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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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20 Abstract

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

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

49

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

52

53 **1. Introduction**

54

55 Mercury (Hg) is a global pollutant, source of major concerns for humans and
56 ecosystems. In this context, the international Minamata Convention on Mercury, adopted in
57 2013 and officially entered into force in August 2017, defines new objectives to mitigate
58 releases of this highly toxic metal to the environment. Specifically, this convention highlights
59 the necessity to develop tools and networks for Hg monitoring to help the implementation and
60 the compliance with set standards (article 15), as well as the necessity to provide baseline data
61 and comparable Hg measurements (article 19) in order to support decision-making and
62 management of Hg (Selin et al., 2018; Chen et al., 2018). This Minamata Convention
63 corroborates the European Water Framework Directive (WFD 2000/60/EC, EC 2000), which
64 previously established a common policy for the management of surface waters and classified
65 Hg as one of the dangerous priority substances. Indeed, Hg under its most toxic form,
66 methylmercury, is easily bioaccumulated and bioamplified along the food chain (Hall et al.,
67 1997; Watras et al., 1998), resulting in high concentrations in ichthyofauna and particularly in
68 top predator fishes (Campbell et al., 2008). By extension, Hg can be a threat to populations

69 whose main food source is fish consumption, for example Amerindian people in French Guiana
70 (Fréry et al., 2001; Cordier et al., 2002; Cardoso et al., 2010). In order to evaluate the chemical
71 status of water bodies in application of the WFD, Environmental Quality Standards were first
72 defined for water (EQS_{water}), then for biota (EQS_{biota}) for persistent, bioaccumulative and toxic
73 priority substances including Hg (WFD 2008/105/EC & 2013/39/EC; EC, 2008a, 2013). The
74 EQS_{biota} is meant for the protection of piscivorous wildlife against secondary poisoning and
75 should protect the aquatic ecosystems and human health. The EQS_{biota} for Hg was set at 0.020
76 $\mu\text{g}\cdot\text{g}^{-1}$ wet weight (ww) and its application is now mandatory through fish monitoring (WFD
77 2013/39/EC; EC, 2013). Although essential in the context of monitoring aquatic environments,
78 this standard is considered very low. In fact, most fishes exceed the EQS_{biota} in temperate
79 freshwater systems (Vignati et al., 2013; Jurgens et al., 2013). In comparison, the human
80 consumption recommendation of the World Health Organization (WHO) and European
81 regulation for fish was set at $0.5 \mu\text{g}\cdot\text{g}^{-1}$ ww (WHO, 1990; EC, 2008b).

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

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

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

114

115 **2. Methods**

116

117 **2.1 Database creation**

118

119 2.1.1 Origin of data

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

126

127 2.1.2 Fish sampling

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

137 For each individual fish, the species was identified, then the wet weight (g), standard and total
138 length (cm) were measured. In addition, because food is the major contamination pathway for
139 Hg in fish (Hall et al., 1997), a specific trophic diet was associated to each fish species.
140 According to the literature (Keith et al., 2000, Le Bail et al., 2000; Durrieu et al., 2005), six
141 types of feeding ecology were retained: (1) strictly herbivorous, feeding exclusively on aquatic

142 plants (macrophytes, phytoplankton algae) or terrestrial materials from the river banks (leaves,
143 flowers, fruits); (2) periphytophagous, consuming periphyton or biofilms on hard substrates
144 (rocks, immersed tree trunks, etc.); (3) benthivorous, ingesting organic detritus and small preys
145 living in the sediment superficial layers; (4) omnivorous, feeding on a variety of food
146 (vegetables, insect larva, crustaceans, mollusks, etc.) according to their availability; (5)
147 carnivorous, eating animal preys of different orders (crustaceans, mollusks, aquatic and
148 terrestrial insects, fish, etc.); and (6) piscivorous, capturing fish of varying size.

149

150 2.1.3 Sampling sites characterisation

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

152 Creeks are shallow (below 1 m depth) and narrow (width less than 10 m) water bodies that flow
153 into rivers. Our study highlighted a difference in the fish species diversity (different species
154 present) between creeks and rivers. Also, some species have a juvenile life-stage in creek and
155 an adult life-stage in river. Likewise, water chemistry has an influence on Hg bioavailability
156 and bioaccumulation (Carmouze et al., 2001); and rivers and creeks differed in water
157 characteristics (Crespy et al., 2015). Therefore, each sampling site was considered according to
158 its hydrographic functioning; and the results are presented separately for creeks and rivers. A
159 total of 217 sites were sampled: 49 for creeks (Figure 1A) and 168 for rivers (Figure 1B). On
160 the rivers within each watershed, to ensure that fish numbers are sufficient by species (i.e., more
161 than five individual fish per site), several sites were grouped when they shared the same
162 geographical position and similar natural or human pressure (see next paragraph). Thus, we
163 defined 48 areas (group of sites) for rivers. Each site was positioned in RGFG95 / UTM zone
164 22N, reference system used in French Guiana.

165

167 In French Guiana, the presence of reference sites for creeks and rivers, not impacted by human
168 activities and especially gold mining, is still unknown. A cross-referencing of current
169 knowledge on risk of Hg contamination in French Guiana through the literature on land use
170 (<http://gisguyane.brgm.fr>) and the absence of significant anthropogenic pressure by field
171 observations (*in situ* expert judgment by R. Vigouroux and R. Maury-Brachet), allowed
172 identifying reference sites. This also allowed discriminating Hg-contaminated sites impacted
173 by gold mining activities, which used Hg for gold amalgamation. We differentiated three types
174 of pressure linked to gold mining: (i) recent gold mining area (< 1 year); (ii) past gold mining
175 (> 1 year); and (iii) sites located in the plume of a recent gold mining area. Other human
176 activities were distinguished: one category with sites under the influence of the Petit Saut
177 hydroelectric dam (Sinnamary watershed) and one more category grouping other human
178 activities (deforestation, agricultural and urban exploitation). Finally, sites under the influence
179 of natural phenomenon, which may have an influence on Hg cycle like swamps and tide, were
180 also identified separately (Figure 1).

181

182 **2.2 Determination of bioindicator fish species**

183 In order to identify the most relevant bioindicator species in agreement with the WFD
184 implementation Guidance documents (EC, 2010, 2014), several criteria were specified: the fish
185 species must (1) be present on one or more sites classified as a reference (at least five individuals
186 per site to allow statistical analysis); (2) have a trophic level equal to or greater than three (=
187 carnivorous, piscivorous), with a well-known and specific diet; (3) be distributed in at least
188 50 % of watersheds indicating a relatively high occurrence; (4) have significantly lower Hg
189 concentrations at reference sites than at Hg-impacted ones. Because of this last criterion, only

190 sites recently impacted by gold mining activities, the most easily identifiable and important
191 human pressure in French Guiana, were considered for the identification of bioindicator species.

