Application of the European Water Framework Directive: Identification of reference sites and bioindicator fish species for mercury in tropical freshwater ecosystems (French Guiana)

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Abstract

Mercury (Hg) is a toxic metal subject to several international regulations. The European Water Framework Directive (WFD) established in 2008 an Environmental Quality Standard for biota (EQS_{biota}) at 0.02 µg.g^{-1} fresh weight. This standard is not always adapted, such as in French Guiana subjected to high natural background Hg levels and intensive illegal gold mining. Therefore, this study focuses on how to apply the WFD for the definition of good chemical status (i.e., EQS_{biota}) in a context of strong and generalized natural and anthropic Hg contamination. Based on Hg concentrations measured in 6208 fish over more than 200 sites between 2004 and 2015, we first aimed at discriminating the natural or anthropogenic influences at each site. Then, as WFD recommends considering only high trophic level fish species as bioindicator species, we selected carnivorous/piscivorous fish species able to significantly accumulate Hg and discriminate reference sites from gold mining polluted sites. Total Hg concentrations measured in fish muscle were mostly above the EQS_{biota} (100% for creeks and 84% for rivers), confirming the unsuitability of the direct application of this standard in French Guiana. Among the studied sites, few potential reference sites were identified: eight sites spread over six different watersheds for creeks, and only two areas (group of sites) both on the Oyapock watershed for rivers. Several relevant bioindicators fish species are proposed: ten species (over 35 species tested) belonging to seven genera on creeks (*Moenkhausia oligolepis*, *Gymnotus carapo*, *Sternopygus macrurus*, *Jupiaba* [*abramoides* + *keithi]*, *Pimelodella [cristata+geryi+macturki*], *Copella carsevennensis*, *Pyrrhulina filamentosa*.), and four species (over 21 species tested) belonging to three genera on rivers (*Acestrorhyncus [micropelis* + *falcatus*], *Hoplias aïmara*, *Ageneiosus inermis).* In order to facilitate field sampling, difficult in such remote hydrosystems, and to improve results interpretation, we tested the possibility to group some of these species. Our results indicate that only *Jupiaba, Moenkhausia, Pimelodella* and *Pyrrhulina* on creeks could be grouped; and the three bioindicators species proposed on rivers could be pooled. Finally, this work proposes *in situ-*

based reference Hg concentrations for selected bioindicator fish species from French Guiana as an alternative to detect Hg-impacted sites and help the application of the WFD in tropical systems.

Keywords: mercury; fish; tropical freshwater; bioindicator; priority substance; Environmental Quality Standard.

1. Introduction

Mercury (Hg) is a global pollutant, source of major concerns for humans and ecosystems. In this context, the international Minamata Convention on Mercury, adopted in 2013 and officially entered into force in August 2017, defines new objectives to mitigate releases of this highly toxic metal to the environment. Specifically, this convention highlights the necessity to develop tools and networks for Hg monitoring to help the implementation and the compliance with set standards (article 15), as well as the necessity to provide baseline data and comparable Hg measurements (article 19) in order to support decision-making and management of Hg (Selin et al., 2018; Chen et al., 2018). This Minamata Convention corroborates the European Water Framework Directive (WFD 2000/60/EC, EC 2000), which previously established a common policy for the management of surface waters and classified Hg as one of the dangerous priority substances. Indeed, Hg under its most toxic form, methylmercury, is easily bioaccumulated and bioamplified along the food chain (Hall et al., 1997; Watras et al., 1998), resulting in high concentrations in ichtyofauna and particularly in top predator fishes (Campbell et al., 2008). By extension, Hg can be a threat to populations whose main food source is fish consumption, for example Amerindian people in French Guiana (Fréry et al., 2001; Cordier et al., 2002; Cardoso et al., 2010). In order to evaluate the chemical status of water bodies in application of the WFD, Environmental Quality Standards were first defined for water (EQS_{water}), then for biota (EQS_{biota}) for persistent, bioaccumulative and toxic priority substances including Hg (WFD 2008/105/EC & 2013/39/EC; EC, 2008a, 2013). The EQS_{biota} is meant for the protection of piscivorous wildlife against secondary poisoning and should protect the aquatic ecosystems and human health. The EQS_{biota} for Hg was set at 0.020 $\mu g.g^{-1}$ wet weight (ww) and its application is now mandatory through fish monitoring (WFD 2013/39/EC; EC, 2013). Although essential in the context of monitoring aquatic environments, this standard is considered very low. In fact, most fishes exceed the EQS_{biota} in temperate freshwater systems (Vignati et al., 2013; Jurgens et al., 2013). In comparison, the human consumption recommendation of the World Health Organization (WHO) and European regulation for fish was set at 0.5 $\mu g.g^{-1}$ ww (WHO, 1990; EC, 2008b).

French Guiana is part of the French territory located in the Amazon basin, therefore subject to the same European WFD regulations as French metropolitan waters. However, the geochemical background for Hg is naturally high in soils of this tropical region (Lechler et al., 2000; Carmouze et al., 2001; Fadini and Jardim, 2001). In addition, French Guiana holds artisanal small scale-gold mining activities (ASGM), which contribute to further increase Hg concentrations in terrestrial and aquatic ecosystems (Durrieu et al., 2005; Guedron et al., 2011; Grimaldi et al., 2015). The hydrosystems in French Guiana consist of about 80 % of creeks (small streams) and 20% of rivers (calculation based on the length of the watercourses, Mourguiart and Linares, 2013). Relatively limited data on Hg concentrations in fishes are available on the creeks, which are the most representative water bodies, although they are particularly affected by human activities like ASGM.

Worldwide, artisanal small scale-gold mining activities are considered as the main contributor to Hg emissions, with 727 tons per year, representing more than 35 % of total anthropogenic emissions of Hg (UNEP 2013). Recent studies showed that only few data are available regarding the impact of ASGM on the environment (WHO, 2016; UNEP, 2013; Eagles-Smith et al., 2018), especially in the Amazon region (Wasserman et al., 2003; Hacon et al., 2008). In such understudied regions, the Minamata Convention highlighted the necessity to obtain data to suitably establish baselines and to integrate research to the policy to efficiently manage the risk assessment at both global and local scales (Selin et al., 2018). This goes through the identification of reference sites, defined as sites undisturbed by human activities and reflecting background levels (Stoddard et al., 2006) and the definition of bioindicator fish species, defined as species reflecting variations in pollution levels between impacted and reference sites (Authman et al., 2015).

In this context, we proposed to contribute to the implementation of the WFD for Hg in tropical freshwaters of French Guiana, by (i) identifying potential reference sites, separately for creeks and rivers, in an Hg-impacted region; (ii) discriminating bioindicator fish species for each hydrosystems based, among others, on the comparison of Hg concentrations in fish from reference sites and recent gold mining sites; (iii) in the end, proposing new *in situ* background Hg concentrations in bioindicator fish as a first solution to identify Hg-impacted sites. For this purpose, a database was constructed by compiling various information (e.g. morphometrics, diet, anthropogenic and natural pressures) and Hg measurements in muscle of 6208 fish caught at 217 sites between 2004 and 2015 within the framework of various research programs.

2. Methods

2.1 Database creation

2.1.1 Origin of data

Data on Hg concentrations in fish of French Guiana were collected on the basis of nine aquatic research and monitoring programs carried out by the University of Bordeaux and the HYDRECO laboratory (France) from 2004 to 2015 (Table S1). During this period, 6208 fishes were sampled in creeks and rivers over French Guiana on the six major watersheds: Maroni, Mana, Sinnamary, Comté, Approuague and Oyapock; and four smaller ones: Iracoubo, Kourou, Macouria and Organabo (Figure 1).

2.1.2 Fish sampling

During all the field campaigns, several fishing techniques were used: rotenone poisoning in creeks, fish lines and nets in rivers. These techniques are complementary and allow catching a higher diversity of fish species with a wide range of size. However, rotenone should no longer be used because it is a non-selective method, causing excessive mortality on aquatic biota, and not adapted for repeatable sampling such as for the WFD monitoring (Bennett et al. 2016). When fish weight was ≤ 1 g ww, the whole fish was used for further analysis; otherwise a part of the dorsal skeletal muscle was collected. Then, samples were preserved in formalin or frozen until Hg analysis. The comparison of the two conservation techniques revealed no significant impact on the Hg concentrations (Maury-Brachet, personal communication).

For each individual fish, the species was identified, then the wet weight (g), standard and total length (cm) were measured. In addition, because food is the major contamination pathway for Hg in fish (Hall et al., 1997), a specific trophic diet was associated to each fish species. According to the literature (Keith et al., 2000, Le Bail et al., 2000; Durrieu et al., 2005), six types of feeding ecology were retained: (1) strictly herbivorous, feeding exclusively on aquatic plants (macrophytes, phytoplankton algae) or terrestrial materials from the river banks (leaves, flowers, fruits); (2) periphytophagous, consuming periphyton or biofilms on hard substrates (rocks, immersed tree trunks, etc.); (3) benthivorous, ingesting organic detritus and small preys living in the sediment superficial layers; (4) omnivorous, feeding on a variety of food (vegetables, insect larva, crustaceans, mollusks, etc.) according to their availability; (5) carnivorous, eating animal preys of different orders (crustaceans, mollusks, aquatic and terrestrial insects, fish, etc.); and (6) piscivorous, capturing fish of varying size.

