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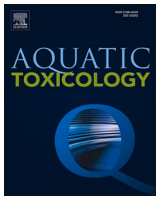

The goby fish *Sicydium* spp. as valuable sentinel species towards the chemical stress in freshwater bodies of West Indies

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ABSTRACT

Implementation of the European Water Framework Directive in tropical areas such as the French West Indies (FWI) requires to select relevant aquatic sentinel species for investigating the ecological status of surface waters. The present work aimed to study the biological response of the widespread fish *Sicydium* spp. towards river chemical quality in Guadeloupe island through a set of proper biomarkers. During a 2-year survey, the hepatic EROD activity, the micronucleus formation and the level of primary DNA strand breaks in erythrocytes were measured respectively as an enzymatic biomarker of exposure and genotoxicity endpoints in fish living upstream and downstream of two chemically-contrasted rivers. Hepatic EROD activity was shown to be variable along the time but always significantly higher in fish from the most contaminated river (Rivière aux Herbes) compared to the low contaminated one (Grande Rivière de Vieux-Habitants). Fish size did not influence EROD activity. Female fish exhibited a lower EROD activity compared to males depending on the catching period. We observed significant temporal variation in micronucleus frequency and primary DNA damage level measured in fish erythrocytes that did not depend on the fish size. Micronucleus frequency and to a lesser extent DNA damage were significantly higher in fish from the Rivière aux Herbes compared to the Grande Rivière de Vieux-Habitants. Our results argue for the interest of using *Sicydium* spp. as sentinel species to assess river quality and chemical pressures in FWI.

1. Introduction

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

Biomonitoring is crucial in environmental management and conservation biology to assess historical trajectories of populations and ecosystem status and to mitigate pressures that impact the most aquatic biodiversity (Santos et al., 2022). For that purpose, fish are commonly used as relevant sentinel species in a large array of environmental risk assessment studies because of their relatively long life-span, their functional role in aquatic ecosystems and their high trophic level that can provide integrated and relevant information regarding availability and effects of environmental stressors (Ortiz-Zarragoitia et al., 2014; Rios et al., 2015; McMillan et al., 2022). Fish sentinel species enable to take into account the overall effects of the complex mixture of pollutants interacting with natural and other anthropogenic stressors to be found in aquatic ecosystems (Colin et al., 2016).

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

Gobioidei, and especially species of the Sicydiinae subfamily, are the most abundant and widely distributed freshwater fish in Caribbean, exhibiting the highest levels of endemism (Keith, 2003; Keith et al., 2011). In Martinique and Guadeloupe islands, *Sicydium punctatum* and *Sicydium plumieri* are widespread amphidromous species, *Sicydium punctatum* being the most abundant one (Lim et al., 2002; Monti et al., 2010; Lejeune et al., 2014). *Sicydium* spp. (namely *S. punctatum* and *S. plumieri*) large ubiquity in FWI rivers is mainly due to their modified sucker-fin enabling them to overcome natural or anthropic obstacles and to resist to heavy water current. *Sicydium* spp. abundance allows quite easy and regular field sampling. Despite their small size, i.e. a maximum body length of 120 mm for *S. punctatum* and 140 mm for *S. plumieri,* respectively (Lim et al., 2002; Monti et al., 2010), it is possible to sample enough tissue for both biological and chemical analyses.

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

Changes in biological responses in fish exposed to contamination can be assessed through the measurement of a large array of biomarkers ranging from molecular through cellular and physiological responses to behavioral changes (Adams, 2001; van der Oost et al., 2003). One of the most widely used biomarkers of exposure to organic pollution in fish for many decades is the measurement of changes in hepatic ethoxyresorufin-*O*-deethylase (EROD) catalytic activity, as a proxy to the subfamily CYP1A (Nilsen et al., 1998; Sarasquete and Segner, 2000). EROD induction in fish is well characterized, the most important modifying factors being fish species, reproductive status and age. EROD activity modulation has been shown as a relevant biomarker in a number of field investigations regarding industrial and urban effluents, contaminated sediments and chemical spills (van der Oost, 2003; Burkina et al., 2015; Santana et al., 2018). Known mechanisms of CYP1A-induced toxicity suggest that modulation of EROD activity considered as a biomarker of exposure to some organic compounds, could also inform about further effects at various levels of biological organization in fish (Nilsen et al., 1998; Whyte et al., 2000; van der Oost et al., 2003). Thus, measurement of EROD activity has been historically recommended in monitoring programs for assessing environmental contamination and effects on fish and remains of great interest (Bucheli and Fent, 1995; Gagnon and Rawson, 2017).

