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1 **The goby fish *Sicydium* spp. as valuable sentinel species towards the chemical stress**
2 **in freshwater bodies of West Indies**

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15

16 **Abstract**

17 Implementation of the European Water Framework Directive in tropical areas such as the
18 French West Indies (FWI) requires to select relevant aquatic sentinel species for
19 investigating the ecological status of surface waters. The present work aimed to study the
20 biological response of the widespread fish *Sicydium* spp. towards river chemical quality in
21 Guadeloupe island through a set of proper biomarkers. During a 2-year survey, the hepatic
22 EROD activity, the micronucleus formation and the level of primary DNA strand breaks in
23 erythrocytes were measured respectively as an enzymatic biomarker of exposure and
24 genotoxicity endpoints in fish living upstream and downstream of two chemically-contrasted
25 rivers. Hepatic EROD activity was shown to be variable along the time but always
26 significantly higher in fish from the most contaminated river (Rivière aux Herbes) compared

27 to the low contaminated one (Grande Rivière de Vieux-Habitants). Fish size did not influence
28 EROD activity. Female fish exhibited a lower EROD activity compared to males depending
29 on the catching period. We observed significant temporal variation in micronucleus frequency
30 and primary DNA damage level measured in fish erythrocytes that did not depend on the fish
31 size. Micronucleus frequency and to a lesser extent DNA damage were significantly higher in
32 fish from the Rivière aux Herbes compared to the Grande Rivière de Vieux-Habitants. Our
33 results argue for the interest of using *Sicydium* spp. as sentinel species to assess river
34 quality and chemical pressures in FWI.

35

36 **Key-words:** fish, sentinel species, biomarker, pollution, West Indies

37

38 **1. Introduction**

39 Rivers are globally amongst the most threatened ecosystems, their integrity being affected
40 by various anthropogenic pressures through multiple water uses (industrial, agricultural,
41 urban activities). These may have an impact on freshwater biodiversity as underlined by
42 significant declines of freshwater fish species observed worldwide over the last decades
43 (Scholz et al., 2012; Tompsett et al., 2014; Galib et al., 2018; Liu et al., 2019; Santos et al.,
44 2021). Assessing accurately the impacts of stressors on freshwater organisms remains
45 clearly a priority in order to favor restoration and conservation of aquatic ecosystems.

46 Biomonitoring is crucial in environmental management and conservation biology to assess
47 historical trajectories of populations and ecosystem status and to mitigate pressures that
48 impact the most aquatic biodiversity (Santos et al., 2022). For that purpose, fish are
49 commonly used as relevant sentinel species in a large array of environmental risk
50 assessment studies because of their relatively long life-span, their functional role in aquatic
51 ecosystems and their high trophic level that can provide integrated and relevant information
52 regarding availability and effects of environmental stressors (Ortiz-Zarragoitia et al., 2014;

53 Rios et al., 2015; McMillan et al., 2022). Fish sentinel species enable to take into account the
54 overall effects of the complex mixture of pollutants interacting with natural and other
55 anthropogenic stressors to be found in aquatic ecosystems (Colin et al., 2016).

56 Thus, the choice of fish sentinel species is of main importance to develop ecological risk
57 assessment approaches in areas where they have not been developed yet, such as in the
58 French West Indies (FWI). So far, fish sentinel species have been mainly used in West
59 Indies to monitor chemical impacts in marine (Dromard et al., 2018; Horricks et al., 2019) and
60 freshwater ecosystems (Labeille et al., 2016). There is still a clear need to select relevant fish
61 sentinel species in order to investigate the ecological status of surface waters as specified by
62 the European Water Framework Directive (EU-WFD, 2000/60/EC; 2008/105/EC). Among the
63 different criteria required to consider an animal species as a key sentinel species, its wide
64 distribution and its abundancy to enable cost-effective routine data collection, a good
65 knowledge of its life-history traits and of its sensitivity to the investigated stressors are of
66 importance (Beeby, 2001).

67 Gobioidae, and especially species of the Sicydiinae subfamily, are the most abundant and
68 widely distributed freshwater fish in Caribbean, exhibiting the highest levels of endemism
69 (Keith, 2003; Keith and Lord, 2011). In Martinique and Guadeloupe islands, *Sicydium*
70 *punctatum* (Perugia, 1896) and *Sicydium plumieri* (Bloch, 1786) are widespread
71 amphidromous species, *Sicydium punctatum* being the most abundant one (Lim et al., 2002;
72 Monti et al., 2010; Lejeune et al., 2016). *Sicydium* spp. (namely *S. punctatum* and *S.*
73 *plumieri*) large ubiquity in FWI rivers is mainly due to their modified sucker-fin enabling them
74 to overcome natural or anthropic obstacles and to resist to heavy water current. *Sicydium*
75 spp. abundance allows quite easy and regular field sampling. Despite their small size, i.e. a
76 maximum body length of 120 mm for *S. punctatum* and 140 mm for *S. plumieri*, respectively
77 (Lim et al., 2002; Monti et al., 2010), it is possible to sample enough tissue for both biological
78 and chemical analyses.

79 In complement to chemical surveys that identify at best only a fraction of environmental
80 pollutants, often those in the so-called « priority lists », ecologically relevant biomarkers
81 enable to assess the exposure and effects of overall pollutants on aquatic organisms (Van
82 der Ost et al., 2003; Wu et al., 2005; Galloway, 2006). Choosing an aquatic species enabling
83 to properly assess biomarker response is a well-known prerequisite in environmental
84 monitoring (Colin et al., 2016). If knowledge base concerning the biological cycle of *Sicydium*
85 spp. such as the sexual maturation process and breeding season are still rather limited, its
86 large distribution and amphidromy life-history (juvenile-return anadromy) have been well
87 described (Keith, 2003; Bell, 2009; Keith and Lord, 2011; Lejeune et al., 2014). After a 2–6-
88 month marine larval phase, *Sicydium* spp. postlarvae move back to the river mouth, swim
89 upstream where they settle to grow, feed and finally spawn several times during their life
90 (Bell, 2009). Due to a relatively high lifespan of many years and to the fact that this bottom
91 dwelling fish mainly feeds on periphyton where lipophilic compounds accumulate, *Sicydium*
92 spp. could match fish sentinel requirements to integrate river chemical quality.

93 Changes in biological responses in fish exposed to contamination can be assessed through
94 the measurement of a large array of biomarkers ranging from molecular through cellular and
95 physiological responses to behavioral changes (Adams, 2001; van der Oost et al., 2003).
96 One of the most widely used biomarkers of exposure to organic pollution in fish for many
97 decades is the measurement of changes in hepatic ethoxyresorufin-*O*-deethylase (EROD)
98 catalytic activity, as a proxy to the subfamily CYP1A (Nilsen et al., 1998; Sarasquete and
99 Segner, 2000). EROD induction in fish is well characterized, the most important modifying
100 factors being fish species, reproductive status and age. EROD activity modulation has been
101 shown as a relevant biomarker in a number of field investigations regarding industrial and
102 urban effluents, contaminated sediments and chemical spills (van der Oost, 2003; Burkina et
103 al., 2015; Santana et al., 2018). Known mechanisms of CYP1A-induced toxicity suggest that
104 modulation of EROD activity considered as a biomarker of exposure to some organic
105 compounds, could also inform about further effects at various levels of biological organization

106 in fish (Nilsen et al., 1998; Whyte et al., 2000; van der Oost et al., 2003). Thus, measurement
107 of EROD activity has been historically recommended in monitoring programs for assessing
108 environmental contamination and effects on fish and remains of great interest (Bucheli and
109 Fent, 1995; Gagnon and Rawson, 2017).

