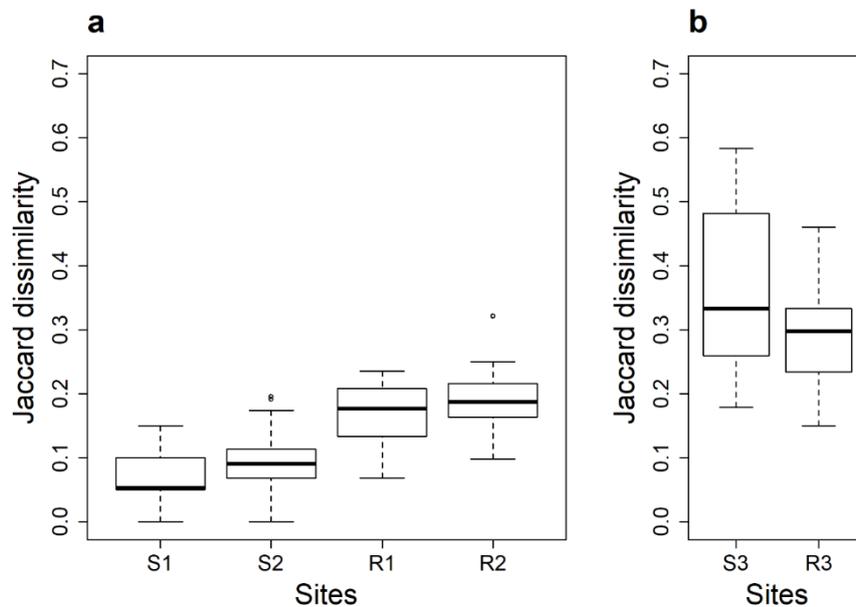


Figure 4: Pairwise Jaccard's distances between replicates for each site. Boxplots summarize species dissimilarity values (n=40 per site) among replicates. (a) Sites sampled under the standard protocol. (b) Sites sampled under the relaxed protocol.

The frequency of detection among the eDNA replicates was not influenced by the species commonness in any site. Indeed, common and rare species were systematically detected in all the replicates (Figure 5). Nevertheless, in the sites sampled under the standard protocol, most of the species that were detected in few eDNA replicates were rare species, given that they were captured in less than 50% of the traditional sampling campaigns. In contrast, using the relaxed

protocol, some common species (occurring in more than 60% of the capture-based sampling campaigns) were only detected in a few eDNA replicates.

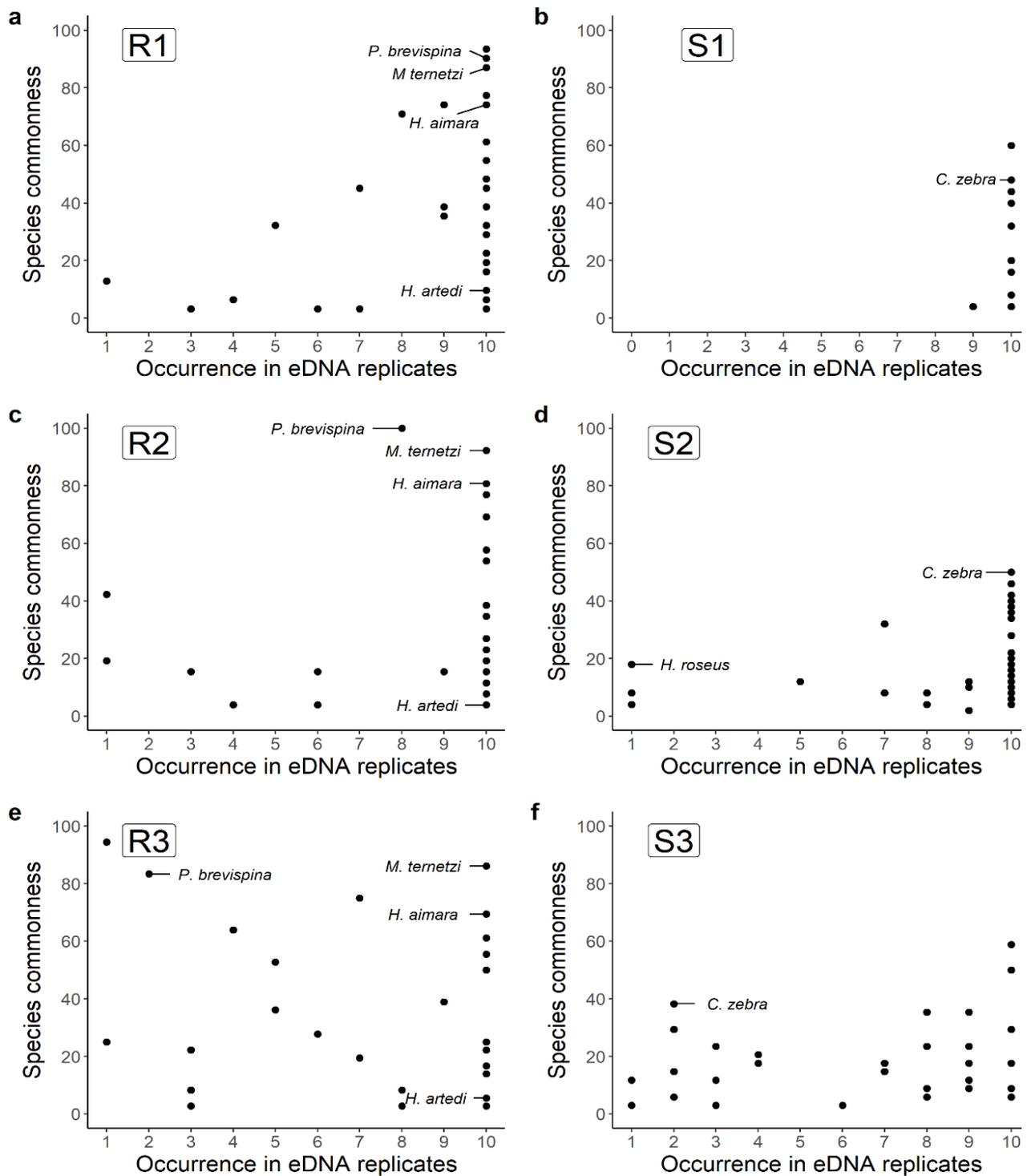


Figure 5: Relationships between the species occurrence in eDNA replicates and the species rarity. Species rarity was measured as the percentage of the occurrence of each species in all the capture-based samples ran in the stream (for stream eDNA sites) or river (for river eDNA sites) stretches of the considered watershed (see methods for details). Some species of interest are indicated on the figure. (a-d) Sites sampled under the standard protocol. (e-f) Sites sampled under the relaxed protocol. River sites are on the left and stream sites are on the right.

### *Distinguishing assemblages*

The first two axes of the NMDS provided a good two dimensional representation of the replicates according to their species composition (Figure 6a), as the stress of the plot was lower than 0.1 (Clarke 1993). The first axis discriminated between river replicates and stream replicates. The ordination distinguished sites, without overlap between replicates from different sites (ANOSIM statistic  $R = 0.996$ ;  $p < 0.001$ , Figure 6b), in spite of a more pronounced dispersion of the replicates collected under the relaxed protocol. Furthermore, the fish composition of the river sites were significantly distinct from those of the stream sites (ANOSIM  $R = 0.996$ ,  $p < 0.001$ , Figure 6c), as shown by the separation of the stream and river sites on the NMDS.

### **Discussion**

The eDNA metabarcoding approach has been claimed as an efficient tool to obtain inventories of aquatic organisms (Valentini *et al.* 2016), but the optimal sampling effort to get those inventories has never been investigated in running waters. Here we show that eDNA replicates not only have a high repeatability on the estimation of species richness but also on the identity of the species detected, which both exhibited slight variations among replicates. Besides, the fish fauna detected in each site was consistent with the one known from each river basin (Planquette *et al.* 1996; Le Bail *et al.* 2000, 2012) giving that the fish fauna of French Guiana is spatially structured into several freshwater ecoregions (Lemopoulos and Covain 2018). Moreover, our results are also consistent with the habitat preferences (streams vs rivers) of Guianese fishes (Planquette *et al.* 1996; Le Bail *et al.* 2000, 2012). The rare erroneous detections (1.8% of the detections) were already reported as the result of an incompleteness in our molecular reference database (Cilleros *et al.* 2018). Indeed, a few species not included in the molecular reference database were erroneously assigned to their closest relative available in the reference database. Furthermore, the fish fauna derived from the eDNA method accounted on average for 71% of the known fauna from each site whereas capture-based methods detected on average 61% of the fauna, making eDNA more efficient than traditional capture based methods. Nevertheless, discrepancies remain between methods, and none can provide an exhaustive image of the local fauna due to the technical limitations of the sampling methods. For instance, capture-based methods are size and species selective (Murphy and

Willis 1996), whereas eDNA detection ability is limited by the completeness of the reference database. Therefore, capture-based and eDNA methods complement each other and should be combined to get the most realistic image of the fauna.

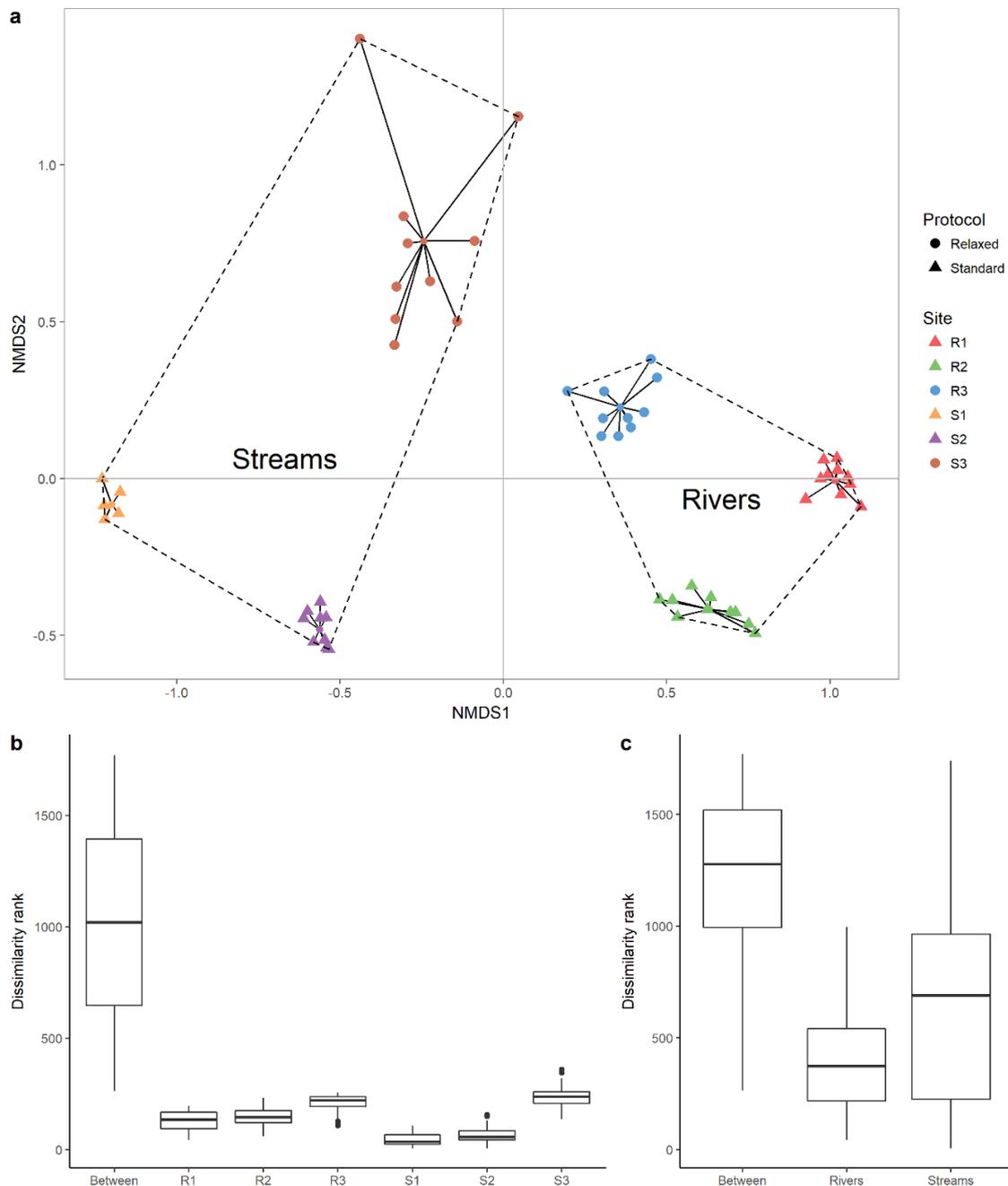


Figure 6: Species composition patterns of the six sites. **(a)** First two axes of the NMDS ordination of the water samples filtered with the standard (triangles) and the relaxed (circles) protocols. The stress of the plot is 0.09. Black segments represents the distance between each replicate and the centroid of their respective site in the two-dimensional space. The dashed lines indicate convex hulls grouping stream and river sites. **(b)** Boxplots indicate the dissimilarity ranks values between and within sites. **(c)** Boxplots indicate the dissimilarity ranks values between and within habitats.

The standard protocol, consisting in the filtration of 34 liters of water, provided little variation in the species richness and in the species composition among replicates. Those trends were more marked in stream sites where replicates gave consistent number and identity of the detected species, with no more than two species differing among replicates. Conversely, in rivers, the differences between replicates reach up to 10 species, suggesting that the sampling effort needed to survey all the detectable species may be less important in streams than in rivers. Certainly, higher species richness is expected in rivers than in streams, given that larger areas are expected to offer more niches and habitat space and potentially host more species and larger population sizes (McGuinness 1984). Indeed, this trend was confirmed in freshwater ecosystems, where species richness increases from upstream to downstream (Oberdorff *et al.* 1993; Cilleros *et al.* 2017). Accordingly, the volume of water needed to get a realistic image of the fauna should increase with the size of the system.

For both stream and river sites, a substantial part of the fish fauna was recovered with only few eDNA replicates using the standard protocol. On average, 87% of the expected fauna from small streams, counting 21 to 48 species, was detected with a single replicate of 34 liters. Adding a second replicate (*i.e.* 68 liters of water) enhanced this detection up to 91%. For river sites, a single replicate was sufficient to detect 67% of the fauna, counting 54 to 60 species, and adding a second replicate enhanced the detection rate up to 74%. In addition, in the four sites most of the species were systematically detected in 100% of the eDNA replicates. This part of the fauna detected in all replicates included both common and rare species. For instance, *Hoplias aimara* or *Myloplus ternetzi*, two common and widespread fish species in French Guiana rivers (Planquette *et al.* 1996), were detected in all the eDNA replicates of all the river sites. Similarly, *Hypopomus artedi* and *Sternopygus macrurus*, although rarely captured in rivers, are known to have colonized all the major watersheds of French Guiana (Planquette *et al.* 1996) and were consistently detected in all of the eDNA replicates of the rivers. In addition, the few species not systematically detected in all the eDNA replicates of a given site were rare species, such as *Hyphessobrycon roseus* in site S2, an uncommon species in French Guiana (Planquette *et al.* 1996; Le Bail *et al.* 2000). This parallels Mächler *et al.* (2016) results, showing that the detection of a rare macro-invertebrate species needs a higher sampling effort than the detection of the common species. Likewise, Lopes *et al.* (2017) showed that increasing sampling effort resulted in an increase of 41% of the detection rates for rare species and of only 8-15% for common species of amphibians in tropical rivers. Consequently, although a trend towards species saturation after 68 liters of water, if the purpose is to exhaustively inventory

the fauna, it will be required to filter more than 68 liters to improve the detection probability of rare species.

Our study offers guidelines to optimize and standardize the volume of filtered water in eDNA studies without reducing the representativeness of the fauna. Previous studies in temperate and less diversified ecosystems, showed a strong heterogeneity in the sampling effort needed to obtain an exhaustive image of the fish fauna. For example, 16 liters of water were sufficient to detect 16 of the 18 historically recorded species in a temperate stream (Olds *et al.* 2016). Similarly, Evans *et al.* (2017) estimated that 5 liters of water are needed to accurately estimate the fish species richness in a small freshwater reservoir colonized by 21 fish species, and (Hänfling *et al.* (2016) considered that filtering 20 liters of water was sufficient to identify 14 of the 16 species inhabiting an English lake. Conversely, Civade *et al.* (2016) and Valentini *et al.* (2016) filtered very large water volumes (up to 6 samples of 34 liters and 6 samples of 60 liters per site, respectively) to detect nearby 20 species in European rivers. We illustrated that filtering intermediates water volumes (2 samples of 34 liters), is sufficient to get a representative picture of the fish fauna inhabiting our sites. Consequently, we recommend using two replicates of approximately 34 liters to sample species rich communities in tropical running waters.

We advise not to reduce the filtering volume per replicate below 34 liters, since reducing filtering volume by 50% (filtering 17 liters instead of 34 liters during 15 minutes instead of 30 minutes) increased the discrepancy between replicates in terms of both species richness and species identity. Moreover, sampling a lower water volume per replicate (relaxed protocol) resulted in replicates missing common species. For instance, *Characidium zebra*, frequently found in Guianese streams (Cilleros *et al.* 2017) or *Poptella brevispina*, occurring in almost all the capture-based samples from the rivers, were not systematically detected with the relaxed protocol (sites S3 and R3, respectively), whereas they were frequently detected in the sites sampled under standard protocol. Therefore, our results underline the need to collect a sufficient volume of water to get reliable and repeatable estimates of fish diversity. It might also be proposed to replace the two replicates by a single filtration of more than 34 liters to reduce the financial costs, but this might be risky due to filter clogging by suspended material. Our trials to increase filtered water volume per replicate, led to damage either the filter, the peristaltic tube or the peristaltic pump. We thus discourage increasing filtered water volume per replicate over 34 liters, with the used materials.

Although 68 liters of water were needed to detect most of the fauna, a single replicate of 34 liters was enough to identify the core of fish assemblages and therefore distinguish between sites and between ecosystem types (stream *versus* rivers). In spite of the close proximity of the sites sampled under the relaxed protocol (only separated by nearby 300 meters), the eDNA data distinguished R3 and S3 sites. Notably, we did not observe any trend toward nestedness of the stream fauna within the riverine fauna. This indicates that even though streams and rivers have been suggested to act as conveyor belts of eDNA (Deiner *et al.* 2016), DNA flowing through the water might not be conserved between distant sites. Therefore, our results reinforce the idea of a detection distance of the eDNA limited to 500 meters in flowing waters, as shown by Jane *et al.* (2015). Forthcoming studies should specify to which extent distance detection of eDNA in the water and species detection rate vary between tropical and temperate ecosystems. Indeed, physiochemical factors such as temperature, pH, conductivity or UV radiation can impact DNA degradation and transport (Pilliod *et al.* 2014; Barnes *et al.* 2014). Nevertheless, our results highlight the ability of eDNA to inventory local species assemblages in tropical running waters, limited up to now to temperate environments (Civade *et al.* 2016; Port *et al.* 2016; Yamamoto *et al.* 2017).

The eDNA approach using the standard sampling protocol deserves to be applied to ecological and conservation studies of highly diverse ecosystems such as tropical waters. Its applicability to Guianese freshwater ecosystems is of particular interest since current fish sampling methods vary among ecosystems, besides being time consuming, destructive and species selective. Indeed, both rotenone sampling in streams and gillnet sampling in rivers are destructive for fishes (Hubert *et al.* 2012), and collect fish from a limited range of habitat resulting in partial images of the fauna (Cilleros *et al.* 2018). In opposition, eDNA sampling was efficient in both streams and large rivers thereby standardizing the potential sampling bias among ecosystems and making possible to compare stream and river samples. Going further in the development of the eDNA inventories requires to complement the reference database to consider more species and to avoid rare, but still existing, false detections. Another forthcoming issue, might be to improve the distinction between closely related species using multiple molecular markers, and by optimizing bioinformatics protocols as proposed by Hänfling *et al.* (2016) and (Evans *et al.* (2017) Moreover, DNA releases may vary among species and affect detection rate, and it would therefore be useful to test for phylogenetic, functional and behavioral signals in species detectability. Finally, as stated before, a plethora of protocols has emerged for every step of the eDNA procedure. For the collection of DNA from water samples,

three common protocols are used: filtration (Valentini *et al.* 2016), precipitation (Ficetola *et al.* 2008) and centrifugation (Klymus *et al.* 2015). The filtration method, consisting on filtering large volumes of water, has proved to yield higher detection rates compared to other methods in both natural ecosystems (Deiner *et al.* 2015) and laboratory conditions<sup>13</sup>. Here we tested one specific filtering system VigiDNA 0.45 µm; SPYGEN, le Bourget du Lac, France, but alternative filtering systems may require different sampling efforts due to differences in filter types and pore sizes (Rees *et al.* 2014; Thomas *et al.* 2018). Therefore, the optimal water volume to obtain robust diversity estimates may vary with the used system and collection method. This highlight the need of forthcoming studies comparing the performance of different filtering systems to gain a more comprehensive view on the performance of the eDNA metabarcoding method in aquatic environments.