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

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

2. Materials and methods

2.1. Study area

Two hydrosystems were chosen according to their contrasted levels of contamination: the Grande Rivière de Vieux-Habitants, one of the least-contaminated river and the Rivière aux Herbes severly contaminated by human activities (urban and agriculture) in Guadeloupe island. For each river an upstream and a downstream station were investigated, called respectively Vm and Vv for the Grande Rivière de Vieux-Habitants, and Hm and Hv for the Rivière aux Herbes (Fig. 1).

2.2. Physico-chemical and ecological status of the rivers

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

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

Physico-chemical quality of water of both rivers was monitored every 2 months during the first year (2019) and 4 times during the

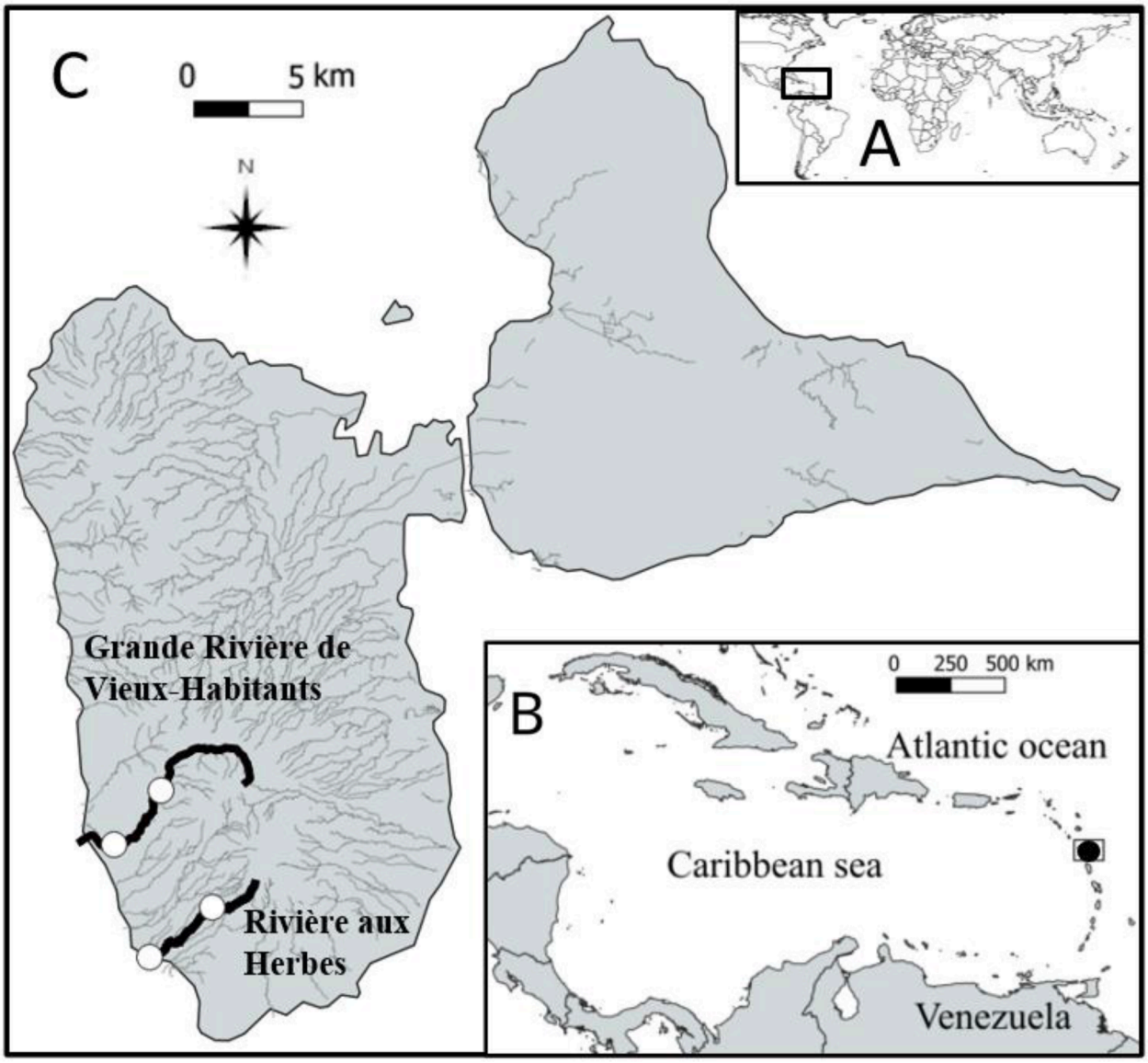

Fig. 1. Localization maps of Guadeloupe island in Caribbean sea (A) and of studied rivers, Grande Rivière de Vieux-Habitants and Rivière aux Herbes (B). Upstream and downstream stations of each river are shown as a white dot.

