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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.

134

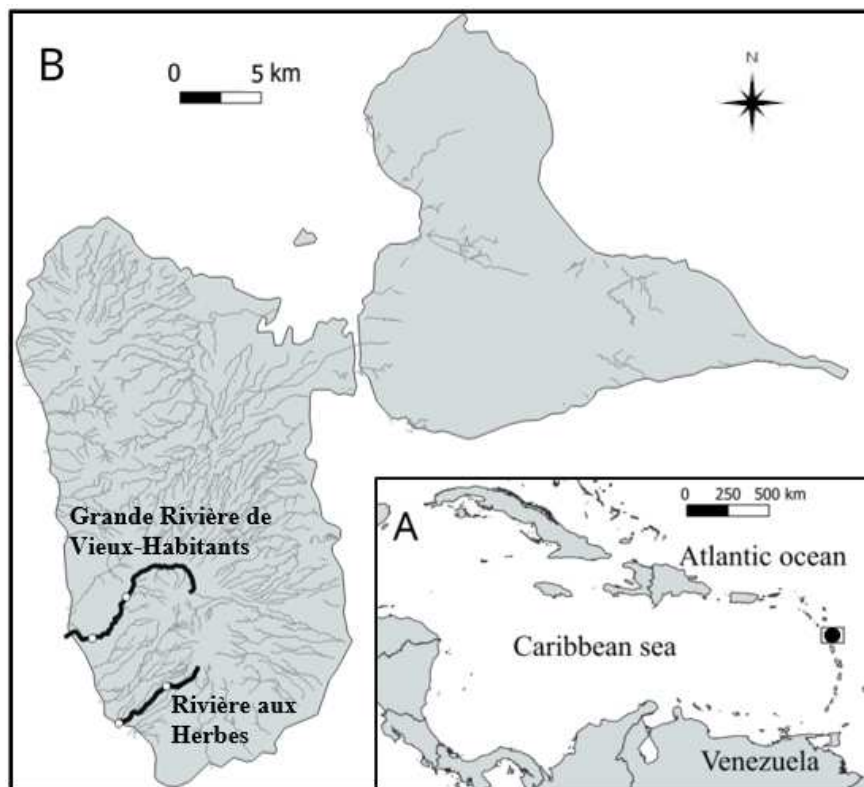
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136

## 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

### 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  $\geq 66$  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  $\mu\text{L}$  of remaining blood sample were diluted in 198  $\mu\text{L}$  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  $\mu\text{L}$  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  $\mu\text{L}$  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  $-20^{\circ}\text{C}$  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.