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

206

207 **2.3 Mercury analysis**

208 Before analysis, samples were dried at 44 °C for 48 hours. All the total Hg concentration data
209 used for this study were measured by the EPOC laboratory (University of Bordeaux) or by the
210 HYDRECO laboratory according to the same standard method (EPA, 1998) using an automated
211 atomic absorption spectrophotometer (AMA 254, Symalab France). Blanks and the same
212 reference materials TORT2 (National Research Council of Canada, lobster hepatopancreas)
213 were systematically used, typically every 10 samples, by both entities to control analytical

214 accuracy. The accuracy averaged respectively 98.2% and 100.5%. The limit of detection of total
215 mercury on AMA 254 is 0.0004 ng. The limit of quantification is 0.010 $\mu\text{g.g}^{-1}$ dw. Total Hg
216 concentrations in biota are expressed in $\mu\text{g.g}^{-1}$ on a wet weight basis; a factor of 5 was applied
217 to convert all Hg results from dry weight to wet weight basis (Maury-Brachet et al., 2006).

218

219 **2.4 Statistical analysis**

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

228

229 **2.5 Cartographic representation and spatial analysis of data**

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

235

236 **3. Results**

237

238 **3.1 Hg concentrations in fish for creeks**

239

240 3.1.1. Dataset presentation

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

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

252

253 3.1.2. Hg concentrations at reference sites in creeks

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

259 (Approuague); Trois-Sauts (Oyapock). For each reference site, average Hg concentration in
260 muscle of all fishes was systematically and statistically lower compared to recent gold mining
261 sites (LSD Fisher test, $p < 0.05$; Table 2). In reference creeks, the lowest Hg concentrations
262 (average Hg \pm standard error for all species) were measured in fish from Trois-Sauts ($0.10 \pm$
263 $0.01 \mu\text{g}\cdot\text{g}^{-1}$, $n=192$), Païra creek ($0.11 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$, $n=53$), Alama creek ($0.11 \pm 0.01 \mu\text{g}\cdot\text{g}^{-1}$,
264 $n=92$) and Montagne Creek ($0.09 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$, $n=10$), with similar Hg level. The other
265 references creeks, Apa ($0.14 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$, $n=39$), Nouvelle France aval ($0.14 \pm 0.01 \mu\text{g}\cdot\text{g}^{-1}$,
266 $n=206$), Saül ($0.15 \pm 0.01 \mu\text{g}\cdot\text{g}^{-1}$, $n=146$) and Galibi ($0.14 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$, $n=26$) showed
267 comparable Hg level, somewhat higher than for the first group of creeks but not statistically
268 different. The most represented trophic guilds in reference creeks belong to high trophic level
269 (≥ 3) with 44 % of omnivorous, 35 % of carnivorous and 12 % of piscivorous (Table 2).

270

271 3.1.3. Identification of bioindicator fish species in creeks

272 Based on the criteria defined in section 2.2 and thanks to the discrimination of reference sites,
273 several species were scrutinized (Table S2). A number of species were definitively eliminated
274 (i.e., "not recommended", total = 13 species), while others would still need additional
275 information (e.g., lack of samples or knowledge on diet) to check their integrative capacity (i.e.,
276 "potential" bioindicator species, total =12). In the end, ten fish species belonging to seven
277 genera were selected as bioindicators: *Moenkhausia oligolepis*, *Gymnotus carapo*, *Sternopygus*
278 *macrurus*, *Jupiaba (abramoides + keithi)*, *Pimelodella (cristata+geryi+macturki)*, *Copella*
279 *carevennensis* and *Pyrrhulina filamentosa*. Their phylogenetic and trophic characteristics as
280 well as their spatial distribution are summarized in Table 3. These bioindicator species represent
281 22 % of all sampled fish.

282 Due to their small size (<15 cm, except for *Sternopygus m.* <25 cm), fish in creeks were not
283 grouped by size class. The comparison of Hg bioaccumulation for each species between
284 reference and recent gold mining impacted sites is shown on Figure 2. All the bioindicator
285 species proposed accumulated significantly more Hg in the Hg-impacted creeks than in
286 reference creeks (Kruskal-Wallis test, $p < 0.05$ for all species). At the reference sites, the lowest
287 average Hg concentration for all bioindicator species was measured in *Copella* (0.031 ± 0.003
288 $\mu\text{g}\cdot\text{g}^{-1}$) and the highest in *Sternopygus* ($0.12 \pm 0.04 \mu\text{g}\cdot\text{g}^{-1}$). Overall, at reference sites, 99% of
289 bioindicator fishes presented Hg concentrations above the $\text{EQS}_{\text{biota}}$ but none exceeded the fish
290 consumption recommendation.

291

292 3.1.4. Grouping of bioindicator fish species in creeks

293 Catching a specific fish species (moreover in sufficient numbers from a statistical point of view)
294 in creeks, and more globally in French Guiana, is quite complicated for two reasons. The first
295 reason is the high specific richness (149 fish species obtained in creeks in the present study,
296 Table 1), associated with a low abundance for most species. Therefore, it requires a significant
297 fishing effort to obtain a representative sample of a specific fish species (Allard et al., 2014);
298 moreover, this is potentially destructive for aquatic biota. In case of creeks, *i.e.* small-size
299 systems, the risk is to disrupt ecosystems greatly by taking a large amount of biomass, which is
300 not acceptable. The second reason is the difficulty of fishing in such isolated areas from a
301 logistical point of view (high cost / benefit ratio). To solve this problem, we tested the
302 possibility of grouping bioindicator species according to criteria defined in section 2.2. In
303 creeks, the four bioindicators *Jupiaba*, *Moenkhausia*, *Pimelodella* and *Pyrrhulina* had a similar
304 trophic diet (Carnivorous- invertivorous, Table 3). At reference sites, these four genera showed
305 no statistical difference in Hg bioaccumulation pattern (from 0.07 ± 0.01 to $0.09 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$)
306 (Figure 2). To test the possibility to group them, we focused on two sites where these four

307 genera were present: the reference creek “Trois Sauts” and the gold mining creek “Chien”
308 sampled during the year 2012. The Hg concentrations were not statistically different between
309 the four bioindicators species, both for the reference creeks (LSD Fisher test, $p > 0.05$) and for
310 recent gold mining sites ($p > 0.05$, Figure 3). Thus, data from these four genera were grouped at
311 each site. Average Hg concentrations were 2.6 times higher for the Hg-impacted creek
312 compared to the reference creek (LSD Fisher test, $p < 0.05$, Figure 3). Moreover, when
313 aggregated, these four bioindicators species cover 100% of the French Guiana watersheds.