### **Acknowledgements**

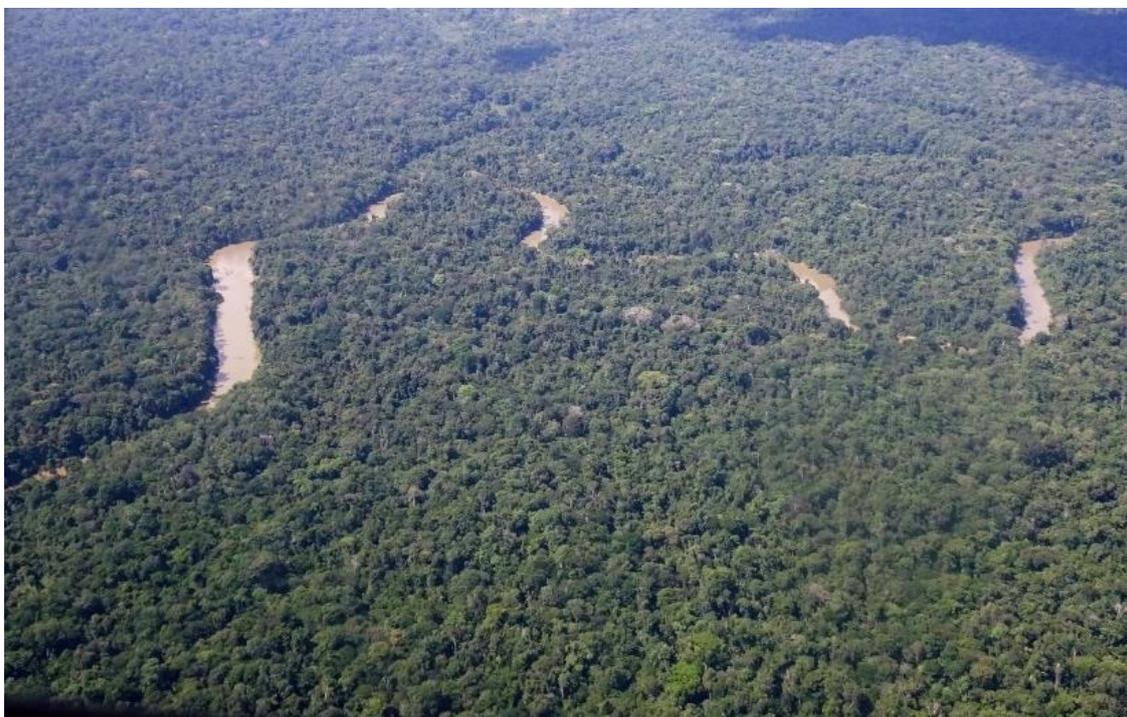
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### **Authors’ contributions**

IC, SB, AV and TD conceived the ideas and designed methodology; AC, IC, PT, RV and SB collected the data; AV and AI conducted the laboratory work; AV and TD conducted bioinformatic analyses; IC and KC analyzed the data; IC and SB led the writing of the manuscript. All authors contributed critically to the drafts.

## *Chapter 3: Spatial extent and strength of anthropogenic impacts on fish biodiversity in rivers*

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### **Low deforestation levels cause harsh and spatially extended biodiversity declines in Amazonian rivers**

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## **Abstract**

Measuring anthropogenic impacts on natural systems is crucial for biodiversity preservation. Such measures are often achieved by determining how local human disturbances affect the fauna from the same locality. In rivers, local biodiversity can also suffer from distant upstream disturbances, as water and materials are transported from headwaters to the ocean. This connectivity of river ecosystems makes pivotal to account for distant upstream disturbances when measuring anthropization impacts on biodiversity. However, the distance to which upstream disturbances influence local fauna remains poorly understood.

Here, we propose a framework to measure the strength and spatial extent of disturbance by analyzing the relationships between local fish fauna diversity and the intensity of disturbances measured within spatial scales ranging from the immediate vicinity of the site to 150 km upstream. Fish assemblages were inventoried using Environmental DNA metabarcoding. Those inventories were used to compute taxonomic (species richness) and functional richness (based on the morphological and ecological characteristics of the species) of species assemblages.

Testing this framework in 50 river sites in French Guiana and Suriname revealed a strong and spatially extended effect (up to 70 km) of distant upstream deforestation on fish fauna. Importantly, less than 5% of deforestation within a range of 70 km upstream from the sites caused a decline of more than 36% of both taxonomic and functional richness of the fish assemblages. The results underline the vulnerability of Amazonian fishes and suggest that human impacts on rivers are often underestimated, and need to be re-evaluated in light of its spatially extended effect.

## Introduction

Measuring the impact of anthropogenic disturbances on natural systems is crucial for the preservation of biodiversity, and the maintenance of the services it provides to human societies (Dirzo and Raven 2003). Such measures are often achieved by determining how human activities affect the local fauna, leading to define the strength of impacts as changes in local biodiversity. Numerous studies have explored the consequences of human-induced impacts on local fauna, including changes in land use (Newbold *et al.* 2015b), hunting and fishing (Myers and Worm 2003; Benítez-López *et al.* 2019) or non-native species (Kuczynski *et al.* 2018b). For instance, Newbold *et al.* (2015) quantified that intensive agriculture or urban expansion reduced on average 40% of site species richness. However, local biodiversity can also suffer from distant impacts. This is particularly true in riverine ecosystems, where directional connectivity transport water from headwaters to the ocean (McCluney *et al.* 2014). Thus, disturbances in one part of a river basin can affect a distant downstream part of that basin. For instance, mountaintop mining has an extended effect on water quality and biodiversity downstream (Palmer *et al.* 2010). Furthermore, the impacts of multiple upstream disturbances can cumulate over the stream network, as illustrated in an Appalachian river, where mountaintop mining modified water quality for more than 10 kilometers downstream, with changes in water quality being proportional to the mining area (Lindberg *et al.* 2011). Therefore, local biodiversity may not only be influenced by local disturbances but also by multiple distant disturbances, which effects may cumulate along the watershed draining a site.

Despite the sensitivity of local habitats and fauna to distant disturbances, river connectivity can also promote species recovery and thus ecosystem resilience downstream from disturbances (McCluney *et al.* 2014). Indeed, less or un-impacted tributaries can transport undisturbed water inputs and thus reduce downstream effects of disturbance by diluting pollutants, as shown in the Amazon river basin where biodiversity threats decreased downstream (Vörösmarty *et al.* 2010). The interplay between the accumulation of multiple upstream impacts and the downstream ecosystem resilience may contribute to the strength of the local impacts experienced by a site. It is therefore pivotal to determine the spatial extent of disturbances to measure properly the strength of their impact on biodiversity.

We here propose a framework to measure the strength and the spatial extent of disturbance impacts in rivers by analysing the relationships between the diversity of local fauna and the intensity of upstream disturbances. We calculated upstream disturbance intensity at

different spatial extents by widening the spatial extent in which disturbance intensity was calculated, from the immediate vicinity of the site (0.5 km upstream) to 150 km upstream (Figure 1). This provided measures of disturbance intensity (here the percentage of deforested surface) for each spatial extent. The larger the spatial extent, the more distant disturbances are integrated in the disturbance variable. Generalized Linear Mixed Models relating fish diversity (species and functional richness) and disturbance intensity were built for each spatial extent. We considered that the most relevant model indicates the representative spatial extent to measure deforestation effects and the slope of the relationship between deforestation and fish diversity was used as a measure of disturbance strength.

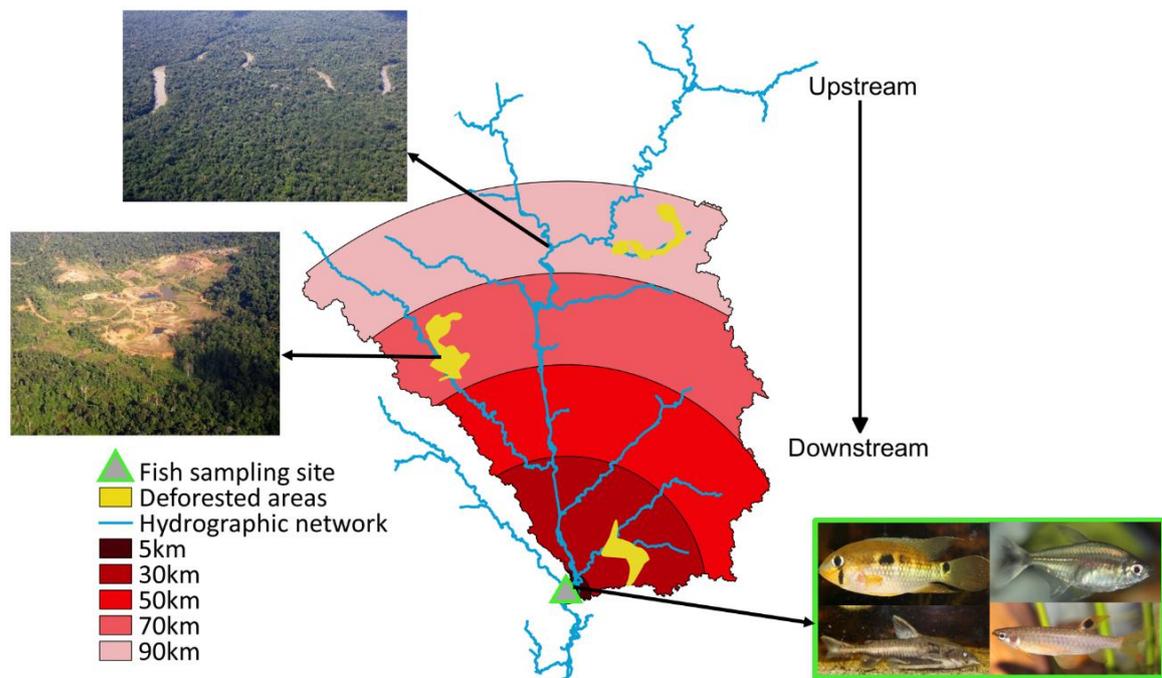


Figure 1: Schematic representation of the measurement of the percentage of deforestation upstream from each fish sampling site for each spatial extent. For clarity, we here illustrated only 5 out of the 14 spatial extents considered in our study. Spatial extents are represented by the surface of the watershed comprised between the fish sampling site and the extent value (here 5 km, 30 km, 50 km, 70 km and 90 km). The fish sampling site is represented by a triangle. The hydrographic network is represented in blue and the watershed boundaries are indicated by a black continuous line. Disturbance surfaces are represented in yellow. For each site, we calculated the percentage of deforested area for each spatial extent. For instance, a 70 km extent of disturbance measures the percentage of disturbed area within the river basin from the biodiversity sampling site to a maximal distance of 70 km upstream from this sampling site.

We applied this framework to rivers of French Guiana, which remain among the most pristine areas on earth, but also face an unprecedented rise of human threats due to deforestation for agriculture, mining and urbanization (Castello *et al.* 2013). Such disturbances are putting at

risk the most diverse freshwater fish fauna on earth, as the Amazonian region hosts about 20% of fish species diversity (Lévêque *et al.* 2008). Studies of fish assemblages in Amazonian rivers remain scarce because common inventory methods (nets, traps, and toxicants) cannot efficiently gather fish data for comprehensive studies without causing massive mortality (Cilleros *et al.* 2018). To overcome this issue, we inventoried local fish assemblages using eDNA, which proved to be efficient in characterizing such species rich ecosystems (Jerde *et al.* 2019; Cantera *et al.* 2019).

## **Materials and methods**

### *Sampling*

Fifty river sites located across the principal rivers of French Guiana were sampled in 2016 and 2017 during the dry season (Figure 6, Chapter 1-IX). Ecologically homogenous sites in rivers wider than 50 meters and deeper than one meter (Strahler orders 3-6, Figure S1) were selected. Following the protocol implemented by Cantera *et al.* (2019) (Chapter 2), we filtered 34 liters of water at each site to collect eDNA and build fish inventories per site. See the materials and methods sections of Chapter 2 for details on field sampling, laboratory procedures and bio-informatic analyses.

### *Deforestation intensity*

For each site, we delineated the upstream sub-basin by applying a Flow Accumulation algorithm to the SRTM Global 30 m Model Elevation (NASA 2013). We delineated 14 spatial extents using buffer areas intersected with the sub-basin area, with distances of 0.5 km, 3 km, 5 km, 10 km, 20 km, 30 km, 40 km, 50 km, 60 km, 70 km, 80 km, 90 km, 120 km and 150 km upstream from each sampling site (See Figure 1). For each spatial extent and for each site, upstream deforestation intensity was quantified by summing deforested surfaces from three data sets obtained from Landsat satellite images.

Information about gold-mined surfaces in French Guiana 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. Given that some sites from the Maroni and Oyapock drainage basins have upstream areas in Suriname and Brazil, we also used the dataset compiled by Rham *et al.* (2017). The

gold-mining intensity was quantified as the percentage of gold-mined surfaces upstream of the sites for each spatial extent.

Forest loss surfaces upstream of the sites were extracted using the Global Forest Change dataset (Hansen *et al.* 2013). This dataset identifies the areas deforested from 2001 to 2017 using global Landsat satellite image at 30 meters spatial scale. To incorporate the areas deforested before 2000, we used the information of tree canopy cover measured in 2000. The pixels having less than 25% of canopy closure were considered deforested, excepting river courses. The deforested surfaces were combined to obtain an estimate of deforestation intensity upstream from the sites for each spatial extent. The deforested surfaces are the results of global human activities on French Guiana: logging, agriculture and human settlements

The percentage of gold-mined and forest loss surfaces upstream of the sampling sites were found to be significantly and highly correlated (Table S1). Therefore, we merged the two datasets to create an integrative disturbance variable that quantifies the percentage of global deforestation upstream from the sampling sites, for each spatial extent area (Table S1). Upstream deforestation intensity was then measured as the percentage of deforested surfaces upstream of the sites, which are the result of gold-mining (46% of deforested surfaces, Table 1), logging, agriculture and human settlements. Hereafter, we relate fish diversity to this variable that represents deforestation intensity upstream of each fish sampling site.

### *Biodiversity measures*

The fish biodiversity of each site was measured through species and functional diversity. Species richness corresponded to the number of detected species in the eDNA sample. This measure, although not exhaustive, provided a more comprehensive image of the fish species richness and species composition than other capture methods (Cantera *et al.* 2019). Morphological and ecological traits of the species detected in each site were used to build a global functional space (see Chapter 1-IX-2 for details). To measure the functional richness (“FRic”, Villéger *et al.* 2008) of each site, we quantified the convex hull volume occupied by co-occurring species in a given site within the global functional space. This measure ranges from 0 to 1, with higher values reflecting high volume occupation and thus high functional diversity.

### *Statistical analyses*

For each spatial extent, we build a specific model to analyze deforestation effects on species and functional richness using Generalized Linear Mixed Models (GLMM) with Poisson distribution for species richness and Linear Mixed Models (LMM) for functional richness. River basin identity and site position in the upstream-downstream river continuum (Strahler order, Figure S1) were included as random effects because site position determines the size of the rivers and thus the hosting capacity of species (Blanchet *et al.* 2010). This allowed the measurement of deforestation impacts as changes in local biodiversity due to upstream deforestation, while controlling for river basin identity and position of the fish sampling site in upstream-downstream gradient. The variables were tested for spatial autocorrelation using Moran's I but the effect was not significant. The models were built using the *lmer* function from the *lme4* package (Bates *et al.* 2015).

First, we assessed the significance and the quality of the models using determination coefficients ( $R^2$  values) to determine which spatial extent provides a better prediction of changes in local biodiversity due to upstream deforestation.  $R^2$  values were calculated using the *r.squaredGLMM* function from the MuMIn package. We used marginal  $R^2$  values which account for the explained variance by fixed variables only as we were interested in the pure effect of deforestation.

The slope of the best model was used as a measure of the strength of deforestation impacts. As a check, we grouped sites according to their Strahler order and distinguished between medium size rivers (Strahler orders 3 and 4), and large rivers (Strahler orders 5 and 6, Figure S1). We then compared the fish diversity between deforested and non-deforested areas in medium and large rivers separately. Deforested sites were those with a percentage of upstream deforested area exceeding 0.4%. Less than 0.4% of deforestation accounted for the natural rate of forest turnover (natural tree falls), and were considered as non-deforested.

## **Results**

Deforestation intensity was on average lower than 6% whatever the spatial extent considered (Figure 2, Table 1). At reduced spatial extents (from 0.5 to 10 km), more than 25% of the sites experienced upstream deforestation intensities higher than 6%, which peaked at more than 20%

for some sites. In contrast, at larger extents, deforestation intensity remained lower than 3% in more than 75% of sites. Therefore, the intensity and the variability of upstream deforestation decreased with increasing spatial extents (Figure 2).

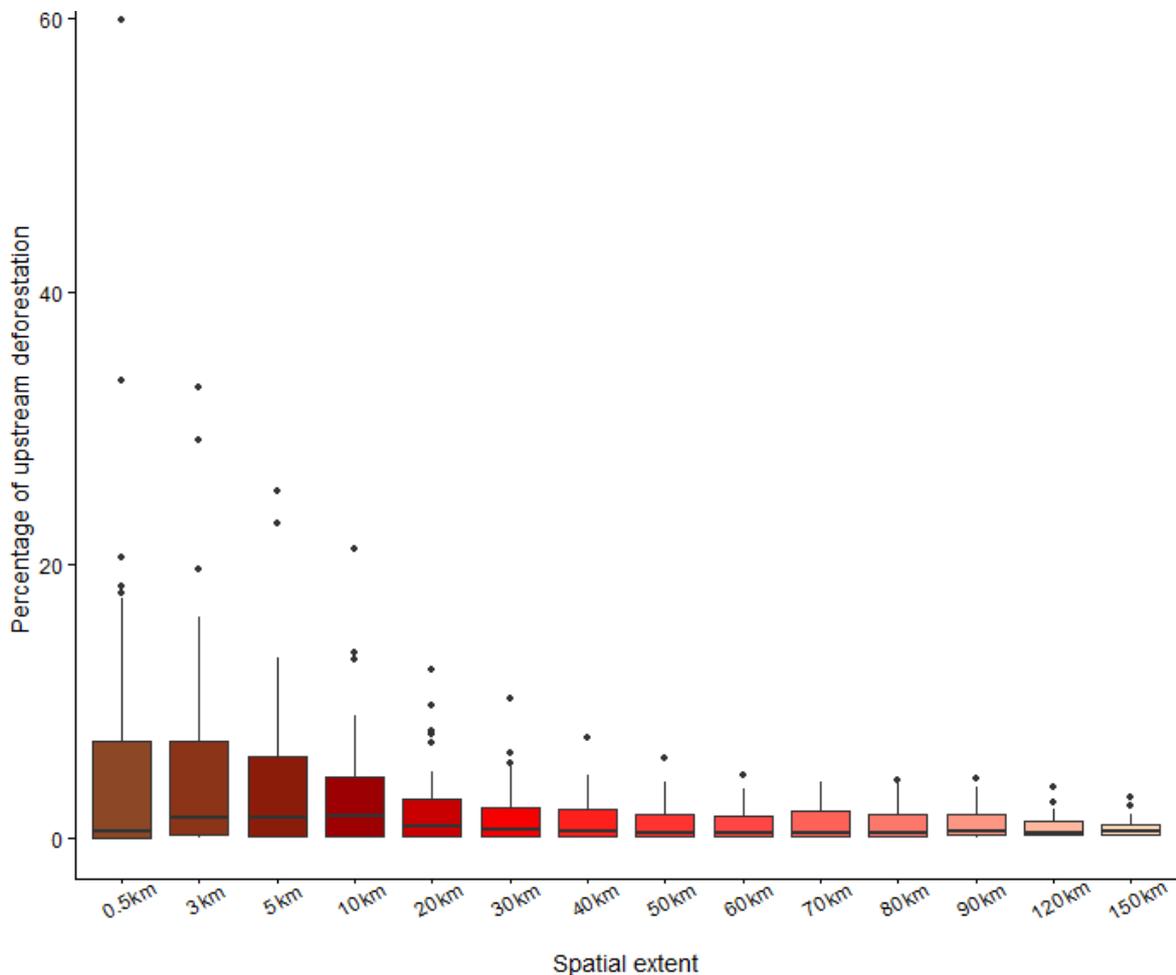


Figure 2: Percentage of deforestation upstream from the sampling sites for each spatial extent. Deforestation intensity is summarized with a boxplot: the central box encompasses the interquartile range, the whiskers represent minimum and maximum deforestation values, and the horizontal line inside the box is the median deforestation. Outliers are represented by circles. Color shades are consistent with the spatial extent, as indicated in Figure 1.

Upstream deforestation intensity had a negative effect on local species richness. This negative effect was significant for all spatial extents, except for deforested surfaces below 0.5 km upstream from the sampling sites (Figure 3A; Table 1). For models considering upstream deforestation at local spatial extents (from 3 to 5km), GLMM quality was low ( $R^2 < 0.2$ ).  $R^2$  values increased from 10 to 30 km and peaked for spatial extents between 40 and 60 km to reach  $R^2$  values of 0.74. (Figure 3A; Table 1). Beyond 60 km,  $R^2$  values lowered but remained higher than at local spatial extents. Such increases of model quality with increasing spatial extent, paired with an increase of the strength of the negative effect of deforestation on species richness (Figure 3C; Table 1). Indeed, the slope values decreased with the spatial extent and maximal negative slope values were reached at 60 and 150 km extents. Interestingly, the models

considering deforestation at small extents did not detect marked species richness decreases through the deforestation gradient (slopes < -0.1). The model accounting for deforestation measured within 60 km upstream from the sampling sites was considered the best model to predict species richness according to upstream deforestation because it had the maximal R<sup>2</sup> and slope values (0.74 and -0.51, respectively).