2.1.3 Sampling sites characterisation

Specificity of the hydrosystem in French Guiana: distinction between creeks and rivers

Creeks are shallow (below 1 m depth) and narrow (width less than 10 m) water bodies that flow into rivers. Our study highlighted a difference in the fish species diversity (different species present) between creeks and rivers. Also, some species have a juvenile life-stage in creek and an adult life-stage in river. Likewise, water chemistry has an influence on Hg bioavailability and bioaccumulation (Carmouze et al., 2001); and rivers and creeks differed in water characteristics (Crespy et al., 2015). Therefore, each sampling site was considered according to its hydrographic functioning; and the results are presented separately for creeks and rivers. A total of 217 sites were sampled: 49 for creeks (Figure 1A) and 168 for rivers (Figure 1B). On the rivers within each watershed, to ensure that fish numbers are sufficient by species (i.e., more than five individual fish per site), several sites were grouped when they shared the same geographical position and similar natural or human pressure (see next paragraph). Thus, we defined 48 areas (group of sites) for rivers. Each site was positioned in RGFG95 / UTM zone 22N, reference system used in French Guiana.

Determination of natural and anthropogenic pressure for each site

In French Guiana, the presence of reference sites for creeks and rivers, not impacted by human activities and especially gold mining, is still unknown. A cross-referencing of current knowledge on risk of Hg contamination in French Guiana through the literature on land use (http://gisguyane.brgm.fr) and the absence of significant anthropogenic pressure by field observations (*in situ* expert judgment by R. Vigouroux and R. Maury-Brachet), allowed identifying reference sites. This also allowed discriminating Hg-contaminated sites impacted by gold mining activities, which used Hg for gold amalgamation. We differentiated three types of pressure linked to gold mining: (i) recent gold mining area (< 1 year); (ii) past gold mining (> 1 year); and (iii) sites located in the plume of a recent gold mining area. Other human activities were distinguished: one category with sites under the influence of the Petit Saut hydroelectric dam (Sinnamary watershed) and one more category grouping other human activities (deforestation, agricultural and urban exploitation). Finally, sites under the influence of natural phenomenon, which may have an influence on Hg cycle like swamps and tide, were also identified separately (Figure 1).

2.2 Determination of bioindicator fish species

In order to identify the most relevant bioindicator species in agreement with the WFD implementation Guidance documents (EC, 2010, 2014), several criteria were specified: the fish species must (1) be present on one or more sites classified as a reference (at least five individuals per site to allow statistical analysis); (2) have a trophic level equal to or greater than three (= carnivorous, piscivorous), with a well-known and specific diet; (3) be distributed in at least 50 % of watersheds indicating a relatively high occurrence; (4) have significantly lower Hg concentrations at reference sites than at Hg-impacted ones. Because of this last criterion, only sites recently impacted by gold mining activities, the most easily identifiable and important human pressure in French Guiana, were considered for the identification of bioindicator species.

Freshwater fish diversity in French Guiana (367 species and 170-190 endemics species; Le Bail et al., 2012) is higher than in metropolitan France (100 species and 2-25 endemics species; Keith et al., 2011), and new species are regularly discovered. In this context, collecting a sufficient number of individuals for each identified bioindicator species at each site appears truly challenging (Allard et al., 2014). Yet, it is a necessary prerequisite to run robust statistical analyses. Despite the three types of complementary fishing techniques used (rotenone, net and line fishing, depending upon the sites), it is difficult to collect the same species at each site and in sufficient numbers for the statistics. During this study, a large variety of fish species were sampled at various sites but, in some cases, only a few individuals per species and/or per site were caught. Therefore, we investigated the possibility and relevance to group the identified bioindicator species based on the following rational: i) Hg contamination pathway being trophic, grouped species must have a similar diet and trophic level; ii) bioindicator species must have similar Hg levels within a sampling site or area, i.e. their physiological responses / sensitivities to Hg pollution must be comparable.

2.3 Mercury analysis

Before analysis, samples were dried at 44 °C for 48 hours. All the total Hg concentration data used for this study were measured by the EPOC laboratory (University of Bordeaux) or by the HYDRECO laboratory according to the same standard method (EPA, 1998) using an automated atomic absorption spectrophotometer (AMA 254, Symalab France). Blanks and the same reference materials TORT2 (National Research Council of Canada, lobster hepatopancreas) were systematically used, typically every 10 samples, by both entities to control analytical accuracy. The accuracy averaged respectively 98.2% and 100.5%. The limit of detection of total mercury on AMA 254 is 0.0004 ng. The limit of quantification is 0.010 $\mu g.g^{-1}$ dw. Total Hg concentrations in biota are expressed in $\mu g.g^{-1}$ on a wet weight basis; a factor of 5 was applied to convert all Hg results from dry weight to wet weight basis (Maury-Brachet et al., 2006).

2.4 Statistical analysis

Factorials ANOVA were used to study the differences in THg concentrations depending on the fish species, sites or areas, and natural or anthropogenic pressure, after checking assumptions of normality (Shapiro test) and homoscedasticity of the error term (Levene test). If the assumption was met, the parametric Fisher's Least Significant Difference (LSD) test was applied. If the assumption was not met, log or box-cox data transformations were used (Peltier et al., 1998), or a Kruskall-Wallis test was performed. In each test, $p<0.05$ was considered significant. Statistical analyses were performed using *STATISTICA* version 12 software (Statsoft, USA).

2.5 Cartographic representation and spatial analysis of data

Map representations were generated with ArcGIS for Desktop 10.3 software (© Esri). The basemaps were realized from (i) BRGM data for gold mining sites before 2006 and for Petit Saut lake (http://gisguyane.brgm.fr), (ii) Carthage database for major rivers and watersheds (http://services.sandre.eaufrance.fr/), and (iii) the SRTM for the field digital model (http://www2.jpl.nasa.gov/srtm/).

3. Results

3.1 Hg concentrations in fish for creeks

3.1.1. Dataset presentation

Table 1 summarizes average Hg concentrations in fish from creeks calculated by trophic guild and influence (types of pressure), and their specific richness associated. Over the 2948 fish sampled in creeks between 2006 and 2015, 149 species were identified unambiguously. Carnivorous fish represented 44% of collected fish, followed by omnivorous (39%), piscivorous (11%), periphytophagous (4%), benthivorous (1%) and herbivorous (1%). Without surprise, piscivorous fishes are the most contaminated (0.234 ± 0.010 $\mu g.g^{-1}$). All fish considered, fish sampled at recent gold mining sites had Hg concentrations two times higher than at reference sites (LSD Fisher test, $p<0.05$).

All years and sites combined, 4% of fish showed Hg concentrations above the fish consumption recommendation (0.5 $\mu g.g^{-1}$ ww, WHO and EU guideline), and 100% of fish had values above the EQS_{biota} set at 0.02 $\mu g.g^{-1}$ ww (WFD).

3.1.2. Hg concentrations at reference sites in creeks

The selection of reference sites for creeks was based on literature, field observations and the comparison of Hg concentrations measured in fish of potential reference sites *vs* Hg-impacted sites. Among the 49 sampling sites, eight creeks were identified as reference sites for Hg, spread over six watersheds (Figure 1A, blue circle): Apa, Alama and Nouvelle France downstream (Maroni); Montagne (Mana); Saül (Sinnamary); Galibi (Kourou); Païra

(Approuague); Trois-Sauts (Oyapock). For each reference site, average Hg concentration in muscle of all fishes was systematically and statistically lower compared to recent gold mining sites (LSD Fisher test, $p<0.05$; Table 2). In reference creeks, the lowest Hg concentrations (average Hg ± standard error for all species) were measured in fish from Trois-Sauts (0.10 ± 0.01 $\mu g.g^{-1}$, n=192), Païra creek (0.11 ± 0.02 $\mu g.g^{-1}$, n=53), Alama creek (0.11 ± 0.01 $\mu g.g^{-1}$, n=92) and Montagne Creek (0.09 ± 0.02 $\mu g.g^{-1}$, n=10), with similar Hg level. The other references creeks, Apa (0.14 ± 0.02 $\mu g.g^{-1}$, n=39), Nouvelle France aval (0.14 ± 0.01 $\mu g.g^{-1}$, n=206), Saül (0.15 ± 0.01 $\mu g.g^{-1}$, n=146) and Galibi (0.14 ± 0.02 $\mu g.g^{-1}$, n=26) showed comparable Hg level, somewhat higher than for the first group of creeks but not statistically different. The most represented trophic guilds in reference creeks belong to high trophic level (≥ 3) with 44 % of omnivorous, 35 % of carnivorous and 12 % of piscivorous (Table 2).

3.1.3. Identification of bioindicator fish species in creeks

Based on the criteria defined in section 2.2 and thanks to the discrimination of reference sites, several species were scrutinized (Table S2). A number of species were definitively eliminated (i.e., "not recommended", total = 13 species), while others would still need additional information (e.g., lack of samples or knowledge on diet) to check their integrative capacity (i.e., "potential" bioindicator species, total =12). In the end, ten fish species belonging to seven genera were selected as bioindicators: *Moenkhausia oligolepis*, *Gymnotus carapo*, *Sternopygus macrurus*, *Jupiaba (abramoides + keithi)*, *Pimelodella (cristata+geryi+macturki*), *Copella carevennensis* and *Pyrrhulina filamentosa.* Their phylogenetic and trophic characteristics as well as their spatial distribution are summarized in Table 3. These bioindicator species represent 22 % of all sampled fish.

Due to their small size (<15 cm, except for *Sternopygus m.* <25 cm), fish in creeks were not grouped by size class. The comparison of Hg bioaccumulation for each species between reference and recent gold mining impacted sites is shown on Figure 2. All the bioindicator species proposed accumulated significantly more Hg in the Hg-impacted creeks than in reference creeks (Kruskal-Wallis test, $p<0.05$ for all species). At the reference sites, the lowest average Hg concentration for all bioindicator species was measured in *Copella* (0.031 ± 0.003 $\mu g.g^{-1}$) and the highest in *Sternopygus* (0.12 ± 0.04 $\mu g.g^{-1}$). Overall, at reference sites, 99% of bioindicator fishes presented Hg concentrations above the EQS_{biota} but none exceeded the fish consumption recommendation.