110 Genotoxicity biomarkers have been shown to be of great value for assessing some relevant
111 effects of contaminants on both marine and freshwater aquatic organisms (Depledge, 1996;
112 Hayashi et al., 1998; Bolognesi and Cirillo, 2014). The consequences of genotoxicity are not
113 limited to carcinogenesis since given the involvement of the genome in the whole of cellular
114 metabolism, exposure to genotoxic substances can affect individual fitness and finally
115 population sustainability (Depledge, 1998; Theodorakis et al., 2000; Jha, 2004; Devaux et al.,
116 2011; Devaux et al., 2015). Among the different biomarkers of genotoxicity used in aquatic
117 ecotoxicology a special attention has been paid to the micronucleus assay (MN) and the
118 single cell gel electrophoresis assay, known as comet assay (CA). These assays provide
119 information about crucial genotoxic events in exposed organisms such as chromosomal
120 aberrations (aneugenicity and clastogenicity) and primary DNA damages (Udroiu, 2006; Jha,
121 2008; Da Rocha et al., 2009; Frenzilli et al., 2009; Devaux and Bony, 2013; de Lapuente et
122 al., 2015). MN and CA differ in that DNA damages detected in the former can not be repaired
123 because of hardly repairable lesions (chromosome breakage or loss and mitotic spindle
124 apparatus malfunction), whereas primary DNA damages revealed by CA (single-strand
125 breaks, alkali-labile sites and cross-linking) are repairable. CA and MN are often used
126 together in environmental studies as they provide complementary information for genotoxic
127 impact assessment (Ali et al., 2009; Polard et al., 2011; Bolognesi and Cirillo, 2014; Colin et
128 al., 2016).

129

130 To explore the interest of *Sicydium* spp. as a sentinel species for environmental assessment
131 in FWI, the present work aimed to study its biological response to pollutants in Guadeloupe
132 island rivers through a set of relevant biomarkers informing about fish exposure and
133 genotoxic consequences.

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137 2. Materials and Methods

138 2.1. Study area

139 Two hydrosystems were chosen according to their contrasted levels of contamination: the

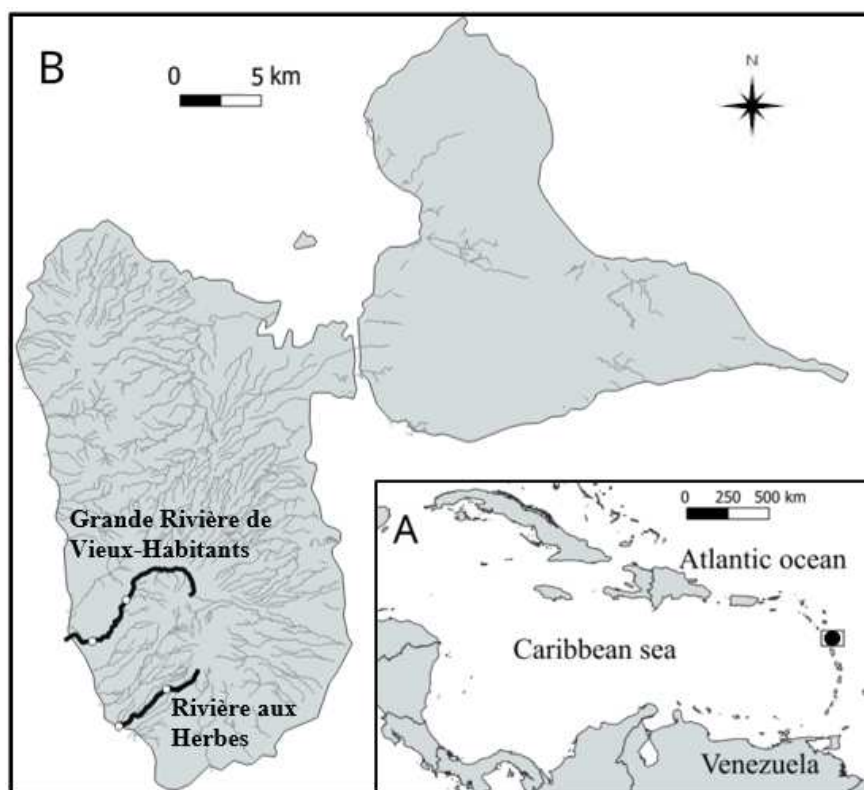
140 Grande Rivière de Vieux-Habitants, one of the least-contaminated river and the Rivière aux

141 Herbes severely contaminated by human activities (urban and agriculture) in Guadeloupe

142 island. For each river an upstream and a downstream station were investigated, called

143 respectively Vm and Vv for the Grande Rivière de Vieux-Habitants, and Hm and Hv for the

144 Rivière aux Herbes (Figure 1).



145

146 Figure 1. Localisation maps of Guadeloupe island in Caribbean sea (A) and of studied rivers,
147 Grande Rivière de Vieux-Habitants and Rivière aux Herbes (B). Upstream and downstream
148 stations of each river are shown as a white dot.
149

149

150 2.2. Physico-chemical and ecological status of the rivers

151 Every 2 months during the first year (2019) and every 3 months during the second year
152 (2020), in situ physico-chemical quality of the 4 sampling stations was monitored using a
153 multiparametric probe (HACH, HQ40d Multi). Chemical contamination was assessed on
154 water column, periphyton and fish (total body burden). A total of 40 samples of each matrix
155 (10 sampling campaigns x 4 sampling stations) have been analyzed along the survey. There
156 were deep-frozen at -20°C before being analyzed at the laboratory. Among a large array of
157 chemical compounds (PCBs, PAHs, pesticides, drugs, heavy metals) and depending on the
158 matrix, 511 compounds have been analyzed in each water sample, 367 compounds in each
159 periphyton sample and 73 compounds in each fish sample.

160 During the first campaign, ecological quality of each station was assessed through the
161 implementation of the Antillean Diatom Index (IDA) as previously described (Monti et al.,
162 2018; Lefrancois et al., 2019). Sampling, preparation and mounting of diatom permanent
163 slides were made in accordance with standard protocol NF T 90-354 and Stoermer et al.
164 (1996).

165 Physico-chemical quality of water of both rivers was monitored every 2 months during the
166 first year (2019) and 4 times during the second year (2020) using a multiparametric probe
167 (HACH, HQ40d Multi).

168 *2.3. Fish sampling and tissue collection*

169 Fish were caught at each station by electrofishing using a 250 V, 40 Hz electric crenulated
170 current (25% duty cycle) at HR stations and a 500 V, 100 Hz one at VR stations (LR-24
171 Smith-Root™ electrofisher), to take into account changes in water conductivity. This
172 procedure was followed each two months in 2019 and each 3 months in 2020, i.e. a total of
173 10 catching campaigns. When possible, fifteen fish of each species (*S. plumieri* and *S.*
174 *punctatum*) divided into 3 size categories 46 to 55 mm, 56 to 65 mm and ≥ 66 mm total body
175 length were caught at each station. After catching, fish were carried back to the laboratory
176 within two hours in air-supplied tanks for further measurements. No *S. plumieri* was caught in
177 the Hm station.

178 After species identification, fish were individually anesthetized using clove-oil (0.03 mL/L in
179 water), measured (total body length) and weighted. Then they were sacrificed by
180 decapitation. A blood drop was collected by using a previously heparinized pipette tip and
181 partially deposited as a smear on an identified glass slide let to dry at room temperature for
182 micronucleus assay. Two μL of remaining blood sample were diluted in 198 μL of
183 cryopreservative buffer (250 mM sucrose, 40 mM trisodium citrate, 5% dimethylsulfoxide, pH
184 7.6 adjusted with 1 M citric acid) in a 500 μL Eppendorf tube previously heparinized,
185 identified and immediately deep-frozen in liquid nitrogen for Comet assay.