second year (2020) using a multiparametric probe (HACH, HQ40d Multi).

2.3. Fish sampling and tissue collection

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

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

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

2.4. Biomarker responses

EROD activity and protein concentration were measured on fish liver S9 homogenate according to the procedure described by Flammarion and Garric (1997) with slight modifications (Noury, 2016).

Micronucleus assay was carried out on fish erythrocytes according to Polard et al. (2011). DNA damage was assessed on fish erythrocytes through the alkaline Comet assay following the procedure described by Singh et al. (1988), slightly modified according to Bony et al. (2008) and by replacing ethidium bromide by Syber Green 1 for DNA staining. Since the aim of the study was to give a global insight of the biomarker response in *Sicydium* spp. as a sentinel species, data of all campaigns have been pooled, apart for EROD activity in order to take into account a possible effect of female sexual maturity on this biomarker response.

2.5. Statistical analysis

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

3. Results

3.1. Physico-chemical and ecological status of the rivers

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

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

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

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

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

3.2. EROD activity

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

3.2.1. EROD activity according to fish species

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

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

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

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

3.2.3. EROD activity according to fish size and sex

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

Effect of fish sex on hepatic EROD activity has been investigated in *Sicydium* spp. from the Grande Rivière de Vieux-Habitants which is the less contaminated river, to limit artefact due to possible endocrine disruption. EROD activity was measured over time in 2019 and 2020 in order to take into account the state maturity of both fish sexes. As previously highlighted, hepatic EROD activity was found to be variable along the time whatever fish sex. A trend for a lower EROD activity in females compared to males was noted in 2019 and 2020, significant differences being observed between both sexes in July 2019, February and May 2020 (Table 2).

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

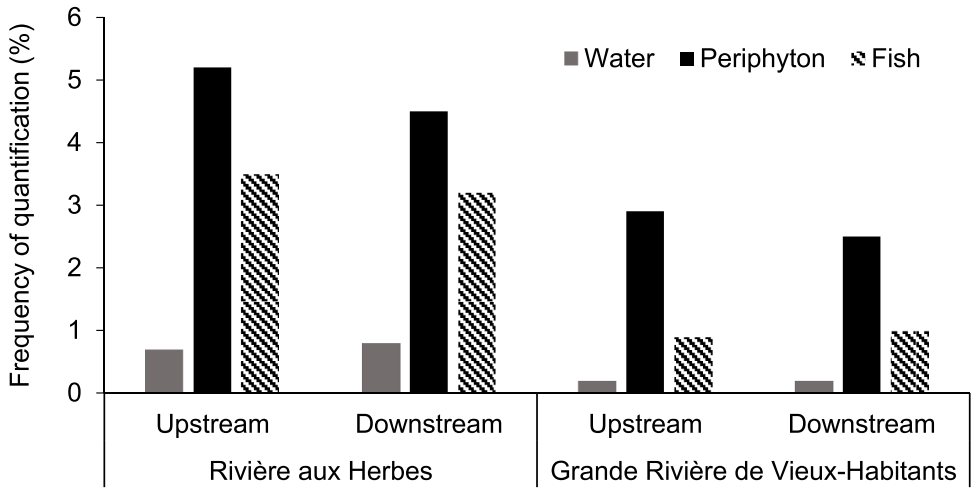

Fig. 2. Frequency of molecule quantification in each matrix analyzed (number of quantified molecules out of the total number of searched molecules per matrix for the 10 sampling campaigns pooled by each river).