3.1.4. Grouping of bioindicator fish species in creeks

Catching a specific fish species (moreover in sufficient numbers from a statistical point of view) in creeks, and more globally in French Guiana, is quite complicated for two reasons. The first reason is the high specific richness (149 fish species obtained in creeks in the present study, Table 1), associated with a low abundance for most species. Therefore, it requires a significant fishing effort to obtain a representative sample of a specific fish species (Allard et al., 2014); moreover, this is potentially destructive for aquatic biota. In case of creeks, *i.e.* small-size systems, the risk is to disrupt ecosystems greatly by taking a large amount of biomass, which is not acceptable. The second reason is the difficulty of fishing in such isolated areas from a logistical point of view (high cost / benefit ratio). To solve this problem, we tested the possibility of grouping bioindicator species according to criteria defined in section 2.2. In creeks, the four bioindicators *Jupiaba, Moenkhausia, Pimelodella* and *Pyrrhulina* had a similar trophic diet (Carnivorous- invertivorous, Table 3). At reference sites, these four genera showed no statistical difference in Hg bioaccumulation pattern (from 0.07 ± 0.01 to 0.09 ± 0.02 $\mu g.g^{-1}$) (Figure 2). To test the possibility to group them, we focused on two sites where these four genera were present: the reference creek "Trois Sauts" and the gold mining creek "Chien" sampled during the year 2012. The Hg concentrations were not statistically different between the four bioindicators species, both for the reference creeks (LSD Fisher test, $p > 0.05$) and for recent gold mining sites ($p > 0.05$, Figure 3). Thus, data from these four genera were grouped at each site. Average Hg concentrations were 2.6 times higher for the Hg-impacted creek compared to the reference creek (LSD Fisher test, $p<0.05$, Figure 3). Moreover, when aggregated, these four bioindicators species cover 100% of the French Guiana watersheds.

Regarding the other biodindicator species, genera *Gymnotus* and *Sternopygus* had a similar trophic diet (piscivorous- invertivorous, Table 3). However, they were not collected at the same sites so they could not be grouped. Finally, *Copella carsevennensis* had significant lower Hg concentration than the other species, excluding the option to pool it with another species.

3.2 Hg concentrations in fish sampled in rivers

3.2.1. Dataset presentation

Over the 3260 fish collected in rivers between 2004 and 2014, 95 species were identified (Table 1). Only 32 fish species are common between rivers and creeks. In rivers, piscivorous fish represented about half of the collected fish (48%), followed by omnivorous (20%), herbivorous (11%), carnivorous (10%), benthivorous (7%) and periphytophagous fish (4%). The trophic guild with the highest Hg concentration was piscivorous fishes (0.509 ± 0.009 $\mu g.g^{-1}$); in contrast, herbivorous fishes are the only species with Hg concentrations below the EQS_{biota}. All fish considered, fish from recent gold mining areas had Hg levels twice higher than at references sites (respectively 0.333 ± 0.009 and 0.150 ± 0.009 $\mu g.g^{-1}$, LSD Fisher test, $p<0.05$).

All years and sites combined, 20% of fish presented Hg concentrations in muscle above the fish consumption recommendation (0.5 $\mu g.g^{-1}$ ww, WHO and EU guideline) and 84 % above the EQS_{biota} (0.02 $\mu g.g^{-1}$ ww, WFD)

3.2.2. Hg concentrations at reference sites in rivers

Among the 48 sampling areas (as a reminder, 168 sampling sites were grouped by their similitude in anthropogenic pressure and geographic position), only two reference areas were identified on the rivers (Figure 1B, blue circle): “upstream Oyapock River (near Trois Sauts)” and “Camopi River (upstream Inipi)”, both situated on the Oyapock watershed. For each reference area, the average Hg concentration in muscle of all fishes was systematically and statically lower compared to those collected at recent gold mining sites (LSD Fisher test, $p<0.05$; Table 4). Mercury concentrations in fish (average ± standard error for all species) were not statistically different between “upstream Oyapock River” (0.15 ± 0.01 $\mu g.g^{-1}$, n=405) and “Camopi River” (0.12 ± 0.02 $\mu g.g^{-1}$ ww, n=35). The distribution of trophic guilds at both reference areas was as follows: 34 % of piscivorous, 18% of omnivorous, 18% of herbivorous, 14 % of periphytophagous, 9% of carnivorous and 7% of benthivorous (Table 4).

3.2.3. Identification of bioindicator fish species in rivers

Several species were examined as candidates as bioindicator species using the criteria detailed in the methodology section (§2.2) and thanks to the preceding discrimination of reference areas. Table S3 presents the species definitively eliminated (i.e., "not recommended", total = 7 species), others which still need additional information to check their integrative capacity (i.e., "potential" bioindicator, total = 4) and the retained species (i.e., "recommended", total: 10, belong to 7 genera). Four fish species belong to three genera were selected as bioindicators: *Acestrorhyncus (micropelis + falcatus*), *Hoplias aïmara* and *Ageneiosus inermis*. Their phylogenetic and trophic characteristics (all piscivorous) as well as their spatial distribution are summarized in Table 5. These bioindicator species represent 30 % of all sampled fish. Each of these species occurred in at least 44% of the sampling areas (*Acestrorhyncus)* and 50% of the watersheds (*Ageneiosus inermis*). A size class was chosen for each species in order to compare Hg concentrations between sites based on the fact that (i) the age and standard length of individuals are generally correlated and (ii) Hg bioaccumulation is influenced by age. Size classes were defined considering only adult fish and using the method of percentiles (i.e., 10% of the most extreme values were eliminated). Therefore, only specimen between between 10 and 30 cm for *Acestrorhyncus (micropelis + falcatus*), 38 and 82 cm for *Hoplias aïmara* and between 20 and 45 cm for *Ageneiosus inermis* were kept for the rest of the study. The comparisons of Hg bioaccumulation for each species between reference and gold mining sites are shown in Figure 4. The three proposed bioindicators species accumulated significantly more Hg at Hg-impacted areas compared to reference areas (Kruskal-Wallis test, $p<0.05$ for all species). For reference areas, Hg concentration (average ± standard error) in *Ageneiosus* (0.27 ± 0.03 $\mu g.g^{-1}$, n=33) was significantly lower than in *Acestrorhyncus* (0.35 ± 0.02 $\mu g.g^{-1}$, n=31) and *Hoplias* (0.36 ± 0.03 $\mu g.g^{-1}$, n=26).

Overall, at reference sites, 100% of bioindicator fish presented Hg concentrations above the EQS_{biota} and 48% also exceeded the fish consumption recommendation.

3.2.4. Grouping of bioindicator fish species in rivers

As explained in section 3.1.4 for creeks, we tested the possibility to group the proposed bioindicator species by sampling area in rivers, in order to strengthen the interpretation of the data for one site or area. In rivers, the three bioindicators *Hoplias*, *Ageneiosus* and *Acestrorhyncus* have a similar diet (strict piscivorous, Table 5). At reference sites, *Acestrorhyncus and Hoplias* showed no statistically difference in Hg bioaccumulation pattern, whereas Hg concentration was somewhat lower in *Ageneiosus,* (about 20% of difference, but not statistically different). To test the possibility to group these three species, two contrasted areas, where they were jointly collected, were selected: the reference area "Oyapock upstream (near Trois-Sauts)"and the gold mining area "Camopi river", both on the Oyapock river watershed, sampled in 2005 and 2006. These two years were selected because a large sampling effort was carried out during this period. Average Hg concentrations measured in the three bioindicators species were not statistically different, both for the reference creeks (LSD Fisher test, $p > 0.05$) and for gold mining sites ($p > 0.05$, Figure 5). Thus, data from these three species were grouped by area. The average Hg concentration was 1.8 times higher for the Hg-impacted area compared to the reference area (LSD Fisher test, $p<0.05$, figure 5). When aggregated, these three bioindicators species are present on 80% of French Guiana watersheds.

4. Discussion

4.1 Difficulties in the application of EQS_{biota} for WFD monitoring

The EQS is defined by the WFD as the maximum concentration of a pollutant or group of pollutants in water, sediment or biota that must not be exceeded in order to protect environmental health and also human health. The EQS_{biota} established for Hg (EC, 2008a) has been questioned ever since (Depew et al., 2012; Vignati et al., 2013). First, it was determined based on secondary poisoning, that is on the maximum Hg concentration absorbed by trophic pathway for 365 days by rhesus monkeys (*Macaca mulatta*) where no effects were observed

(NOEC -No Observed Effect Concentration) and with a ten-fold safety factor applied. Second, the WFD emphasized the importance of the trophic level when selecting bioindicators species, and stated that only fish species with a trophic position ≥3 (carnivorous/piscivorous) should be considered (EC, 2014). However, even when considering aquatic environments not exposed to Hg, carnivorous/piscivorous fish often exceed the EQS_{biota}, thereby systematically declassifying most water bodies (Vignati et al. 2013; Jürgens et al., 2013; Nguetseng et al., 2015; Fliedner et al., 2016). In the present study, among the 6208 fish sampled in rivers and creeks of French Guiana, almost all had Hg concentrations higher than the EQS_{biota}, regardless of the anthropogenic influences associated with the sampling sites. All years and sites included, the Hg concentrations were up to 30 times higher than the EQS_{biota} for piscivorous fish, and about 10 times higher for carnivorous, omnivorous and benthivorous species. Nonetheless, most Hg concentrations were below the threshold of human consumption (0.5 $\mu g.g^{-1}$ ww; WHO and EC), except for the piscivorous species. This work highlights the unsuitability of the direct application of the EQS_{biota} in French Guiana.