186 Fish were dissected under a Stemi 2000 Zeiss™ stereomicroscope. Liver was removed,
187 weighted and stored in a previously identified 500 μL Eppendorf tube and immediately deep-
188 frozen in liquid nitrogen for EROD activity and TBARs measurement. Gonads were removed
189 and fixed in Finefix® for sex determination through histological analyses. At each sampling
190 campaign, fish of both species and from the same sampling station were pooled after
191 dissection, wrapped in an aluminium foil and stored at -20°C for chemical analyses.

192 *2.4. Biomarker responses*

193 EROD activity and protein concentration were measured on fish liver S9 homogenate
194 according to the procedure described by Flammarion and Garric (1997) with slight
195 modifications (Noury, 2016). Micronucleus assay was carried out on fish erythrocytes
196 according to Polard et al. (2011). DNA damage was assessed on fish erythrocytes through
197 the alkaline Comet assay following the procedure described by Singh et al. (1988), slightly
198 modified according to Bony et al. (2008) and by replacing ethidium bromide by Syber Green
199 1 for DNA staining. Since the aim of the study was to give a global insight of the biomarker
200 response in *Sicydium* spp. as a sentinel species, data of all campaigns have been pooled,
201 apart for EROD activity in order to take into account a possible effect of female sexual
202 maturity on this biomarker response.

203 *2.5. Statistical analysis*

204 EROD activity data were ln-transformed for normality. Data analysis for EROD activity and
 205 for micronucleus assay was processed using ANOVA and post-hoc Tukey's test according to
 206 Flammarion and Garric (1997) and Polard et al. (2011), respectively. Since the distribution of
 207 DNA damage measured by the Comet assay did not follow a Gaussian distribution as
 208 described earlier (Bauer et al., 1998), both Kruskal-Wallis and Mann-Whitney non-
 209 parametric tests were used for data analysis.

210 3. Results

211 3.1. Physico-chemical and ecological status of the rivers

212 Annual water conductivity average at the Rivière aux Herbes was 300 $\mu\text{S}/\text{cm}$ downstream
 213 (Hv) and 100 $\mu\text{S}/\text{cm}$ upstream (Hm) respectively, higher than the one upstream and
 214 downstream of the Grande Rivière de Vieux-Habitants measured in the range of 70-90
 215 $\mu\text{S}/\text{cm}$. Annual water temperature average was around 23°C for both rivers, except the Hv
 216 station exhibiting a higher 25°C mean water temperature. Annual average of dissolved
 217 oxygen content and of oxygen saturation level in water were around 8 mg/L and 95 %
 218 respectively for both rivers, excepted the Hm station exhibiting lower values of 7.4 mg/L and
 219 91 % respectively.

220 IDA scores highlight a difference in ecological quality between the 2 rivers but not between
 221 the upstream and downstream stations of the same river (Table 1). The Grande Rivière de
 222 Vieux-Habitants appears to be in good ecological condition according to the diatom index.
 223 The Rivière aux Herbes shows a degraded ecological state, which could testify to a
 224 contamination by organic matter and/or notable nutrients.

225 Table 1. IDA score measured at each sampling river station.

		IDA score (marked out of 20)	Ecological quality according IDA	Number of diatom taxons found	Percentage of diatom taxons not involved in IDA calculation
Grande Rivière de Vieux-Habitants	Upstream (Vm)	19	good	35	12
	Downstream (Vv)	19	good	28	7

Rivière aux Herbes	Upstream (Hm)	17.6	medium	29	12
	Downstream (Hv)	16.1	medium	30	9

226

227 When taking into account all the quantified compounds (i.e. showing a concentration over the
 228 quantification limit of analysis) in each matrix along the survey, the two rivers exhibited
 229 different patterns of contamination regarding both upstream and downstream stations (Figure
 230 2). Global contamination of the Rivière aux Herbes appears higher than the one of the
 231 Grande Rivière de Vieux-Habitants whatever the matrix, the most contaminated profile
 232 concerns fish and periphyton matrices.

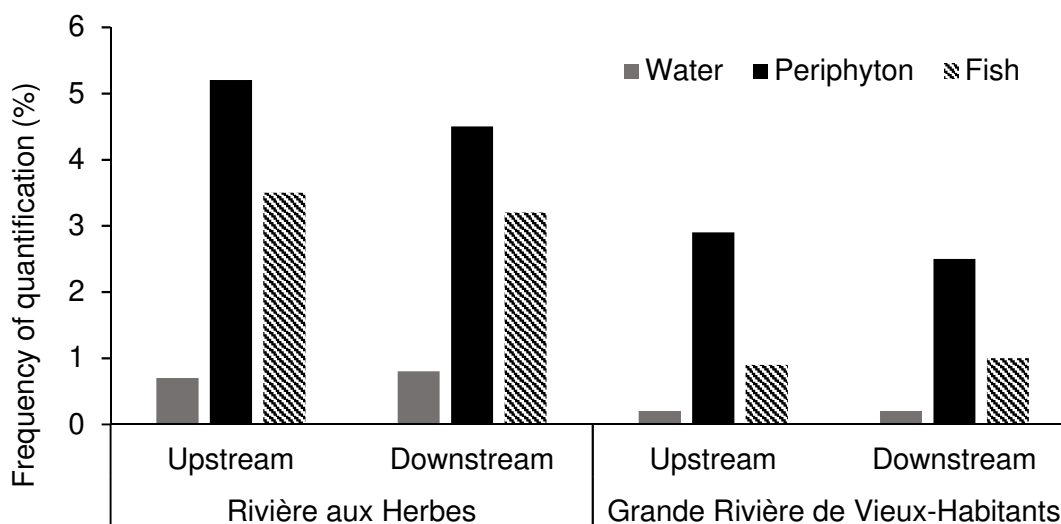
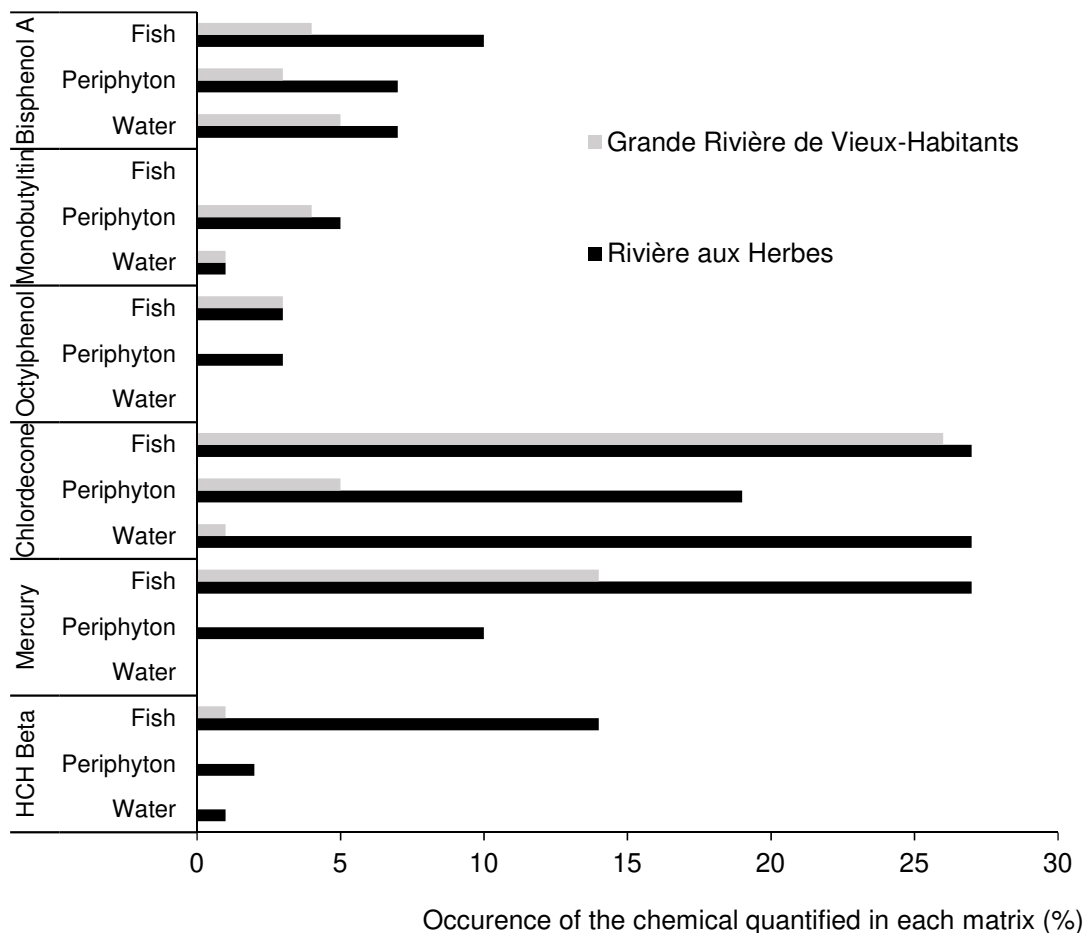