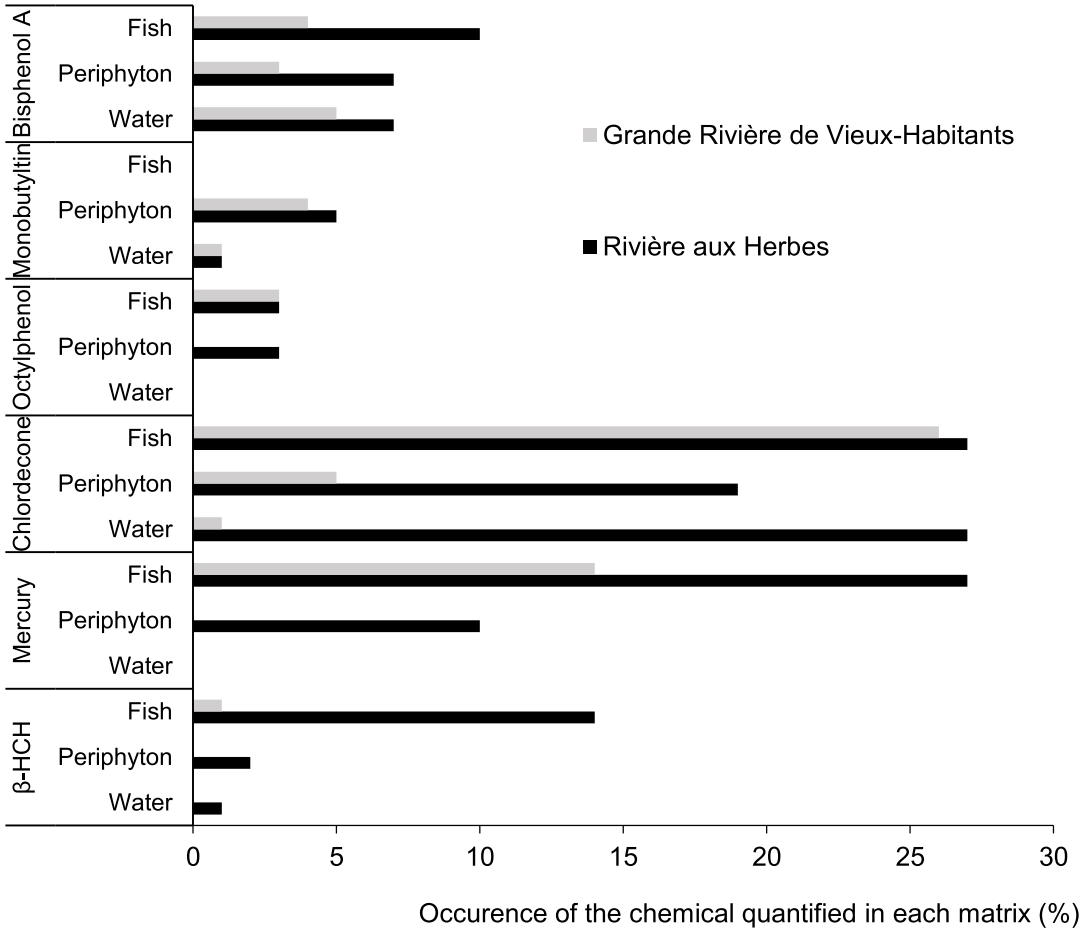

Fig. 3. Occurence percentage regarding the 6 chemicals quantified common to the 3 matrices from the two rivers. Percentage was calculated by summing each of the 6 compounds when quantified versus the total number of compounds analyzed per matrix for all the 10 sampling campaigns.

3.3. Micronucleus formation

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

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

No significant difference in micronucleus frequency was shown between the two fish species from the same river and between the fish of the same species caught upstream and downstream of each river. Micronucleus frequency was significantly higher in fish from the Rivière aux Herbes compared to those from the Grande Rivière de Vieux-Habitants, whatever the species and the river station (Fig. 8).