With the introduction of EQS for priority pollutants, the European Commission showed its willingness to protect the environment by banning the environmental inequalities that may exist between countries. However, as demonstrated here and in other studies (Crane and Babut, 2007), some limitations in its application appear at a regional scale. Indeed, the characteristics of ecoregions or sites are not taken into account in the present application of this EQS_{biota}, whereas background concentrations for Hg could vary depending on local geology. An evolution of the European EQS_{biota} would be required, with a greater emphasis on the collection of field data, rather than solely relying on extrapolation from laboratory data.

In this context, this study proposes an alternative to detect Hg-impacted sites using new *in situ*-based reference or background Hg concentrations adapted for tropical freshwaters of French Guiana.

4.2 Well known factors responsible of high Hg levels in fish of French Guiana: role of the pedo-climatic context and gold mining activity

In the Amazonian region, Hg concentrations in soils are naturally higher compared to boreal and temperate areas (Roulet and Lucotte, 1995; Carmouze et al., 2001; Guédron et al., 2006), due to high atmospheric depositions during the latest millions of years (Théveniaut and Freyssinet, 1999). The background Hg level in river sediments of the whole of French Guiana was estimated at 0.108 ± 0.042 µg. g^{-1} dw, depending on particle size (Laperche et al., 2014). Due to this particular lithology, Hg naturally accumulated in soils constitutes an important reservoir that can be mobilized through natural or anthropogenic erosion (deforestation, gold-mining…), leading to an increase in terrestrial Hg export to aquatic systems. Indeed, ASGM are a real cause for concern in French Guiana since the 1850s due to the release of large amounts of naturally Hg-rich particles into the hydrosystems by soil erosion and the direct release of Hg into the environment due to the gold recovery process (Grimaldi et al., 2015). The use of Hg for gold mining, banned since 2006 in Europe, is still widely used by illegal miners. Monitoring campaigns carried out by the Amazonian Park of French Guiana (PAG, 2017) and the French National Forestry Office (ONF) showed an increase in illegal gold mining activities since 2013 in French Guiana. Such an increase is explained by local cultural factors and by economic market trends with the increase of the global price of gold (Swenson et al., 2011; Asner et al., 2013). In the present study, gold mining activity was the main anthropogenic pressure represented among the studied sites (Figure 1). Indeed, a concordance in the occurrence of the gold mining activity (recent and old activity) with the BRGM (French geological survey) gold mining data was evidenced (grey zone in Figure 1). In rivers, average Hg concentrations are two fold higher in all piscivorous fish from recent gold mining-impacted areas than those of reference sites (0.567 ± 0.014 µg.g^{-1}, n=692, min-max=0.03-2.82 versus 0.343 ± 0.015 µg.g^{-1}, n=148, min-max=0.04-0.91). Here, no size classes of fish were taken into account for comparison with literature because it is rarely documented (excepted in the study of Bastos et al., 2015). Mercury levels in piscivorous or carnivorous fish of other gold mining-impacted regions in the Amazon basin are of the same order of magnitude as our results (Myster et al., 2018). In the Madeira river basin, an Hg-impacted area, Hg levels in carnivorous (n=461) and piscivorous (n=597) fish ranged from 0.051 to 1.242 µg. g^{-1} (Bastos et al., 2015). Malm et al. (1995) reported high values in carnivorous fish collected from the upper part of the river system (impacted by gold mining activities) with an average value of 0.69 µg.g^{-1} (SD not available, min-max= 0.15-3.8 µg.g^{-1}, n=43). In the lower part of the Tapajos, far from gold mining activities, in areas which could be considered as "almost non-impacted" (Santarém), average Hg concentration in the same carnivorous species decreased to 0.19 µg.g^{-1} (SD not available, min-max= 0.05-0.55 µg.g^{-1}, n=17). Likewise, on gold mining impacted area of the Tapajos basin, Lino et al. (2018) measured Hg concentrations from 0.4 to 1.51 µg.g^{-1} in carnivorous fish (n=35, Hg average).

Research on ASGM was mainly realized in temperate regions, resulting in a lack of data available in tropical regions (Chen et al., 2018). Pacyna et al. (2016) highlighted that Hg emissions sources are relatively well-quantified for some sectors such as industrial and energy, but larger uncertainties are associated to other sources such as ASGM. In the Minamata convention, the identification and the characterization of the risk assessment associated with ASGM is one of the defined priorities (article 7). In this study, efforts realized for the identification of sites under the influence of gold mining activities is a first step to help the implementation of EQS_{biota} under the WFD in French Guiana, and more globally, to the detection of Hg-impacted sites linked to ASGM using bioindicator species.

4.3 Identifiying reference sites is a difficult task in such impacted environment

In French Guiana, we were confronted to the difficulty to find truly pristine areas, especially in the context of extended illegal gold mining activities for many years. The history of human activities (gold mining, agriculture, deforestation…) is poorly known in a large part of the territory. For example, it is difficult to determine if a site has already been prospected for gold mining and, if so, for how long and to what extent. The classification of the reference sites proposed here is therefore bound to evolve, also with the gain of future knowledge. For creeks, we identified eight reference sites over the 49 studied sites, located on six watersheds. For rivers, only two areas (groups of sites) over the 48 sampled areas could be identified unambiguously as reference; both are located on the Oyapock watershed. In fact, these two river areas have previously been identified as reference areas for Hg in sediment (Laperche et al., 2014). Existing literature shows rather well how challenging it is to find true reference sites in Amazonia. Indeed, most studies focused only on Hg polluted areas and compared measured concentrations in fish to the WHO guideline; whereas only few studies worked on "natural" sites as showed in the review of Kasper et al. (2018) in Amazonia. The originality of our work is to present a comprehensive study in tropical environment enabling the identification of reference sites for Hg and the determination of Hg background levels in fish for such a large territory.

4.4 Identification of bioindicator species: a useful and original work

In this study, for the same genus, several species presented different patterns of Hg bioaccumulation. This could be explained by difference in their physiology, even if they are genetically close. For example, in creeks, *Moenkhausia chrysargyrea*, *M. colletti* and *M. surinamensis* did not accumulate significantly more Hg between Hg-impacted and reference sites, whereas *M. oligolepis* appeared as a good bioindicator, species (Table S2). Indeed, sympatric species may show different feeding behaviors, resulting in contrasting bioaccumulation patterns. Due to this species-dependent bioaccumulation of Hg, our sampling and data investigation was performed at species level, which is difficult in such tropical ecosystems with a rich biodiversity and a low biomass (Cilleros et al., 2016).

Some studies provide data on Hg bioaccumulation in fish in the Amazonian region but it is often restricted to few taxa (Mol et al., 2001; Berzas-Nevado et al., 2010; Pouilly et al., 2013; Souza-Araujo et al., 2016). Our database provides comprehensive and consistent information on Hg bioaccumulation in such ecosystems and allowed to identify several species, according to a set of criteria, to be proposed as bioindicator species. Only one previous study (Bouvier et al., 2015) has identified three potential bioindicator species for Hg in French Guiana creeks based on 110 individuals and 15 species: *Bryconops affinis* and *sp* (n=60), *Bryconamericus guyanensis* (n=8) and *Sternopygus macrurus* (n=7). *Sternopygus macrurus* appears as the only common bioindicator species with those identified in the present study. *Bryconops affinis* was not identified as an integrator of Hg pollution and *Bryconamericus guyanensis* was not sufficiently represented in the database of our study.

In this study, it appeared relevant to group data on *Jupiaba* spp., *Moenkhausia* spp., *Pimelodella* spp. and *Pyrrhulina* spp. at each site. *Gymnotus carapo* prefers clear waters to the turbid waters found in gold mining areas. This species was therefore not found at recent gold mining-impacted sites and was excluded from the list of potential bioindicator species. Nevertheless, we believe that this species remains a relevant bioindicator because i) of its high abundance in freshwaters of French Guiana, ii) of its wide distribution in all watersheds and iii) its absence highlights an alteration of the structure of the fish community at recent gold mining sites. Indeed, previous studies in French Guiana demonstrated that gold mining activities impacted fish communities assemblage (Allard et al., 2016), in addition to increase Hg concentrations in fish (Durrieu et al., 2005).

For rivers, four fish species were selected as potential bioindicators (Table 5). They correspond to those proposed by Bouvier et al. (2015) who based his study on 268 individuals belonging to 15 species on the main watersheds of French Guiana. Moreover, Durrieu et al. (2005) published a specific study on *Hoplias aimara* and they recommended this species as a bioindicator for Hg due to its ubiquity, its sedentary behaviour (Junk 1985; Menezes and Vazzoler 1992; Planquette et al., 1996) and its capacity to accumulate significantly Hg at impacted sites (Durrieu et al., 2005). Other bioindicator species were proposed in rivers, such as *Curimatida Cyprinoides* (Dominique et al., 2007), a widely distributed species in the whole Amazonian basin. However, its trophic level < 3 (detritivorous/benthivorous species) is not compatible with the criteria of the WFD, so we did not consider this species as a potential bioindicator.

The relatively high background concentrations of Hg measured in fish muscle in freshwaters caused by exposure to naturally Hg rich water or sediments, could be the result of acclimation or natural selection processes linked to the development of a set of regulation of uptake and detoxification processes, such as metallothionein proteins (Chan et al., 2002; Gentès et al. 2015). Thus, the average Hg concentrations measured in muscle of the proposed bioindicator species at reference sites could be considered as reference thresholds in French Guiana (Table 6). For each defined bioindicator species/genera or group of species, a concentration at a given site significantly higher than this reference threshold would indicate Hg contamination.