<i>Spatial extent</i>	<i>Gold-mining</i>	<i>Others human activities</i>	<i>Global deforestation (%)</i>	<i>Species richness models</i>			<i>Functional richness models</i>		
	<i>Deforestation (%)</i>	<i>Deforestation (%)</i>		Slope	p.value	R2m	Slope	p.value	R2m
0.5 km	0.0	5.5	5.5	-0.02	0.09	0.02	0.01	0.62	0
3 km	0.1	4.9	5.0	-0.07	<b>&lt;0.01</b>	0.15	-0.01	0.65	0
5 km	0.3	3.6	3.8	-0.1	<b>&lt;0.01</b>	0.19	-0.02	0.28	0.02
10 km	0.9	2.2	3.0	-0.17	<b>&lt;0.01</b>	0.42	-0.04	<b>0.03</b>	0.1
20 km	0.8	1.2	2.0	-0.29	<b>&lt;0.01</b>	0.66	-0.09	<b>0.01</b>	0.3
30 km	0.6	0.9	1.5	-0.38	<b>&lt;0.01</b>	0.73	-0.12	<b>&lt;0.01</b>	0.37
40 km	0.5	0.7	1.3	-0.43	<b>&lt;0.01</b>	<b>0.74</b>	-0.14	<b>&lt;0.01</b>	0.37
50 km	0.5	0.6	1.1	-0.48	<b>&lt;0.01</b>	<b>0.74</b>	-0.16	<b>&lt;0.01</b>	0.37
60 km	0.4	0.6	1.0	-0.51	<b>&lt;0.01</b>	<b>0.74</b>	-0.17	<b>&lt;0.01</b>	0.4
70 km	0.4	0.5	1.0	-0.45	<b>&lt;0.01</b>	0.66	-0.18	<b>&lt;0.01</b>	<b>0.45</b>
80 km	0.4	0.5	0.9	-0.45	<b>&lt;0.01</b>	0.62	-0.2	<b>&lt;0.01</b>	0.44
90 km	0.5	0.5	1.0	-0.43	<b>&lt;0.01</b>	0.56	-0.19	<b>&lt;0.01</b>	0.36
120 km	0.4	0.5	0.8	-0.4	<b>&lt;0.01</b>	0.31	-0.2	<b>0.01</b>	0.24
150 km	0.3	0.4	0.7	-0.56	<b>0.03</b>	0.64	-0.17	0.05	0.17

Table 1: Mean percentage of upstream deforested surfaces due to gold-mining, mean percentage of upstream deforested surfaces due to other human activities and mean global percentage of upstream deforested surfaces are also indicated for each spatial scale. Results of the mixed models relating fish diversity (species and functional richness) and global deforestation intensity for the 14 spatial extents. For each spatial extent, a specific model was built using GLMM with Poisson distribution for species richness and LMM for functional richness. River basin identity and site position in the upstream-downstream river continuum (Strahler order) were included as random effects. Significant p-values and most relevant R<sup>2</sup> are indicated in bold.

Functional richness was significantly and negatively linked to upstream deforestation intensity when considering deforestation from 10 km to 120 km upstream from the sampling sites. Model quality peaked for R<sup>2</sup> values around 0.4 corresponding to spatial extents from 60 to 80 km (Figure 3B; Table1). The effect of deforestation was maximal at large spatial extents (beyond 70 km) with slope values higher than -0.18 (Figure 3D; Table 1). For this diversity facet, the model measuring deforestation intensity within a spatial extent of 70 km upstream from the fish sampling sites was considered the best model (R<sup>2</sup> = 0.45 and slope = -0.18).

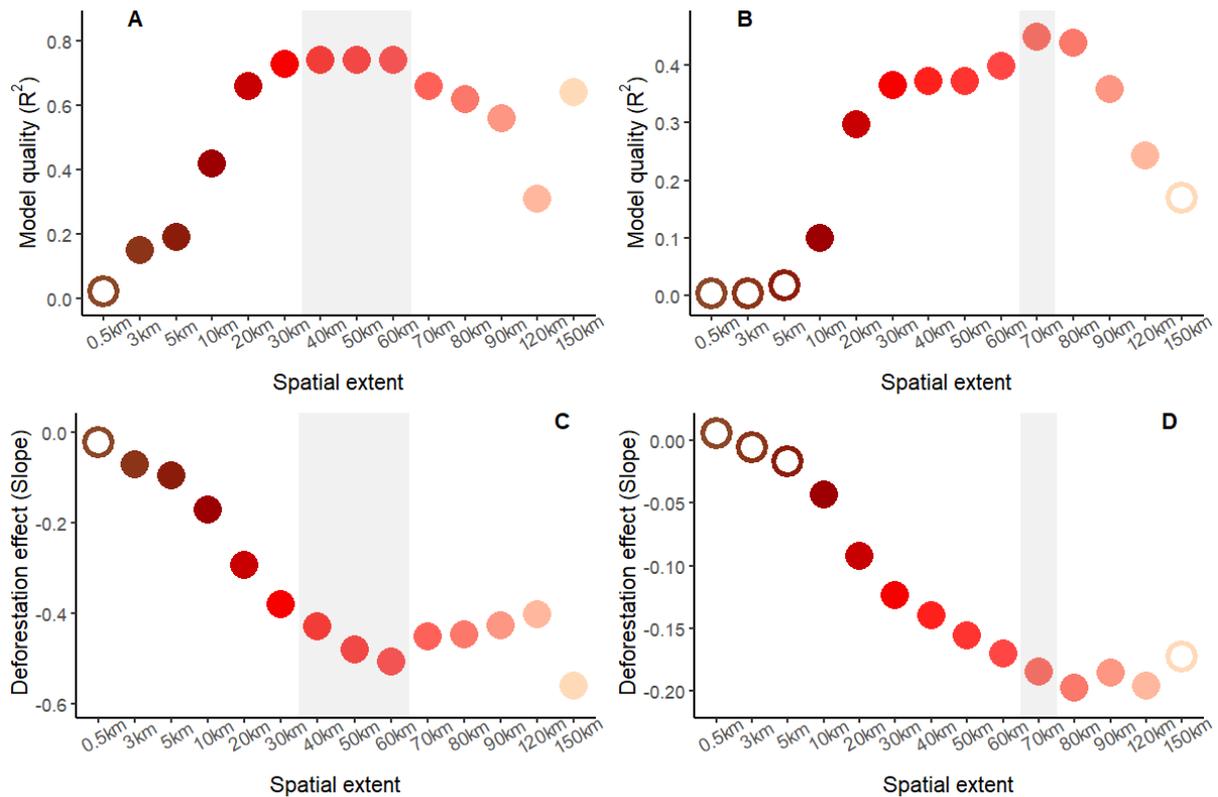


Figure 3: Results of the mixed models relating fish diversity and the percentage of deforested area upstream from the sampling sites for each spatial extent. R<sup>2</sup> values (A, B) represent the model quality of the models and the slope values (C, D) represent the strength of the effect of deforestation on species richness (A, C) and functional richness (B, D). For each spatial extent, a specific model accounting for site network position and basin identity as random effects was built. Significant models ( $p < 0.05$ ) are indicated by filled circles. Non-significant models ( $p > 0.05$ ) are indicated by open circles (see Table 1 for details). Color shades are consistent with the spatial extent. The grey vertical bars indicate the most relevant models.

At the most relevant spatial extents, deforestation intensity ranged from 0 to 4.6%. Along the deforestation gradients, both species and functional richness showed marked decreases (Figure 4A and B), despite some variability depending on sites. Overall, both functional and species richness decreased according to the position of the sites within the river upstream-downstream gradient measured using the Strahler stream order (Strahler 1957) (Figure 4C&D, Figure S1). Nevertheless, within sites with similar Strahler orders, we recorded a significant decline of fish diversity in deforested areas due to anthropogenic activities (deforestation intensity  $> 0.4\%$ ) compared to non-deforested areas (deforestation intensity  $< 0.4\%$ ). Those decreases were significant for both species richness (Kruskal-Wallis test:  $\chi^2 = 8.36$  and  $p < 0.01$  for medium size rivers;  $\chi^2 = 11.63$  and  $p < 0.01$  for large rivers) and functional richness (Kruskal-Wallis test:  $\chi^2 = 10.26$  and  $p\text{-value} < 0.01$  for medium size rivers;  $\chi^2 = 8.82$  and  $p < 0.01$  for large rivers). On average, we report a loss of 36% of species richness

(37.5% and 34.4% for medium and large rivers, respectively) and 38% of functional richness (37.6 and 39.6% for medium and large rivers respectively) in deforested areas.

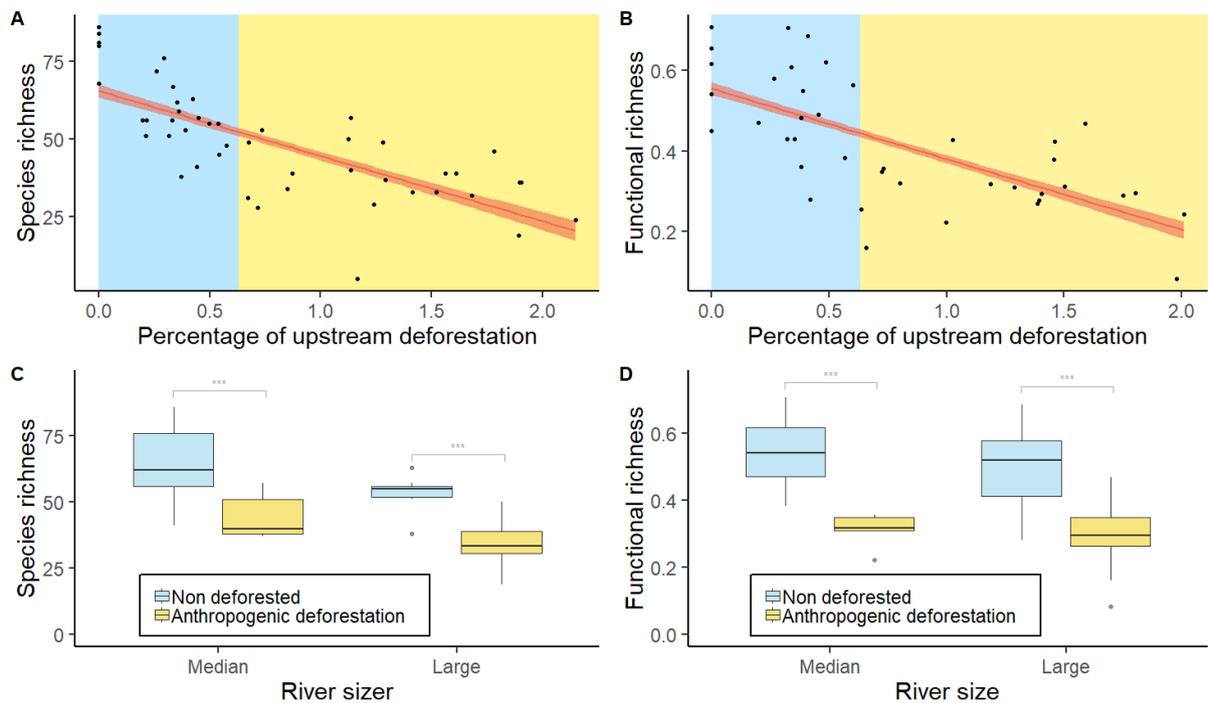


Figure 4: Effects of upstream deforestation on fish diversity. The species richness (A) and functional richness (B) are represented according to percentage of upstream deforestation. Fitted values of the mixed models accounting for site network position and basin identity are shown with red solid lines and 95% confidence intervals with light red shades. Deforestation corresponds to the percentage of deforested area at the most relevant spatial extents for each diversity facet (60 and 70 km for species and functional richness, respectively). Deforestation values were squared root transformed for a better representation (Real deforestation values ranged from 0 to 4.3%). Sites were subjected to anthropogenic deforestation when deforested area exceed 0.4% of the upstream area (see methods). Losses of species richness (C) and of functional richness (D) due to deforestation when accounting for the network position of sites are represented using boxplots for medium (stream order 3 and 4) and large sized rivers (stream order 5 and 6). Significant differences between low and high deforestation intensities were tested using Kruskal Wallis tests (\*\*\*)  $p < 0.01$ .

## Discussion

Rivers are among the most threatened ecosystems with deforestation severely threatening biodiversity (Carpenter *et al.* 2011). Studies of local diversity change following local deforestation in Amazonian rivers revealed shifts in taxonomic and functional without species richness declines (Brosse *et al.* 2011; Allard *et al.* 2016; Arantes *et al.* 2018; Leitão *et al.* 2018). However, the potential cumulative effects of upstream distant disturbances in large spatial extents was not considered. Here we show that deforestation can affect biodiversity beyond local effects, revealing an extended and cumulative effect of distant upstream deforestation on fish biodiversity.

The effect of deforestation on fish diversity reported here peaked when considering disturbance extents from 60 to 70 km upstream of the sampling sites. Notably, the deforestation intensities measured at these extents were less marked and less variable than at local extents (from 0.5 to 10 km), in which deforestation intensities ranged from 0 to 60% of the upstream area (Figure 2). This suggests that even though the intensity of deforestation is more marked at local extents, it does not properly predict nearby biodiversity decline. In contrast, considering upstream deforestation at larger extents, capturing both local and more distant deforestation effects, makes deforestation a strong predictor of fish biodiversity. Therefore, deforestation effects on fish fauna cumulated downstream up to 70 km. Such cumulative downstream effects of deforestation have been reported for water chemistry but this result was directly related to an increase of disturbed surface with increasing spatial extent (Lindberg *et al.* 2011). Here, despite a declining percentage of deforested surface with increasing spatial extents, we report a stronger decline of biodiversity with deforestation intensity over large extents. Moreover, this suggests that previous studies linking local deforestation to local biodiversity missed an important part of deforestation impacts on fish fauna. For instance, Brosse *et al.* (2011) and measured Allard *et al.* (2016) the impact of gold-mining and forestry induced deforestation on Guianese fish diversity and failed to detect a decline of fish species diversity, paralleling our results when reduced extents are considered. We can therefore suppose that a stronger impact of deforestation might have been detected if a more relevant spatial extent had been considered.

Measuring the impact of deforestation intensity at the most relevant spatial extent (60 km for species richness and 70 km for functional richness) on local biodiversity revealed a drastic decline of biodiversity along the deforestation gradient. Slight deforestation intensities (< 4.3% of the upstream area) caused on average a 36% and 38% decline in species and functional richness respectively, testifying for a drastic negative effect of upstream deforestation. We highlight a particular vulnerability of the Amazonian freshwater fish fauna, and of the functions it supports, to slight environmental changes. This parallels studies of coral reef and tropical forest mammal vulnerability which show that low levels of fishing and hunting were responsible for a decline in taxonomic and functional diversity (D'agata *et al.* 2014; Benítez-López *et al.* 2019). Similarly, negative threshold responses (*i.e.* a point where there is an abrupt negative change of an ecological variable (Scheffer *et al.* 2009)), were found at low disturbance intensities for several aquatic taxa. For instance, negative threshold responses in Amazonian fish (Brejão *et al.* 2018) and temperate diatom assemblages (Smucker *et al.* 2013) were detected at moderate deforestation levels (<20% of deforested watershed). Such

deforestation percentages were higher than those measured in our study, but they included the whole upstream river basin, and did not consider a potential ecosystem resilience for the most distant sites. They may therefore overestimate the response threshold. For instance, a decline in the relationship between fish diversity and upstream deforestation was detected beyond 80 km, indicating that beyond this distance, disturbances are less influential on the downstream fauna.

The considerable loss of river fish biodiversity documented here is the result of deforestation driven by several anthropogenic activities (agriculture, human settlements and gold-mining). These activities are known to influence water quality through organic matter release and/or increases in the suspended sediment load (Castello and Macedo 2016), thus affecting fish diversity via different pathways. Nearly 46% of the deforested surfaces are due to gold-mining (Table 1), which severely damages water quality due to the massive release of suspended sediments (Hammond *et al.* 2007), and has detrimental effects on fishes (Mol and Ouboter 2004; Allard *et al.* 2016). However, disentangling the effects of gold-mining, agriculture and urbanization on downstream fish diversity is complicated as downstream assemblages collect different anthropogenic inputs.

The demonstrated vulnerability of Amazonian fauna is of particular interest given the unprecedented deforestation rates throughout the Amazonian forest (Malhi *et al.* 2008). In the Guyana shield, deforestation caused by gold-mining is rapidly expanding whilst representing a relatively low deforestation surface across the region (Rham *et al.* 2017). Ongoing increases in human disturbances of Amazonian ecosystems, due to demographic increases, mining and agriculture (Castello *et al.* 2013) are prone to further affect Amazonian freshwater fauna, through cumulative impacts over large parts of the upstream drainage. We therefore call for future studies and conservation practices to not only consider local disturbances on fauna but also consider disturbances accumulating upstream. This is of paramount importance to avoid underestimating deforestation effects on riverine fauna, and to capture the actual human impacts on aquatic ecosystems.

## Supplementary information

<i>Spatial extent</i>	<i>p-value</i>	<i>Correlation coefficients</i>
0.5 km	NA	NA
3 km	0.571	-0.082
5 km	0.291	0.152
10 km	<0.01	0.755
20 km	<0.01	0.766
30 km	<0.01	0.76
40 km	<0.01	0.815
50 km	<0.01	0.836
60 km	<0.01	0.865
70 km	<0.01	0.893
80 km	<0.01	0.912
90 km	<0.01	0.922
120 km	<0.01	0.94
150 km	<0.01	0.964

Table S1: Correlations between the percentage of gold-mined areas and deforested areas upstream of the sampling sites for each spatial extent.

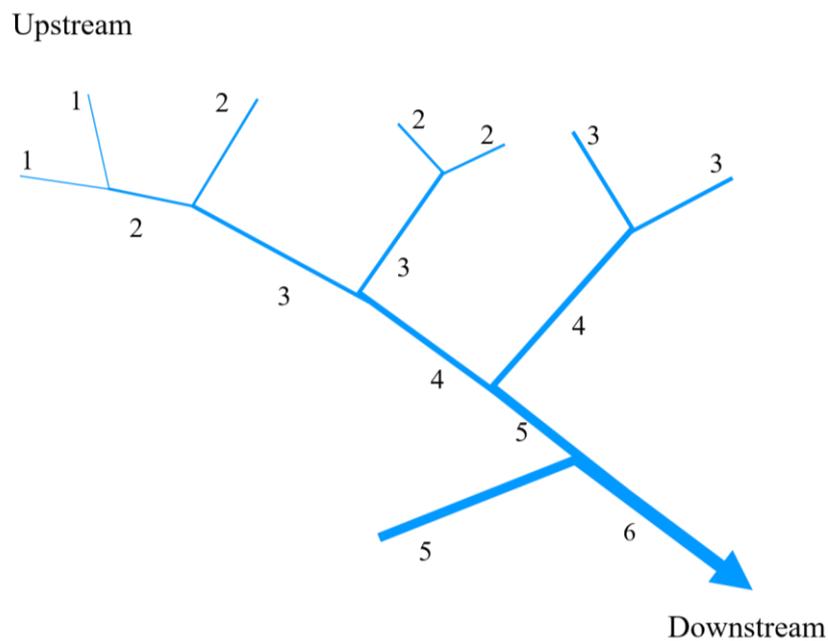


Figure S1: Diagram illustrating Strahler river classification. The headwaters without any confluence are first order streams. A river reach a second order at the confluence of two first order streams. At a confluence of two streams with the same order, the downstream segment order increases by one. At a confluence, if the two streams are not of the same order then the highest numbered order is maintained on the downstream segment. Orders 1 and 2 account for streams and small rivers, orders 3 and 4 for medium sized rivers and orders 5 and 6 to large rivers. Orders over 6 account for very large rivers.

## Chapter 4: How anthropogenic impacts modify diversity patterns and ecological processes?

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### Environmental context determines the effect of anthropogenic disturbances on Neotropical fish assemblages

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## Abstract

Environmental gradients, induced by natural or disturbed conditions, structure diversity patterns and may therefore influence local ecological processes that shape local communities, such as environmental filtering and biotic interactions. The variation of diversity patterns and processes was analysed along a deforestation gradient and the upstream-downstream gradient.

We sample 50 river sites and 37 stream sites across French Guiana using environmental DNA. Rivers and streams sites were considered separately given that they associated fauna and environment markedly differ. Deforested surfaces upstream from the fish sampling sites were extracted from spatial data to create a global deforestation variable that integrates the effects urbanization, agriculture, gold-mining and logging. Ecological processes were assessed by confronting the observed relationships between taxonomic and functional diversity to null models simulating random species assembly. This permitted to test the hypothesis that deforestation constitutes a strong environmental filter and therefore drive assemblages toward non-random functional clustering.

In streams, diversity patterns were more influenced by the upstream-downstream gradient than by the deforestation gradient and the opposite was found for the ecological processes. The strength of environmental filtering increased along the deforestation gradient. In rivers, the deforestation gradient affected significantly both species and functional richness but not the ecological processes. In opposition, a trend towards limiting similarity along the upstream-downstream gradient was observed. Our results highlight the complexity of deforestation impacts on Amazonian biodiversity, as they reveal a context-dependency of deforestation impacts.

## Introduction

In the current global trend of reorganization of local communities, both natural ecological processes and anthropogenic disturbances are shaping biological diversity patterns across the world (Sax and Gaines 2003). Understanding the relative role of natural and anthropogenic processes, which determine the assembly of species onto communities, is the baseline to assess the depth of the anthropogenic impacts on biodiversity. Community structure results from a combined effect of local environmental conditions and biotic interactions and ecological theory predicts that at local scale, two main deterministic processes drive community structure (Weiher and Keddy 1999): environmental filtering (Keddy 1992) and limiting similarity (MacArthur and Levins 1967). While assemblages ruled mainly by limiting similarity harbor species with different ecological strategies due to competitive exclusion, under environmental filtering, assemblages have more ecologically similar species than expected randomly due to a limited availability of environmental niches. Those two processes may act simultaneously to shape communities and their relative importance may vary according to environmental gradients. Indeed, the majority of ecosystems are directly affected by environmental gradients, which have been reported to shape community structure in terrestrial communities. For instance, it has been shown that limiting similarity dominated community assembly at low altitudes while environmental filtering dominated at high altitudes for tropical hummingbirds (Graham, Parra, Rahbek, & McGuire, 2009) and temperate bees (Hoiss *et al.* 2012).