Fig. 2. Frequency of molecule quantification in each matrix analyzed (number of quantified molecules out of the total number of searched molecules per matrix for the 10 sampling campaigns pooled by each river).

233

234 Such a trend was confirmed when focusing on 6 chemical compounds common to the 3
 235 matrices from the Rivière aux Herbes and the Grande Rivière de Vieux-Habitants (Figure 3).
 236 It shows a general trend for a higher occurrence of these compounds in samples analyzed
 237 along the survey from the Rivière aux Herbes compared to the Grande Rivière de Vieux-
 238 Habitants.



239

240 Fig. 3. Occurrence percentage regarding the 6 chemicals quantified common to the 3
 241 matrices from the two rivers. Percentage was calculated by summing each of the 6
 242 compounds when quantified versus the total number of compounds analyzed per matrix for
 243 all the 10 sampling campaigns.
 244

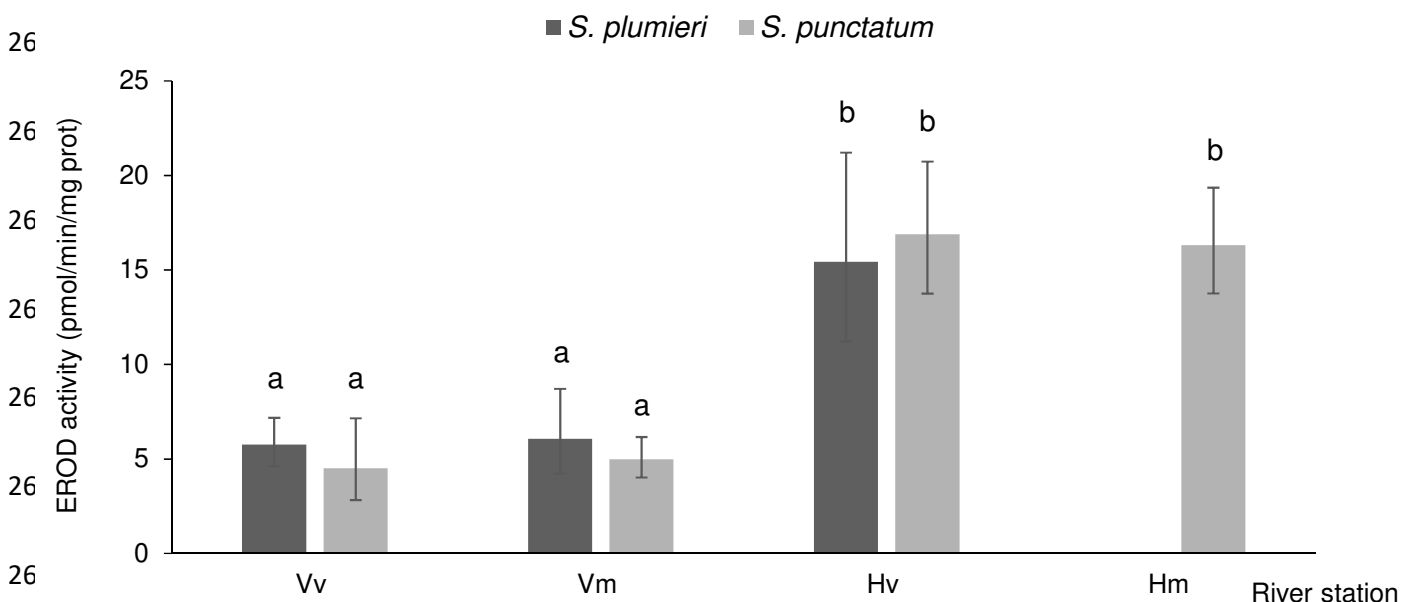
245 To sum up, the Rivière aux Herbes appears to be more chemically and ecologically
 246 degraded than the Grande Rivière de Vieux-Habitants.

247 *3.2. EROD activity*

248 Since hepatic EROD activity can vary according to the chemical contamination of the river by
 249 a large array of pollutants (PCBs, HAPs, halogenated pesticides, heavy metals...) but also
 250 according to the fish species, size (used here as a proxy of age) and sex, effect of these
 251 parameters on EROD activity was investigated.

252 3.2.1. EROD activity according to fish species

253 Figure 4 illustrates EROD activity levels measured in liver of both fish species caught
 254 downstream and upstream of the two rivers. It has to be noted that no *S. plumieri* have been
 255 caught at the upstream station of the Rivière aux Herbes (Hm) all along the survey. No
 256 significant difference in EROD activity was observed between *S. plumieri* and *S. punctatum*
 257 from the same station Vv, Vm or Hv. EROD activity varied between 3 to 20 pmol/min/mg
 258 protein according to the catching station. Since no difference in EROD activity was
 259 evidenced between both species and between stations from the same river, EROD activity
 260 data of fish of both species from the same river were pooled for river comparison.



26 Fig. 4. Hepatic EROD activity in *Sicydium plumieri* (PLU) and *Sicydium punctatum* (PUN). Vv =
 26 Grande Rivière de Vieux-Habitants downstream station; Vm = Grande Rivière de Vieux-Habitants
 26 upstream station; Hv = Rivière aux Herbes downstream station; Hm = Rivière aux Herbes upstream
 26 station. EROD activity is expressed as the geometric mean \pm 95 percent confidence interval. Means
 270 that do not share a letter are significantly different (post-hoc Tukey's test, $p < 0.01$).
 271 PLU: n=92 (Vv) ; n=41 (Vm) ; n=55 (Hv). PUN: n=22 (Vv) ; n=98 (Vm) ; n=91 (Hv) ; n=131 (Hm).

271 3.2.2. Comparison of EROD activity in *Sicydium* spp. from both rivers along the time

272 EROD activity was significantly higher in liver of fish living in the Rivière aux Herbes
 273 compared to those from the Rivière de Vieux-Habitants, except for the fish caught in August
 274 2020 (Figure 5). EROD activity showed a trend for lower values in May and July 2019 and
 275 May 2020, although not significant.