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

318

319 **3.2 Hg concentrations in fish sampled in rivers**

320

321 3.2.1. Dataset presentation

322 Over the 3260 fish collected in rivers between 2004 and 2014, 95 species were identified (Table
323 1). Only 32 fish species are common between rivers and creeks. In rivers, piscivorous fish
324 represented about half of the collected fish (48%), followed by omnivorous (20%), herbivorous
325 (11%), carnivorous (10%), benthivorous (7%) and periphytophagous fish (4%). The trophic
326 guild with the highest Hg concentration was piscivorous fishes ($0.509 \pm 0.009 \mu\text{g}\cdot\text{g}^{-1}$); in
327 contrast, herbivorous fishes are the only species with Hg concentrations below the $\text{EQS}_{\text{biota}}$. All
328 fish considered, fish from recent gold mining areas had Hg levels twice higher than at references
329 sites (respectively 0.333 ± 0.009 and $0.150 \pm 0.009 \mu\text{g}\cdot\text{g}^{-1}$, LSD Fisher test, $p < 0.05$).

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

333

334 3.2.2. Hg concentrations at reference sites in rivers

335 Among the 48 sampling areas (as a reminder, 168 sampling sites were grouped by their
336 similitude in anthropogenic pressure and geographic position), only two reference areas were
337 identified on the rivers (Figure 1B, blue circle): “upstream Oyapock River (near Trois Sauts)”
338 and “Camopi River (upstream Inipi)”, both situated on the Oyapock watershed. For each
339 reference area, the average Hg concentration in muscle of all fishes was systematically and
340 statically lower compared to those collected at recent gold mining sites (LSD Fisher test,
341 $p < 0.05$; Table 4). Mercury concentrations in fish (average \pm standard error for all species) were
342 not statistically different between “upstream Oyapock River” ($0.15 \pm 0.01 \mu\text{g}\cdot\text{g}^{-1}$, $n=405$) and
343 “Camopi River” ($0.12 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$ ww, $n=35$). The distribution of trophic guilds at both
344 reference areas was as follows: 34 % of piscivorous, 18% of omnivorous, 18% of herbivorous,
345 14 % of periphytophagous, 9% of carnivorous and 7% of benthivorous (Table 4).

346

347 3.2.3. Identification of bioindicator fish species in rivers

348 Several species were examined as candidates as bioindicator species using the criteria detailed
349 in the methodology section (§2.2) and thanks to the preceding discrimination of reference areas.
350 Table S3 presents the species definitively eliminated (i.e., "not recommended", total = 7
351 species), others which still need additional information to check their integrative capacity (i.e.,
352 "potential" bioindicator, total = 4) and the retained species (i.e., "recommended", total: 10,

353 belong to 7 genera). Four fish species belong to three genera were selected as bioindicators:
354 *Acestrorhyncus (micropelis + falcatus)*, *Hoplias aimara* and *Ageneiosus inermis*. Their
355 phylogenetic and trophic characteristics (all piscivorous) as well as their spatial distribution are
356 summarized in Table 5. These bioindicator species represent 30 % of all sampled fish. Each of
357 these species occurred in at least 44% of the sampling areas (*Acestrorhyncus*) and 50% of the
358 watersheds (*Ageneiosus inermis*). A size class was chosen for each species in order to compare
359 Hg concentrations between sites based on the fact that (i) the age and standard length of
360 individuals are generally correlated and (ii) Hg bioaccumulation is influenced by age. Size
361 classes were defined considering only adult fish and using the method of percentiles (i.e., 10%
362 of the most extreme values were eliminated). Therefore, only specimen between between 10
363 and 30 cm for *Acestrorhyncus (micropelis + falcatus)*, 38 and 82 cm for *Hoplias aimara* and
364 between 20 and 45 cm for *Ageneiosus inermis* were kept for the rest of the study. The
365 comparisons of Hg bioaccumulation for each species between reference and gold mining sites
366 are shown in Figure 4. The three proposed bioindicators species accumulated significantly more
367 Hg at Hg-impacted areas compared to reference areas (Kruskal-Wallis test, $p < 0.05$ for all
368 species). For reference areas, Hg concentration (average \pm standard error) in *Ageneiosus* (0.27
369 $\pm 0.03 \mu\text{g}\cdot\text{g}^{-1}$, $n=33$) was significantly lower than in *Acestrorhyncus* ($0.35 \pm 0.02 \mu\text{g}\cdot\text{g}^{-1}$, $n=31$)
370 and *Hoplias* ($0.36 \pm 0.03 \mu\text{g}\cdot\text{g}^{-1}$, $n=26$).

371 Overall, at reference sites, 100% of bioindicator fish presented Hg concentrations above the
372 $\text{EQS}_{\text{biota}}$ and 48% also exceeded the fish consumption recommendation.

373

374 3.2.4. Grouping of bioindicator fish species in rivers

375 As explained in section 3.1.4 for creeks, we tested the possibility to group the proposed
376 bioindicator species by sampling area in rivers, in order to strengthen the interpretation of the

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

391

392 **4. Discussion**

393

394 **4.1 Difficulties in the application of EQS_{biota} for WFD monitoring**

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

401 (NOEC -No Observed Effect Concentration) and with a ten-fold safety factor applied. Second,
402 the WFD emphasized the importance of the trophic level when selecting bioindicators species,
403 and stated that only fish species with a trophic position ≥ 3 (carnivorous/piscivorous) should be
404 considered (EC, 2014). However, even when considering aquatic environments not exposed to
405 Hg, carnivorous/piscivorous fish often exceed the EQS_{biota}, thereby systematically declassifying
406 most water bodies (Vignati et al. 2013; Jürgens et al., 2013; Nguetseng et al., 2015; Fliedner et
407 al., 2016). In the present study, among the 6208 fish sampled in rivers and creeks of French
408 Guiana, almost all had Hg concentrations higher than the EQS_{biota}, regardless of the
409 anthropogenic influences associated with the sampling sites. All years and sites included, the
410 Hg concentrations were up to 30 times higher than the EQS_{biota} for piscivorous fish, and about
411 10 times higher for carnivorous, omnivorous and benthivorous species. Nonetheless, most Hg
412 concentrations were below the threshold of human consumption ($0.5 \mu\text{g}\cdot\text{g}^{-1}$ ww; WHO and EC),
413 except for the piscivorous species. This work highlights the unsuitability of the direct
414 application of the EQS_{biota} in French Guiana.