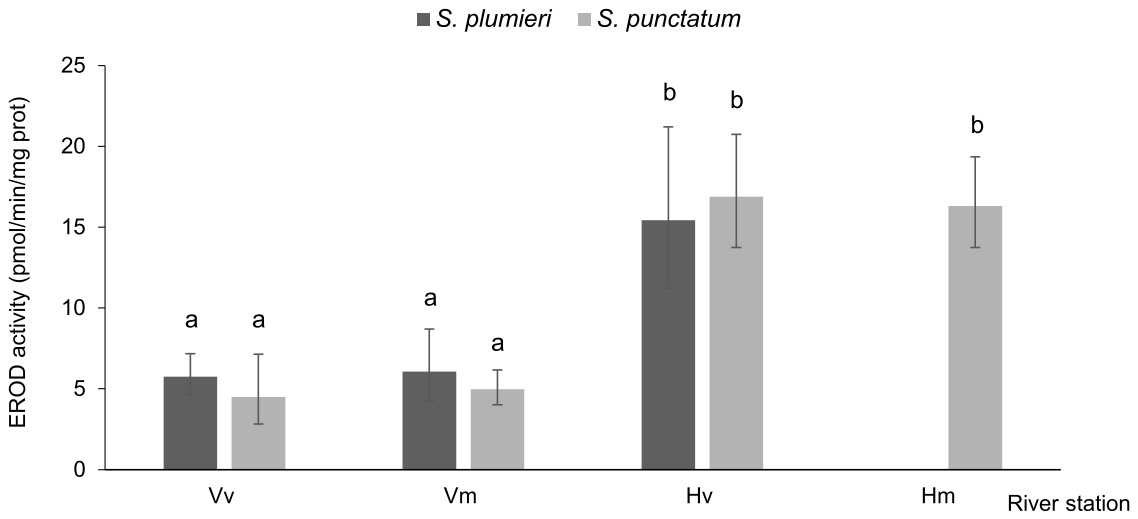

Fig. 4. Hepatic EROD activity in *Sicydium plumieri* (PLU) and *Sicydium punctatum* (PUN). Vv = Grande Rivière de Vieux-Habitants downstream station; Vm = Grande Rivière de Vieux-Habitants upstream station; Hv = Rivière aux Herbes downstream station; Hm = Rivière aux Herbes upstream station. EROD activity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

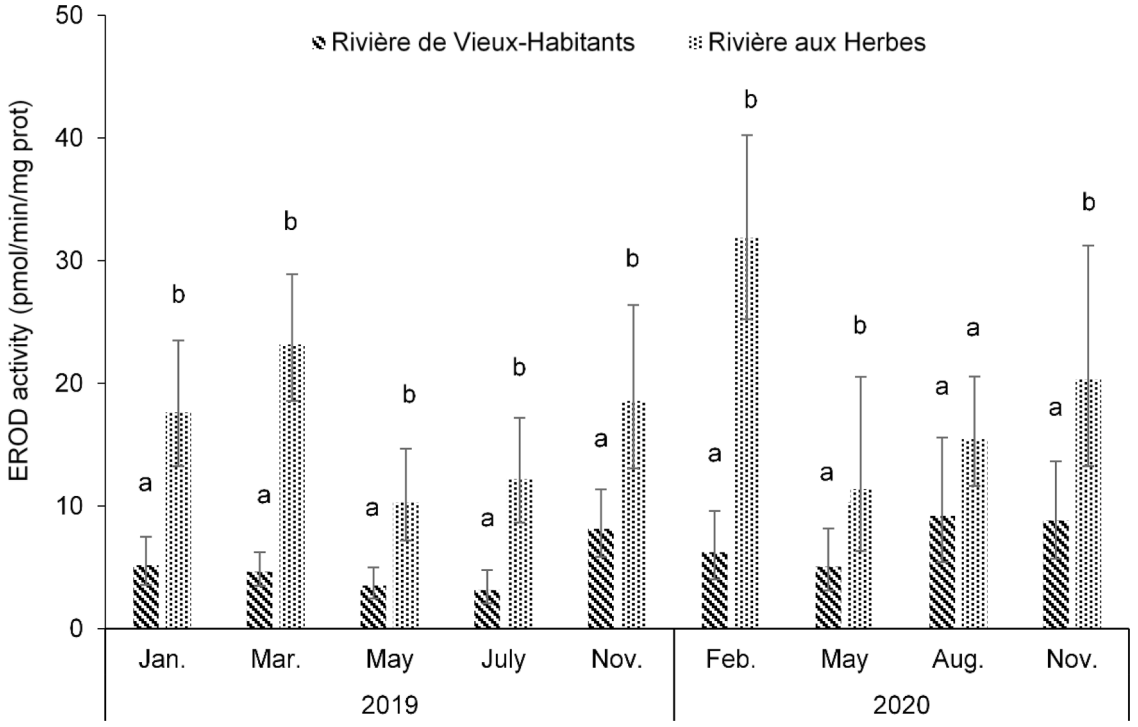

Fig. 5. Comparison of hepatic EROD activity in *Sicydium* spp. from the two rivers at the different catching campaigns. EROD activity is expressed as the geometric mean ± 95 percent confidence interval. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$).