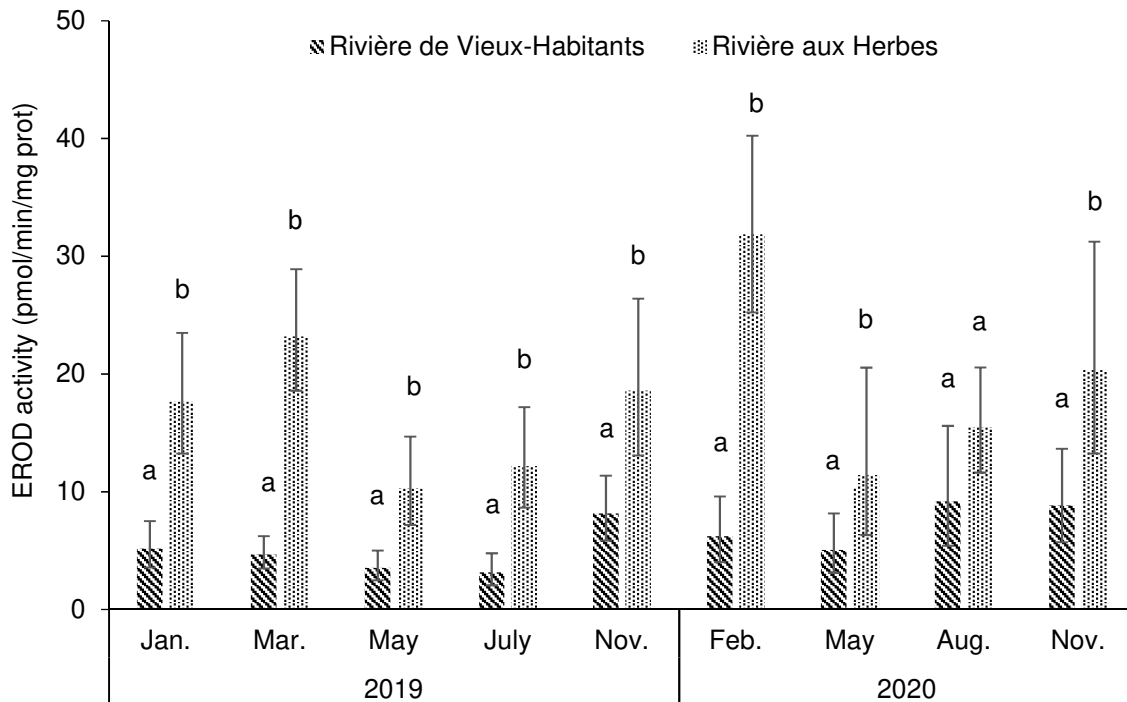


Fig. 5. Comparison of hepatic EROD activity in *Sicydium* spp. from the two rivers at the different catching campaigns. EROD activity is expressed as the geometric mean \pm 95 percent confidence interval. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

276

277 EROD activity induction factor, i.e. the ratio of EROD activity measured in fish from the
 278 Rivière aux Herbes versus that from the fish caught in the Grande Rivière de Vieux-
 279 Habitants, ranged from 2.3 to 5.1 (Figure 6).

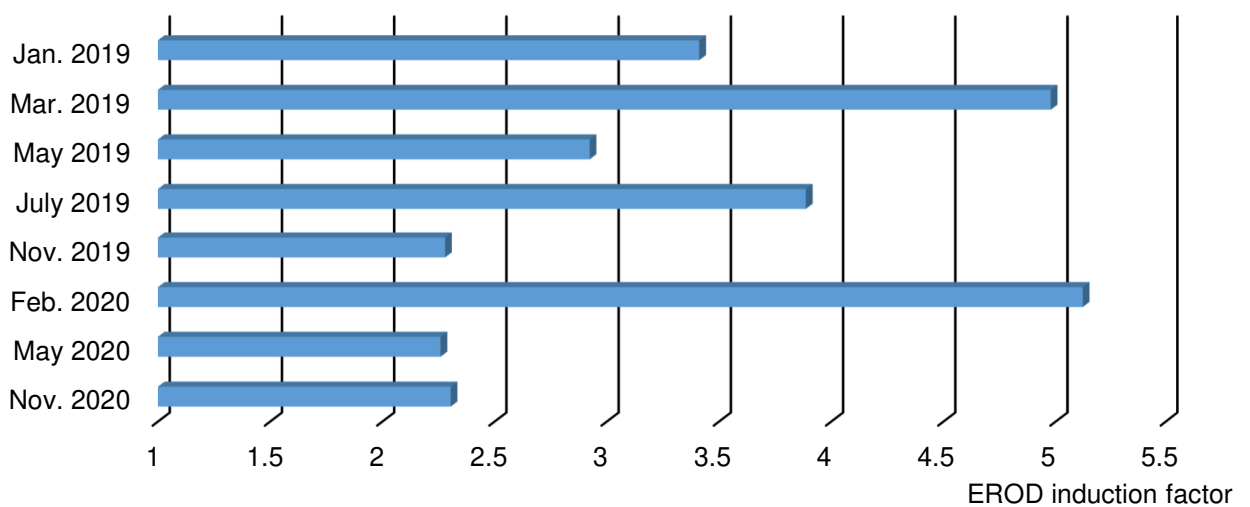
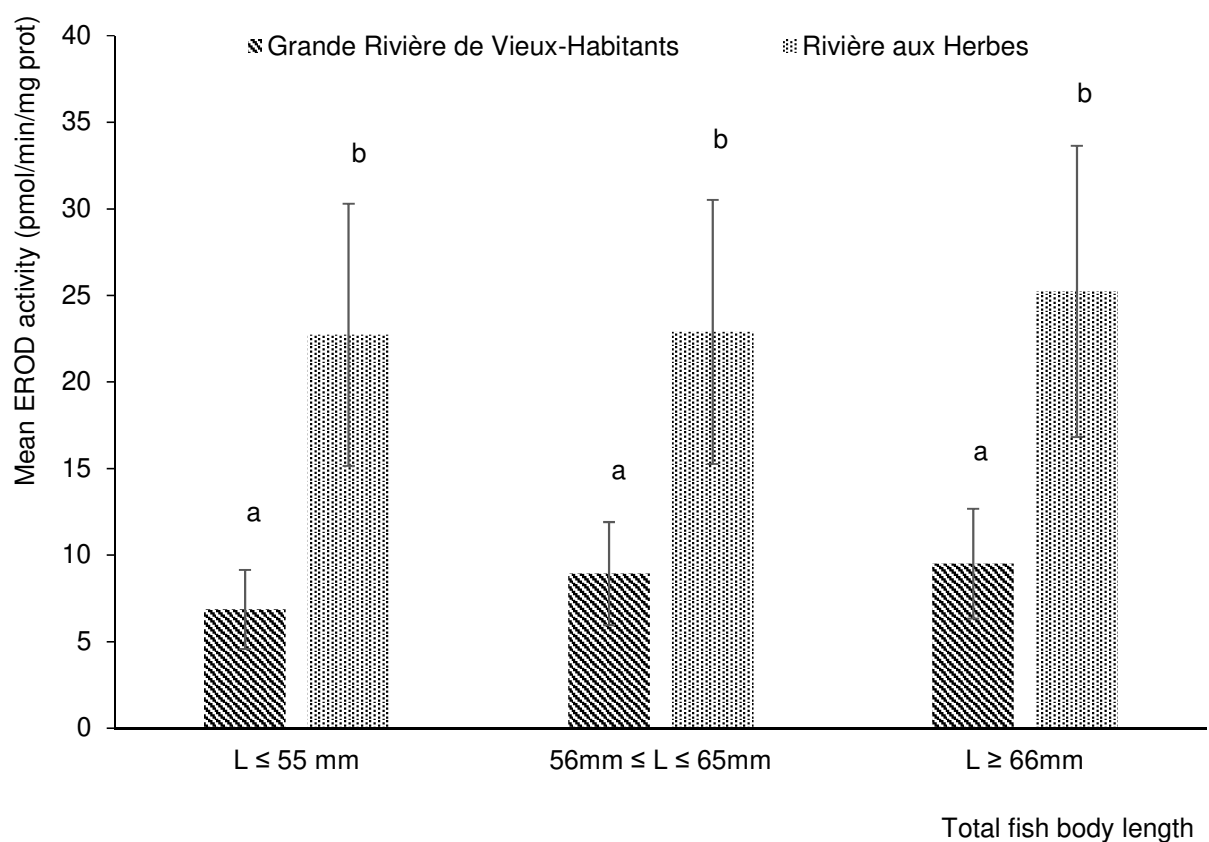