415 With the introduction of EQS for priority pollutants, the European Commission showed its
416 willingness to protect the environment by banning the environmental inequalities that may exist
417 between countries. However, as demonstrated here and in other studies (Crane and Babut,
418 2007), some limitations in its application appear at a regional scale. Indeed, the characteristics
419 of ecoregions or sites are not taken into account in the present application of this EQS_{biota},
420 whereas background concentrations for Hg could vary depending on local geology. An
421 evolution of the European EQS_{biota} would be required, with a greater emphasis on the collection
422 of field data, rather than solely relying on extrapolation from laboratory data.

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

426 **4.2 Well known factors responsible of high Hg levels in fish of French Guiana: role of the**
427 **pedo-climatic context and gold mining activity**

428 In the Amazonian region, Hg concentrations in soils are naturally higher compared to boreal
429 and temperate areas (Roulet and Lucotte, 1995; Carmouze et al., 2001; Guédron et al., 2006),
430 due to high atmospheric depositions during the latest millions of years (Théveniaut and
431 Freyssinet, 1999). The background Hg level in river sediments of the whole of French Guiana
432 was estimated at $0.108 \pm 0.042 \mu\text{g. g}^{-1} \text{ dw}$, depending on particle size (Laperche et al., 2014).
433 Due to this particular lithology, Hg naturally accumulated in soils constitutes an important
434 reservoir that can be mobilized through natural or anthropogenic erosion (deforestation, gold-
435 mining...), leading to an increase in terrestrial Hg export to aquatic systems. Indeed, ASGM
436 are a real cause for concern in French Guiana since the 1850s due to the release of large amounts
437 of naturally Hg-rich particles into the hydrosystems by soil erosion and the direct release of Hg
438 into the environment due to the gold recovery process (Grimaldi et al., 2015). The use of Hg
439 for gold mining, banned since 2006 in Europe, is still widely used by illegal miners. Monitoring
440 campaigns carried out by the Amazonian Park of French Guiana (PAG, 2017) and the French
441 National Forestry Office (ONF) showed an increase in illegal gold mining activities since 2013
442 in French Guiana. Such an increase is explained by local cultural factors and by economic
443 market trends with the increase of the global price of gold (Swenson et al., 2011; Asner et al.,
444 2013). In the present study, gold mining activity was the main anthropogenic pressure
445 represented among the studied sites (Figure 1). Indeed, a concordance in the occurrence of the
446 gold mining activity (recent and old activity) with the BRGM (French geological survey) gold
447 mining data was evidenced (grey zone in Figure 1). In rivers, average Hg concentrations are
448 two fold higher in all piscivorous fish from recent gold mining-impacted areas than those of
449 reference sites ($0.567 \pm 0.014 \mu\text{g.g}^{-1}$, $n=692$, min-max=0.03-2.82 versus $0.343 \pm 0.015 \mu\text{g.g}^{-1}$,
450 $n=148$, min-max=0.04-0.91). Here, no size classes of fish were taken into account for
451 comparison with literature because it is rarely documented (excepted in the study of Bastos et

452 al., 2015). Mercury levels in piscivorous or carnivorous fish of other gold mining-impacted
453 regions in the Amazon basin are of the same order of magnitude as our results (Myster et al.,
454 2018). In the Madeira river basin, an Hg-impacted area, Hg levels in carnivorous (n=461) and
455 piscivorous (n=597) fish ranged from 0.051 to 1.242 $\mu\text{g}\cdot\text{g}^{-1}$ (Bastos et al., 2015). Malm et al.
456 (1995) reported high values in carnivorous fish collected from the upper part of the river system
457 (impacted by gold mining activities) with an average value of 0.69 $\mu\text{g}\cdot\text{g}^{-1}$ (SD not available,
458 min-max= 0.15-3.8 $\mu\text{g}\cdot\text{g}^{-1}$, n=43). In the lower part of the Tapajos, far from gold mining
459 activities, in areas which could be considered as “almost non-impacted” (Santarém), average
460 Hg concentration in the same carnivorous species decreased to 0.19 $\mu\text{g}\cdot\text{g}^{-1}$ (SD not available,
461 min-max= 0.05-0.55 $\mu\text{g}\cdot\text{g}^{-1}$, n=17). Likewise, on gold mining impacted area of the Tapajos
462 basin, Lino et al. (2018) measured Hg concentrations from 0.4 to 1.51 $\mu\text{g}\cdot\text{g}^{-1}$ in carnivorous fish
463 (n=35, Hg average).

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

473 **4.3 Identifying reference sites is a difficult task in such impacted environment**

474 In French Guiana, we were confronted to the difficulty to find truly pristine areas, especially in
475 the context of extended illegal gold mining activities for many years. The history of human
476 activities (gold mining, agriculture, deforestation...) is poorly known in a large part of the

477 territory. For example, it is difficult to determine if a site has already been prospected for gold
478 mining and, if so, for how long and to what extent. The classification of the reference sites
479 proposed here is therefore bound to evolve, also with the gain of future knowledge. For creeks,
480 we identified eight reference sites over the 49 studied sites, located on six watersheds. For
481 rivers, only two areas (groups of sites) over the 48 sampled areas could be identified
482 unambiguously as reference; both are located on the Oyapock watershed. In fact, these two river
483 areas have previously been identified as reference areas for Hg in sediment (Laperche et al.,
484 2014). Existing literature shows rather well how challenging it is to find true reference sites in
485 Amazonia. Indeed, most studies focused only on Hg polluted areas and compared measured
486 concentrations in fish to the WHO guideline; whereas only few studies worked on “natural”
487 sites as showed in the review of Kasper et al. (2018) in Amazonia. The originality of our work
488 is to present a comprehensive study in tropical environment enabling the identification of
489 reference sites for Hg and the determination of Hg background levels in fish for such a large
490 territory.