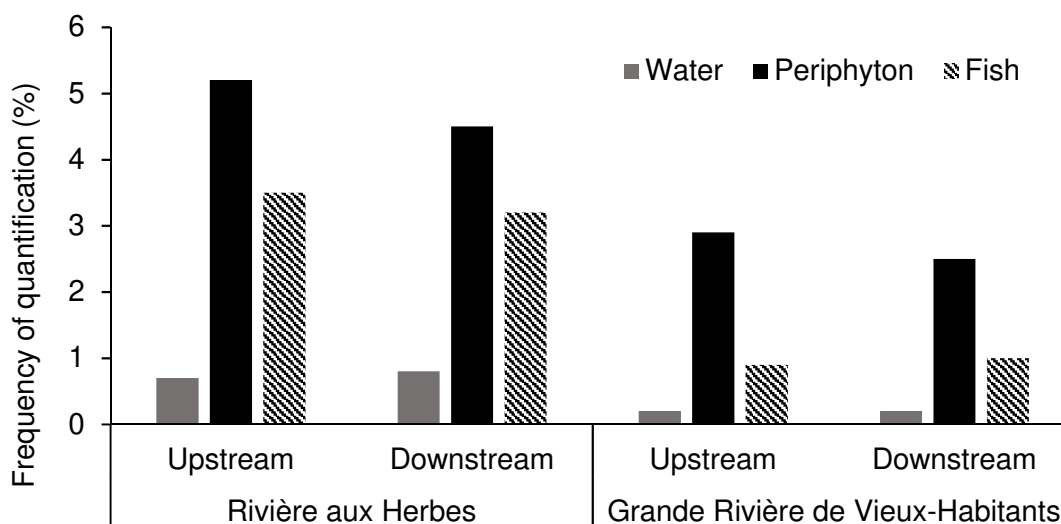
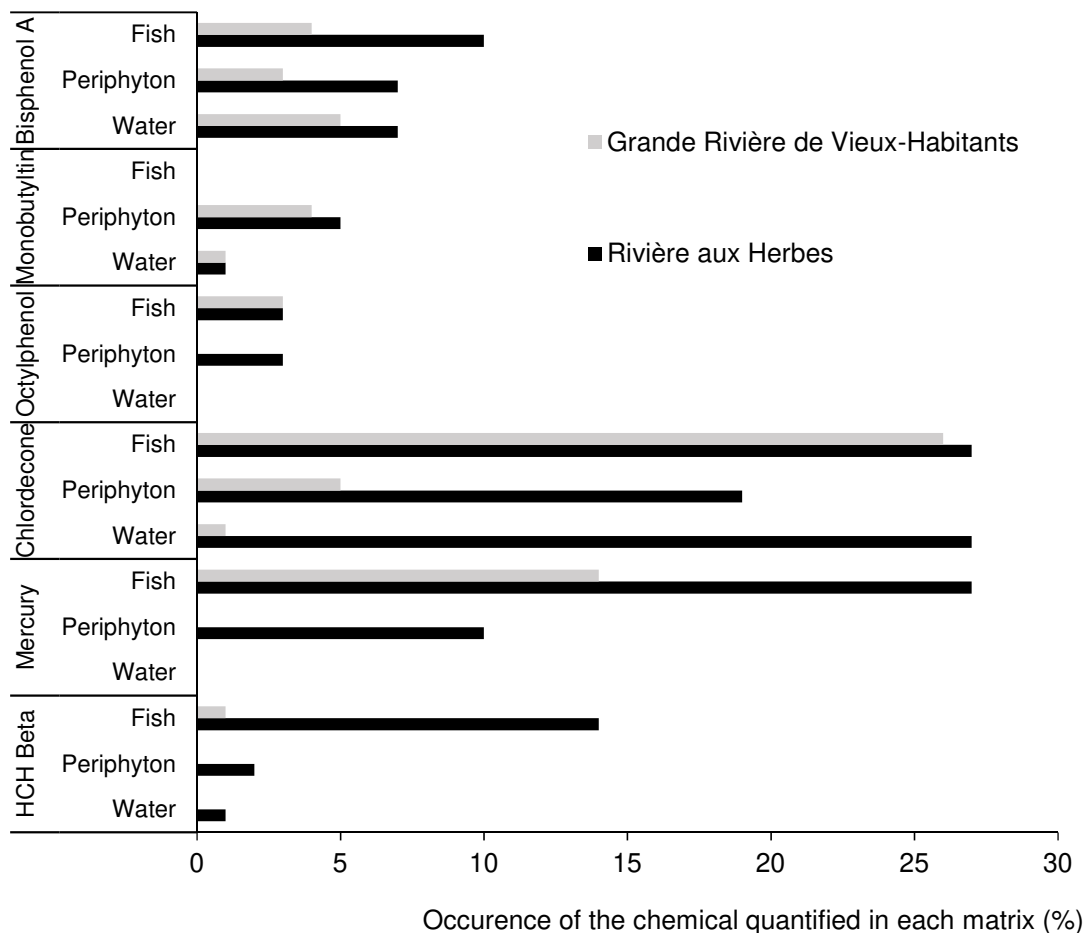


Fig. 2. Frequency of molecule quantification in each matrix analyzed (number of quantified molecules out of the total number of searched molecules per matrix for the 10 sampling campaigns pooled by each river).