5. Conclusion

This study provides an important enhancement of the database on Hg in fish species for the WFD in tropical ecosystems of French Guiana. First, by defining anthropogenic and natural influences associated with each of 200 sampling sites. Among them, only eight for creeks and two for rivers were identified as references sites, demonstrating the wide impact of anthropogenic activities, especially gold mining, on French Guiana surface waters. Several bioindicator fish species (ten species identified for creeks and three for rivers) were proposed to help decision-makers to discriminate the chemical status for Hg in French Guiana freshwaters. Moreover, some of these bioindicators species could be grouped to facilitate field sampling and improve the robustness of results. Average Hg concentrations in bioindicators species (grouped or not) measured at reference sites are interpreted as the no-effect observed tissue concentrations expected to be protective for the ichtyofauna and are proposed as local background or threshold to discriminate Hg-impacted sites from pristine areas. This approach is a first step toward establishing future Hg environmental guidance threshold for the ichtyofauna. Furthermore, the importance to distinguish hydrosystems, here creeks and rivers, was clearly demonstrated in our study and should not be neglected in future research. A regular monitoring at reference sites appears essential to check the Hg contamination trends of these sites. Additional prospections should be realized on non-impacted systems to try to identify other reference sites.

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Legend of Figures

Figure 1. Location of study sites for creeks (triangle, n=49) and study areas for rivers (circle, n=48). blue: reference (n=8/2 for creeks/rivers); red: recent gold mining area (n=3/22); black: plume of a recent gold mining area (n= 0/3); yellow: past gold mining area (> 1 year) (n=15/8); dark green: influence of the Petit Saut hydroelectric dam (n=0/3); green: pink: other human activities (deforestation, agricultural and urban exploitation, n=23/5); brown: swamp (n=0/3); velvet: tide (n=0/2). Gray area: gold mining area before 2006 (BRGM source).

Figure 2. Average Hg concentrations (µg.g^{-1} ww) in fish muscle for each of the seven bioindicator species/genus at reference sites and Hg-impacted sites (recent gold mining sites) in creeks (all watersheds combined). n: number of samples. *: statistical difference between two conditions for each bioindicator species/genus (Kruskall-Wallis test, p<0.05).

Figure 3. Average Hg concentrations (µg.g^{-1} ww) in *Jupiaba* (*J. abramoides* + *J. keithi*), *Moenkhausia oligolepis*, *Pimelodella* (*P. cristata* + *P. geryi* + *P.macturki*) and *Pyrrhulina filamentosa* at the reference creek "Trois Sauts" and the creek impacted by recent gold mining activities "Chien"; and after grouping genus for each site. Error bars represent standard errors, n: number of samples. Letters indicate statistical differences between sites for each bioindicator species/genus (p<0.05).

Figure 4. Average Hg concentrations (µg.g^{-1} ww) in fish muscle for each of the three bioindicator species/genus at reference sites and recent gold mining influence in rivers (all watersheds combined). n: number of samples. *: statistical difference between two conditions for each bioindicator species/genus (Kruskall-Wallis test, p<0.05).

Figure 5. Average Hg concentrations (µg.g^{-1} ww) in (A) *Acestrorhyncus (A. falcatus + A. microlepis), Ageneiosus inermis* and *Hoplias aïmara* in the reference area "Oyapock upstream (near Trois Sauts)" and the creek impacted by recent gold mining activities "Camopi River"; and after grouping genus for each area.

Legend of Tables

Table 1. Hg concentrations (µg.g^{-1} ww) and specific richness (R) by feeding ecology and by natural or anthropic influence, in creeks and rivers. Average ± standard error; n: number of samples.

Table 2. Average Hg concentrations (µg.g^{-1} ww) in fish muscle and average standard length of fish for each reference creek classified by feeding ecology. Average ± standard error; Min: Minimum, Max: Maximum; n: number of samples.

Table 3. Characteristics of the seven bioindicator species/genus identified in creeks and their occurrence and distribution in French Guiana watersheds. Fish standard length and weight (wet weight) are indicated as average ± standard error; n: number of sample; +: occurrence of species/genus in watersheds according to this study; +: occurrence of species/genus in watersheds according to the literature (Le bail et al. 2012) but not in this study; % total WS: percentage of total occurrence of bioindicator species/genus in the different watersheds (this study + literature, in percentage). Distribution in creeks: percentage of occurrence of bioindicator species/genus in creeks classified by natural or anthropogenic influences compared to the total number of sampled creeks. % total creeks: percentage of occurrence of bioindicator species/genus in each creek compared to the total number of creeks.

Table 4. Average Hg concentrations (µg.g^{-1} ww) in fish muscle and average standard length of fish for each reference area (groups of stations classified by trophic guild). Average ± standard error; Min: Minimum, Max: Maximum; n: number of samples.

Table 5. Characteristics of the three bioindicator species/genus identified in rivers and their occurrence and distribution in French Guiana watersheds. Fish standard length and weight (wet weight) are indicated as average ± standard error; n: number of sample; +: occurrence of species/genus in watersheds according to this study; +: occurrence of species/genus in

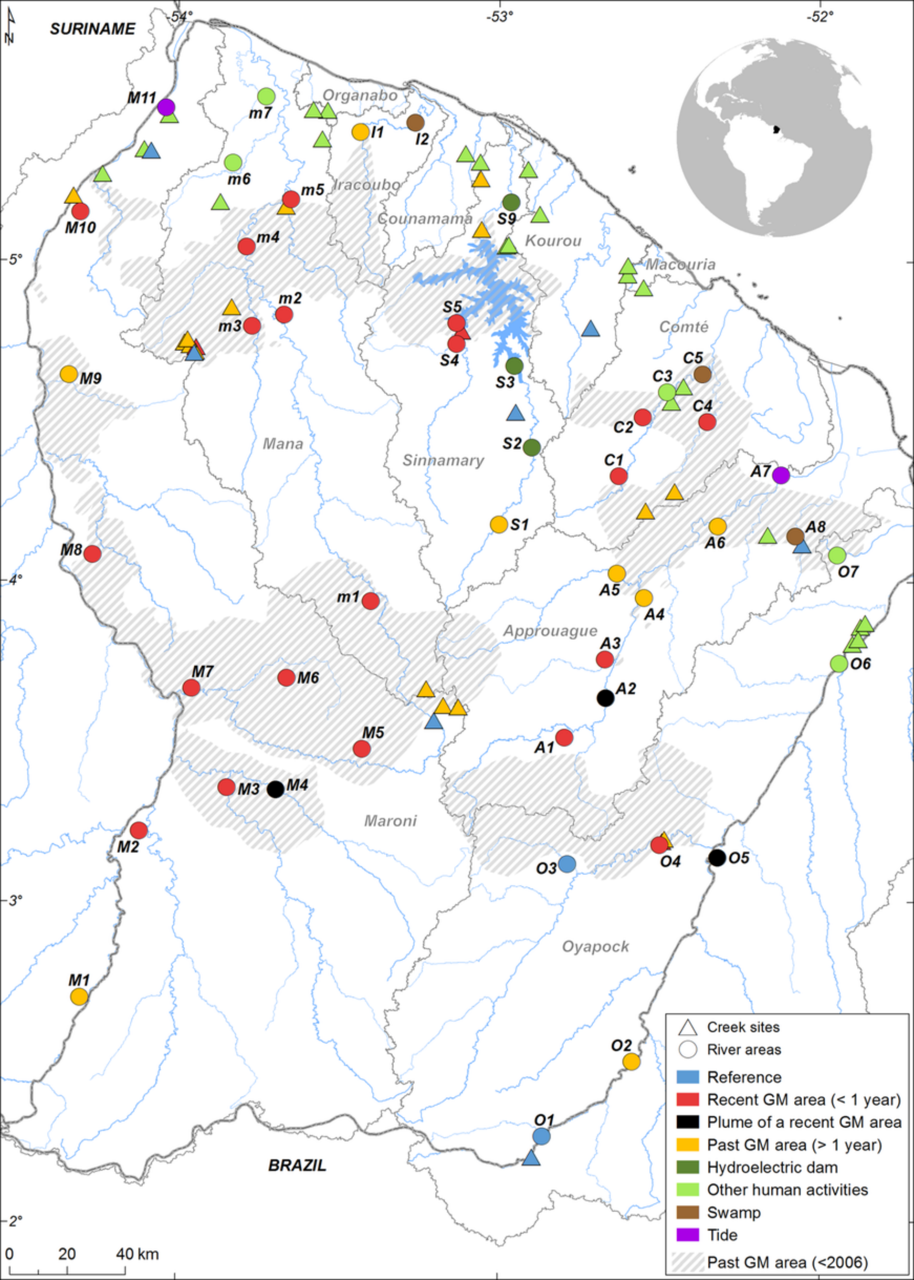
watersheds according to the literature (Le bail et al. 2012) but not in this study; % total WS: percentage of total occurrence of bioindicator species/genus in the different watersheds (this study + literature, in percentage). Distribution in rivers: percentage of occurrence of bioindicator species/genus in areas (grouping of sites) classified by natural or anthropogenic influences compared to the total number of sampled areas. % total rivers: percentage of occurrence of bioindicator species/genus in each area compared to the total number of areas.

Table 6. Average Hg concentrations (µg.g^{-1} ww) in muscle of bioindicator species from the reference sites. n: number of samples, results ± standard error.