491 **4.4 Identification of bioindicator species: a useful and original work**

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

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

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

522 For rivers, four fish species were selected as potential bioindicators (Table 5). They correspond
523 to those proposed by Bouvier et al. (2015) who based his study on 268 individuals belonging
524 to 15 species on the main watersheds of French Guiana. Moreover, Durrieu et al. (2005)
525 published a specific study on *Hoplias aimara* and they recommended this species as a

526 bioindicator for Hg due to its ubiquity, its sedentary behaviour (Junk 1985; Menezes and
527 Vazzoler 1992; Planquette et al., 1996) and its capacity to accumulate significantly Hg at
528 impacted sites (Durrieu et al., 2005). Other bioindicator species were proposed in rivers, such
529 as *Curimatida Cyprinoides* (Dominique et al., 2007), a widely distributed species in the whole
530 Amazonian basin. However, its trophic level < 3 (detritivorous/benthivorous species) is not
531 compatible with the criteria of the WFD, so we did not consider this species as a potential
532 bioindicator.

533 The relatively high background concentrations of Hg measured in fish muscle in freshwaters
534 caused by exposure to naturally Hg rich water or sediments, could be the result of acclimation
535 or natural selection processes linked to the development of a set of regulation of uptake and
536 detoxification processes, such as metallothionein proteins (Chan et al., 2002; Gentès et al.
537 2015). Thus, the average Hg concentrations measured in muscle of the proposed bioindicator
538 species at reference sites could be considered as reference thresholds in French Guiana (Table
539 6). For each defined bioindicator species/genera or group of species, a concentration at a given
540 site significantly higher than this reference threshold would indicate Hg contamination.

541

542 **5. Conclusion**

543 This study provides an important enhancement of the database on Hg in fish species for the
544 WFD in tropical ecosystems of French Guiana. First, by defining anthropogenic and natural
545 influences associated with each of 200 sampling sites. Among them, only eight for creeks and
546 two for rivers were identified as references sites, demonstrating the wide impact of
547 anthropogenic activities, especially gold mining, on French Guiana surface waters. Several
548 bioindicator fish species (ten species identified for creeks and three for rivers) were proposed
549 to help decision-makers to discriminate the chemical status for Hg in French Guiana

550 freshwaters. Moreover, some of these bioindicators species could be grouped to facilitate field
551 sampling and improve the robustness of results. Average Hg concentrations in bioindicators
552 species (grouped or not) measured at reference sites are interpreted as the no-effect observed
553 tissue concentrations expected to be protective for the ichthyofauna and are proposed as local
554 background or threshold to discriminate Hg-impacted sites from pristine areas. This approach
555 is a first step toward establishing future Hg environmental guidance threshold for the
556 ichthyofauna. Furthermore, the importance to distinguish hydrosystems, here creeks and rivers,
557 was clearly demonstrated in our study and should not be neglected in future research. A regular
558 monitoring at reference sites appears essential to check the Hg contamination trends of these
559 sites. Additional prospections should be realized on non-impacted systems to try to identify
560 other reference sites.

561

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563

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569

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783

784 Legend of Figures

785

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

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

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

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

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

810

811 **Legend of Tables**

812 Table 1. Hg concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ ww) and specific richness (R) by feeding ecology and by
813 natural or anthropic influence, in creeks and rivers. Average \pm standard error; n: number of
814 samples.

815 Table 2. Average Hg concentrations ($\mu\text{g}\cdot\text{g}^{-1}$ ww) in fish muscle and average standard length of
816 fish for each reference creek classified by feeding ecology. Average \pm standard error; Min:
817 Minimum, Max: Maximum; n: number of samples.

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

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

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

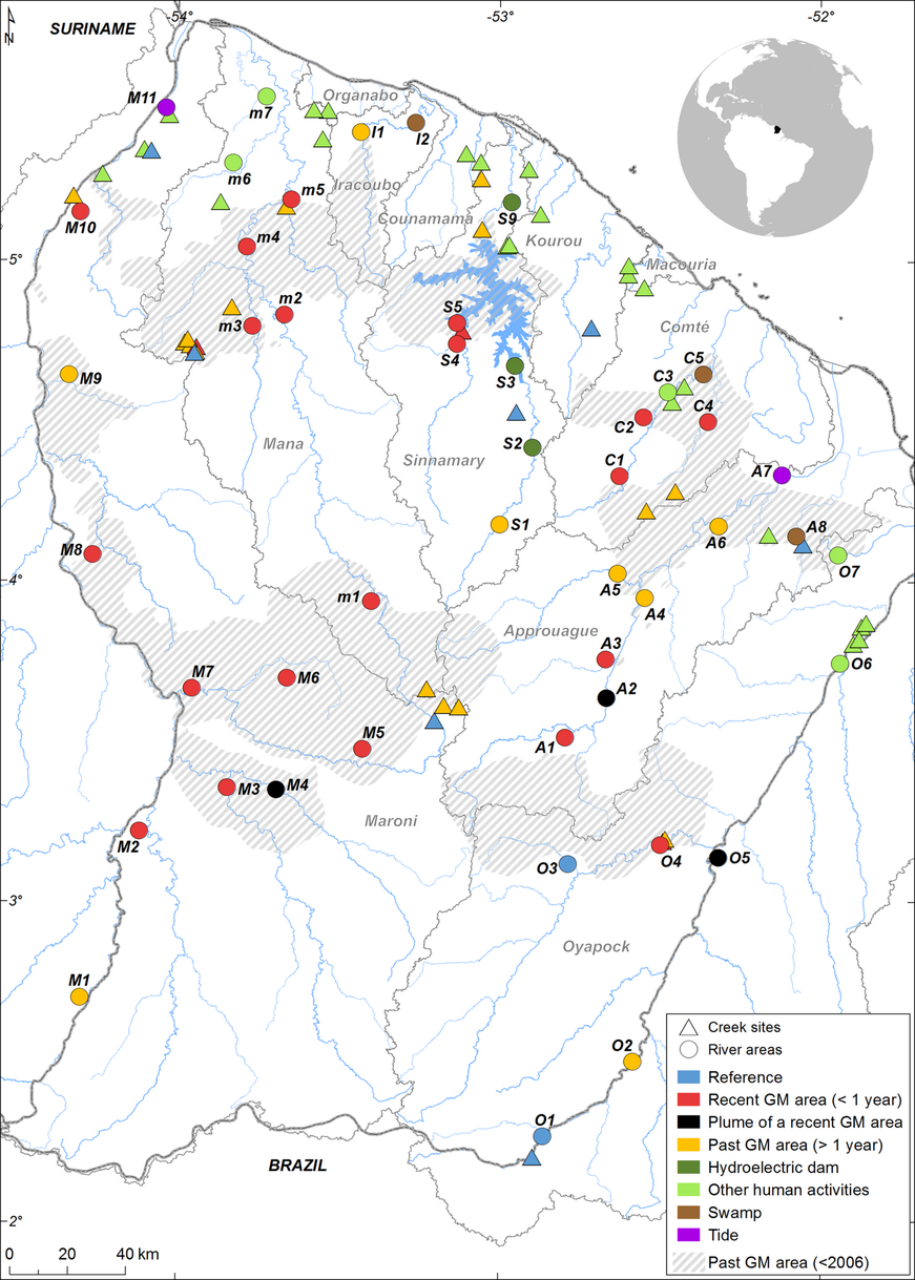
835 watersheds according to the literature (Le bail et al. 2012) but not in this study; % total WS:
836 percentage of total occurrence of bioindicator species/genus in the different watersheds (this
837 study + literature, in percentage). Distribution in rivers: percentage of occurrence of
838 bioindicator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