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

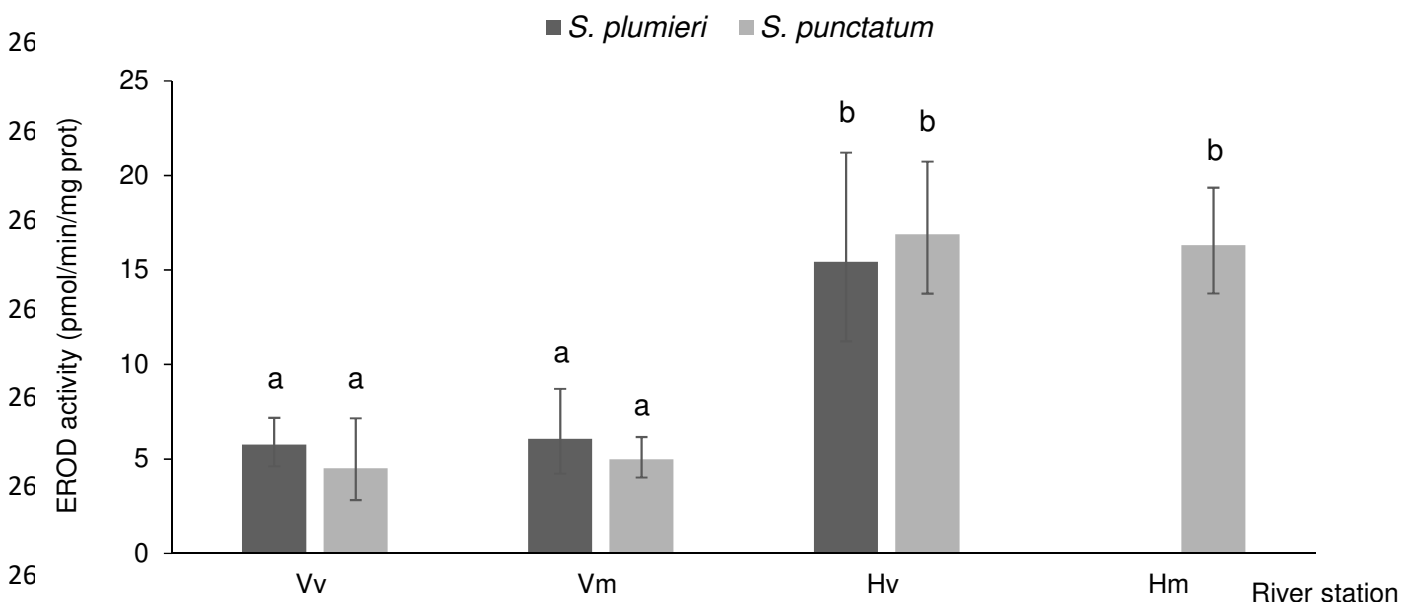
245 To sum up, the Rivière aux Herbes appears to be more chemically and ecologically  
 246 degraded than the Grande Rivière de Vieux-Habitants.

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  
26 that do not share a letter are significantly different (post-hoc Tukey's test,  $p < 0.01$ ).  
27 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.