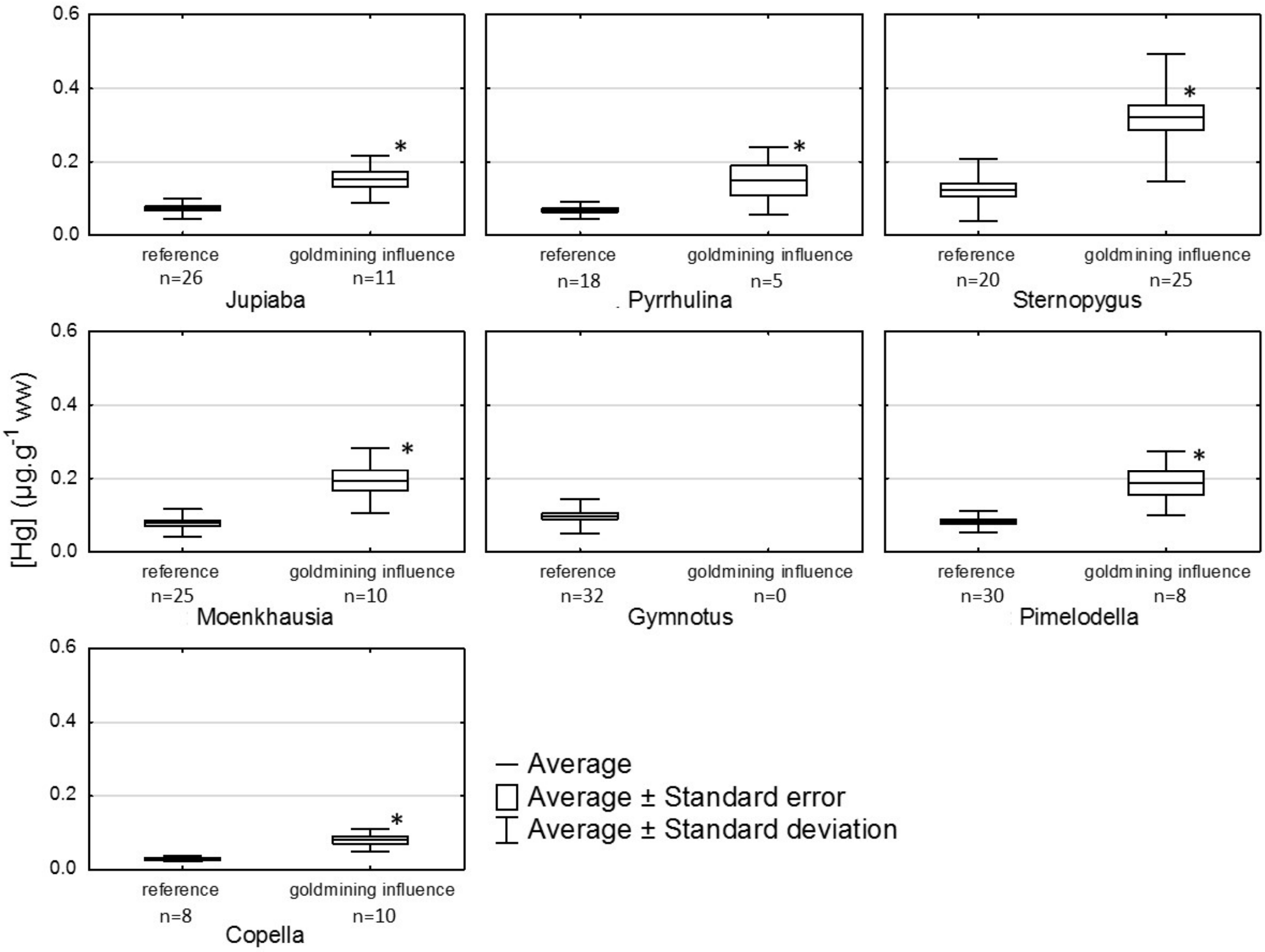
Table S1. Origins of the data used in this study: Projects with financial support, organism responsible of the data collection and sampling years.

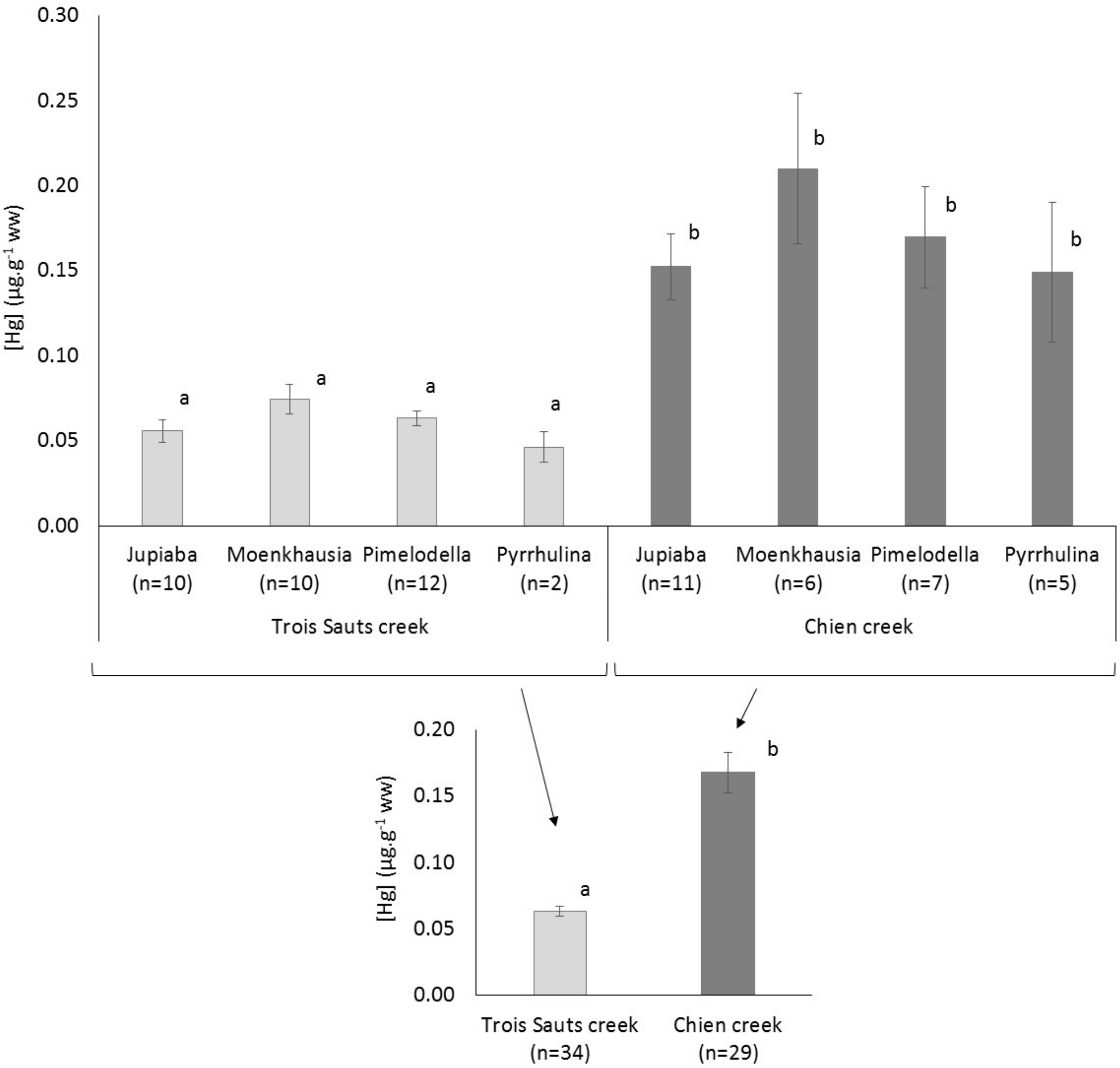
Table S2. Summary of recommended, potential and not recommended bioindicator species, according to criteria defined for creeks. ✓: criterion met; ≈: moderately respected criterion (trophic guild not (so) specific, trophic level < 3 or fish not detected in small watersheds); **?**: criterion impossible to evaluate (lack of data to conclude); “empty”: criterion not met.

Table S3. Summary of recommended, potential and not recommended bioindicator species, according to criteria defined for rivers. ✓: criterion met; ≈: moderately respected criterion (trophic guild not (so) specific, trophic level < 3 or fish not detected in small watersheds); **?**: criterion impossible to evaluate with our data (lack of data to conclude); “empty”: criterion not met. Size class based on standard length.