The assembly of communities in freshwater ecosystems might be particularly sensitive to environmental gradients as those systems are complex networks strongly influenced by directional connectivity due to movement of the water from upstream headwaters to the ocean (McCluney *et al.* 2014; Moore 2015). The position of a locality in the upstream-downstream gradient influences environmental conditions and thus the community structure of the aquatic fauna inhabiting this locality (Vannote *et al.* 1980; Grenouillet *et al.* 2004). This pattern, primarily formalized in temperate rivers, was then expanded to tropical ecosystems (Ibañez *et al.* 2009; Cilleros *et al.* 2017). Habitat size and complexity increase along the upstream-downstream gradient, generally leading to an increase in species richness and changes on species composition along the gradient. Therefore, as diversity patterns change along the gradient, the relative importance of ecological processes shaping communities may also change along this gradient. Streams communities located in the headwater part of hydrological networks are isolated and have been found to be strongly influenced by abiotic conditions such

as water velocity, substrate type, dissolved oxygen, water temperature and transport of particulate organic matter (Poff 1997). Moreover, streams have high variability in abiotic conditions and thus stream communities are expected to have a low number of species able to tolerate those conditions (Jackson *et al.* 2001). All of these features suggest that environmental filtering may preponderantly shape the structure of stream communities by excluding functions not adapted to those harsh environments. In opposition, environmental stability, habitat size and complexity increase downstream promoting high species richness (Willis *et al.* 2005) in large rivers. As riverine communities are less influenced by abiotic conditions, limiting similarity may govern community structure in those communities by promoting the coexistence of species with different traits. Therefore, the importance of ecological processes ruling aquatic communities may progressively change along the upstream-downstream gradient. Specifically, environmental filters may have a main role in upstream communities while limiting similarity may be predominant in downstream large rivers. Indeed, fish communities in Brazilian streams were mostly structured by environmental filtering resulting in a significant functional homogeneity. In contrast, a coexistence of more functionally dissimilar species was found in communities inhabiting downstream rivers (Carvalho and Tejerina-Garro 2015).

Progressive changes on the type and/or intensity of the ecological processes shaping community structure have also been reported under human disturbances. Through an expected decline of species richness under human disturbances, trait diversity can decrease more than expected under a random selection of extirpated species. This might suggest that human disturbances filter out species with similar traits, indicating an increase of environmental filtering (Webb *et al.* 2002). In contrast, the disturbance can also drive the community toward less functional similarity between species by increasing competitive interactions (Webb *et al.* 2002). The few works exploring the relationships between those facets in the context of human disturbances showed contrasting results. In temperate environments, functional diversity decreased faster than taxonomic diversity under global change (Kuczynski and Grenouillet 2018) and land use gradients (Gutiérrez-Cánovas *et al.* 2015) revealing an increase in environmental filtering processes. Contrastingly, in a Neotropical lowland stream, high functional diversity was associated with deforested streams and that was suggested to be the result of new conditions favoring species with particular traits (*e.g.*, detritivorous, species inhabiting stream margins and tolerant to hypoxia) (Teresa and Casatti 2012), relaxing therefore environmental filtering effects.

Amazonian streams and rivers host the most diverse freshwater fish fauna on earth (*c.a.* 20% of global fish species diversity, Lévêque *et al.* 2008) and provide significant goods and services (Castello and Macedo 2016). However, those ecosystems are facing unprecedented levels of deforestation impacts due to increasing agriculture, mining and urbanization (Castello *et al.* 2013). Those activities are polluting freshwater systems and altering their hydrology and physico-chemical conditions (Castello and Macedo 2016; Leitão *et al.* 2018). Consequently, it is urgent to assess the impacts of deforestation on the ecological processes shaping aquatic communities to understand if the degradation of Amazonian biodiversity has consequences on the processes ruling local species assembly.

Such approaches were until now limited by our constrained ability to get relevant fish inventories in Neotropical rivers and streams, but the recent development of environmental metabarcoding techniques made possible to get fast and relevant fish inventories in Neotropical freshwaters (Zinger *et al.* 2020). This was particularly verified in French Guiana where eDNA metabarcoding has been proven to be efficient in characterizing species assemblages in both streams and rivers (Cilleros *et al.* 2018; Jerde *et al.* 2019; Cantera *et al.* 2019). Although fish fauna was inventoried using the same eDNA protocols, we here considered separately streams and rivers fish separately because deforestation may affect the two ecosystems differently. While stream ecosystems mainly suffer from local deforestation (Dedieu *et al.* 2014, 2015; Allard *et al.* 2016), due to the limited spatial extend of their drainage basin (a few square kilometers), rivers act as recipient for deforestation effects cumulating from substantial upstream distances (several hundreds of square kilometers, see Chapter 3).

The aim of this study is to define how diversity patterns and processes vary along a deforestation gradient and along the upstream-downstream gradient (natural *versus* disturbance gradients). We hypothesized that: (1) environmental filtering govern community assembly in small streams with the strength of the process progressively decreasing along the upstream-downstream gradient (See Figure 1A), because increasing the distance to the source accounts for an increase of habitat diversity (Willis *et al.* 2005). Conversely, limiting similarity is predicted to be the predominant processes ruling community assembly in large rivers, because of a saturation of habitat diversity (Willis *et al.*, 2005), and the strength of this process should increase from the upstream to the downstream of rivers (See Figure 1C). (2) Environmental filtering should govern community assembly under high deforestation levels because in deforested sites, the degraded conditions may filter species according to their traits. In contrast, in undisturbed sites, the higher resource availability and habitat complexity will promote the

coexistence of functionally distinct species (Willis *et al.* 2005), and therefore reduce the environmental filtering effect. However, the strength of this process will differ between rivers and streams. As streams communities are expected to be mainly ruled by environmental filtering, we expect that the strength of this processes will increase along the deforestation gradient (See Figure 1B). For river communities, we expect a progressive transition from limiting similarity as the predominant process to environmental filtering, when increasing the anthropogenic disturbances summarized by the deforestation intensity (See Figure 1D).

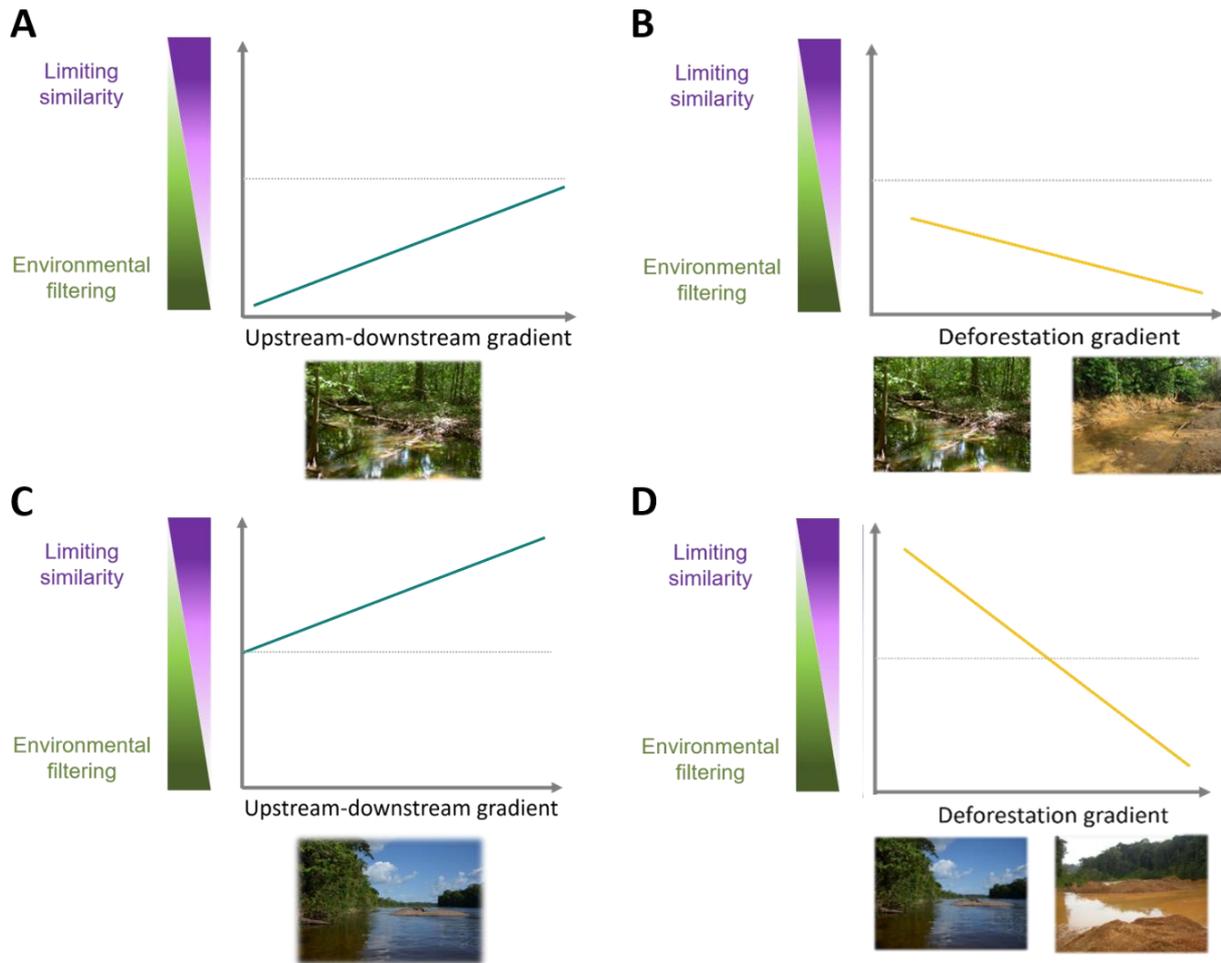


Figure 1: Conceptual framework illustrating our hypotheses about the changes in the relative importance of ecological processes (limiting similarity vs environmental filtering) across the environmental gradients (upstream-downstream and deforestation). Dashed horizontal lines indicate an equal strength of the two processes. (A, C) Contribution of the processes along the upstream-downstream gradient for streams and rivers, respectively. (B, D) Contribution of the processes along a deforestation gradient for streams and rivers, respectively.

## Materials and Methods

### *Sampling sites and deforestation intensity*

Sampling was undertaken at 37 stream and 50 river sites located across nine river basins of French Guiana (Figure 6, Chapter 1-IX). Stream sites were less than 10 meters wide and 1 meter depth while river sites were wider than 30 meters and deeper than 1 meter. Following the protocol implemented by Cantera et al. (2019), we filtered 34 liters of water at each site to collect eDNA. See the materials and methods section of Chapter 2 for details on field sampling, laboratory procedures and bio-informatic analyses.

For each sampled site, we calculated deforestation intensity as the percentage of deforested surfaces upstream from each site following the same method used in Chapter 3. For streams, buffer areas were delineated with a distance of 0.5 km upstream from each sampling site. For rivers, buffer distance was of 70 km as it was found as the appropriate spatial extent to measure deforestation impacts on fish functional diversity in large Guianese rivers (Chapter 3).

### *Fish species and functional diversity*

For each site, species inventories based on presence/absence were build based on the assigned sequences. The fish biodiversity of each site was measured through species and functional diversity. Species richness corresponded to the number of detected species in the eDNA sample. This measure, although not exhaustive, provided a more comprehensive image of the fish species richness and species composition than other capture methods (Cantera *et al.* 2019). Morphological and ecological traits of the species detected in each site were used to build a global functional space (see Chapter 1-IX-2 for details). Among the 187 detected species, traits were available for 178 species for the morphological data (95% of the total number of detected species) and for 182 species for ecological data (97%). Moreover, to assess the differences on species composition due to upstream deforestation, Jaccard dissimilarity values between sites were calculated for each habitat separately and ordinated using non-metric multidimensional scaling.

### *Null models to assess ecological processes*

The relations between functional and species diversity were analyzed using null models (Gotelli and Graves 1996) to determine if taxonomic and functional changes are paired. Given that trait

diversity is directly influenced by taxonomic diversity, it is important to control this effect. The number of detected species were fixed for each site and the species identity were randomized 999 times. By doing so, 999 null values of functional richness were generated per site. Then, we compared observed functional richness to the one expected by chance by calculating standardized effect size (SES) values per site. SES values correspond to the difference between the observed functional richness and the mean of the 999 null values of functional richness divided by the standard deviation of the 999 null values. Negative values of SES indicate that the functional diversity is lower than expected by chance given the observed taxonomic diversity and thus that environmental filtering is predominant. In contrast, positive values of SES indicate that functional richness is higher than expected under random assembly, indicating that limiting similarity is governing community structure.

### *Statistical analyses*

Linear Mixed Models were used to test the effects of the upstream-downstream and deforestation gradients on diversity patterns and processes. The upstream-downstream gradient was coded using the basin surface area upstream from the sampling site considering that the area increases from upstream to downstream. Diversity patterns corresponded to species composition (measured by the variation of sites along NMDS axes 1 and 2), species richness and functional richness. Diversity processes were measured with the SES values. For each diversity pattern and the SES values (response variables), we built a specific model in which the upstream-downstream and deforestation gradients were scaled fixed variables. The variables were test for spatial autocorrelation using Moran's I, and the effect was not significant. The effect of the interaction between the two gradients was also assessed. Basin identity was included as a random effect, to control for differences in fish species between river basins. The models were built using the *lmer* function from the lme4 package (Bates et al. 2015) in R (R Core Team 2016). Rivers and streams sites were considered separately (see Introduction).

## Results

### *Species composition*

Once controlling for basin identity, the upstream-downstream gradient affected significantly species composition of fish communities along the NMDS axis 1, for both stream and river sites (Table 1). Moreover, the deforestation gradient significantly influenced the species composition of fish communities along the NMDS axis 2, for both stream and river sites (Table 1). Stream communities under high deforestation levels were scattered in a limited portion of the NMDS plane (positive values of NMDS axis 2), indicating that stream fish communities affected by deforestation are constituted of similar species assemblages regardless of the basin identity (Figure 2A). Contrastingly, river communities under high levels of deforestation differed in species composition, as they were more dispersed in the NMDS plane (Figure 2B).

<i>Habitat</i>	<i>Response variable</i>	<i>Upstream-downstream gradient effect</i>		<i>Deforestation gradient</i>	
		<i>Slope</i>	<i>p-value</i>	<i>Slope</i>	<i>p-value</i>
<i>Streams</i>	Species richness	<b>6.18</b>	<b>0.021</b>	2.25	0.24
	Functional richness	<b>0.070</b>	<b>0.043</b>	-0.004	0.48
	SES	0.157	0.5	<b>-0.529</b>	<b>&lt;0.01</b>
	NMDS axis1	<b>-0.33</b>	<b>0.03</b>	<b>-0.05</b>	<b>0.68</b>
	NMDS axis2	-0.04	0.71	<b>0.25</b>	<b>0.008</b>
<i>Rivers</i>	Species richness	-7.028	0.065	<b>-11.345</b>	<b>0.001</b>
	Functional richness	-0.052	0.102	<b>-0.076</b>	<b>&lt;0.01</b>
	SES	<b>0.480</b>	<b>0.044</b>	-0.136	0.501
	NMDS axis1	<b>0.34</b>	<b>&lt;0.01</b>	0.02	0.71
	NMDS axis2	-0.04	0.71	<b>-0.13</b>	<b>0.03</b>

Table 1: Results of the linear mixed models. For each response variable, a specific model was build controlling by basin identity as random effect.

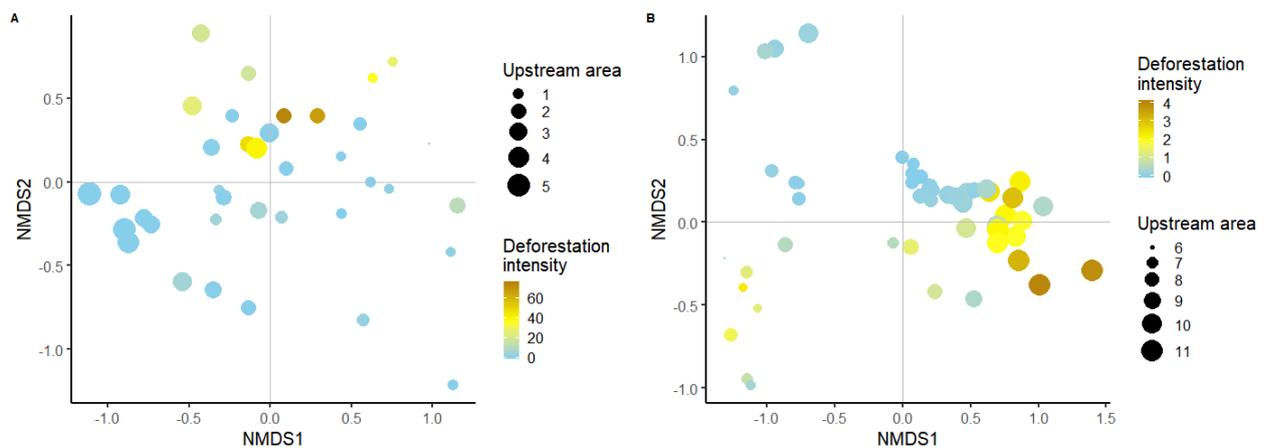


Figure 2: NMDS ordination of the sites based on species composition in stream (A) and river (B) fish communities. Deforestation intensity in each site was indicated as a blue to brown color shade. Upstream area was log transformed.

### *Species and functional richness*

On average, we found a higher species richness and functional richness in rivers communities than in streams communities (Table 2). The upstream-downstream gradient had a significant effect on diversity patterns for stream communities (Table 1): species and functional richness increased from upstream to downstream (Figure 3). In contrast, the effect of deforestation was not significant on stream communities (Table 1). Opposite patterns were found for riverine communities, as the upstream-downstream gradient had not a significant influence on diversity patterns (Table 1), but the effect of deforestation was significantly negative for both species and functional richness (Figure 4, Table 1).

<i>Habitat</i>	<i>Response variable</i>	<i>Mean</i>	<i>Standard deviation</i>	<i>Mean percentage of upstream deforested surfaces</i>
<i>Streams</i>	Species richness	28.25	11.7	
	Functional richness	0.22	0.16	9.1 (0 -67%)
	SES	-0.23	1.02	
<i>Rivers</i>	Species richness	48.76	17.06	
	Functional richness	0.40	0.13	1% (0 -4.3%)
	SES	0.00	0.79	

Table 2: Mean and standard deviation of species richness, functional richness and SES values for each habitat. The mean percentage of upstream deforested surfaces for each habitat is also indicated.

### *Ecological processes*

In streams communities, the mean of the SES values was negative despite a large standard deviation (Table 2). In river communities the mean values of SES values was null. The effect of the upstream-downstream gradient was not significant for the SES values (Table 1), in spite of a slight trend towards increasing values of SES downstream (Figure 4). Although, the majority of the SES values were within the neutral interval [-1.75 to 1.75], the SES values significantly decreased along the deforestation gradient (Table 1). At low deforestation intensities, SES values exhibited a marked heterogeneity but this variability declined with increasing deforestation intensity (Figure 4). At high deforestation levels, SES values were all negative, indicating a trend towards species having lower functional richness than expected randomly with increasing deforestation intensity.

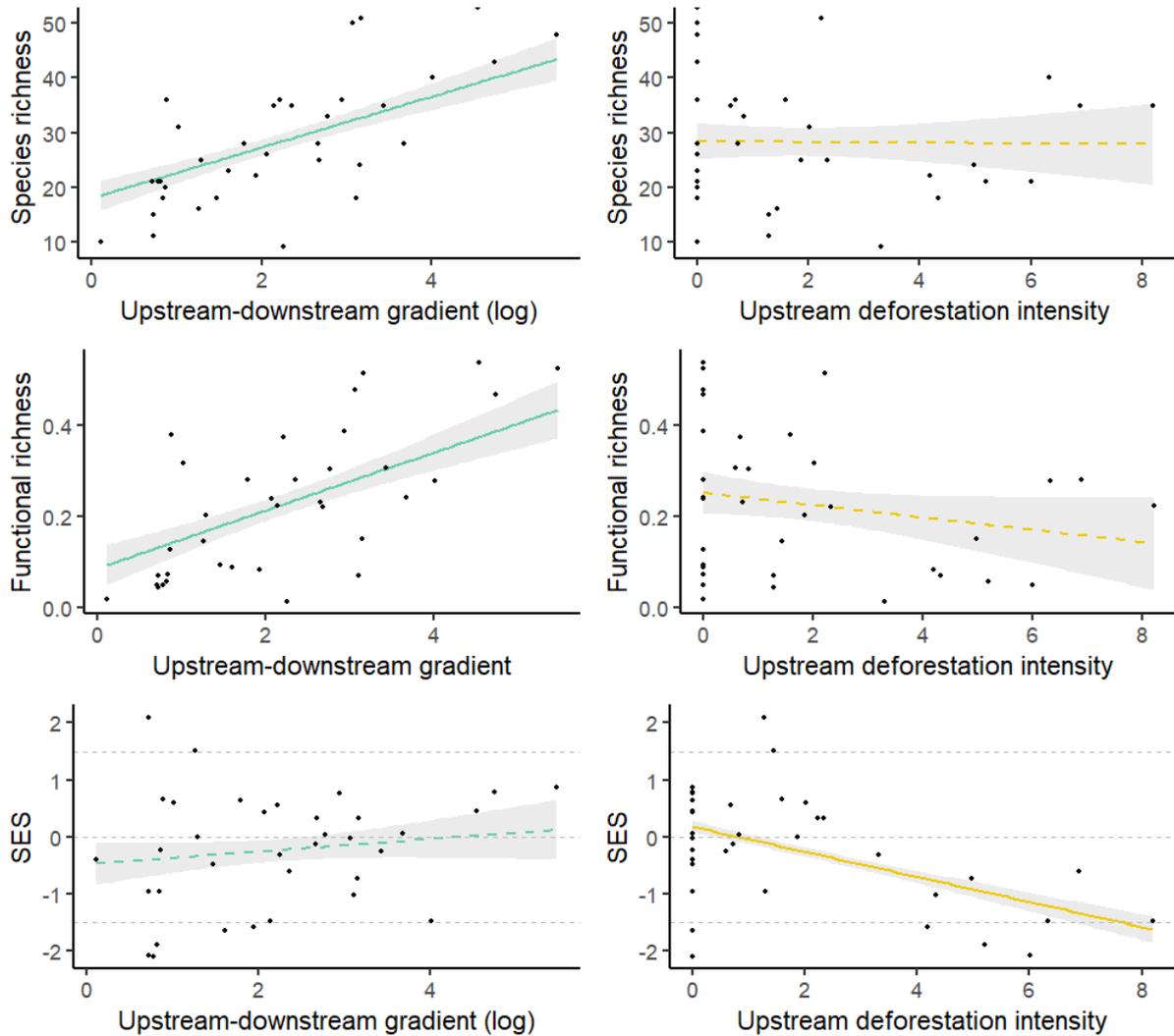


Figure 3: Effects of the upstream-downstream gradient (left) and the deforestation gradient (right) on species richness (A, B), functional richness (C, D) of fish communities for stream sites, and standardized effect size (SES) values of functional diversity (E, F). The sign of the SES value indicates if the functional diversity is lower (negative) or higher (positive) than expected by chance given the observed species richness. Fitted values of the mixed models are shown with solid lines for significant effects and 95% confidence intervals are indicated with grey shades. Dashed lines represent non significant effects. For a better representation, deforestation values were squared root transformed (real deforestation values ranged from 0 to 67%) and the basin surface areas upstream from the sampling were log transformed.