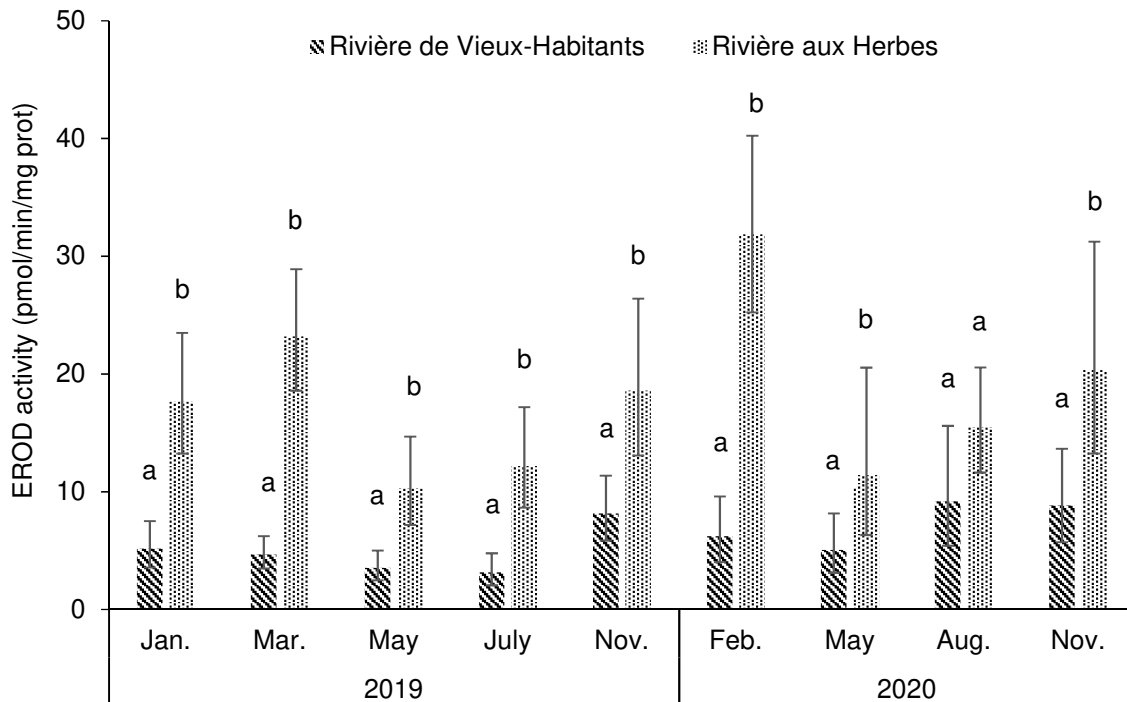


Fig. 5. Comparison of hepatic EROD activity in *Sicydium* spp. from the two rivers at the different catching campaigns. EROD activity is expressed as the geometric mean  $\pm$  95 percent confidence interval. Means that do not share a letter are significantly different (Tukey's test,  $p < 0.05$ ).

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).

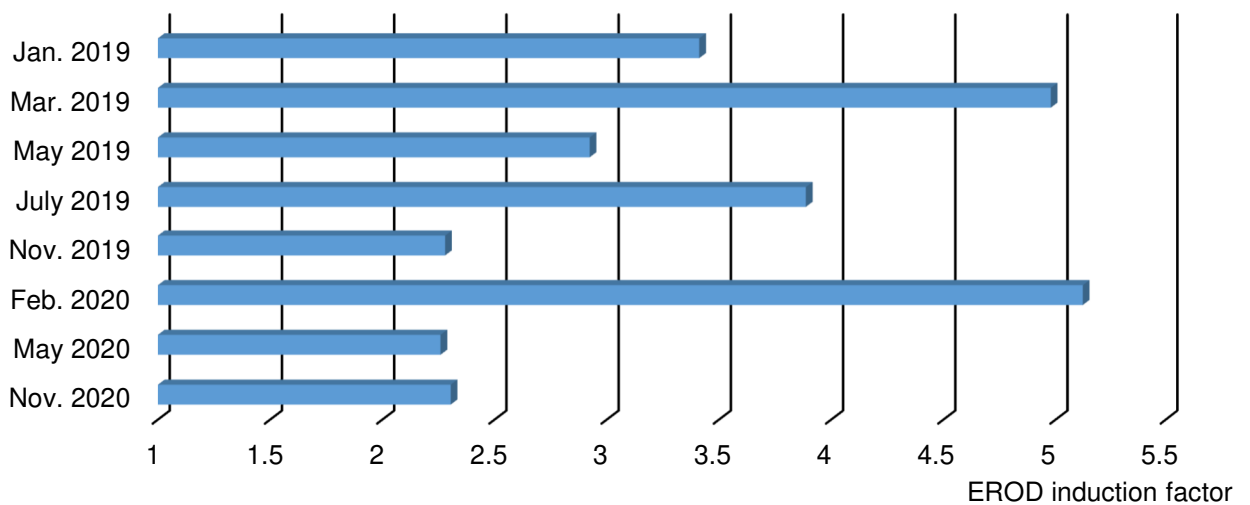
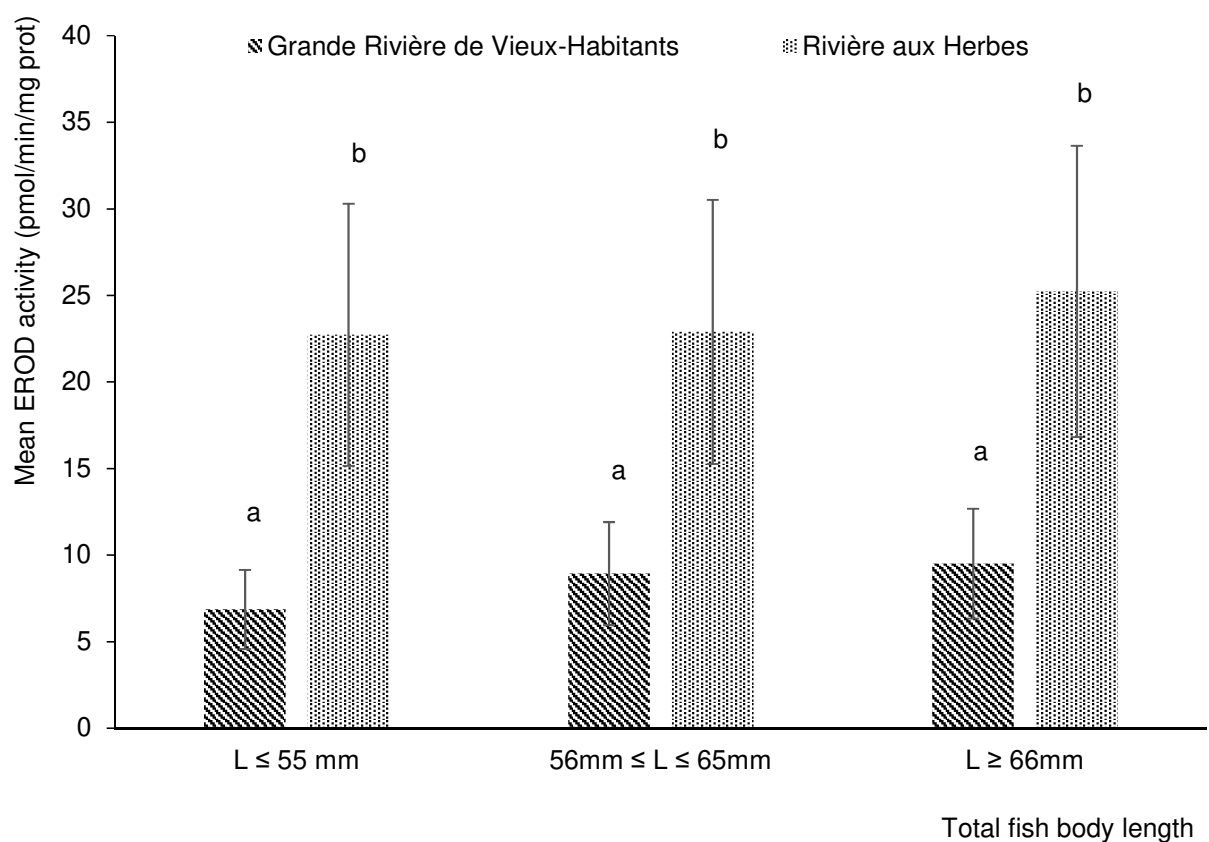