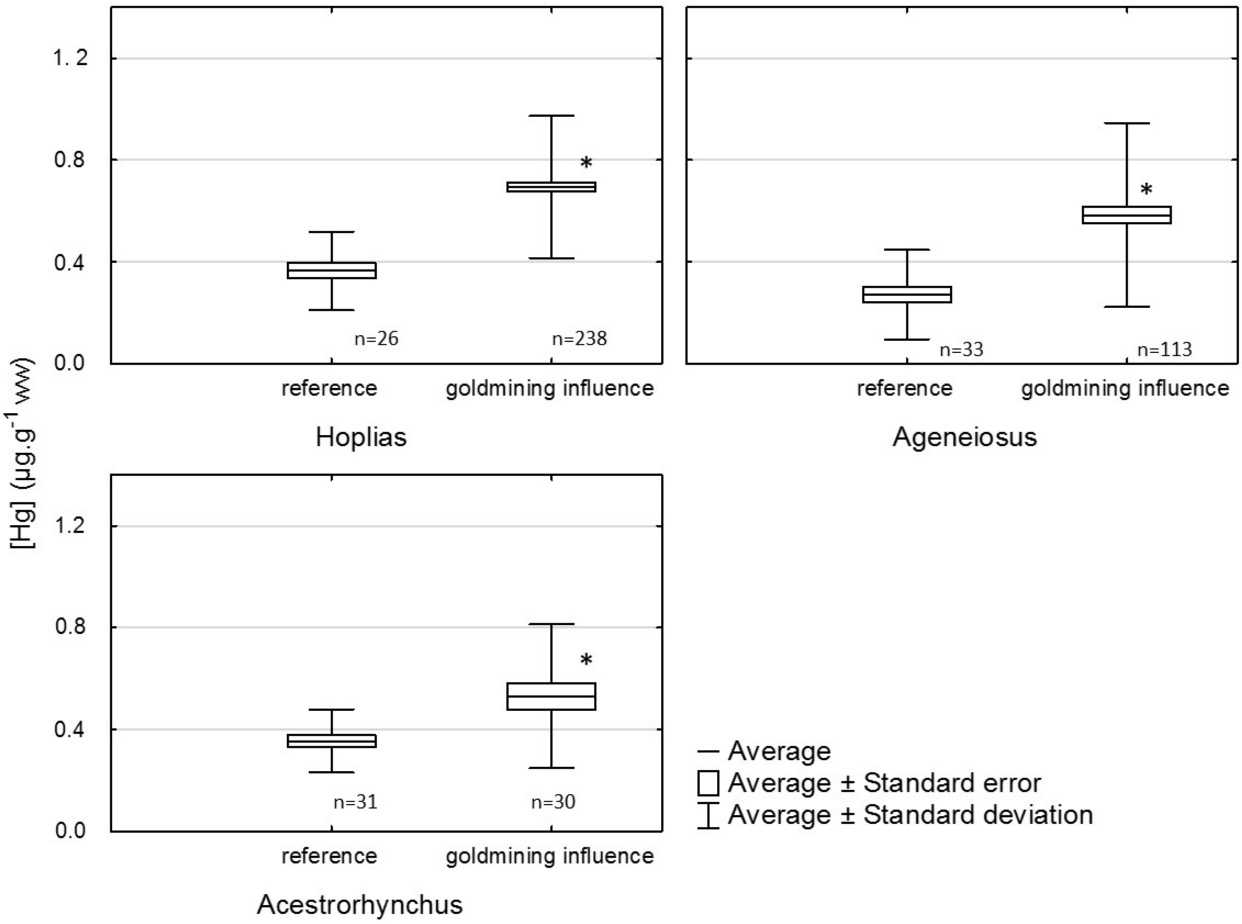


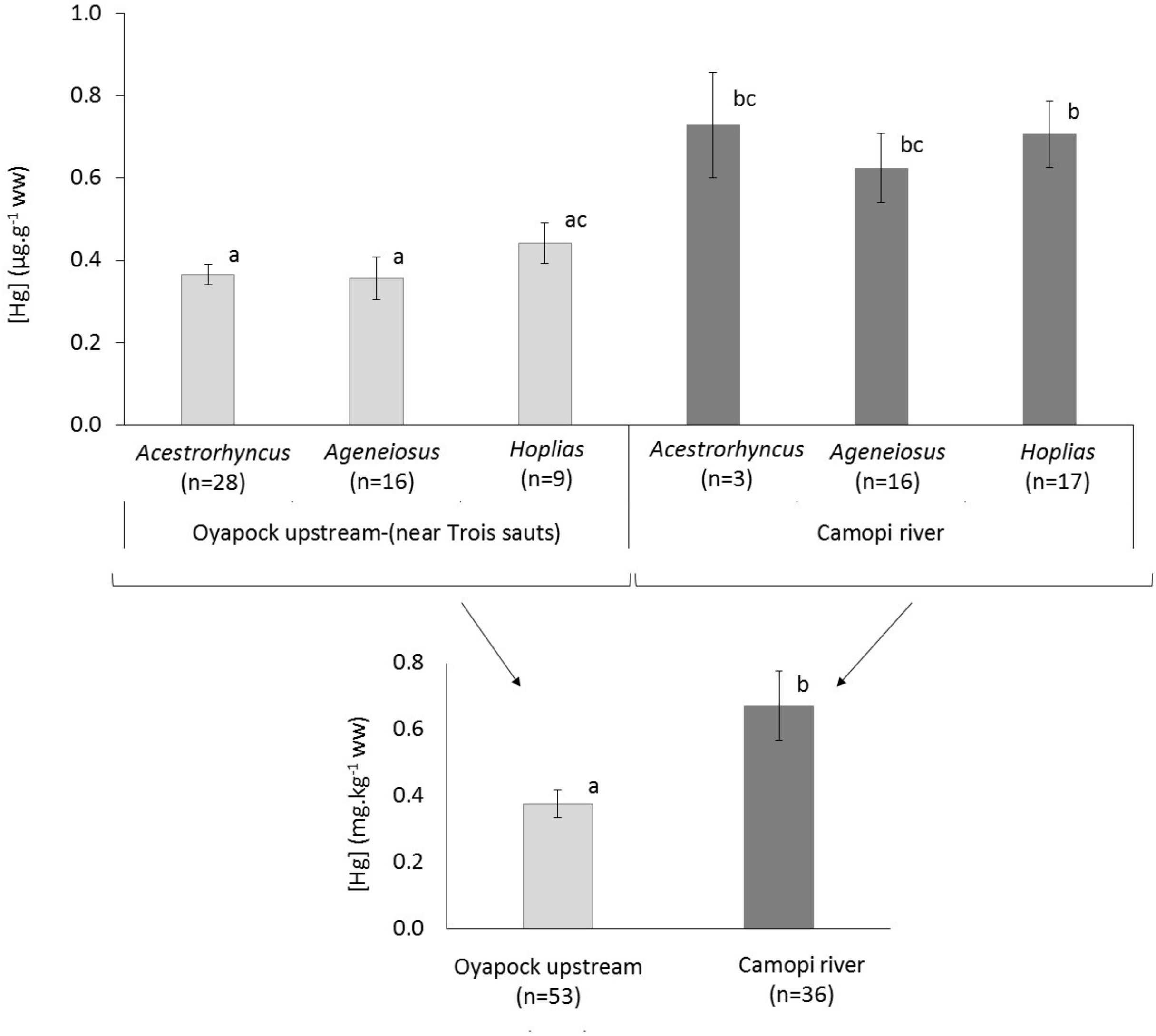
SURINAME
BRAZIL
-54°
-53°
-52°
5°
4°
3°
2°
N
Organabo
Iracoubo
Counamama
Kourou
Macouria
Comté
Mana
Sinnamary
Approuague
Maroni
Oyapock
M1
M2
M3
M4
M5
M6
M7
M8
M9
M10
M11
m1
m2
m3
m4
m5
m6
m7
I1
I2
S1
S2
S3
S4
S5
S9
C1
C2
C3
C4
C5
A1
A2
A3
A4
A5
A6
A7
A8
O1
O2
O3
O4
O5
O6
O7
0
20
40 km
Creek sites
River areas
Reference
Recent GM area (< 1 year)
Plume of a recent GM area
Past GM area (> 1 year)
Hydroelectric dam
Other human activities
Swamp
Tide
Past GM area (<2006)


[Hg] (µg.g-1 ww)
0.6
0.4
0.2
0.0
reference
n=26
goldmining influence
n=11
Jupiaba
reference
n=18
goldmining influence
n=5
Pyrrhulina
reference
n=20
goldmining influence
n=25
Sternopygus
reference
n=25
goldmining influence
n=10
Moenkhausia
reference
n=32
goldmining influence
n=0
Gymnotus
reference
n=30
goldmining influence
n=8
Pimelodella
reference
n=8
goldmining influence
n=10
Copella
Average
Average ± Standard error
Average ± Standard deviation



0.30
0.25
0.20
0.15
0.10
0.05
0.00
[Hg] (µg.g-1 ww)
a
a
a
a
b
b
b
b
Jupiaba (n=10)
Moenkhausia (n=10)
Pimelodella (n=12)
Pyrrhulina (n=2)
Jupiaba (n=11)
Moenkhausia (n=6)
Pimelodella (n=7)
Pyrrhulina (n=5)
Trois Sauts creek
Chien creek
0.20
0.15
0.10
0.05
0.00
[Hg] (µg.g-1 ww)
a
b
Trois Sauts creek (n=34)
Chien creek (n=29)


[Hg] (µg.g-1 ww)
1. 2
0.8
0.4
0.0
n=26
n=238
reference
goldmining influence
Hoplias
n=33
n=113
Ageneiosus
1.2
n=31
n=30
Acestrorhynchus
Average
Average ± Standard error
Average ± Standard deviation



[Hg] (µg.g-1 ww)
1.0
0.8
0.6
0.4
0.2
0.0
a
a
ac
bc
bc
b
Acestrorhyncus (n=28)
Ageneiosus (n=16)
Hoplias (n=9)
Acestrorhyncus (n=3)
Ageneiosus (n=16)
Hoplias (n=17)
Oyapock upstream-(near Trois sauts)
Camopi river
[Hg] (mg.kg-1 ww)
0.8
0.6
0.4
0.2
0.0
a
b
Oyapock upstream (n=53)
Camopi river (n=36)

	Creeks			Rivers		
Feeding ecology	N	R	[Hg] ($\mu g.g^{-1}$ ww)	N	R	[Hg] ($\mu g.g^{-1}$ ww)
Piscivorous	326	19	0.234±0.010	1515	23	0.509±0.009
Carnivorous	1282	44	0.179±0.004	325	17	0.184±0.012
Omnivorous	1157	56	0.156±0.003	565	32	0.146±0.007
Periphytophagous	122	21	0.171±0.014	174	6	0.046±0.009
Benthivorous	40	7	0.203±0.019	220	12	0.144±0.008
Herbivorous	21	2	0.065±0.010	461	5	0.011±0.005
Influence						
Reference	764		0.123±0.003	440		0.150±0.009
Recent gold mining	301		0.235±0.011	1491		0.333±0.009
Plume of gold mining	-		-	238		0.148±0.013
Past gold mining	687		0.213±0.007	533		0.237±0.013
Dam	-		-	279		0.503±0.024
Other human activities	1196		0.171±0.003	220		0.297±0.019
Swamp	-		-	45		0.570±0.077
Tide	-		-	14		0.264±0.032
Total	**2948**	**149**		**3260**	**95**	