Observed micronucleus frequency remained in all cases at a low level < 1‰.

3.3.2. Micronucleus frequency according to fish size

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

3.4. Primary DNA damage in fish erythrocytes

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

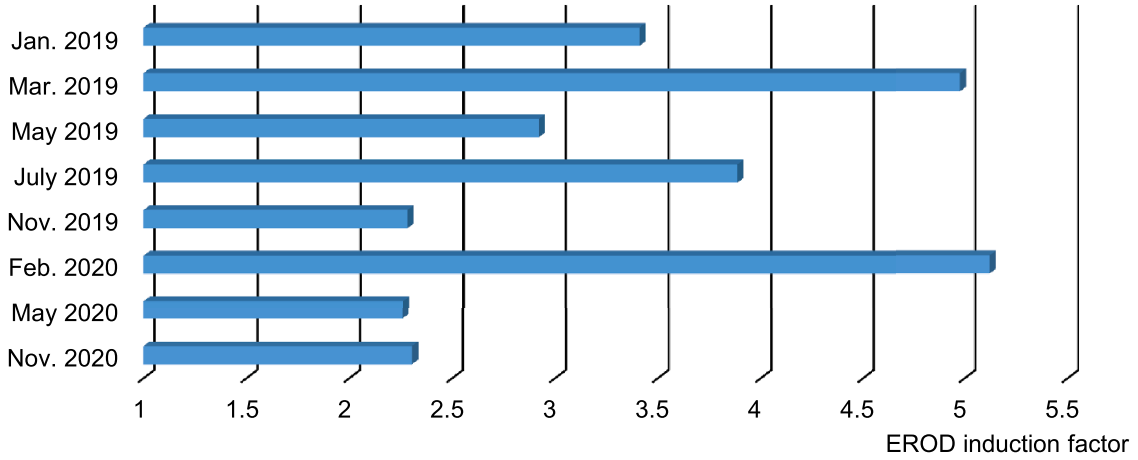

Fig. 6. Induction factor of EROD activity measured in *Sicydium* spp. from the Rivière aux Herbes compared to those from the Grande Rivière de Vieux-Habitants.

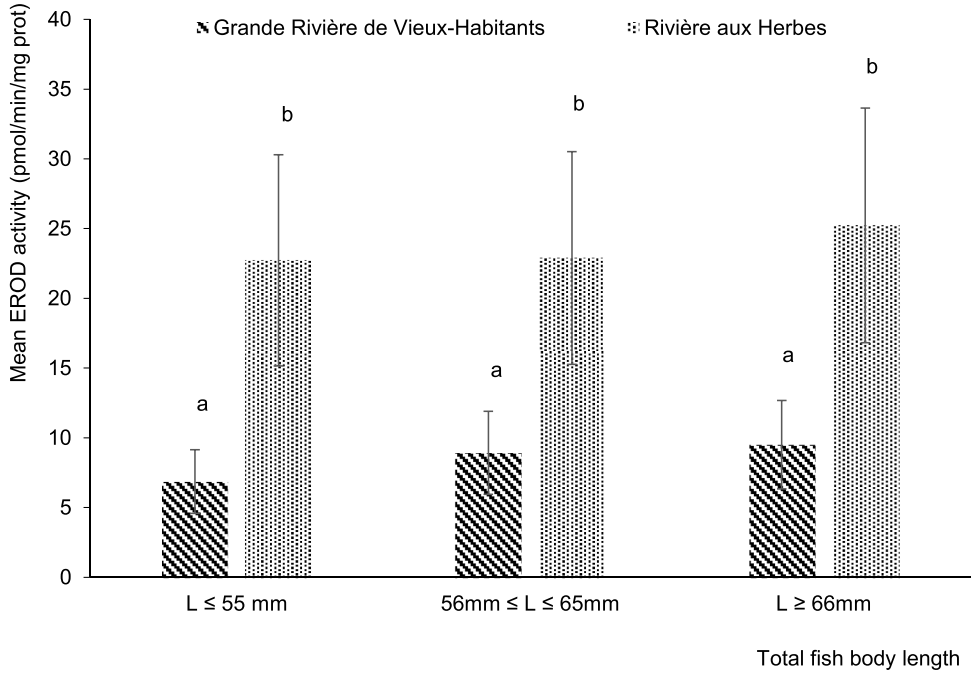

Fig. 7. Mean EROD activity in liver of *Sicydium* spp. according to total fish body length. Means that do not share a letter are significantly different (Tukey's test, $p < 0.05$). Grande Rivière de Vieux-Habitants: $n = 34$ ($L \leq 55$ mm), $n = 68$ (56 mm $\leq L \leq$ 65 mm), $n = 130$ ($L \geq 66$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

Table 2
Hepatic EROD activity in liver of male and female *Sicydium* spp. from the Grande Rivière de Vieux-Habitants. EROD activity (pmol/min/mg protein) is expressed as mean ± standard deviation (number of fish). EROD activity was compared between both fish sex at each sampling date. Means that do not share a letter are significantly different (Student *t*-test, $p < 0.05$).