Fig. 6. Induction factor of EROD activity measured in *Sicydium* spp. from the Rivière aux Herbes compared to those from the Grande Rivière de Vieux-Habitants.

280

281 3.2.3. EROD activity according to fish size and sex

282 Total body length of *Sicydium* spp. was used as a proxy of age since it is an indeterminate
283 grower, to assess a possible effect of age on EROD activity. Mean value of EROD activity
284 has been calculating by pooling data from all the measured fish of both species and from all
285 sampling campaigns, sorted in 3 size classes: total body length $L \leq 55$ mm, $56 \text{ mm} \leq L \leq 65$
286 mm, $L \geq 66$ mm. No significant effect of the total body length of *Sicydium* spp. on the level of
287 EROD activity was demonstrated (Figure 7).



288 Fig. 7. Mean EROD activity in liver of *Sicydium* spp. according to total fish body length. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$). Grande Rivière de Vieux-Habitants: $n=34$ ($L \leq 55$ mm), $n=68$ ($56 \text{ mm} \leq L \leq 65$ mm), $n=130$ ($L \geq 66$ mm); Rivière aux Herbes: $n=63$ ($L \leq 55$ mm), $n=90$ ($56 \text{ mm} \leq L \leq 65$ mm), $n=124$ ($L \geq 66$ mm).

289 Effect of fish sex on hepatic EROD activity has been investigated in *Sicydium* spp. from the
290 Grande Rivière de Vieux-Habitants which is the less contaminated river, to limit artefact due
291 to possible endocrine disruption. EROD activity was measured over time in 2019 and 2020 in
292 order to take into account the state maturity of both fish sexes. As previously highlighted,

293 hepatic EROD activity was found to be variable along the time whatever fish sex. A trend for
 294 a lower EROD activity in females compared to males was noted in 2019 and 2020, significant
 295 differences being observed between both sexes in July 2019, February and May 2020 (Table
 296 2).

297 Table 2.

298 Hepatic EROD activity in liver of male and female *Sicydium* spp. from the Grande Rivière de
 299 Vieux-Habitants. EROD activity (pmol/min/mg protein) is expressed as mean \pm standard
 300 deviation (number of fish). EROD activity was compared between both fish sex at each
 301 sampling date. Means that do not share a letter are significantly different (Student *t*-test, $p <$
 302 0.05).

		EROD activity (pmol/min/mg protein)	
Sampling date		male	female
2019	Jan.	10.12 \pm 4.27 ^a (3)	9.14 \pm 3.78 ^a (24)
	Mar.	7.12 \pm 2.27 ^a (17)	6.02 \pm 1.81 ^a (15)
	May	4.29 \pm 1.02 ^a (10)	5.12 \pm 2.16 ^a (14)
	July	5.13 \pm 1.53 ^a (13)	3.77 \pm 1.41 ^b (9)
	Nov.	11.64 \pm 2.42 ^a (16)	9.93 \pm 3.33 ^a (8)
2020	Feb.	13.86 \pm 2.02 ^a (4)	6.63 \pm 1.49 ^b (8)
	May	9.14 \pm 1.99 ^a (4)	5.52 \pm 1.90 ^b (9)
	Aug.	16.65 \pm 5.02 ^a (3)	15.20 \pm 3.57 ^a (12)
	Nov.	15.07 \pm 3.05 ^a (5)	11.38 \pm 2.71 ^a (7)

303

304 3.3. Micronucleus formation

305 Other factors than pollutant exposure may contribute to the formation of micronuclei in
 306 erythrocytes of fish such as fish species and size (correlated to age). Thus, a possible effect
 307 of these parameters on micronucleus formation was also investigated.

308

309

310 3.3.1. *Micronucleus frequency according to the fish species and the river*

311 No significant difference in micronucleus frequency was shown between the two fish species
312 from the same river and between the fish of the same species caught upstream and
313 downstream of each river. Micronucleus frequency was significantly higher in fish from the
314 Rivière aux Herbes compared to those from the Grande Rivière de Vieux-Habitants,
315 whatever the species and the river station (Figure 8). Observed micronucleus frequency
316 remained in all cases at a low level < 1‰.

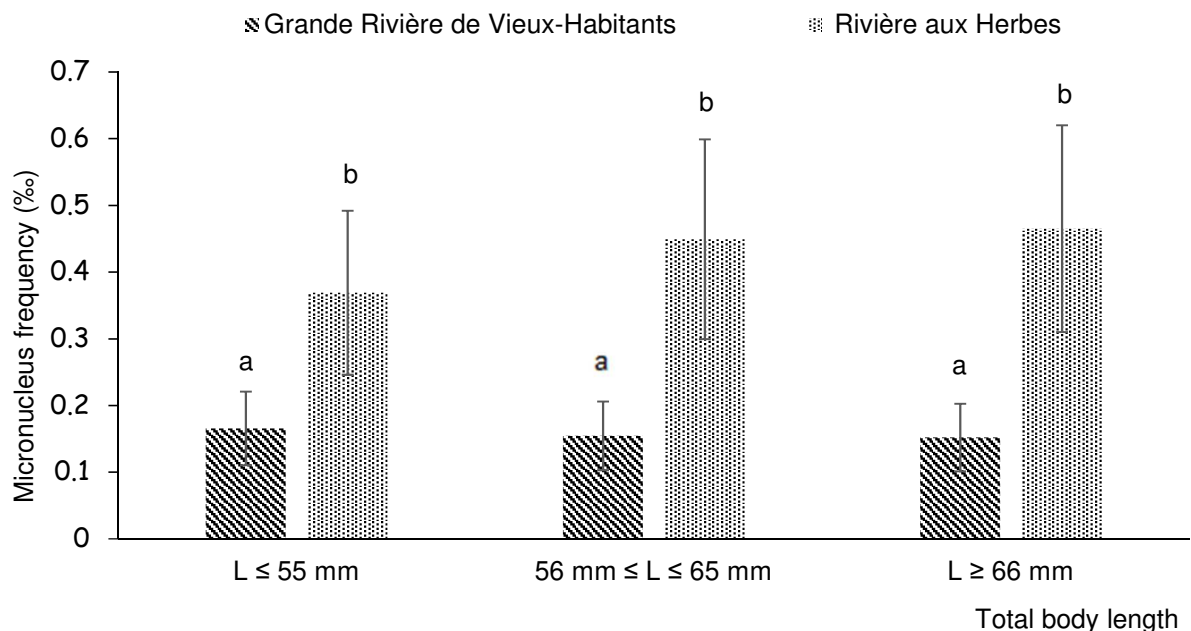


Fig. 8. Micronucleus frequency in *Sicydium* spp. circulating erythrocytes according to total body length. Results are expressed as mean ± standard deviation. Means that do not share a letter are significantly different (post-hoc Tukey's test, $p < 0.05$).