In riverine communities, the deforestation gradient effect on SES values was not significant, whereas the effect of the upstream-downstream gradient was significant. SES values increased from upstream to downstream suggesting a trend towards communities having higher functional richness than expected given the number of species in downstream communities. Finally, the effect of the interaction between deforestation and the upstream-downstream gradient was not significant for any response variable.

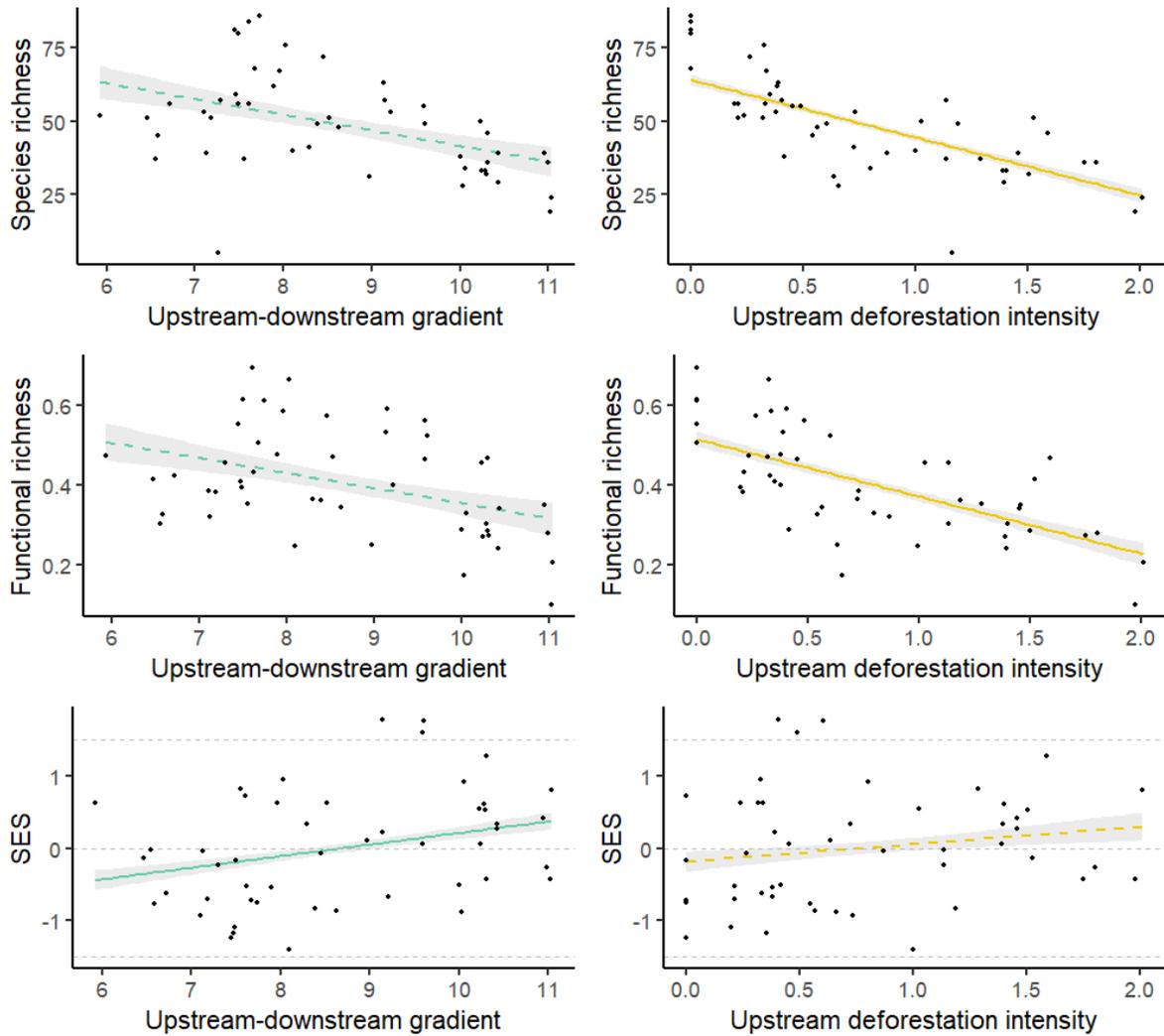


Figure 4: Figure 3: Effects of the upstream-downstream gradient (left) and the deforestation gradient (right) on species richness (A, B), functional richness (C, D) of fish communities for river sites. Standardized effect size (SES) values of functional diversity (E, F). The sign of the value indicates if the functional diversity is lower (negative) or higher (positive) than expected by chance given the observed species richness. Fitted values of the mixed models are shown with solid lines for significant effects and 95% confidence intervals are indicated with grey shades. Dashed lines represent non significant effects. Deforestation values were squared root transformed (real deforestation values ranged from 0 to 4%) and the basin surface areas upstream from the sampling were log transformed.

## Discussion

Environmental gradients, be they natural or generated by anthropic disturbances, structure diversity patterns. This is a fairly well known trend in temperate rivers and streams (Grenouillet *et al.* 2004; Buisson *et al.* 2008). It has also been verified in tropical rivers (Cilleros *et al.* 2017), although tropical ecosystems benefitted from much less attention than their temperate counterparts. We here confirm such trends, but interestingly show that taxonomic and

functional changes in diversity across environmental gradients also account for changes in ecological processes shaping local communities.

In stream communities, the percentage of deforested surfaces upstream from the fish sampling sites ranged from 0 to 75%. This impact resulted in differences in community composition according to the deforestation level. Furthermore, the dissimilarity in species identity between high deforested communities was low (Figure 3), even between stream sites belonging to different watersheds. This means that highly disturbed communities are composed of the same set of species, whereas natural streams show a strong species dissimilarity. Nevertheless, the changes on species composition did not result in significant losses of taxonomic and functional richness of stream communities. Similarly, other studies in the region failed to detect an effect of deforestation on species richness but reported changes on species composition (Bojsen and Barriga 2002; Brosse *et al.* 2011; Allard *et al.* 2016; Prudente *et al.* 2017). Such discrepancy in species turnover between non-deforested and deforested streams indicate changes on ecological processes. The significantly negative effect of the deforestation gradient on SES values validate our hypothesis stipulating that the strength of environmental filtering increases with deforestation intensity (Figure 1B). Such a tendency has already been suggested in temperate streams for both fish and invertebrate communities (Gutiérrez-Cánovas *et al.* 2015; Kuczynski and Grenouillet 2018), but has not, to date, been reported in species rich tropical streams. This finding illustrates that the degraded conditions in deforested sites may filter out species according to their traits resulting in assemblages with ecologically similar species. Indeed, deforestation in Amazonian streams was found to mainly affect the physical structure of the streambed through reductions in bottom complexity and bed stability (Leitão *et al.* 2018). Additionally, the deforestation measured in our study is mainly due to gold-mining which have pronounced detrimental effects on stream physico-chemical conditions and streambed physical structure (Dedieu *et al.* 2015). Consequently, deforestation filters out species according to their habitat use and species associated with the benthic compartment might be more vulnerable than pelagic ones.

For river communities, upstream deforestation intensity was lower, compared to streams (from 0 to 4%), but was more extended over a large part of the upstream drainage basin (Chapter 3). Nevertheless, this low deforestation intensity significantly modified species composition and drive a marked erosion of both species and functional richness along the deforestation gradient. This parallels and extends the few studies assessing human impact on large rivers

illustrating a drastic effect of human impacts on biodiversity and ecosystem functioning (Vörösmarty *et al.* 2010). Despite a significant species and functional richness loss and contrary to our expectations (Figure 1C), ecological processes were not affected by the deforestation gradient. Therefore, the observed functional erosion is the result of random species loss, regardless of their functional traits. This finding suggests that in rivers, the observed decline of biodiversity is not due to habitat loss but rather to a chronic effect of pollution, which affects all of the species in the same way, without taxonomic or functional distinction. This type of chronic effect was indeed reported in increases of suspended matter contents downstream from mining sites for rivers in French Guiana but also in North America (Palmer *et al.* 2010; Lindberg *et al.* 2011; Gallay *et al.* 2018). Finally, the lack of deforestation effects on ecological processes should be considered with caution, because deforestation intensity remained low (less than 4% of the upstream drainage basin considered), and species extirpations are known to occur under extreme conditions (Mouillot *et al.* 2013b).

Contrary to our expectations, not all the diversity patterns and processes were influenced by the upstream-downstream gradient. In fish communities inhabiting streams, species and functional richness both increased from upstream to downstream. For species richness, the same pattern was found in Guianese streams by Cilleros *et al.* (2017) with capture-based fish inventories. Moreover, habitat structural diversity was found to increase from upstream to downstream and promote local species richness (Cilleros *et al.* 2017). Therefore, the increase of functional diversity observed along the upstream-downstream gradient is the result of an addition of species. However, those species are not more functionally dissimilar than expected randomly, as SES values did not increase along the upstream-downstream gradient. Therefore, even if functional richness is increasing from upstream to downstream it is paired with an increase of species richness and therefore not associated to a higher than expected increase in functional traits. The unexpected lack of global predominance of environmental filtering in stream communities (as hypothesized in Figure 1A) can be the result of either a predominance of neutral processes or an equal contribution of limiting similarity and environmental filtering. This suggests that the streams sampled in French Guiana may exhibit less harsh conditions than expected. Indeed, Cilleros *et al.* (2017) found that the environment explained poorly the variability on the species composition of Guianese stream communities. Moreover, at the regional scale, those communities are more structured by dispersal limitation than by environmental filtering (Cilleros *et al.* 2016), highlighting again a weak role of the abiotic conditions.

In river communities, the lack of an upstream-downstream gradient on diversity patterns was unexpected, because such trend is recognized as an almost universal pattern for rivers (Vannote *et al.* 1980; Osborne 2002; Allan and Ibañez Castillo 2009). Indeed, deforestation impacts may be altering the expected gradient of increasing diversity along the upstream-downstream gradient. Indeed, the percentage of deforested surfaces was positively correlated with the upstream-downstream gradient on rivers sites ( $r=0.8$ ;  $p<0.01$ ), which is explained by the fact that Guianese human population is mainly concentrated in the coastal zone and around large rivers. In other side, converging with an expectation of a relaxation of environmental filters in downstream habitats (Figure 1A), SES values increased significantly from upstream to downstream in river sites. Even though, limiting similarity did not governed community assembly in rivers (the SES values were within the neutral interval), we found a trend towards species having more different traits than expected randomly in downstream sites. This result combined with the lack of environmental filtering on deforested rivers advocates for dispersal processes gaining importance in downstream rivers compared to environmental factors (Henriques-Silva *et al.* 2019). According to the network position hypothesis, the central position of rivers facilitates the dispersion of species and may promote mass effects (Schmera *et al.* 2018), which means that under high colonization rates, species can temporarily occupy habitat patches that are not suitable for them (Pulliam 1988). Thus, the low contribution of environmental process mediated by the high dispersal rates of species in rivers may be compensating the diversity erosion induced by deforestation. Answering this hypothesis is currently difficult because abundance data is needed to detect mass effect processes, and neither traditional fish inventory methods nor eDNA metabarcoding is able to provide relevant abundance data in Guianese streams (Cilleros *et al.* 2018). Further studies might therefore investigate the potential of eDNA to provide fish abundance, as highlighted by Zinger *et al.* (2020), which will provide a finer assessment of community assembly, functional structure and the impact of human activities (Mouillot *et al.* 2013b; Cadotte and Tucker 2017).

Despite above cited uncertainties in fish inventories, the deforestation gradient and the upstream-downstream gradient had consistent contrasting effects on rivers and streams as well as on diversity patterns and processes. Such results reveal a context-dependency of deforestation impacts on Amazonian biodiversity, rivers and streams therefore exhibiting distinct responses to perturbation. Communities inhabiting streams suffered from direct effects of deforestation and gold extraction, which has drastic consequences on water quality and the physical structure of the river bed by reducing the complexity of bed bottom and stability

(Hammond et al. 2007; Dedieu et al. 2014; Leitão et al. 2018). These physical changes caused a strengthening of environmental filters, which did not reduce taxonomic or functional diversity but reduced species dissimilarity between sites. However, Guianese streams host endemic species that occupy specific habitats and have limited dispersal capacities (e.g. genera *Harttiella*, *Lithoxius*, *Melanocharacidium*, *Farlowella*). Those species, some of which are already listed as endangered by the IUCN (IUCN 2017), are probably the first to suffer from physical habitat degradation. Consequently, “species-centered” conservation measures seem necessary to preserve the diversity of stream fauna. Conversely, fish communities inhabiting rivers face more indirect disturbances. The lack of an effect of the disturbance gradient on ecological processes highlights a chronic decline in diversity where the entire species assemblages are affected, generating random local species extirpations. Therefore, a conservation approach centered on the protection of the whole community would be relevant in rivers.

## Chapter 5: How anthropogenic impacts modify on functional structure?

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### Differential responses to deforestation on fish functional structure between large and small tropical rivers.

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## Abstract

Quantifying biodiversity responses to anthropogenic disturbances is fundamental to assess the severity of human impacts. Functional diversity allows a deeper assessment of anthropogenic impacts on natural ecosystems because functional traits are more tightly linked to ecosystem processes than taxonomic diversity. However, the functional diversity is multifaceted and the different facets can have different responses to human impacts. The aim of this study was to describe the multifaceted effects of anthropogenic impacts on the functional structure of freshwater fish communities.

We sample 50 river sites and 37 stream sites across French Guiana using environmental DNA. Rivers and streams sites were considered separately given that they associated fauna and environment markedly differ. Deforested surfaces upstream from the fish sampling sites were extracted from spatial data to create a global deforestation variable that integrates the effects urbanization, agriculture, gold-mining and logging. Functional spaces were built using morphological and ecological traits of the detected fish species in our eDNA samples to measure different functional indexes and assess the multifaceted effect of deforestation on functional diversity.

In streams communities, deforestation affected significantly the functional evenness and the functional identity but not the functional richness or the functional specialization. This results in modifications in the internal structure of the functional space with fish communities overrepresented by pelagic detritivorous while underrepresented by benthic phytophagous species, but remaining functionally specialized. In rivers, deforestation was not significantly related to the functional specialization or evenness of communities but had a negative effect on functional richness and the positive relationship between the upstream-downstream gradient and those indices. Finally, deforestation did not to modify trait composition, which was more structured by the upstream-downstream gradient. We observed a global simplification in a multifaceted way in the functional diversity of fish communities under high levels of deforestation but the pathways were different between streams and rivers.

## Introduction

Natural ecosystems are facing increasing and unprecedented anthropogenic impacts that are eroding the diversity of biological communities (Barnosky *et al.* 2011). Quantifying biodiversity responses to anthropogenic disturbances is fundamental as biodiversity maintains the functionality of ecosystems (Tilman *et al.* 2006; Mouillot *et al.* 2011) and therefore the multitude of ecosystem services they provide to human societies (Cardinale *et al.* 2012).

Biodiversity is a multi-faceted concept with each facet providing complementary information (Le Bagousse-Pinguet *et al.* 2019). Among the different facets, taxonomic diversity corresponds to the number of species occurring in a community whereas the functional facet captures the variety of morphological, ecological, behavioral and physiological traits among species within a community (Villéger *et al.* 2017). In the last decades, functional diversity has been claimed as a more appropriate tool to assess the impact of anthropogenic activities on natural ecosystems (Mouillot *et al.* 2013b), as functional diversity is more closely linked to ecosystem processes than taxonomic diversity (Cadotte *et al.* 2011; Mori *et al.* 2013). This suggests that functional changes will better relate alterations in community structure to changes in ecological processes that maintain ecosystem functions and will therefore better quantify the depth of human impacts on natural ecosystems. Indeed, previous studies have reported more pronounced functional changes than taxonomic changes as a result of disturbances regardless of the ecosystems or taxa considered. For instance, the introduction of non-native fish to rivers across the world over the past two centuries resulted in functional changes 10 times higher than taxonomic changes (Toussaint *et al.* 2018). Likewise, ocean acidification resulted in losses of functional diversity twice as high than taxonomic diversity in benthic marine communities.

The functional structure of communities is also multifaceted and can be represented in a multidimensional space built constructed by ordinating species based on trait distances and multivariate analyses (Villéger *et al.* 2008). In this multidimensional space, the axes correspond to functional traits or to synthetic traits summarizing several raw traits. Hence, species are located according to their trait values within the functional space and communities are represented in terms of the functional abilities of both the entire community and the component species (Mouillot *et al.* 2013b). The functional structure of a community can describe several facets of the functional diversity (see Villéger *et al.* 2008, 2010; Mouillot *et al.* 2013 for more details):

- i) The amount of the traits supported by all of the species co-occurring in a given community. This facet is commonly called “Functional richness” and represents the multidimensional volume occupied by the community within the functional space.
- ii) How traits are supported by the species in a given community and are distributed within the functional space. This facet is commonly called “Functional evenness” and represents the internal structure of the multidimensional volume of the community.
- iii) The identity of the traits supported by all of the species co-occurring in a given community. This facet is commonly called “Functional identity” and consists on the localization of a given community along the axis of the multidimensional space.

Those facets provide different information about the functional diversity supported by a given community. For instance, coral reef fish communities exhibit a high diversity of but low evenness, as species were found to be packed into a few traits combinations, leaving the majority of traits without redundancy (Mouillot *et al.* 2014; D’agata *et al.* 2016). Notably, the different facets of functional structure can respond differently to human disturbances. Indeed, functional evenness was highly impacted by fragmentation and habitat loss in tropical communities of birds and trees, but the functional richness remained unchanged. It is thus important to assess the multifaceted responses of functional structure under disturbance because even if one component remain unchanged, the others components can have different responses.

Deforestation is one of the major causes of ecosystem degradation, especially in Amazonian forest environments (Hansen *et al.* 2010; Gibbs *et al.* 2010; Morris 2010), where rivers and streams are facing growing rates of deforestation due to agricultural expansion, mining and logging (Castello *et al.* 2013). It is thus mandatory to define the functional structure of freshwater communities to understand the relations among community structure, trait diversity, and ecosystem functioning. Figure 1A represents the functional structure of a hypothetical non-impacted community within the global functional space (constructed with all of the species present in a set of sites). We propose three non-exclusive hypotheses describing the impacts on functional structure due to deforestation. Environmental filtering theory predicts that communities will progressively became functionally simplified along disturbance gradients (Mouillot *et al.* 2013b) resulting in:

- i) Disturbed communities showing a lower range of traits (lower functional richness) than non-deforested ones (Figure 1B).

- ii) Deforested communities experiencing higher trait packing in the functional space than non-deforested ones. This results in deforested communities having low functional redundancy and thus low functional evenness in the distribution of the species within the functional space (Figure 1C).
- iii) Deforested communities having distinct functional traits from those non-deforested ones and thus different functional identity values along the axis (Figure 1D).

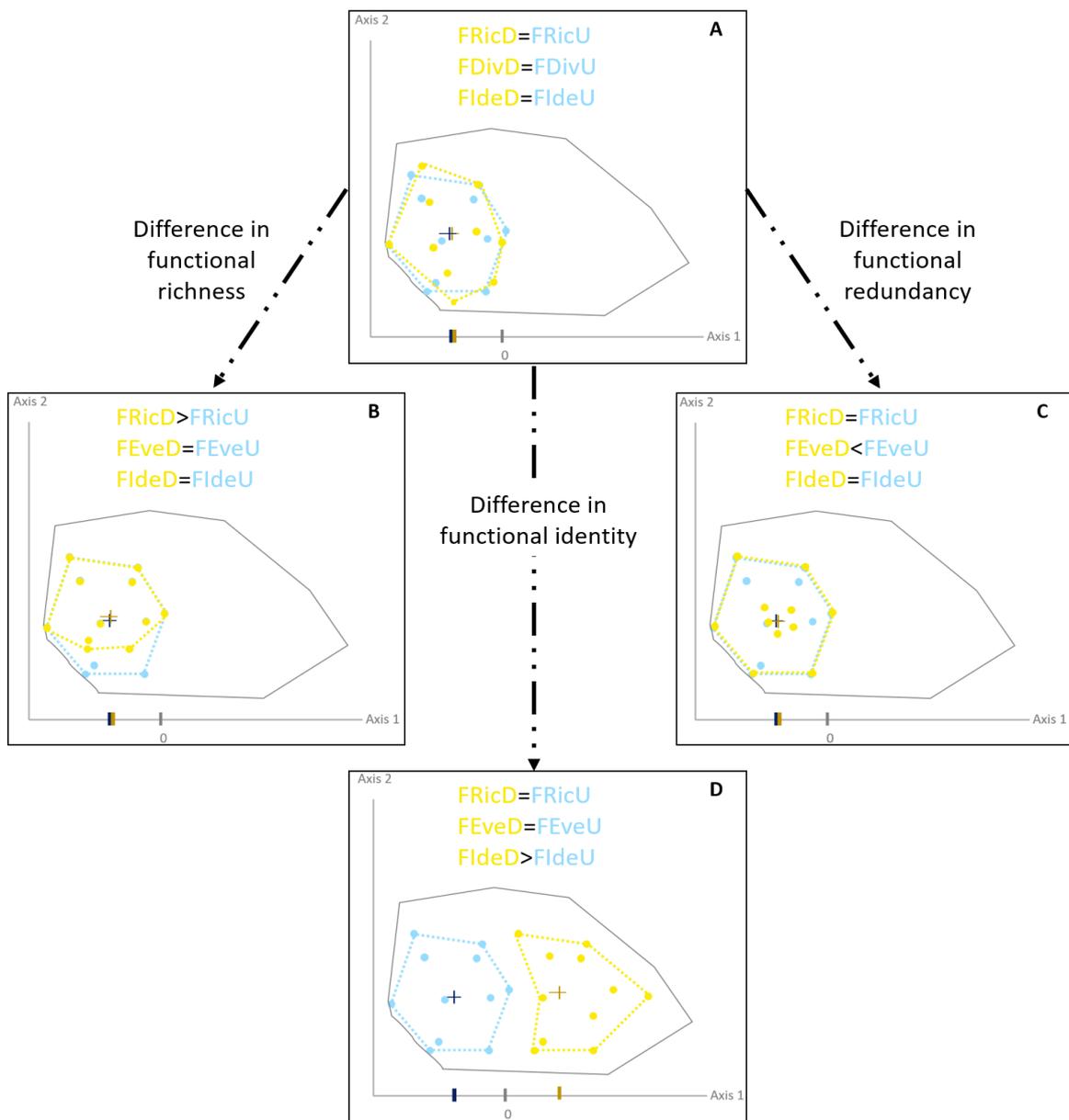


Figure 1: Illustration of the potential multifaceted effects of human impacts on functional structure. The global functional space is delimited with grey solid lines. The functional space of hypothetical communities are delimited by dashed lines with blue representing the non-deforested communities and yellow the deforested ones. Species are represented with dots and the Community barycenter is indicated with a cross. Three non-exclusive changes from the functional space of a hypothetical community (A) are proposed: (B) Decrease on functional richness due to trait losses. (C) Shifts in the identity of traits reflected by changes in the average position of the community along the ordination axis. (D) Species are less unevenly distributed within the functional space, with few species having extreme trait values and many generalists' species.