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

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

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

3.4.2. *Primary DNA damage level according to fish size*

No effect of the total body length of *Sicydium* spp. on the DNA damage level was observed whatever the river (Fig. 9).

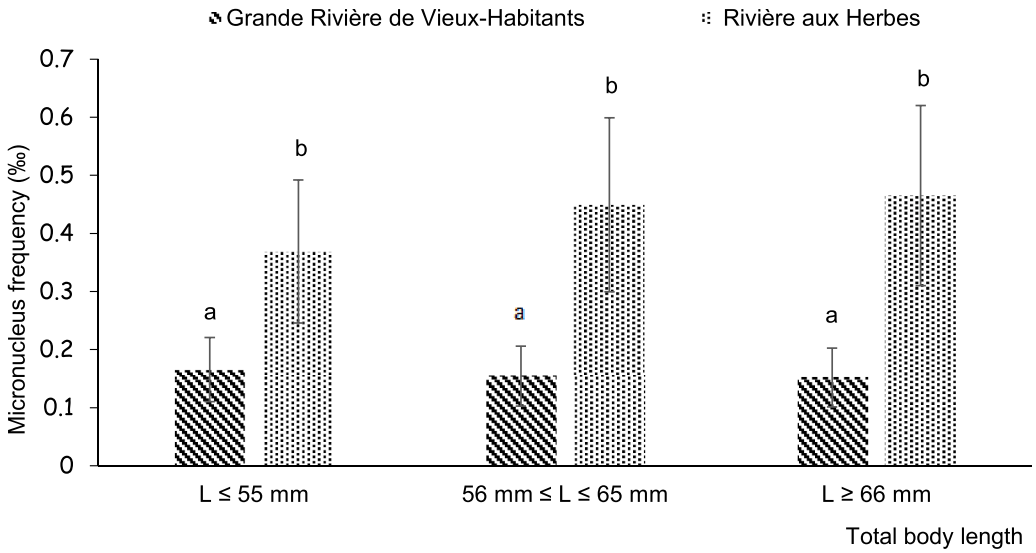

Fig. 8. Micronucleus frequency in *Sicydium* spp. circulating erythrocytes according to total body length. Results are expressed as mean ± standard deviation. Means that do not share a letter are significantly different (post-hoc Tukey's test, $p < 0.05$).

Table 3
Level of DNA damage in circulating erythrocytes of *Sicydium plumieri* and *Sicydium punctatum* caught upstream and downstream of both rivers (Grande Rivière de Vieux-Habitants and Rivière aux Herbes). Data are shown as the tail intensity percentage, mean ± standard error of the mean (*n*= number of fish). Means that do not share a letter are significantly different (Mann-Whitney *U* test, $p < 0.05$).

	Rivière de Vieux-Habitants		Rivière aux Herbes	
Fish species	Station	% Tail intensity	Station	% Tail intensity
Sicydium plumieri	Vv	12.86 ± 2.97^{a} (126)	Hv	12.40 ± 1.96^{a} (91)
	Vm	12.18 ± 2.58^{a} (59)		
Sicydium punctatum	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)

4. Discussion

4.1. Enzymatic biomarker: hepatic EROD activity

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

No relationship between fish age measured here as the proxy total body length and EROD activity was highlighted. The relationship between fish size or age and hepatic EROD activity remains controversial since it depends on biotic/abiotic parameters. On one hand, larger fish can accumulate more hydrophobic contaminants along the exposure time leading to higher EROD activity levels compared to smaller fish. As shown by Hugla et al. (1995) in the common barbel *Barbus barbus* and by Sleiderink et al. (1995) in the dab *Limanda limanda* living in