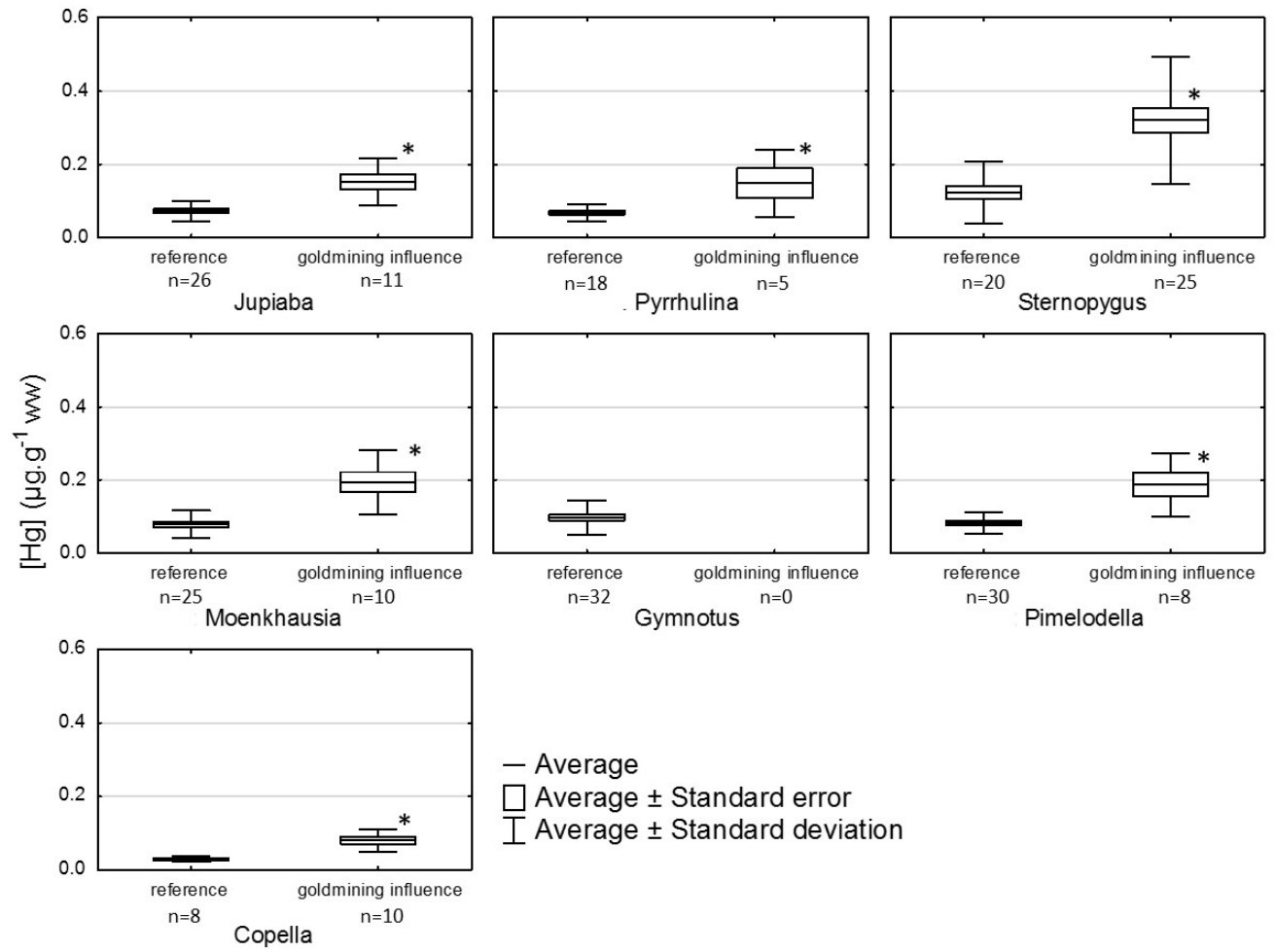
841 Table 6. Average Hg concentrations ($\mu\text{g.g}^{-1}$ ww) in muscle of bioindicator species from the
842 reference sites. n: number of samples, results \pm standard error.