We applied this framework to assess the impacts of deforestation on the functional structure of freshwater fish communities inhabiting tropical forests. Local fish assemblages in rivers and streams across French Guiana were inventoried using eDNA. This method allows to efficiently gather fish data for comprehensive studies without causing massive fish mortality and has been proven to be efficient in characterizing species rich ecosystems, such as Guianese streams and rivers (Cilleros *et al.* 2018; Jerde *et al.* 2019; Cantera *et al.* 2019). We assessed stream and large river communities separately to ensure environmental and faunistic homogeneity of the fish assemblages among sites. Moreover, deforestation may affect the two ecosystems differently because streams are directly impacted by deforestation whereas rivers act as recipient for deforestation effects cumulating from substantial upstream distances (Chapter 3).

## **Materials and methods**

### *Sampling sites and deforestation intensity*

Sampling was undertaken in 37 stream and 50 river sites located across nine river basins of French Guiana (see Figure 6 in Chapter 1-IX). Following the protocol implemented by Cantera *et al.* (2019), we filtered 34 liters of water at each site to collect eDNA. See the materials and methods section of Chapter 2 for details on field sampling, laboratory procedures and bio-informatic analyses.

For each sampled site, we calculated deforestation intensity as the percentage of deforested surfaces upstream from each site following the same method used in Chapter 3. For streams, buffer areas were delineated with a distance of 0.5 km upstream from each sampling site. For rivers, buffer distance was of 70 km, as it was found as the appropriate spatial extent to measure deforestation impacts on fish functional diversity in large Guianese rivers (Chapter 3).

### *Fish species and functional diversity*

For each site, inventories based on the presence/absence of species were build based on the assignment of the obtained sequences in our eDNA replicates. The fish biodiversity of each site was measured through species and functional diversity. Species richness per site corresponded

to the number of detected species in the eDNA sample. This measure, although not exhaustive, provided a more comprehensive image of the fish species richness and species composition than other capture methods (Cantera *et al.* 2019).

Morphological and ecological traits of the species detected in each site were used to build a global functional space (see Chapter 1-IX for details). Among the 187 detected species, traits were available for 178 species for the morphological data (95% of the total number of detected species) and for 182 species for ecological data (97%). Based on the position of species and communities within the multidimensional functional space, functional indices were calculated to describe the functional structure of fish communities using the function `multidimFD` available online (<http://villeger.sebastien.free.fr/Rscripts.html>). See (Villéger *et al.* 2008; Mouillot *et al.* 2013b) for more details.

The amount of traits supported by fish communities was measured using Functional richness (“FRic”), which corresponds to the convex hull volume occupied by co-occurring species for a given community in the functional space. This index ranges from 0 to 1, with higher values reflecting high volume occupation of the community and thus high diversity of traits. In addition, trait composition was measured using Functional identity (“Fide”), which is the mean position of the community in each ordination axis and calculated as the average PCoA scores of the species present in a community. This index reflects trends on the identity of traits displayed by the species present in a given community. Differences in Fide values between deforested and non-deforested communities will reflect qualitative impacts of deforestation in the types of traits. Moreover, the regularity of species’ distributions within the internal structure of the functional space was quantified using Functional evenness (“FEve”). High values suggest greater regularity of species distribution. Finally, functional specialization (“FSpe”) was also quantified. This index measures the extent of functionally unique species present in a community relative to the regional pool of species. This index is measured as the mean Euclidean distance between each species and the average position of all species (i.e. the barycenter) in the functional space. FSpe decreases when a community is dominated by generalist’s species (species close to the center of the functional space) and increases when a community is dominated by specialist’s species (species with extreme trait combinations).

## *Data analysis*

Linear Mixed Models were used to test if upstream deforestation significantly affects the functional structure of fish communities. For each functional index (response variables), we built a specific model in which the upstream-downstream and deforestation gradients were scaled fixed variables. The variables were tested for spatial autocorrelation using Moran's I but the effect was not significant. The effect of the interaction between the two variables was also assessed and basin identity was included as a random effect, to control for the regional pool context (Le Bail *et al.* 2012). The models were built using the *lmer* function from the *lme4* package (Bates *et al.* 2015). Rivers and streams sites were considered separately given that stream and river fauna and environment markedly differ (Allard *et al.* 2016; Cilleros *et al.* 2017).

To define how the impacts on the functional indices translate into impacts on trait composition and identify the traits that were significantly affected by deforestation, the function *envifit* from the *vegan* package was used. The function fits variables (here traits) onto the PCoA ordination in order to identify strong or weak correlations between traits and the ordination axes. A determination coefficient ( $R^2$ ) was calculated to assess the strength of those correlations. Traits having high  $R^2$  correspond to strong predictors of the ordination axis. In addition, p-values were calculated by comparing if the observed  $R^2$  values were significantly higher than permuted  $R^2$  values, based on 999 random permutations of the data. To quantify the contribution of the continuous traits, they were transformed onto vectors with its direction according to the correlation type with the axes (positive or negative) and the length of the vectors proportional to the strength of the correlation between the axis and the trait ( $R^2$  values). For categorical variables, average ordination scores were computed for each category of the traits to locate the different categories within the functional spaces.

## **Results**

### *Functional richness and species distribution on the functional space*

In stream communities, the effect of upstream deforestation on functional richness and functional specialization was not significant (Table 1) but was significant on functional evenness ( $p=0.001$ , slope=  $-0.006$ ). The functional evenness of communities significantly

decreased with deforestation intensity. The upstream-downstream gradient only had a significantly effect on functional richness (Table 1). Finally, the interaction between deforestation and the upstream-downstream gradient was not significant for any index (Table 1).

Habitat	Functional index	Upstream-downstream gradient		Deforestation effect		Interaction	
		Slope	p-value	Slope	p-value	Slope	p-value
Streams	Functional richness	<b>0.055</b>	<b>0.026</b>	-0.004	0.732	0.022	0.670
	Functional evenness	-0.002	0.687	<b>-0.006</b>	<b>0.001</b>	0.010	0.280
	Functional specialization	0.006	0.175	-0.001	0.565	0.006	0.513
Rivers	Functional richness	-0.057	0.28	<b>-0.143</b>	<b>0.001</b>	0.024	0.42
	Functional evenness	<b>0.036</b>	<b>0.006</b>	0.016	0.078	<b>-0.027</b>	<b>0</b>
	Functional specialization	<b>0.021</b>	<b>0.001</b>	0.004	0.391	<b>-0.008</b>	<b>0.043</b>

Table 1: Results of the linear mixed models relating upstream deforestation and the upstream-downstream gradient to the functional indices in river and stream sites. For each index, a specific model was build controlling by basin identity.

In river communities, functional evenness and functional specialization significantly increased along the upstream-downstream gradient (Table 1). The effect of upstream deforestation was only significant on functional richness (Table 1). Increasing deforestation induced a steep decrease of functional richness. Nonetheless, the interaction between the upstream-downstream gradient and deforestation had a significant negative effect on functional evenness and functional specialization (Table 1).

### *Trait composition*

In order to assess if deforestation influences trait composition in fish communities, we analyze the relationships between deforestation intensity and the functional identity of communities.

For stream sites, deforestation had a significant effect on FIde values along the PCoA1 ( $p=0.003$ , slope= 0.006). As the intensity of upstream deforestation increased, FIde values increased along the PCoA1 (Figure 4B). Moreover, the upstream-downstream gradient had a significant effect on FIde values along the PCoA2 ( $p=0.013$ , slope= 0.013). FIde values increased along this axis from upstream to downstream. The gradients had no effect on the FIde values along the other axes and the interaction between the gradients had non-significant effect on FIde.

<i>Measured trait</i>	<i>Measured trait</i>	<i>p-value</i>	<i>R2</i>	<i>Axis.1</i>	<i>Axis.2</i>
Morphological traits	Ed/Hd	<b>0.001</b>	0,36	0,92	-0,39
	Bbl/Bl	<b>0.001</b>	0,18	-0,44	0,90
	Mo/Bd	<b>0.001</b>	0,56	0,52	-0,85
	Jl/Hd	<b>0.001</b>	0,26	0,38	-0,93
	Eh/Bd	<b>0.001</b>	0,75	-0,90	0,43
	Bl/Bd	<b>0.001</b>	0,20	-0,90	0,43
	Hd/Bd	<b>0.001</b>	0,64	-0,95	0,32
	Pfi/Bd	<b>0.001</b>	0,40	-0,15	-0,99
	Pfl/Bl	0.247	0,03	-0,98	-0,21
	Cfd/CPd	<b>0.001</b>	0,30	0,37	0,93
	Maximum body length	0.502	0,02	-0,87	-0,49
Ecological traits	Motility	<b>0.001</b>	0,50		
	Gregariousness	<b>0.001</b>	0,36		
	Water column position	<b>0.001</b>	0,53		
	Prefered substrate	<b>0.001</b>	0,23		
	Territorial	<b>0.001</b>	0,32		

Table 2: Contribution of each the trait to the axes of the functional space of stream communities. Only the axes significantly related to deforestation and the upstream-downstream gradient were included (i.e. PCoA 1 and PCoA 2, see results). The table shows the determination coefficient  $R^2$  of the correlation between each trait and the ordination, p-values based on random permutations of the data indicate if observed  $R^2$  are higher than  $R^2$  with randomly permuted data and the direction cosines of the continuous variables on the PCoA axes.

Relating the ordination based on PCoA1 and PCoA2 axes with functional traits showed that in stream communities, all the traits except maximum body length and pectoral fin (“Pfl/Bl”) were significantly related with the ordination (Table 2). The first axis of the functional space was mainly characterized by differences between benthic and pelagic species. Benthic species associated to hard substrates and exhibiting a sedentary, solitary and territorial behavior had lower PCoA1 values, whereas pelagic and benthopelagic species with mobile, gregarious and non-territorial behavior had higher PCoA1 values. For the morphological variables, eye vertical position “Eh/Bd”, body lateral shape “Hd/Bd” and body elongation “Bl/Bd” were negatively correlated to the PCoA1, while the eye size “Ed/Hd” was positively correlated to this axis (Table 2). The PCoA axis 2 was negatively positively with pectoral fin position “Pfi/Bd”, oral gape position “Mo/Bd” and maxillary length “Jl/Hd” (Table 2). Conversely, relative barbell length “Bbl/Bl” and caudal peduncle throating “Cfd/CPd” were positively correlated to this axis. Figure 4 illustrates that high deforested communities tend to be less represented by elongated “Bl/Bd” species having big heads “Hd/Bd” and relatively small eyes “Ed/Hd” positioned toward the top of the head “Eh/Bd” (positive PCoA1 scores, Figure 4). These species mostly belong the Loricaridae family, which are benthic algae feeders. Conversely, under high deforestation levels, sites were characterized by a dominance of pelagic

detritivorous species. Furthermore, upstream communities were characterized by species having small caudal peduncle throttling “CFd/CPd”, a basal mouth pectoral fin “PFi/Bd” and oral gape positioned towards the top of the head (Figure 4).

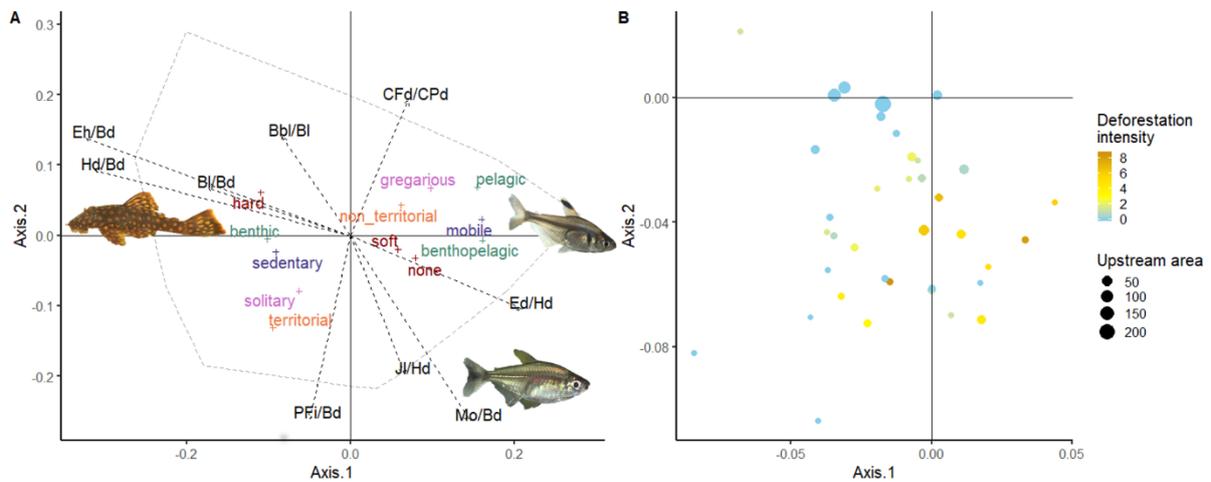


Figure 3: The functional space build with the axes significantly impacted by deforestation and the upstream-downstream gradient in stream communities. (A) Trait structure within the functional space. Only traits significantly related to the ordination are indicated (see Table 2 and results). Black arrows indicate the direction and the strength of the correlation between morphological continuous traits and the PCoA axes. The mean average position of each category is indicated by colored text corresponding to the five ecological categorical traits. Fish illustrations indicate typical morphologies for the different areas of the morphological planes. (B) Location of the fish communities within the functional space.

For river sites, we did not find any significant effect of upstream deforestation on the functional identity of fish communities. Conversely, the upstream-downstream gradient had a significant effect on functional identity along all of the five axes ( $p < 0.05$ ).

## Discussion

Amazonian rivers and streams are highly diverse, but are facing multiple and increasing human impacts that are affecting Amazonian biodiversity (Castello *et al.* 2013). Here we show that anthropogenic impacts in French Guiana modify the functional diversity of freshwater fish communities by affecting not only the amount of functional traits but also their identity and their distribution within the functional space. Following the environmental filtering theory, we hypothesized that highly deforested communities facing harsh conditions will exhibit more ecologically similar traits among the remaining species. Overall, we observed a simplification of the functional structure of communities under high levels of deforestation, but the pathways to this simplification were different between streams and rivers.

The percentage of deforested surfaces upstream from the fish communities in stream sites ranged from 0 to 75% (mean=9%). In spite of this considerable impact, the functional

richness of stream communities was not significantly affected by upstream deforestation, rejecting therefore our first hypothesis (see Figure 1B). However, as expected according to our second hypothesis (see Figure 1C), the functional evenness of communities significantly decreased under deforestation. As deforestation percentages increased, the resulting environmental degradations may filter out some species according to their traits, leaving some parts of the functional space underrepresented while other parts are overrepresented. Similarly, increases in functional redundancy due to local and watershed scale deforestation were reported for fish communities in Amazonian streams of Brazil (Bordignon *et al.* 2015; Leitão *et al.* 2018). Nevertheless, deforestation was not significantly related to the functional specialization of communities. This suggests that the conditions encountered in deforested sites are not particularly disfavoring species with extreme trait combinations (*i.e.* in the extremes of the functional space), which should explain why the functional richness was not significantly eroded. Therefore, our results point that high deforestation levels lead to modifications in the internal structure of the functional space but fish communities remain functionally specialized. The persistence of specialized species may be explained by the low percentages of upstream deforested surfaces recorded in our study sites. Indeed, 81% of our sites experienced less than 20% of upstream deforestation, which was the threshold proposed by Brejão *et al.* (2018) to observe abrupt responses to deforestation for Amazonian fishes. Thus, the functional changes reported here may be early warnings of more drastic functional responses and suggest that if deforestation intensity continues to increase in streams, the influence of environmental filtering might be stronger and fish communities would lose species with extreme trait values and be less functionally diverse.

Deforestation had also a significant effect on the functional identity of fish communities inhabiting stream sites (confirming hypothesis 3, Figure 1D). Importantly, the deforestation gradient was significantly related with PCoA1 axis and the upstream-downstream gradient with the PCoA2. This illustrates that, in those sites, the deforestation gradient explained more the variation in trait composition than the upstream-downstream gradient. A shift from communities dominated by benthic species towards communities dominated by pelagic detritivorous species along the deforestation gradient was detected, paralleling shifts observed in marine fish communities under global warming in both tropical and temperate ecosystems (McLean *et al.* 2019a). Deforestation and gold-mining were found to drastically affect the physical structure of stream bottoms through reductions in bottom complexity and bed stability in Amazonian streams (Hammond *et al.* 2007; Leitão *et al.* 2018). This will disproportionately

affect species associated with the benthic compartment. In addition, most benthic species absent from high-deforested sites feed on algae. Similarly, Allard *et al.* (2016) found that phytophagous species were underrepresented in logged sites in French Guiana using capture-based inventories. In fact, logging and gold-mining were reported to increase the turbidity and fine particle siltation in streams (Hammond *et al.* 2007; Brosse *et al.* 2011; Dedieu *et al.* 2014), leading to negative effects on algal growth (Tudesque *et al.* 2012) and thereby reducing food availability for algae feeders. Therefore, the anthropogenic activities assessed here may be arising environmental filters related to food availability and habitat alteration, thereby explaining the shift from a dominance of benthic phytophagous to pelagic detritivorous species along the deforestation gradient. Moreover, this shift may drive the observed decreases on functional evenness. As the deforestation intensity increases, benthic phytophagous species may be disfavored while pelagic detritivorous fish would be favored, which would result on species disproportionally packing into traits related with the pelagic compartment and the detritivorous guild. This shift can lead to consequences on ecosystem functioning, such as nutrient cycling (Cao *et al.* 2018) and the regulation of plant communities, especially when considering that local deforestation might lead to increases of aquatic vegetation due to shading decreases (Leitão *et al.* 2018).

In opposition with streams, river sites experienced low levels of deforestation intensity (from 0 to 4%, mean= 1%) but it was enough to significantly impact functional richness. As expected in our first hypothesis (Figure 1B), a marked erosion of functional richness was observed along the deforestation gradient. Nonetheless, we did not found direct significant effects of deforestation on the functional evenness or functional specialization, but deforestation had a negative effect on the positive relationship between the upstream-downstream gradient and those indices. Indeed, the functional evenness and functional specialization of fish communities significantly increased from upstream to downstream. Environmental stability, habitat size and complexity increase from upstream to downstream (Willis *et al.* 2005), driving an relaxation of abiotic filters and promoting an increasing resource availability along the upstream-downstream gradient. Thus, the observed increases of functional evenness and functional specialization from upstream to downstream, may be mediated by a limiting similarity process that governs community structure in riverine habitats and promotes the coexistence of species with different traits (Carvalho and Tejerina-Garro 2015). However, deforestation seems to be disrupting this pattern, as it had a negative effect on this positive relationship. This suggests that if deforestation levels are higher than the levels

observed here, the effect of the upstream-downstream gradient on the functional structure on fish communities will be completely disrupted. This interception with the upstream-downstream gradient may come from the fact that deforestation intensity increases from upstream to downstream ( $r=0.8$ ;  $p<0.01$ ), as the Guianese human population is mainly concentrated in the coastal zone and around large rivers. Finally, in river communities, deforestation did not have a significant effect on trait composition or on the relationship between functional identity and the upstream-downstream gradient (Hypothesis 3 rejected, Figure 1D). Since the upstream-downstream gradient had significant effects on the functional identity along all axes of the PCoA, trait composition should be primarily governed by the upstream-downstream gradient in these habitats.

In general, the functional structure of freshwater fish communities in our sites was affected by deforestation but the responses were different between river and stream communities. Moreover, the deforestation responses differ among the different facets of the functional diversity. Thus, we advocated that the functional structure of communities should be assessed in a multifaceted way. The functional richness of fish communities was significantly eroded with increasing deforestation in rivers sites (confirming hypothesis 1, Figure 1B) but not in stream sites (rejecting hypothesis 1, Figure 1B). Additionally, deforestation affected significantly the functional evenness of stream communities, resulting in communities unrepresented by benthic herbivore species compared to pristine sites (confirming hypothesis 2, Figure 1C). In rivers, deforestation did not directly affect the functional evenness of the communities but affected the positive relationship between the upstream-downstream gradient and functional evenness. Finally, deforestation affected the functional identity of fish communities in stream sites (confirming hypothesis 3, Figure 1C) but not in river communities. Our findings strongly support that vulnerable traits (which may vary with the type of disturbance) represented here by benthic herbivores can be locally extirpated, leaving the related functions unaccomplished and this will severely hamper the functioning of ecosystems.