Watersheds	Reference creeks	n	[Hg] ($\mu g.g^{-1}$ ww)	Min [Hg]	Max [Hg]	Standard length (cm)	Min SL	Max SL
Oyapock	**Trois Sauts**	**192**	**0.099±0.005**	**0.002**	**0.332**	**10.9±0.5**	**1.9**	**38.0**
	Piscivorous	31	0.143±0.011	0.054	0.298	13.8±1	7.5	28.4
	Carnivorous	55	0.093±0.008	0.022	0.326	14.3±1.2	1.9	38.0
	Omnivorous	82	0.096±0.007	0.031	0.332	8.5±0.3	3.4	14.4
	Periphytophagous	22	0.066±0.007	0.018	0.133	7.6±0.6	3.0	11.0
	Herbivorous	2		0.002	0.053		3.8	15.2
Approuague	**Pai?ra**	**53**	**0.106±0.009**	**0.018**	**0.347**	**7.2±0.6**	**2.1**	**22.5**
	Piscivorous	10	0.124±0.012	0.074	0.201	11.5±1.1	5.3	17.8
	Carnivorous	34	0.098±0.01	0.018	0.25	6.8±0.8	3.1	22.5
	Omnivorous	9	0.116±0.031	0.056	0.347	4.2±0.6	2.1	7.4
Mana	**Montagne**	**10**	**0.088±0.011**	**0.046**	**0.141**	**4.2±0.2**	**3.2**	**5.2**
	Carnivore	10	0.088±0.011	0.046	0.141	4.2±0.2	3.2	5.2
Maroni	**Alama**	**92**	**0.106±0.006**	**0.038**	**0.290**	**6.8±0.3**	**2.0**	**16.0**
	Piscivorous	2		0.217	0.262		7.5	8.2
	Carnivorous	36	0.132±0.011	0.038	0.290	6.1±0.6	2.0	16.0
	Omnivorous	48	0.084±0.005	0.039	0.169	7.3±0.4	2.6	15.0
	Periphytophagous	6	0.086±0.006	0.068	0.101	6.6±0.3	5.9	8.0
	Apa	**39**	**0.144±0.015**	**0.039**	**0.412**	**4.9±0.4**	**1.7**	**11.1**
	Piscivorous	4	0.228±0.068	0.084	0.412	7.5±2.2	2.5	11.1
	Carnivorous	13	0.096±0.013	0.049	0.194	5.1±0.4	2.9	8.0
	Omnivorous	22	0.157±0.02	0.039	0.335	4.3±0.4	1.7	8.1
	Nouvelle France aval	**206**	**0.138±0.007**	**0.002**	**0.614**	**8.1±0.5**	**1.3**	**55.0**
	Piscivorous	31	0.244±0.024	0.088	0.614	11.2±1.9	4.0	55.0
	Carnivorous	59	0.103±0.009	0.029	0.381	9.7±1.3	2.0	41.2
	Omnivorous	101	0.131±0.008	0.040	0.463	6.1±0.2	1.3	10.5
	Periphytophagous	3	0.189±0.033	0.156	0.254	9.9±3	4.0	13.0
	Benthivorous	2		0.085	0.147		7.4	7.9
	Herbivorous	10	0.072±0.016	0.002	0.135	7.9±0.9	4.2	14.6
Kourou	**Galibi**	**26**	**0.143±0.022**	**0.053**	**0.451**	**6.3±1.2**	**2.1**	**32.9**
	Carnivore	14	0.155±0.031	0.053	0.398	8.1±2.2	2.1	32.9
	Omnivore	10	0.136±0.038	0.073	0.451	4.6±0.8	2.3	9.7
	Periphytophagous	2		0.084	0.102		2.7	3.0
Sinnamary	**Saül**	**146**	**0.146±0.008**	**0.028**	**0.512**	**7.8±0.4**	**2.2**	**37.5**
	Piscivorous	10	0.216±0.023	0.081	0.291	15.3±3.1	3.1	37.5
	Carnivorous	44	0.144±0.018	0.028	0.512	7.8±0.9	2.8	35.1
	Omnivorous	67	0.153±0.011	0.037	0.444	6.8±0.4	2.2	18.2
	Periphytophagous	19	0.111±0.022	0.048	0.466	7.9±0.3	5.3	10.0
	Herbivorous	6	0.075±0.005	0.055	0.091	6.7±0.8	3.5	9.3
Total		**764**						

In color

Phylogenetic classification				Feeding ecology	Biometrics			Occurrence in watersheds											Distribution in creeks (%)				
Order	Family	Genus	Species	Diet	Length ± SE (cm)	Weight ± SE (g)	n	Maroni	Mana	Iracoubo	Sinnamary	Kourou	Comté	Approuague	Oyapock	Organabo	Macouria	% total WS	Reference	Recent goldmining	Other human activities	other natural influences	% total creeks
Characiformes	Characidae	*Moenkhausia*	*oligolepis*	carnivorous- invertivorous	6.7±0.1	11.3±0.7	93	+	+	+	+	+	+	+	+			80	3	2	13	0	37
		Jupiaba	-	carnivorous- invertivorous	7.3±0.2	11.7±0.8	77												4	1	12	0	35
			abramoides				62	+	+		+		+	+	+			60					
			keithi				15	+	+		+		+	+	+			60					
	Lebiasinidae	*Copella*	*carsevennensis*	carnivorous- invertivorous	3.0±0.1	0.37±0.03	74	+	+		+	+	+	+	+	+		80	2	1	11	0	29
		Pyrrhulina	*filamentosa*	carnivorous- invertivorous	5.4±0.2	2.8±0.2	101	+	+	+	+	+	+	+	+	+	+	100	5	1	19	0	53
Siluriformes	Heptapteridae	*Pimelodella*	-	piscivorous- invertivorous	9.2±0.4	11.7±1.4	98												4	2	13	0	39
			cristata				78	+	+	+	+	+	+	+	+			80					
			geryi				15	+	+	+	+	+	+	+	+			80					
			macturki				5	+	+					+	+			40					
Gymnotiformes	Gymnotidae	*Gymnotus*	*carapo*	piscivorous- invertivorous	14.6±0.6	15.5±1.7	126	+	+	+	+	+	+	+	+	+	+	100	7	0	25	0	65
	Sternopygidae	*Sternopygus*	*macrurus*	piscivorous- invertivorous	24.5±0.9	35.1±2.9	98	+	+	+	+	+	+	+	+			80	4	2	12	0	37
						Total	**667**																

Watersheds	Reference areas	n	Hg] (µg.g^{-1} ww)	Min [Hg]	Max [Hg]	Standard length (cm)	Min SL	Max SL
Oyapock	**Oyapock upstream (near Trois sauts)**	**405**	**0.152±0.009**	**0.00001**	**0.911**	**24±0.8**	**2.25**	**100.0**
	Piscivorous	138	0.35±0.016	0.043	0.911	34.6±1.7	2.25	100.0
	Carnivorous	29	0.085±0.008	0.015	0.181	18.1±2.3	7.0	47.0
	Omnivorous	71	0.094±0.006	0.024	0.221	15.6±1	6.5	41.0
	Periphytophagous	60	0.029±0.002	0.003	0.088	15.4±0.3	10.0	21.8
	Benthivorous	28	0.084±0.012	0.020	0.341	23.6±2.1	9.0	37.0
	Herbivorous	79	0.004±0	0.00001	0.014	21.7±0.9	8.5	40.5
	Camopi river (Upstream Inipi)	**35**	**0.121±0.02**	**0.001**	**0.427**	**17.1±1.6**	**5.7**	**41.0**
	Piscivorous	10	0.25±0.024	0.141	0.427	27±3.1	14.0	41.0
	Carnivorous	11	0.117±0.034	0.006	0.400	11.8±1.1	7.8	20.0
	Omnivorous	9	0.04±0.004	0.021	0.062	13.4±2.7	5.7	29.7
	Benthivorous	3	0.022±0	0.022	0.023	15.3±4.2	7.3	21.5
	Herbivorous	2		0.001	0.003		12.3	19.5
Total		**440**						

In color

Phylogenetic classification				Feeding ecology	Biometrics			Occurrence in watersheds											Distribution in rivers (%)				
Order	Family	Genus	Species	Diet	Length ± SE (cm)	Weight ± SE (g)	n	Maroni	Mana	Iracoubo	Sinnamary	Kourou	Comté	Approuague	Oyapock	Organabo	Macouria	% total WS	Reference	Recent goldmining	Other human activities	other natural influences	% total rivers
Characiformes	**Characidae**	*Acestrorhyncus*	*falcatus*	piscivorous	18.0±0.3	78.4±4.9	37	+	+	+	+	+	+	+	+			80	4	15	23	2	44
			microlepis				135	+	+	+	+		+	+	+			70					
	Erythrinidae	*Hoplias*	*aimara*	piscivorous	56.3±0.4	4030.4±86.6	549	+	+	+	+		+	+	+			70	4	38	35	6	83
Siluriformes	**Ageneiosidae**	*Ageniosus*	*inermis*	piscivorous	28.9±0.3	379.9±14.5	253	+	+				+	+	+			50	4	33	23	2	63
						Total	**974**																

Water system	Species	n	[Hg] (µg.g^{-1} ww)
RIVERS	*Acestrorhynchus (falcatus,microlepis); Ageneiosus inermis; Hoplias aimara*	90	0.33±0.03
CREEKS	*Jupiaba (abramoides, keithi);Moenkhausia oligolepis; Pimelodella (cristata, geryi, macturki); Pyrrhulina filamentosa*	93	0.08±0.01
	Copella carsevennensis	5	0.03±0.004
	Gymnotus carapo	21	0.09±0.02
	Sternopygus macrurus	20	0.12±0.04