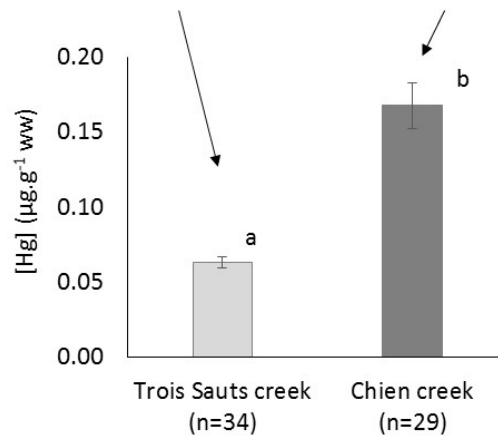
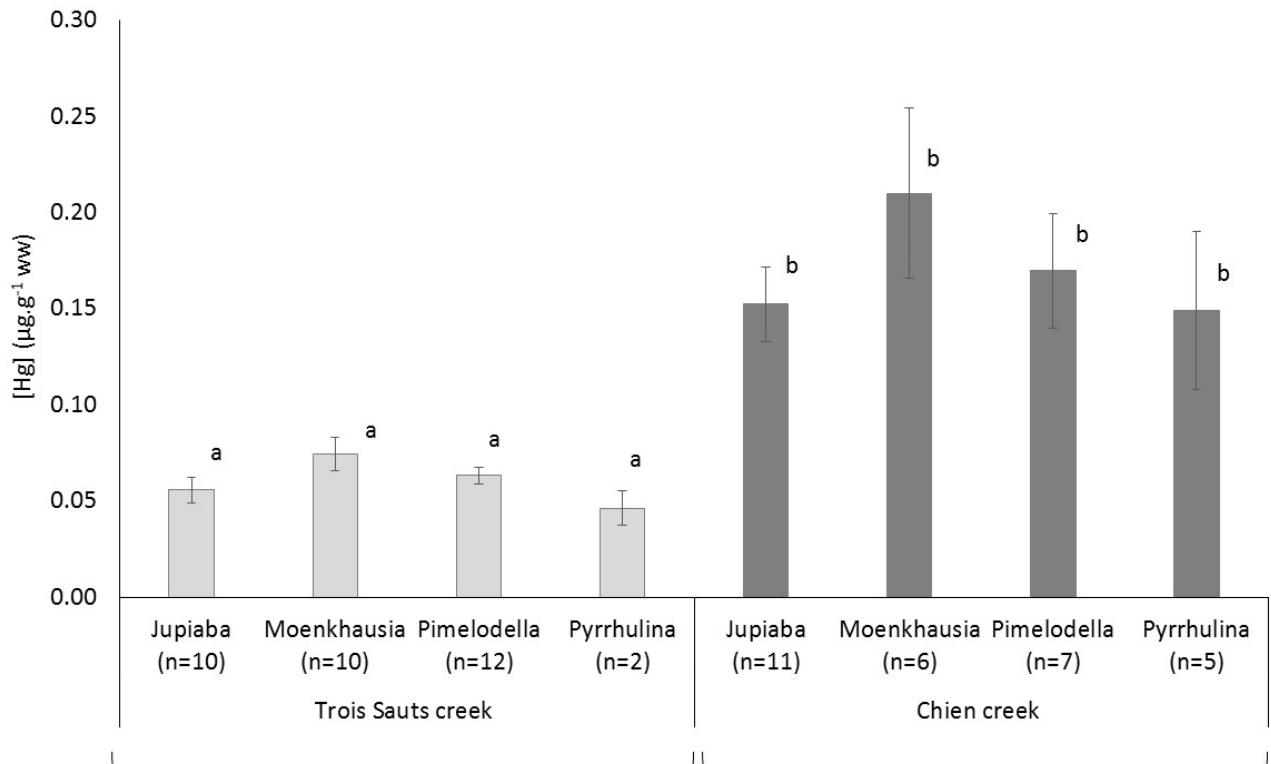
843 Table S1. Origins of the data used in this study: Projects with financial support, organism
844 responsible of the data collection and sampling years.

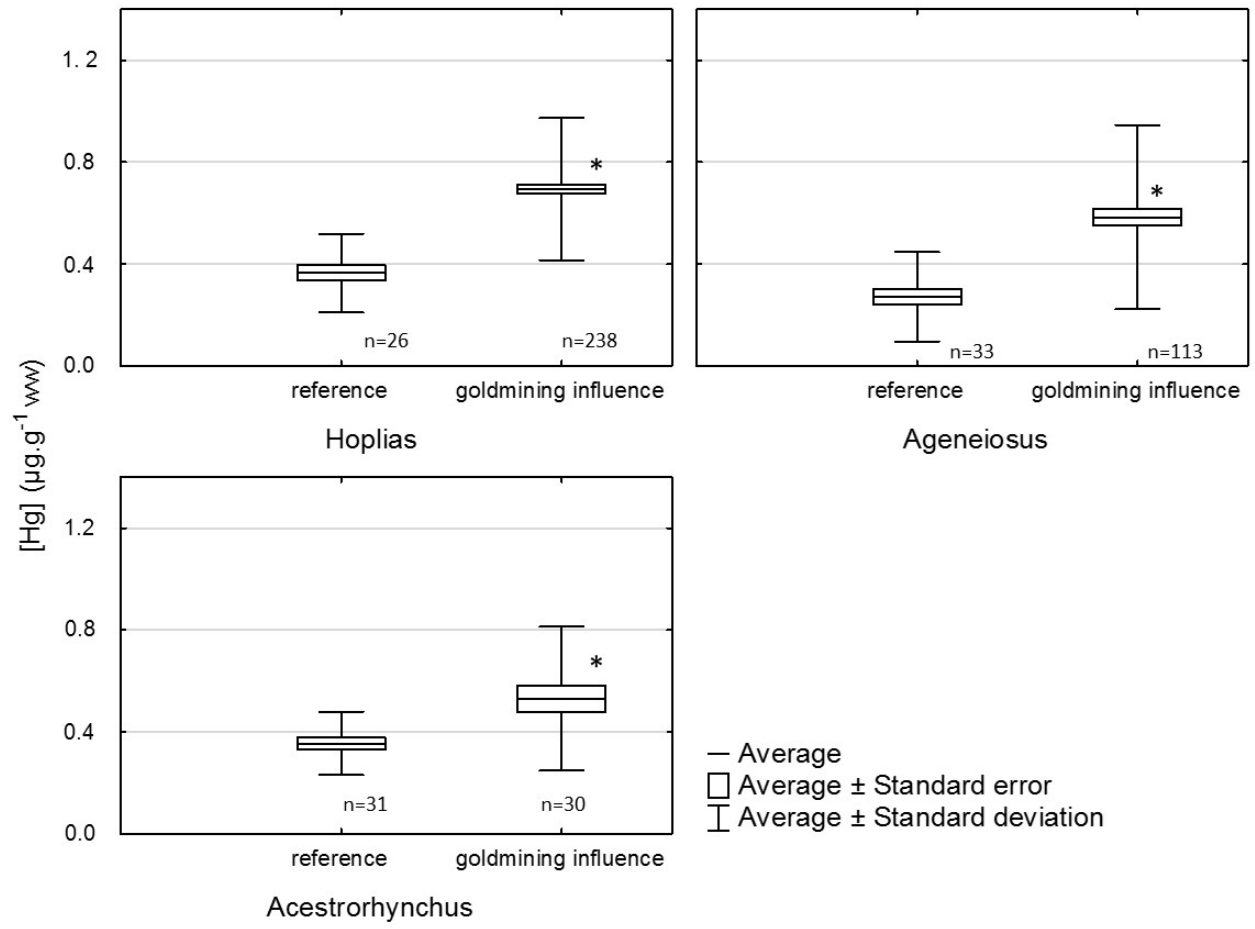
845 Table S2. Summary of recommended, potential and not recommended bioindicator species,
846 according to criteria defined for creeks. ✓: criterion met; ≈: moderately respected criterion
847 (trophic guild not (so) specific, trophic level < 3 or fish not detected in small watersheds); ?:
848 criterion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