317 3.3.2. *Micronucleus frequency according to fish size*

319 Since no difference in micronucleus frequency was observed between fish species and river
320 station, data from both species and both stations of a river were pooled to study a possible
321 effect of fish size on micronucleus formation. No effect of fish size was demonstrated in
322 *Sicydium* spp. from both rivers (Figure 8).

323

324 3.4. Primary DNA damage in fish erythrocytes

325 Primary damage in erythrocyte nuclear DNA was monitored through the alkaline Comet
 326 assay. As previously highlighted for the other biomarkers studied in this work, the level of
 327 primary DNA damage stemming from a large array of nuclear events (DNA strand breakage,
 328 alkali-labile sites, incomplete excision repair sites, cross-linking, unstable DNA adducts...)
 329 can vary according to the level of river pollution, the fish species and size. Effect of these
 330 parameters on primary DNA damage has been thus investigated.

331 3.4.1. Primary DNA damage level according to fish species and river

332 No difference in the average level of DNA damage was observed between fish species and
 333 between stations of the same river, except for fish from the upstream station of the Rivière
 334 aux Herbes that exhibited a significantly higher DNA damage than those caught at the
 335 downstream station (Table 3). DNA damage in erythrocytes of *Sicydium* spp. from the
 336 Rivière aux Herbes was significantly higher with a tail intensity percentage of 16.13 ± 3.16
 337 ($n= 423$) compared to that of fish from the Grande Rivière de Vieux-Habitants 12.24 ± 3.57
 338 ($n= 379$) (Table 3).

339 Table 3

340 Level of DNA damage in circulating erythrocytes of *Sicydium plumieri* and *Sicydium*
 341 *punctatum* caught upstream and downstream of both rivers (Grande Rivière de Vieux-
 342 Habitants and Rivière aux Herbes). Data are shown as the tail intensity percentage, mean \pm
 343 standard error of the mean ($n=$ number of fish). Means that do not share a letter are
 344 significantly different (Mann-Whitney *U* test, $p < 0.05$).

Fish species	Rivière de Vieux-Habitants		Rivière aux Herbes	
	Station	% Tail intensity	Station	% Tail intensity
<i>Sicydium plumieri</i>	Vv	12.86 ± 2.97^a (126)	Hv	12.40 ± 1.96^a (91)
	Vm	12.18 ± 2.58^a (59)		
<i>Sicydium punctatum</i>	Vv	12.00 ± 3.26^a (45)	Hv	14.64 ± 1.91^a (111)
	Vm	11.25 ± 2.22^a (149)	Hm	18.40 ± 2.57^b (221)

345

346

347 3.4.2. Primary DNA damage level according to fish size

348 No effect of the total body length of *Sicydium* spp. on the DNA damage level was observed

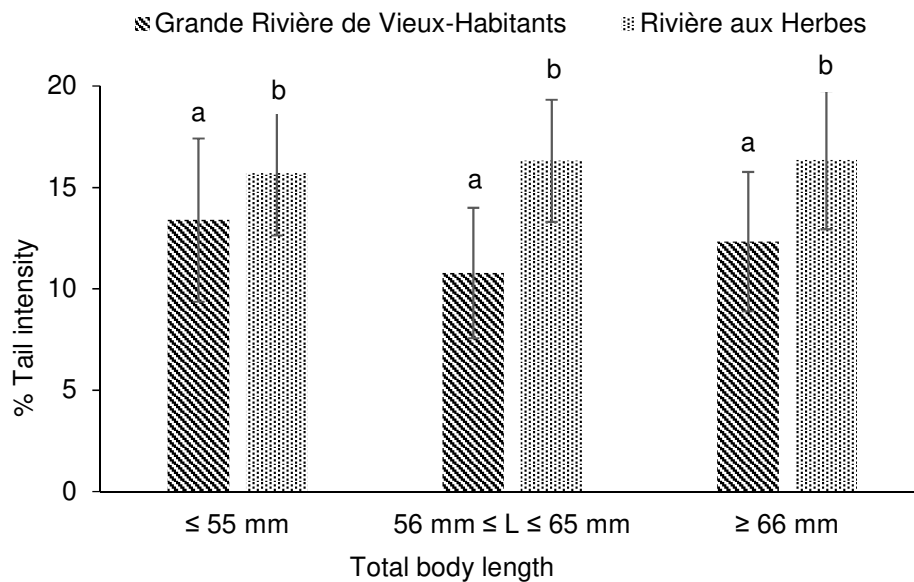


Fig. 9. Mean DNA damage in *Sicydium* spp. erythrocytes according to total body length. Means that do not share a letter are significantly different (Mann Whitney *U* test, $p < 0.05$).

349 whatever the river (Figure 9).

350

351 **4. Discussion**

352 4.1. Enzymatic biomarker: hepatic EROD activity

353 To our knowledge, hepatic EROD activity measurement in *Sicydium* spp. liver has never
354 been reported in literature. In the present work, EROD activity was found in both *S. plumieri*
355 and *S. punctatum* from the low contaminated Grande Rivière de Vieux-Habitants to be in the
356 same range than the one measured in other tropical freshwater fish living in low anthropized
357 areas, such as the yellow acara *Aequidens metae* (Corredor-Santamaria et al., 2021), the
358 blackfin pacu *Colossoma macropomum* (Sadauskas-Henrique et al., 2017) or the striped
359 prochilod *Prochilodus lineatus* (Lunardelli et al., 2018).

360 No relationship between fish age measured here as the proxy total body length and EROD
361 activity was highlighted. The relationship between fish size or age and hepatic EROD activity
362 remains controversial since it depends on biotic/abiotic parameters. On one hand, larger fish
363 can accumulate more hydrophobic contaminants along the exposure time leading to higher
364 EROD activity levels compared to smaller fish. As shown by Hugla et al. (1995) in the
365 common barbel *Barbus barbus* and by Sleiderink et al. (1995) in the dab *Limanda limanda*
366 living in PCB-contaminated sites, adult fish exhibited a significantly higher EROD activity
367 than juveniles. On the contrary, Couillard et al. (2004) reported that EROD activity declined
368 with fish size and age in Atlantic tomcod *Microgadus tomcod* exposed to a complex mixture
369 of contaminants in the St. Lawrence Estuary polluted by tributyltin, heavy metals and
370 chlorinated organic compounds. These authors hypothesized that EROD activity in older fish
371 could decrease as a result of cellular alterations, like neoplastic liver lesions associated with
372 aging or chronic exposure to pollutants, as also reported in winter flounder *Pleuronectes*
373 *americanus* (Wall et al., 1998) and flounder *Platichthys flesus* (Köhler & Jürgen Pluta, 1995).
374 In the present work, hepatic EROD activity in *Sicydium* spp. females was lower than in males
375 at 3 sampling dates along the survey. EROD activity downregulation in fish spawning
376 females has been frequently reported in the literature as a consequence of an increase in the
377 steroid hormone 17 β -estradiol at the end of the reproductive cycle that acts as an antagonist
378 of this activity (Navas and Segner, 2000; Burkina et al., 2015). This should be clarified in
379 *Sicydium* spp. through a better knowledge of its sexual maturation process since with a view
380 to measuring this biomarker in *Sicydium* spp. for environmental assessment, it would be
381 important to discriminate EROD data from both sexes.