Fig. 6. Induction factor of EROD activity measured in *Sicydium* spp. from the Rivière aux Herbes compared to those from the Grande Rivière de Vieux-Habitants.

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 <sup>a</sup> (3)	9.14 $\pm$ 3.78 <sup>a</sup> (24)
	Mar.	7.12 $\pm$ 2.27 <sup>a</sup> (17)	6.02 $\pm$ 1.81 <sup>a</sup> (15)
	May	4.29 $\pm$ 1.02 <sup>a</sup> (10)	5.12 $\pm$ 2.16 <sup>a</sup> (14)
	July	5.13 $\pm$ 1.53 <sup>a</sup> (13)	3.77 $\pm$ 1.41 <sup>b</sup> (9)
	Nov.	11.64 $\pm$ 2.42 <sup>a</sup> (16)	9.93 $\pm$ 3.33 <sup>a</sup> (8)
2020	Feb.	13.86 $\pm$ 2.02 <sup>a</sup> (4)	6.63 $\pm$ 1.49 <sup>b</sup> (8)
	May	9.14 $\pm$ 1.99 <sup>a</sup> (4)	5.52 $\pm$ 1.90 <sup>b</sup> (9)
	Aug.	16.65 $\pm$ 5.02 <sup>a</sup> (3)	15.20 $\pm$ 3.57 <sup>a</sup> (12)
	Nov.	15.07 $\pm$ 3.05 <sup>a</sup> (5)	11.38 $\pm$ 2.71 <sup>a</sup> (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‰.