## *General discussion*

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Amazonian rivers and streams host enormous levels of species diversity, but are also facing increasing and unprecedented anthropogenic impacts (Barlow *et al.* 2018). This work attempted to understand the depth of those impacts in Guianese streams and rivers using an integrative community ecology approach with an innovative and non-invasive sampling method.

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### *1) Optimization of the eDNA method for sampling species-rich communities*

This study provides guidelines for the application of eDNA in running waters and, specially, for standardizing and optimizing eDNA-based fish inventories in species-rich ecosystems, without reducing the representativeness of the fauna. Collecting eDNA from 34 liters of water was sufficient to obtain a good characterization of fish communities (87% of the expected fauna) in both stream and river sites. Increasing eDNA sampling effort to 68 liters of water enhanced detection rate by up to 91%. Higher sampling efforts only identified a few additional species. Consequently, if the purpose is to exhaustively inventory the fauna, it will be necessary to filter more than 68 liters to improve the detection probability of rare species.

Nevertheless, the core of fish communities is efficiently detected when collecting eDNA from a single sample of 34 liters of water, and such inventories were i) more efficient and less biased by fish morphology than traditional capture-based methods and ii) sufficient to distinguish fish assemblages among sites and to identify ecologically relevant patterns.

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### *2) Definition of the spatial extent and strength of anthropogenic impacts on biodiversity*

The anthropogenic impacts measured in this study had an extended effect on fish biodiversity. Deforestation impacts cumulated up to 70 kilometers upstream from fish sampling sites and caused steep declines of both species and functional diversity. Indeed, less than 5% of

deforested area within a buffer zone up to 70 kilometers away upstream of the fish sampling site, has caused a decline of more than 30% of fish biodiversity. We advocate that measuring deforestation intensity (here the percentage of deforested surfaces upstream from the sites) at large extent, capturing both local and more distant deforestation effects, explains better deforestation impacts on fish biodiversity.

Distant and low levels of anthropogenic impacts were linked to considerable erosions of fish diversity, suggesting that the impact of deforestation in rivers, often measured at the vicinity of the site, has been strongly underestimated in previous studies.

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3) *How anthropogenic impacts modify diversity patterns and ecological processes?*

Fish biodiversity patterns and processes presented different responses to human activities depending on the habitat. In stream communities, the strength of environmental filtering became more important in highly deforested sites resulting in assemblages with ecologically similar species. In rivers, in spite of a drastic erosion of functional and taxonomic diversity along the deforestation gradient, these communities were less influenced by environmental filtering. In contrast, the high habitat size, complexity and stability of downstream large rivers may be promoting fish functional complementary and thus compensating the potential environmental filters that deforestation can arise.

The deforestation gradient had contrasting effects on rivers and streams as well as on diversity patterns and processes, revealing the complexity of the impacts of anthropogenic activities on Amazonian biodiversity.

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4) *How anthropogenic impacts modify diversity the functional structure of fish communities?*

A global simplification of the functional structure of communities under high levels of deforestation was observed. The functional richness was significantly eroded with increasing deforestation in rivers sites but not in stream sites. Deforestation leads to an overrepresented of stream communities by pelagic detritivorous species while they were

underrepresented by benthic phytophagous species. In rivers, deforestation did not impacted significantly the functional specialization and evenness of communities but had a negative effect on the positive relationship between the upstream-downstream gradient and those indices.

Anthropogenic impacts modified the functional structure of fish communities in a multifaceted way and the responses of the different functional facets varied between streams and rivers.

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## **I) Environmental DNA, a tool to assess human impacts on Amazonian aquatic biodiversity**

Gathering realistic biodiversity inventories is a prerequisite to measure the strength of human impacts on biodiversity. Describing Amazonian biodiversity is challenging given the wide range of species diversity and the strong proportion of rare species (Leitão *et al.* 2016; Barlow *et al.* 2018). In French Guiana, sampling freshwater fishes is particularly challenging since current fish sampling methods vary among ecosystems, besides being destructive and species selective (see Chapter 1). We developed an eDNA metabarcoding procedure, which was validated and optimized. Our results might nevertheless be considered with caution because the measure of fish biodiversity in tropical rivers and streams cannot be exhaustive. Nevertheless, in the Chapter 1, we demonstrated that this method is equivalent, and even more effective than traditional methods to inventory fish fauna in Guyanese streams and rivers. Additionally, the method provided a realistic image of fish communities and exhibited a high repeatability in terms of species richness and identity. Finally, eDNA sampling was efficient in both streams and rivers, standardizing potential sampling bias among ecosystems and making possible to compare stream and river samples.

One uncertainty of the method lies in the distance detection of species in the water. Indeed, DNA in the water can be transported downstream along the river network. The distance detection was assessed in temperate ecosystems and the obtained estimations display a high variability among studies. Deiner *et al.* (2016) claimed a distance detection at the watershed scale, but these results were partly due to biases in the bioinformatic treatment of the data (Taberlet *et al.* 2018). Furthermore, a more recent study showed that eDNA of abundant species can be detected up to 130 km downstream, whereas the detection of less abundant species was restricted to a few kilometers, suggesting that abundant species are detected farther downstream than rare ones. Moreover, the introduction of caged animals in streams revealed that eDNA was detected 5 m but not 50 m downstream from caged salamander (Pilliod *et al.* 2014) and up to 1 km downstream from caged trout (Wilcox *et al.* 2016). In addition, DNA from a lacustrine fish species was detected up to 3 km downstream from the outlet of a lake by Civade *et al.* (2016), and Deiner and Altermatt (2014) detected two lake invertebrate species up to 12.3 km downstream from a lake. Similarly, our results on Chapter 2 showed that the method was able to discriminate between the fauna of a main course site from the fauna of an affluent site located nearby 300m upstream, without any trend toward nestedness of the stream fauna within the riverine fauna as claimed in other studies (Deiner *et al.* 2016; Cilleros *et al.* 2018).

Finally, this work highlights the ability of the method to distinguish among sites, ecosystems types (rivers *versus* streams) and therefore to inventory local species assemblages in tropical running waters, which was limited up to now to temperate environments (Civade *et al.* 2016; Port *et al.* 2016; Yamamoto *et al.* 2017). The eDNA metabarcoding approach has been claimed as a promising tool to measure biodiversity. Several studies tested its reliability (Evans *et al.* 2017; Lopes *et al.* 2017; Civade *et al.* 2016; Hänfling *et al.* 2016; Olds *et al.* 2016; Valentini *et al.* 2016) and advocated that the method provides similar or more complete species inventories. However, its application in ecological and conservation studies has not been widely exploited. Our results showed that the method can be used to sample disturbed communities and was able to discriminate between deforested and non-deforested sites (Chapters 2, 3 and 4).

## **II) Anthropogenic impacts on fish communities in Amazonian streams and rivers**

A community ecology approach was used to define how deeply anthropogenic activities affect diversity patterns and ecological processes of Amazonian fish communities, in which the connectivity of freshwater ecosystems was considered. Deforested surfaces from spatial data were extracted to create a global deforestation variable that integrates the effects of urbanization, agriculture, gold-mining and logging. Globally, we observed differences on species composition and a simplification of the functional structure of communities under high levels of deforestation, but the pathways to this simplification were different between streams and rivers.

In stream communities, the taxonomic and functional richness of fish communities were mainly shaped by the upstream-downstream gradient, while ecological processes were mainly influenced by the deforestation gradient. The direct and detrimental impacts of human activities on stream habitats resulted on changes on species composition, without significantly decreasing species and functional richness (Chapter 4). Those alterations were mediated by the environmental filtering process that gained strength in highly deforested sites. The analyses of the functional structure of fish communities allowed to deepen those findings and revealed that the increasing environmental filtering affected the internal structure of the functional space without affecting the functional richness (Chapter 5). Indeed, species were less evenly distributed within the functional space, leaving traits related with the pelagic compartment and

the detritivorous guild overrepresented, whereas benthic phytophagous species were underrepresented. Therefore, the anthropogenic activities assessed here may be arising environmental filters related to food availability and habitat alteration.

For river communities, we found that the effects of upstream deforestation are cumulating along the river network (Chapter 3) resulting in a marked erosion of fish diversity in downstream large rivers. Indeed, distant and low deforestation (<5% of deforested area upstream from the sampling sites) caused a decline of more than 30% of fish species and functional diversity (Chapter 3). This extended effect on biodiversity may be the result of an accumulation of alterations related with water and habitat conditions, as it has been largely reported for chemistry changes induced by mountain top mining (Palmer *et al.* 2010; Lindberg *et al.* 2011; Hitt and Chambers 2014). Nonetheless, this perturbation was not determinant on the ecological processes shaping fish communities in rivers, which were more influenced by the upstream-downstream gradient than by the deforestation gradient (Chapter 4). Contrary to our expectations, deforestation did not lead to an increase of environmental filters in these habitats. Furthermore, the lack of predominance of environmental filtering under high deforestation levels found in Chapter 4 advocates that in spite of the diversity erosion, the ecological rule shaping fish communities remains unchangeable. The Chapter 5 confirmed that the observed functional erosion might be the result of random species loss, regardless of their traits. Indeed, deforestation did not have a significant effect on trait composition, which was more influenced by the upstream-downstream gradient, nor on the functional evenness or functional specialization of river communities. This parallels with the results of Chapter 4, where we demonstrated that the ecological processes were more influenced by the upstream-downstream gradient than by deforestation. Nevertheless, deforestation had a negative effect on the pattern of increasing functional evenness and functional specialization from upstream to downstream. Deforestation intensity increases upstream from downstream, as Guianese human population is mainly concentrated in the coastal zone and around large rivers. Thus, deforestation can perturb this pattern towards more functionally simplified communities (less specialized and less evenly distributed within the functional space) in the highly disturbed downstream rivers.

Rivers and streams responded differently to the effects of anthropogenic activities in terms of both diversity patterns and processes. The way in which streams and rivers are impacted by human activities varies in French Guiana. Streams are often directly impacted by

gold-mining, logging and agriculture, which results in high disturbance levels, as we observed in our study (0 to 75% of upstream deforested surfaces). Moreover, their small size make them more prone to be complete damaged after disturbance. Indeed, deforestation and gold-mining in Amazonian streams was found to severely modify stream water quality, due to the massive release of suspended sediments (Hammond *et al.* 2007), and the physical structure of the streambed, through reductions in bottom complexity and bed stability (Leitão *et al.* 2018). In contrast, rivers may act as a recipients of diffused upstream impacts, excepting for large cities which directly affect rivers (Vörösmarty *et al.* 2010). Accordingly, we pointed out that the measured disturbances affected fish biodiversity beyond local effects (Chapter 3). Indeed upstream deforested surfaces located between 30 and 70 km away from our sampling sites, were still related to detrimental changes on biodiversity and our models better predict declines in fish biodiversity by measuring deforested surfaces over large spatial extents, capturing both local and large disturbances. Interestingly, in spite of being higher and more variable, local deforestation did not influence local biodiversity descriptors. Therefore, riverine biodiversity is impacted by distant upstream disturbance that cumulated downstream.

Furthermore, streams and rivers differ in environmental conditions and the ecological processes ruling community assembly (see the introduction of Chapter 4 for details). Small stream communities are mostly influenced by abiotic conditions, which converges with the significant impacts of deforestation observed in assembly processes (Chapter 4) and functional structure (Chapter 5). In opposition, downstream large rivers are less shaped by environmental conditions, paralleling with the lack of environmental filtering that we found in Chapter 4. Moreover, according to the network position hypothesis (Schmera *et al.* 2018; Henriques-Silva *et al.* 2019), those habitats are highly influenced by spatial processes due to the central position of rivers, which means that under high colonization rates, species can temporarily occupy habitat patches that are not suitable for them (Pulliam 1988). Thus, the low contribution of environmental process mediated by the high dispersal rates of species in rivers may maintain functionally diverse communities in spite of the loss of functional richness induced by deforestation. Correspondingly, in the Chapters 4 and 5, we observed that the upstream-downstream gradients have more strong effects on diversity patterns and community assembly than the deforestation gradient.

### III) Conclusion

Throughout the different Chapters, our findings strongly support the complexity of anthropogenic impacts on Amazonian biodiversity. Besides habitat-dependent responses, anthropogenic impacts modified the biodiversity of freshwater fish communities in a multifaceted way. Thus, we advocated that the sole consideration of local disturbances (Chapter 3) and/or one diversity facet can mask deeper impacts of anthropogenic disturbances on fish communities (Chapters 3, 4 and 5). Indeed, as many other studies, we found that the impacts of local deforestation in streams communities were masked by changes on species composition. This converges with previous studies that failed to detect changes in species richness of stream fish communities under deforestation or gold-mining (Chapter 1 section IV). Nonetheless, we revealed that anthropogenic activities in French Guiana's streams had deeper impacts on fish communities than reported before, as they modified the ecological processes ruling assembly.

This work underlined the vulnerability of tropical fauna to slight environmental changes, even in relatively well-preserved regions, such as Guianese forests. These findings are aligned with the high vulnerability of tropical ecosystems exposed in the Chapter 1 (section II). Importantly, this study highlights the vulnerability of the functions supported by Amazonian biodiversity as well as the vulnerability of the whole functioning of the ecosystem, considering the strong link between species traits and ecosystem functioning (see Chapter 1 Section I). In the Chapter 5, we observed that even if stream communities remained functionally diverse under high levels of deforestation, the trait structure changed strongly. Indeed, benthic herbivores are prone to be locally extirpated leaving their related functions unaccomplished and this may strongly hamper the ecosystem functions, such as nutrient cycling and the regulation of plant communities.

In light of the spatially extended and cumulative effect of human activities reported in Chapter 3 and the interactions between the upstream-downstream gradient and the deforestation gradient (Chapter 5), we call for future studies and conservation practices to not only consider local disturbances on fauna. This will avoid underestimating deforestation effects on riverine fauna, and will capture the actual human impacts on aquatic ecosystems. This also means that the way in which conservation policies are planned should be re-evaluated too and re-oriented towards a catchment based framework (Castello *et al.* 2013). For instance, protected areas should take into account the spatially extended impacts of human activities in freshwater systems.

### III) Perspectives

#### 1) Methodological perspectives

Although we were able to distinguish communities separated only by 300m (Chapter 1), forthcoming studies should explicitly define the distance detection of species in tropical rivers and streams. As mentioned before the distance detection displays a high variability among studies and all of the studies that assessed this issue explicitly were performed on temperate ecosystems. Unfortunately, their findings cannot be transposable to tropical waters, as tropical and temperate streams have distinct physical and chemical characteristics and these differences affect the rate of DNA degradation (Barnes *et al.* 2014). Moreover, comparing eDNA detections with the known distribution range of the species requires precise inventories. However, the current state of knowledge about the distribution range of Guinness species is very limited. In part, because of the inefficiency of traditional sampling methods. One solution is to assess the distance detection as the distance in which downstream communities became significantly dissimilar from one upstream community. This measure will be more a measure of the ability to distinguish communities than a species distance detection itself. Sites located linearly along the upstream-downstream gradient of a river can be used to test if the eDNA is transported:

- i) at the watershed scale (Figure 2a) as suggested by Deiner *et al.* (2016), which means that there is no significant dissimilarity between the red dot and all of the upstream sites.
- ii) at intermediates distances (Figure 2b), according to Deiner and Altermatt (2014).
- iii) at low distances (Jane *et al.* 2015; Civade *et al.* 2016), following and Pilliod *et al.* (2014) Wilcox *et al.* (2016).

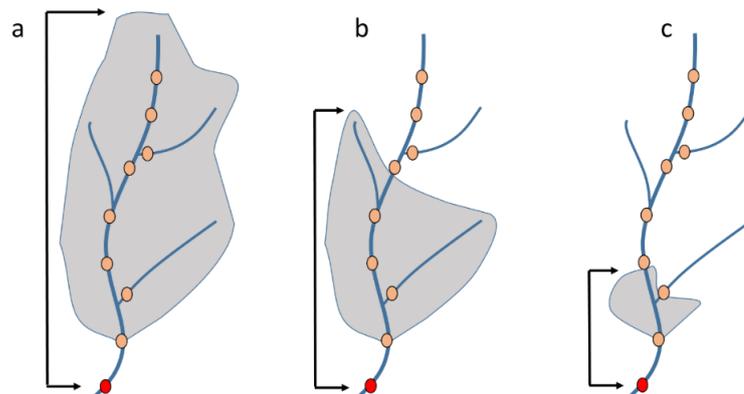


Figure 1: Illustration of the assessment of distance detection based on dissimilarities on species composition

Moreover, as discussed in the Chapter 4, we failed to report an increasing pattern of fish species richness and functional richness along the upstream-downstream gradient in large rivers. One explanation is that the eDNA method is failing to detect all the species on downstream habitats. This can be explained by the fact that downstream large rivers have more diverse habitats and higher species richness comparing to small rivers. Consequently, if the method has limited distance detection, not all the habitats may be extensively sampled with one eDNA replicate. Indeed, in the Chapter 2, we observed that species accumulation curves saturated faster in streams than in rivers and that stream replicates exhibited less variability than river replicates. This suggests that water volume should be adapted to the size of the sampled water system and that in very large rivers located downstream, the sampling effort should be higher than in upstream streams.

Finally, an important pitfall of this work is the inability to have information about the abundance of species. Indeed, deforestation effects in our study can be underestimated as species extirpations will only occur in extreme conditions (Mouillot *et al.* 2013b). This suggests that, the fact that a species was detected in one site, does not imply that the species has sustainable population sizes. Including the abundance of species may better reveal environmental filtering effects and thus provide a finer assessment of community assembly, functional structure and the impact of human activities (Mouillot *et al.* 2013b; Cadotte and Tucker 2017). It has been proposed that the number of reads can be an estimate of the abundance of individuals. However, the amount of free DNA on the water may also depend on the size of the fish, the behavior (for instance, mobile species might release more DNA than settle species), the metabolism, the proximity of the fish to the filter, etc. Thus, at the moment, it is complicated to measure species abundance with eDNA data. Assessing the release rate of each species in experimental designs would be ideal to have insights into the correlation between reads and abundance but it is impossible. One possible solution is to assess the release rate of some ecological groups (*e.g.* small vs. big, mobile vs sedentary) to define if the release rate varies considerable between ecological traits.

## 2) *Theoretical perspectives*

Given the observed significant effect of the upstream-downstream gradient on the diversity patterns of river communities (Chapter 4) and the known importance of dispersal processes in downstream areas (Henriques-Silva *et al.* 2019), it will fruitful to explicitly add large scale

processes to the assessment of the impact of human activities on fish communities. An approach, in which we compare beta-diversity patterns of taxonomic and functional diversity among communities, can provide insights into the effect of deforestation on ecological processes shaping riverine communities at large scale. For instance, we can test the network position hypothesis (Schmera *et al.* 2018; Henriques-Silva *et al.* 2019) in our sites and test if we obtained different results between deforested and non-deforested sites.

The findings reported here apply only to one taxonomic group, freshwater fishes. A comparative study using a multi taxa approach will allow extending our conclusions to different compartments of the aquatic ecosystem. Moreover, it will allow a better understanding how human activities are affecting the global ecosystem and its functioning. For instance, we can expect that more pronounced responses would be obtained if we use the same approach with aquatic invertebrates or algae, which are less mobile. Contrastingly, if we compare our results with taxa that occasionally use aquatic ecosystems and are therefore less dependent, such as birds and mammals, we might expect less pronounced responses. Such approach is feasible using eDNA data as the extracted DNA can be used to identify other taxa if a reference data base is accessible.

### 3) *Towards the development of a fish-based index of biotic integrity using eDNA*

This study enabled the validation and optimization of the eDNA method as a tool for assessing the strength of human impacts on fish communities across the rivers and streams of French Guiana. As stated before, a reliable definition of the distance detection will considerably improve the application of the eDNA sampling method in studies evaluating the ecological impacts of human activities on diversity but also for biodiversity monitoring and conservation goals, such as the development of an index of biotic integrity. This tool consists on the analysis of the characteristics of biological communities to assess the quality of aquatic habitats. The development of biotic indices is a prerequisite to systematically and uniformly assess the quality of ecosystems and their potential degradation by human disturbance. Biotic indices measure the condition of a given stream or river and are rely on comparisons between an undisturbed reference situation and the observed situation. Those biotic indices are often based on the prediction of species occurrences derived from species distribution models (Oberdorff *et al.* 2001). Such approaches require a comprehensive sampling of fish communities to have many sites covering different environmental variables and then build reliable models. The

development of a biotic index is of a particular interest in French Guiana, as a French territory, it must comply with European regulations aimed at developing surveillance programmes on water quality. However, all attempts to develop a fish-based index of biotic integrity have failed due to low quality of the models, which may be partly explained by the inefficiency of the current sampling. Thus, eDNA may be an efficient and affordable solution for performing compressive samplings across the territory and improving the quality of the models to build a biotic index.

#### *4) Integrating social dimensions on the assessment of human impacts on rivers*

Over this work, the diversity of rivers has been described through different facets: the diversity of species hosted by the rivers, the diversity of traits carried by those species, the diversity of habitats that support those species and the diversity of the responses to anthropogenic disturbances. However, there is another diversity facet, which is even at the origin of anthropogenic impacts, the diversity of relationships between human populations and rivers. For instance, French Guiana represents an intercultural mosaic, this diversity is reflected in the diversity of the relationships between the human populations and rivers (see the epilogue below). This diversity facet must be taken into account in the assessment of anthropization effects on rivers and more importantly, in management and conservation strategies. Human populations depend on river systems and exploit their resources and these relationships will have an impact on river ecosystems. The intensity and type of the impact will vary according to the relationship considered and they will also determine the strengths of the impacts on the biodiversity inhabiting the rivers. Another characteristic to be taken into account is that certain human activities will induce biodiversity alterations and this will in turn affect the human populations that depend on the river resources (e.g. fishing).