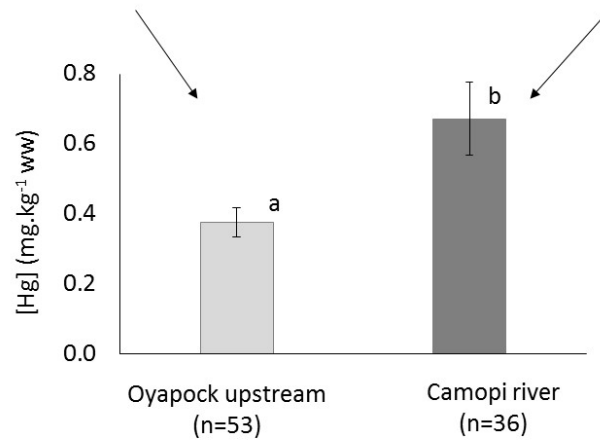
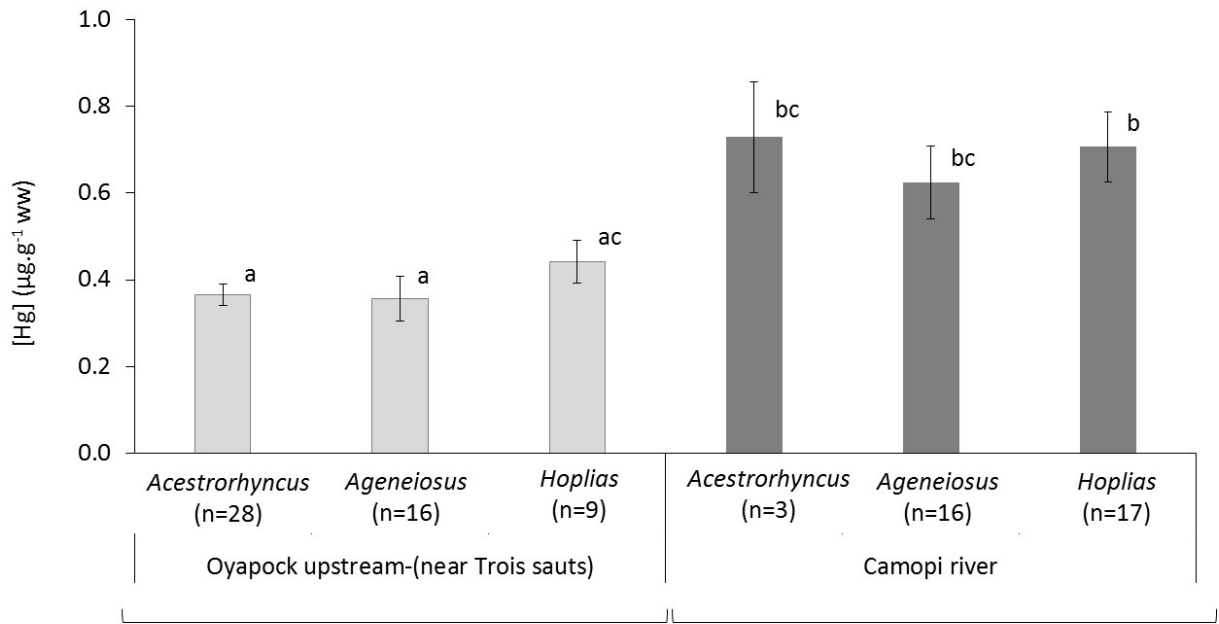
849 Table S3. Summary of recommended, potential and not recommended bioindicator species,
850 according to criteria defined for rivers. ✓: criterion met; ≈: moderately respected criterion
851 (trophic guild not (so) specific, trophic level < 3 or fish not detected in small watersheds); ?:
852 criterion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











Feeding ecology	Creeks			Rivers		
	N	R	[Hg] ($\mu\text{g.g}^{-1}$ ww)	N	R	[Hg] ($\mu\text{g.g}^{-1}$ 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] ($\mu\text{g}\cdot\text{g}^{-1}\text{ ww}$)	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	<i>Moenkhausia</i>	<i>oligolepis</i>	carnivorous- invertivorous	6.7±0.1	11.3±0.7	93	+	+	+	+	+	+	+	+			80	3	2	13	0	37	
		-			7.3±0.2	11.7±0.8	77												4	1	12	0	35	
		<i>Jupiaba</i>	<i>abramoides keithi</i>	carnivorous- invertivorous			62	+	+		+		+	+	+				60					
	Lebiasinidae	<i>Copella</i>	<i>carsevensis</i>	carnivorous- invertivorous	3.0±0.1	0.37±0.03	74	+	+		+	+	+	+	+	+	+		80	2	1	11	0	29
		<i>Pyrrhulina</i>	<i>filamentosa</i>	carnivorous- invertivorous	5.4±0.2	2.8±0.2	101	+	+	+	+	+	+	+	+	+	+	+	100	5	1	19	0	53
Siluriformes	Heptapteridae	-			9.2±0.4	11.7±1.4	98												4	2	13	0	39	
		<i>Pimelodella</i>	<i>cristata geryi macturki</i>	piscivorous- invertivorous			78	+	+	+	+	+	+	+	+	+			80					
							15	+	+	+	+	+	+	+	+	+			80					
							5	+	+						+	+			40					
Gymnotiformes	Gymnotidae	<i>Gymnotus</i>	<i>carapo</i>	piscivorous- invertivorous	14.6±0.6	15.5±1.7	126	+	+	+	+	+	+	+	+	+	+	100	7	0	25	0	65	
	Sternopygidae	<i>Sternopygus</i>	<i>macrurus</i>	piscivorous- invertivorous	24.5±0.9	35.1±2.9	98	+	+	+	+	+	+	+	+	+		80	4	2	12	0	37	

Total 667

Watersheds	Reference areas	n	Hg] ($\mu\text{g}\cdot\text{g}^{-1}$ 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	<i>Acestrorhyncus</i>	<i>falcatus</i>	piscivorous	18.0±0.3	78.4±4.9	37	+	+	+	+	+	+	+	+	+	+	80	4	15	23	2	44
			<i>microlepis</i>				135	+	+	+	+		+	+	+	70							
	Erythrinidae	<i>Hoplias</i>	<i>aimara</i>	piscivorous	56.3±0.4	4030.4±86.6	549	+	+	+	+		+	+	+			70	4	38	35	6	83
Siluriformes	Ageneiosidae	<i>Ageniosus</i>	<i>inermis</i>	piscivorous	28.9±0.3	379.9±14.5	253	+	+				+	+	+			50	4	33	23	2	63
							Total	974															

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