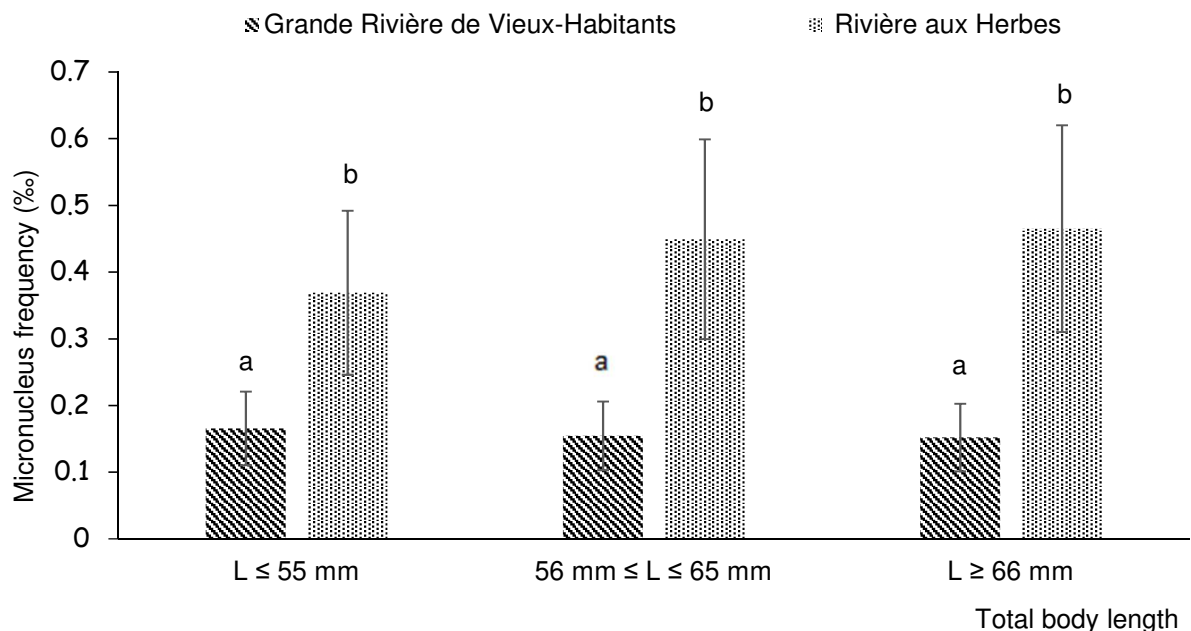


Fig. 8. Micronucleus frequency in *Sicydium* spp. circulating erythrocytes according to total body length. Results are expressed as mean  $\pm$  standard deviation. Means that do not share a letter are significantly different (post-hoc Tukey's test,  $p < 0.05$ ).

317 3.3.2. Micronucleus frequency according to fish size  
318

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  $16.13 \pm 3.16$   
 337 ( $n= 423$ ) compared to that of fish from the Grande Rivière de Vieux-Habitants  $12.24 \pm 3.57$   
 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	$12.86 \pm 2.97^a$ (126)	Hv	$12.40 \pm 1.96^a$ (91)
	Vm	$12.18 \pm 2.58^a$ (59)		
<i>Sicydium punctatum</i>	Vv	$12.00 \pm 3.26^a$ (45)	Hv	$14.64 \pm 1.91^a$ (111)
	Vm	$11.25 \pm 2.22^a$ (149)	Hm	$18.40 \pm 2.57^b$ (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

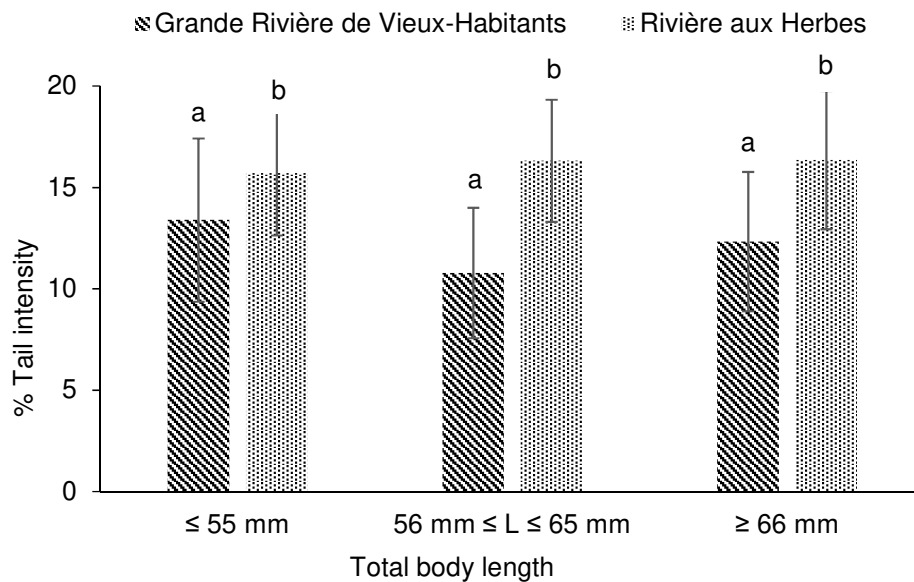


Fig. 9. Mean DNA damage in *Sicydium* spp. erythrocytes according to total body length. Means that do not share a letter are significantly different (Mann Whitney *U* test,  $p < 0.05$ ).

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 $\beta$ -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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