## *Epilogue : Les peuples et les rivières, une autre facette de diversité*

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Au cours de ce travail, la diversité des rivières a été décrite à travers de différentes facettes: la diversité en espèces abrités par les rivières, la diversité des traits portés pas ces espèces, la diversité des habitats qui abritent ces espèces et la diversité des réponses aux perturbations anthropiques. Cependant, il y a une autre facette de diversité, qui est même à l'origine des impacts anthropiques. C'est la diversité des relations entre les peuples et les rivières. La Guyane Française représente un mosaïque interculturel et cette diversité se reflète dans la diversité des relations entre les peuples et les rivières.

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*Les rivières sont source d'énergie.* Depuis 1994, le barrage hydroélectrique de Petit Saut a été construit sur le Fleuve Sinnamary. Ce barrage alimente en énergie surtout les villes de la région côtière.



Lac artificiel du barrage de Petit Saut (Sinnamary).

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*Les rivières sont source de vie et transport.* Sur les fleuves de l'Oyapock et du Maroni, les peuples amérindiens et noirs-marrons se sont installés. Ces fleuves sont fortement parcourus par des pirogues, puisqu'elles représentent le seul moyen de transport. En outre, ces peuples dépendent du fleuve pour se nourrir et pour avoir de l'eau.



Habitations au bord de la rivière Camopi (Affluent de l'Oyapock).

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*Les rivières sont source d'irrigation pour l'agriculture.* Les Hmongs, Laotiens réfugiés politiques se sont installés à la fin des années 1970 autour des cours d'eau de la Comte et fournissent la Guyane en fruits et légumes.

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*Les rivières sont source d'or.* Les cours d'eau guyanais possèdent beaucoup d'or. L'accroissement du prix de l'or a entraîné une multiplication de l'orpaillage légale et illégale. L'extraction d'or se fait partout dans la Guyane, mais c'est sur la Mana que l'entreprise Montagne d'or s'est installé.



Vue aérienne d'un chantier d'orpaillage illégal (haut) et ses conséquences sur la turbidité de l'eau (bas). Source : Le Parc Amazonien de Guyane

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## *Appendix*

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# Aquatic eDNA for monitoring French Guiana biodiversity

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## Abstract

## Background

Environmental DNA [eDNA] metabarcoding has recently emerged as a non-destructive alternative to traditional sampling for characterising species assemblages.

## New information

We here provide a consistent dataset synthesising all eDNA sampling sites in French Guiana to date. Field collections have been initiated in 2014 and have continued until 2019. This dataset is however a work in progress and will be updated after each collecting campaign. We also provide a taxon by site matrix for fishes presence / absence as inferred from eDNA. Our aim is to allow a transparent communication to the stakeholders and provide the foundation for a monitoring programme based on eDNA. The latest version of the dataset is publicly and freely accessible through the CEBA geoportal (<http://vmcebagndev.ird.fr>) or through the French Guiana geographic portal (<https://www.geoguyane.fr>).

## Keywords

South America, French Guiana, metabarcoding, environmental genomics

## Introduction

French Guiana is an overseas territory of France located on the north-eastern coast of South America. With ca. 84,000 km<sup>2</sup> (the size of Austria), it represents the largest outermost region of Europe. About 96% of its surface is covered by undisturbed primary rainforest. Due to its location in a tropical humid environment, the territory harbours a very dense hydrographic network. This network is comprised of 112,000 km of water bodies and is divided into 8 drainage basins flowing south-north (Mourguiart and Linares 2013). As opposed to Amazonia *sensu stricto*, where all the basins are connected to the Amazon, French Guiana basins are all disconnected and independently lead to the Atlantic Ocean. The two largest basins, the Maroni and the Oyapock, are boundaries with Suriname and Brazil, respectively. A total of 20% of the network is represented by rivers (Strahler order > 3) while the remaining 80% correspond to streams less than 10 m large and less than 1 metre deep.

As a European territory, French Guiana must comply with European regulations aiming at developing surveillance programmes on water quality (Directive 2000/60/EC). This directive was translated into French law (n°2004-338) mainly under article R212-22 of the environment code and the “Law on water and aquatic environment” (n°2006-1772). For the territory of French Guiana, several surveillance programmes have been set up for the time periods 2010-2015 and 2016-2021. This has resulted in a characterisation of both reference physico-chemical environments and biological communities, as well as practical tools (e.g. biological indices) to evaluate and monitor water quality. A set of sites have been defined under the “Surveillance Control Network” and the “Operational Control Network” that are monitored on a yearly basis.

However, quantifying the composition of species assemblages in Amazonian aquatic systems remains difficult because species inventories are harmful to the fauna. Indeed, sampling fish in small streams consists in the use of toxicant (rotenone) that kill all the fishes within the stream reach (Allard et al. 2014). In rivers, gill nets are used and cause lethal injuries to the fishes entangled in the nets (Murphy and Willis 1996). Such destructive sampling no longer complies with ethics and European laws. Non-destructive methods, such as diving and electrofishing are not efficient in those streams and rivers due to their low water conductivity and their high turbidity (Allard et al. 2014, Melki 2016). As a consequence, collecting data on entire assemblages is almost impossible using traditional sampling methods, which act as a barrier to scientific advances on ecosystem structure and function and associated applied issues on biodiversity conservation and management.

Since 2014, we used a non-destructive alternative to traditional fish sampling by characterising species assemblages using environmental DNA [hereafter eDNA]

metabarcoding (Taberlet et al. 2018, Taberlet et al. 2012). eDNA consists of collecting DNA released by organisms directly into the water. Environmental DNA sequences are then compared to reference molecular databases to assign sequences to species. This method has been shown to efficiently characterise fish faunas in temperate rivers (Civade et al. 2016, Valentini et al. 2016) and has recently been successfully applied in French Guiana (Cilleros et al. 2019, Cantera et al. 2019). We here provide a consistent dataset synthesising all eDNA sampling sites in French Guiana to date. We also provide a taxon by site presence/absence matrix for the fish fauna. Our aim is to allow a transparent communication to the stakeholders and provide the foundation for a monitoring programme based on eDNA.

## Project description

**Title:** Aquatic eDNA samples in French Guiana

**Personnel:** Personnel involved in data acquisition (by alphabetic order): Sébastien Brosse, Isabel Cantera, Axel Cerdan, Kévin Cilleros, Jean-Baptiste Decotte, Gaël Grenouillet, Amaia Iribar, Jérôme Murienne, Pierre Taberlet, Pablo Tedesco and Régis Vigouroux.

**Study area description:** Collecting trips have been conducted in various locations throughout French Guiana.

**Design description:** This dataset was developed to provide the foundation for a biodiversity monitoring programme based on eDNA but also to better understand the impact of human activities on aquatic biodiversity. Locations were thus selected to maximise the geographic coverage of rivers and streams, taking into account undisturbed sites but also sites under human disturbances (close to villages, close to gold mining sites etc.).

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## Sampling methods

**Study extent:** Sampling sites were located throughout French Guiana Fig. 1.

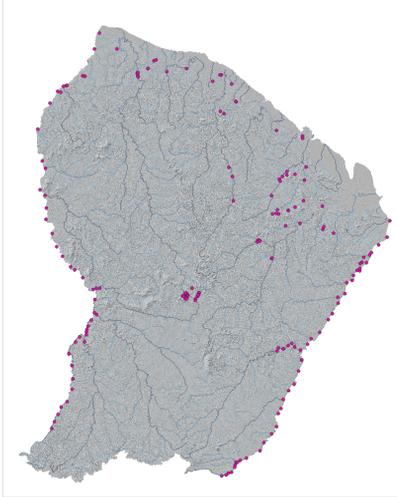


Figure 1. [doi](#)

Localisation of the environmental DNA sampling sites.

**Sampling description:** We collected eDNA samples from November 2014 to 2019. For sampling, laboratory and bioinformatic protocols, we followed Valentini et al. (2016) from 2014 to 2016 and Pont et al. (2018) since 2016. For each sample, we used a filtration kit made of a sterile, single use filtration cartridge (Enviroteck HV; Pall Corporation, Ann Arbor, MI, USA and VigiDNA 0.45  $\mu\text{m}$ ; SPYGEN, le Bourget du Lac, France), a peristaltic pump (Vampir Sampler; Bürkle GmbH, Bad Bellingen, Germany) and sterile, single-use tubing. All the materials were handled with sterile gloves. Initial sampling (2014-2015) was performed using a 1 micrometre filtration cartridge (Enviroteck HV; Pall Corporation, Ann Arbor, MI, USA) but 0.45 micron capsules (VigiDNA 0.45  $\mu\text{m}$ ; SPYGEN, le Bourget du Lac, France) have been used as standard since 2016. Most of the samples consisted of 30 minutes water filtration using a portable battery powered peristaltic pump (Vampir sampler, Bürkle, Germany), but in a few sites, filtration time was reduced to 15 minutes. A single sample per site was collected during initial sampling (2014-2015). Cantera et al. (2019) collected 10 replicate samples in 6 selected sites and showed that two replicate samples per site provided a realistic species list while limiting sampling costs. Two replicate samples were therefore collected in each site since 2016.

**Quality control:** The operator always remained downstream from the filtration area and stayed on the bank (for small streams) or on emergent rocks (for larger streams and rivers). For sites located along the same river course, we sampled downstream to upstream to avoid contamination by eDNA transported by the boat (for rivers) or clothes.

Geographical coordinates were obtained using a GPSmap 64S device (Garmin) or similar. Such devices report coordinates accuracy using the CEP50 (Circular Error Probability), meaning that there is only 50% probability that a reported position would be within a distance of X metres to the real position. Considering other sources of GPS errors (such as ionosphere delay and signal multi-path), we estimate the accuracy of the coordinates to be around 30 metres at a 95% confidence level under dense forest cover.

**Step description:** At each site, we placed the input part of the tubing in a high-flow part of the watercourse. Sampling was achieved in rapid hydromorphological units to ensure an optimal homogenisation of the water throughout the water column. Water was pumped ca. 20 cm below the surface and each filtration lasted 30 min (except for a few sites where filtration time was 15 minutes). Each sample results from the filtration of ~34 l of water (~17 litres when filtration time was 15 minutes). At the end of the filtration, we emptied the filtration capsule of water, filled it with 150 ml of preservation buffer (Tris-HCl 0.1 M, EDTA 0.1 M, NaCl 0.01 M and N-lauroyl sarcosine 1%, pH 7.5–8) and stored it in the dark in individual sterile plastic bags. Samples were then stored at room temperature before DNA extraction. Preliminary tests demonstrated that the preservation buffer was suitable for room temperature storage up to a month. Information on DNA extraction, amplification and sequencing, as well as subsequent bioinformatic pipelines, can be found in Cilleros et al. (2019) and Cantera et al. (2019).

Site scale variables were measured directly in the field at the sampling location. Width was measured using a decameter for small streams (less than 15 metres width and 1 metre depth) and using an electronic telemeter (Bushnell Sport 850) for larger rivers. Water depth was measured using a graduated stick in small streams and a depth sounder (Plastimo echotest II) in larger rivers. Turbidity was measured using a Eutech Instrument Turbimeter (TN-100). Temperature, O<sub>2</sub> saturation, O<sub>2</sub> and pH were measured using a WTW 3420 field multimeter. Geographical coordinates were obtained using a GPSmap 64S device (Garmin) or similar. Elevation was derived for the geographic coordinates using the SRTM30 dataset.

## Geographic coverage

**Description:** The sampling area is delimited by the current administrative boundaries of the French Guiana territory. To the East, the Oyapock river delimits the frontier with Brazil. To the West, the Maroni river delimits the frontier with Suriname. This is an important detail as the delimitation of the territory has not been constant throughout history and a large portion of northern Brazil was disputed between France and Brazil during the 19th century. Even though French Guiana is an overseas territory of France, all occurrences are considered as belonging to the French Guiana "country" to comply with the ISO 3166-1 standard.

**Coordinates:** 2.00000 and 6.00000 Latitude; -51.5000 and -54.5000 Longitude.

## Taxonomic coverage

**Description:** The dataset provides information on eDNA sampling sites and fishes presence/absence as inferred from metabarcoding analyses (Cilleros et al. 2019). DNA extracted from the sampling cartridge could, in theory, be used for amplifying any taxonomic group, depending on the downstream molecular biology protocols. Local metabarcoding reference databases for French Guiana biodiversity are currently available for mammals (Kocher et al. 2017b, Kocher et al. 2017a) and insects (Talaga et al. 2017, Kocher et al. 2016), but additional databases are under active development for other groups as well.

## Temporal coverage

**Notes:** 2014-2019

## Usage rights

**Use license:** Creative Commons Public Domain Waiver (CC-Zero)

**IP rights notes:** Users of this resource should comply with the CEBA data sharing agreement available here: [www.labex-ceba.fr/assets/CEBA\\_Data\\_Sharing\\_Agreement\\_nov2013.pdf](http://www.labex-ceba.fr/assets/CEBA_Data_Sharing_Agreement_nov2013.pdf)

## Data resources

**Data package title:** Aquatic eDNA for monitoring French Guiana biodiversity

**Resource link:** <http://vmcebagn-dev.ird.fr/geonetwork/srv/eng/search?eng#|5617a9ff-d0aa-48a9-b2c2-cb7fd5b92692>

**Alternative identifiers:** 5617a9ff-d0aa-48a9-b2c2-cb7fd5b92692

**Number of data sets:** 2

**Data set name:** Aquatic\_eDNA\_[date]

**Data format:** ESRI Shapefile (a spreadsheet in "tab separated value" format is also provided for compatibility).

**Description:** This dataset provides detailed information on sampling sites and sampling events. The latest version of the dataset is available on the CEBA geoportal (<http://vmcebagn-dev.ird.fr>) under reference 5617a9ff-d0aa-48a9-b2c2-cb7fd5b92692.

Column label	Column description
Site code	A unique identifier of the site that could be used for downstream analyses (optional).
Site name	The name of the sampling location.
Site description	The original textual description of the site.
Drainage Basin	The name of the drainage basin (either Oyapock, Arouague, Comte, Sinamary, Organabo, Iracoubo, Mana, Maroni).
Latitude	The geographic Latitude (in decimal degrees, WGS84) of the sampling point.
Longitude	The geographic Longitude (in decimal degrees, WGS84) of the sampling point.
Elevation	Altitude in metres above sea level inferred from the geographic coordinates and the SRTM30 dataset.
Watercourse class	The watercourse class inferred a posteriori based on the BD Carthage dataset.
Event date	The date of the sampling event.
Disturbance	Level of disturbance at the site (either Reference for undisturbed site, gold mining, ancient gold mining, agriculture and/or urbanisation). Estimated a priori.
Depth	Water depth in metres (measured at the sampling site).
Width	Watercourse width (in metres) measured at the sampling site.
Conductivity	Water conductivity (in micro Siemens) measured at the sampling site using a WTW 3420 field Multiparameter fitted with a TetraCon 925 conductivity probe
Temperature	Water temperature (in degree Celcius) measured at the sampling site.
pH	Water pH measured at the sampling site using a WTW 3420 field Multiparameter fitted with a SenTix 940-3 pH probe.
Turbidity	Turbidity (in NTU) measured at the sampling site by a EUTECH TN-100 field turbidimeter.
O <sub>2</sub>	O <sub>2</sub> (in milligram per litre) measured at the sampling site using a WTW 3420 field Multiparameter fitted with a FDO925 Oxygen probe.
O <sub>2</sub> saturation	O <sub>2</sub> saturation (in percent) measured at the sampling site using a WTW 3420 field Multiparameter fitted with a FDO925 Oxygen probe.
Salinity	Water salinity measured at the sampling site using a WTW 3420 field Multiparameter
Time	Filtering time (in minutes)
Filter	Filter size (in micrometres)
Nb_replicates	Number of replicates
replicatX	For each replicate, the unique filter identifier

Data set name: Aquatic\_eDNA\_fishData\_[date]

**Data format:** Spreadsheet in "tab separated value"

**Description:** This dataset provides a taxon by site matrix, made after sequences assignment to the reference database (Cilleros et al. 2019). For taxa described at the genus level or higher, the number of included species is indicated within parentheses. The latest version of the dataset is available on the CEBA geoportal (<http://vmcebagndev.ird.fr>) under reference 5617a9ff-d0aa-48a9-b2c2-cb7fd5b92692.

## Acknowledgements

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## *Abstract*

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Natural ecosystems are facing increasing anthropogenic impacts that alter the diversity of biological communities. Amazonian rivers and streams shelter a unique and vast biodiversity. Yet, they are facing unprecedented deforestation levels due to human activities, such as mining, logging and agriculture. It is therefore urgent to deeply understand how human impacts affect biological communities in these ecosystems. The majority of studies addressing this issue were conducted in small streams and documented changes on species composition, but not responses on local species richness. This work proposes a community ecology approach, in which the connected nature of freshwater ecosystems is considered, to define how deforestation affects diversity patterns, but also the ecological processes shaping fish communities. Environmental DNA (eDNA), a non-invasive sampling method was used to equally sampling fish communities in streams and rivers across French Guiana. Deforested surfaces from spatial data were extracted to create a global deforestation variable that integrates the effects of urbanization, agriculture, gold-mining and logging.

This work has optimized and validated the use of eDNA to assess the effects of human activities on species-rich ecosystems, such as tropical streams and rivers. The method showed high replicability, as well as the ability to distinguish local fish communities, habitats and disturbed sites from pristine sites. Moreover, we show that deforestation affected fish biodiversity beyond local effects and reveal an extended effect of distant upstream deforestation on downstream fish biodiversity. Distant and low-intensity deforestation caused a decline of over 30% in taxonomic and functional richness of riverine fish communities. Nonetheless, this perturbation was not determinant on the ecological processes shaping fish communities in rivers nor on the trait composition, which were more influenced by the upstream-downstream gradient than by the deforestation gradient. In stream communities, deforestation leads to changes in species composition, without a significant decrease in species or functional richness. These alterations were mediated by environmental filtering which was reinforced in highly deforested sites. As a result, species were less evenly distributed within the functional space, leaving the traits related to the benthic and phytophagous guild underrepresented while overrepresented by pelagic detritivorous.

Our findings strongly support the complexity of deforestation impacts on Amazonian biodiversity. Besides, context-dependent responses, the diversity of freshwater fish communities responded to deforestation in a multifaceted way. This work underlined the vulnerability of tropical fauna to slight environmental changes, even in relatively well-preserved region, such as French Guiana.

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**Keywords:** Neotropical fish | Amazonian rivers | Community ecology | Taxonomic diversity | Functional diversity | Deforestation | Assembly rules.

**Auteure :** Isabel Cantera

**Titre :** Effet de l'anthropisation sur les communautés des poissons dans les cours d'eau Guyanais. Approche par ADN environnemental

**Directeur de thèse :** Sébastien Brosse

**Lieu et date de la soutenance :** Université Paul Sabatier, le 20 Décembre 2019

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Les écosystèmes naturels subissent des impacts anthropiques croissants qui altèrent la diversité des communautés biologiques. Les cours d'eau amazoniens abritent une biodiversité unique et conséquente, mais sont confrontés à des taux de déforestation sans précédent. Il est donc urgent de comprendre comment l'anthropisation affecte la biodiversité dans ces écosystèmes. Ce travail propose une approche en écologie des communautés pour définir comment l'anthropisation affecte les patrons de diversité, mais aussi les processus écologiques qui façonnent les communautés de poissons. Une méthode d'échantillonnage non-invasive, l'ADN environnemental (ADNe), a été utilisée pour inventorier les communautés de poissons dans les ruisseaux et les grands fleuves en Guyane française. Des surfaces déforestées ont été cartographiées à partir de données spatiales pour créer une variable de déforestation globale qui intègre les effets de l'urbanisation, l'agriculture et l'exploitation aurifère et forestière.

Ce travail a, premièrement, permis d'optimiser et valider l'utilisation de l'ADNe pour évaluer l'impact humain sur les écosystèmes riches en espèces, tels que les rivières tropicales. La méthode a montré une reproductibilité élevée, ainsi qu'une capacité à distinguer les communautés de poissons, les habitats et les sites perturbés des sites non perturbés. De plus, nous avons montré que la déforestation affecte la biodiversité au-delà des effets locaux et avons mis en évidence un effet étendu de la déforestation sur la biodiversité en aval. Cet impact lointain a provoqué un déclin de plus de 30% de la diversité taxonomique et fonctionnelle des communautés de poissons qui habitent les fleuves. Cependant, cette perturbation n'a pas été déterminante sur les processus écologiques qui façonnent les communautés de poissons, ni sur la composition des traits. Dans les petits ruisseaux, la déforestation a entraîné des modifications dans la composition spécifique, sans diminuer le nombre d'espèces ni la richesse fonctionnelle. Ces altérations ont été induites par un rôle prépondérant des filtres environnementaux sur l'assemblage des communautés. En conséquence, les espèces étaient moins uniformément réparties dans l'espace fonctionnel, laissant des traits liés au compartiment benthique et au régime phytophage sous-représentés alors que les espèces pélagiques et détritivores étaient surreprésentées.

Nos résultats montrent la complexité des réponses de la biodiversité à la déforestation. Outre des réponses dépendantes de l'habitat, la déforestation a modifié la biodiversité des communautés de poissons d'eau douce à travers différentes facettes. Finalement, ce travail a souligné la vulnérabilité de la faune tropicale à des légers changements environnementaux, même dans des régions relativement bien préservées, comme la Guyane Française.

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**Mots-clés :** Poissons Néotropicaux | Fleuves amazoniens | Écologie des communautés | Diversité taxonomique | Diversité fonctionnelle | Déforestation | Règles d'assemblage.

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**Discipline administrative :** Ecologie

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