382 Along the 2-year survey, EROD activity was most of the time higher in fish from the Rivière
383 aux Herbes compared to those caught in the Grande Rivière de Vieux-Habitants, in
384 accordance with the different pattern of contamination of the two rivers. EROD activity
385 induction was over a 2-fold value even reaching in March 2019 and February 2020 a 5-fold
386 value. This result is in accordance with those described in other studies on tropical fish
387 exposed to a large array of pollutants. For example, Corredor-Santamaria et al. (2021)

388 recently reported a 4-fold increase in hepatic EROD activity of *Aequidens metae* exposed to
389 oil spill in a Columbian river, suggesting a fish response to PAHs exposure. A 3-fold EROD
390 induction was found in the liver of *Prochilodus lineatus* caged in a Brazilian river receiving
391 leather tannery effluents (Lunardelli et al., 2018). Authors underlined the possible role of
392 organic contaminants in the tannery effluents in EROD activity induction. However, as stated
393 by many authors, it would be illusory to attribute the observed enzymatic induction to a given
394 pollutant, keeping in mind that EROD activity plays a key role in biotransformation of many
395 pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls,
396 polychlorinated dibenzo - dioxins and - furanes, drugs and organo-chlorinated pesticides that
397 are encountered in the present study (Goksøyr and Förlin, 1992; Whyte et al., 2000; Van der
398 Oost, 2003). Thus, in order to assess *Sicydium* spp. response in the field after exposure to
399 chemically complex contaminations, hepatic EROD induction could be used as a sensitive
400 biomarker of exposure. In this perspective and as underlined in other fish species
401 (Flammarion & Garric,1997), further studies are required to precise reference levels of
402 hepatic EROD activity in *Sicydium* spp., as a first step for future biomonitoring in West Indies.

403

404 4.2. Genotoxicity biomarkers: micronucleus formation and primary DNA damages

405 As previously highlighted for hepatic EROD activity, genotoxicity biomarkers such as
406 micronucleus formation or primary DNA damages have never been studied in *Sicydium* spp.
407 No difference in erythrocyte micronucleus frequency was found in our study between the two
408 sympatric species *S. plumieri* and *S. punctatum* living in the same river. Micronucleus
409 frequency in *Sicydium* spp. from the low contaminated Grande Rivière de Vieux-Habitants
410 was in the range of 0-0.3 ‰. This is in accordance with the baseline micronucleus frequency
411 reported by many authors in fish to be in the average range of 0-1‰, even if a large
412 interspecies variability has been described (Al-Sabti and Metcalfe, 1995; Bolognesi and
413 Hayashi, 2011). A significant 4-5 fold increase in micronucleus frequency was shown in fish
414 erythrocytes from the Rivière aux Herbes compared to that of fish from the Grande Rivière
415 de Vieux-Habitants. After a 1 to 3 weeks exposure of fathead minnow (*Pimephales*

416 *promelas*) to water samples from a river receiving petrochemical effluents, a 3-7 fold
417 increase in micronuclei in peripheral erythrocytes was registered, depending both on the water
418 sampling area and exposure duration time (Torres de Lemos et al., 2007). In the same way,
419 De Flora et al. (1993) demonstrated a 2-3 fold increase in micronucleus frequency in
420 peripheral erythrocytes of trout engaged for 1 to 3 weeks in the Po River contaminated by a
421 variety of organic pollutants. Fish response was time-dependent as well, with the highest
422 micronucleus increase observed after one week of exposure. Authors hypothesized a
423 possible role of erythrocyte turn-over because damaged cells tend to be removed from the
424 organism faster than undamaged cells. Micronucleus frequency in peripheral erythrocytes
425 must be considered as the result of the balance between the formation of micronucleated
426 cells and their elimination, direct or through apoptosis (Udroiu, 2006; Polard et al., 2011).
427 Fish size used as a fish age proxy did not affect micronucleus formation in the present study,
428 meaning that micronucleus formation was not influenced by fish age. Since in the present
429 study *Sicydium* spp. was exposed continuously in the field to chemical contamination,
430 although likely variable along the time, it could mean that a steady-state between erythrocyte
431 turn-over and micronucleus formation was already reached in the smallest (youngest) fish
432 having a 46 mm total body length. In order to improve assay sensitivity for further
433 investigations it would be interesting to count both micronuclei and other nuclear
434 abnormalities (ENAs) as recommended by Colin et al. (2016).

435 Increase in primary DNA damages in fish measured through the alkaline version of the
436 Comet assay is considered as an ubiquitous biomarker in genotoxicity assessment, often
437 pointed out as being more sensitive than micronucleus formation and responsive to a
438 broader array of contaminants (Jha, 2008; Ali et al., 2009; Da Rocha, 2009; Polard et al.,
439 2011). The level of DNA damage was shown to be higher only in *Sicydium* spp. from the
440 upstream station of the Rivière aux Herbes compared to fish from the two stations of the less
441 contaminated Grande Rivière de Vieux-Habitants. Moreover, it remained limited to a 1.3-fold
442 increase. The high value of DNA damage measured in erythrocytes of fish from the Grande
443 Rivière de Vieux-Habitants (around 12 % tail intensity) argues for a chemically degraded

444 state of this river detected by this sensitive biomarker. Indeed, as demonstrated in our
445 previous studies when using the same comet assay protocol, basal level of DNA damage
446 measured in erythrocytes of other fish species such as brown trout (*Salmo trutta fario*),
447 European minnow (*Phoxinus phoxinus*), European perch (*Perca fluviatilis*) and white fish
448 (*Coregonus lavaretus*) living in pristine areas, was around 5% tail intensity (Bony et al., 2008;
449 Sotton et al., 2012a, 2012b). This could have accounted for the low difference in DNA
450 damage level measured in erythrocytes of fish from the two rivers contrary to other studies
451 reporting higher increase in fish erythrocyte DNA damage when comparing fish response
452 from low and heavily contaminated rivers (Devaux et al., 1998; Winter et al., 2004;
453 Deutschmann et al., 2016; Kolarević et al., 2016). As stated with the other genotoxicity
454 endpoint, no difference regarding DNA damage level was observed between both sympatric
455 *Sicydium* species. Fish size considered here as a fish age proxy did not influence the
456 response of this biomarker as well. Effect of fish age on the extent of DNA damage remains
457 controversial since it closely depends on many factors like fish species and characteristics of
458 exposure in terms of intensity and duration (Akcha et al., 2004; Wirzinger et al., 2007; Santos
459 et al., 2016). As highlighted for micronucleus formation, continuous exposure of *Sicydium*
460 spp. in the river could explain that fish size (age) does not affect the biomarker response
461 resulting from the balance between the genotoxicity pressure and DNA repair capacities of
462 fish (Kienzler et al., 2013).

463

464 **5. Conclusion**

465 Results of the present work argue for the interest of using *Sicydium* spp. as sentinel species
466 for environmental assessment in West Indies freshwater bodies. Because of their similar
467 responses toward the environment, both sympatric species *Sicydium punctatum* and
468 *Sicydium plumieri* are good candidates for biomonitoring programs that implement
469 biomarkers of exposure and effects. In complement to the induction of hepatic EROD activity
470 and genotoxicity endpoints, assessment of oxidative stress through lipid peroxidation would

471 be of great value as shown in our first tests (data not shown) but still requires some technical
472 improvements in *Sicydium* spp. for a routine use.

473

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