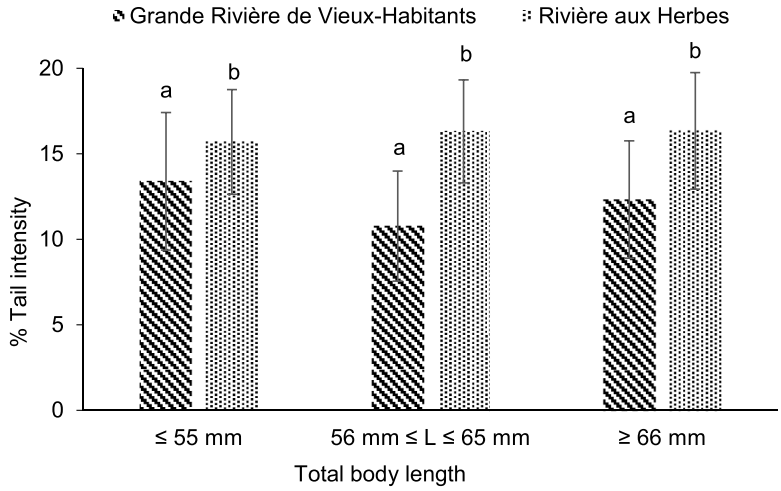

Fig. 9. Mean DNA damage in *Sicydium* spp. erythrocytes according to total body length. Means that do not share a letter are significantly different (Mann Whitney *U* test, $p < 0.05$).

PCB-contaminated sites, adult fish exhibited a significantly higher EROD activity than juveniles. On the contrary, Couillard et al. (2004) reported that EROD activity declined with fish size and age in Atlantic tomcod *Microgadus tomcod* exposed to a complex mixture of contaminants in the St. Lawrence Estuary polluted by tributyltin, heavy metals and chlorinated organic compounds. These authors hypothesized that EROD activity in older fish could decrease as a result of cellular alterations, like neoplastic liver lesions associated with aging or chronic exposure to pollutants, as also reported in winter flounder *Pleuronectes americanus* (Wall et al., 1998) and flounder *Platichthys flesus* (Köhler & Jürgen Pluta, 1995). In the present work, hepatic EROD activity in *Sicydium* spp. females was lower than in males at 3 sampling dates along the survey. EROD activity downregulation in fish spawning females has been frequently reported in the literature as a consequence of an increase in the steroid hormone 17β-estradiol at the end of the reproductive cycle that acts as an antagonist of this activity (Navas and Segner, 2000; Burkina et al., 2015). This should be clarified in *Sicydium* spp. through a better knowledge of its sexual maturation process since with a view to measuring this biomarker in *Sicydium* spp. for environmental assessment, it would be important to discriminate EROD data from both sexes.

Along the 2-year survey, EROD activity was most of the time higher in fish from the Rivière aux Herbes compared to those caught in the Grande Rivière de Vieux-Habitants, in accordance with the different pattern of contamination of the two rivers. EROD activity induction was over a 2-fold value even reaching in March 2019 and February 2020 a 5-fold value. This result is in accordance with those described in other studies on tropical fish exposed to a large array of pollutants. For example, Corredor-Santamaria et al. (2021) recently reported a 4-fold increase in hepatic EROD activity of *Aequidens metae* exposed to oil spill in a Columbian river, suggesting a fish response to PAHs exposure. A 3-fold EROD induction was found in the liver of *Prochilodus lineatus* caged in a Brazilian river receiving leather tannery effluents (Lunardelli et al., 2018). Authors underlined the possible role of organic contaminants in the tannery effluents in EROD activity induction. However, as stated by many authors, it would be illusory to attribute the observed enzymatic induction to a given pollutant, keeping in mind that EROD activity plays a key role in biotransformation of many pollutants, such as polycyclic aromatic hydrocarbons, polychlorinated biphenyls, polychlorinated dibenzo - dioxins and - furanes, drugs and organo-chlorinated pesticides that are encountered in the present study (Goksøyr and Förlin, 1992; Whyte et al., 2000; Van der Oost, 2003). Thus, in order to assess *Sicydium* spp. response in the field after exposure to chemically complex contaminations, hepatic EROD induction could be used as a sensitive biomarker of exposure. In this perspective and as underlined in other fish species (Flammarion and Garric,1997), further studies are required to precise reference levels of hepatic EROD activity in *Sicydium* spp., as a first step for future biomonitoring in West Indies.

4.2. Genotoxicity biomarkers: micronucleus formation and primary DNA damages

As previously highlighted for hepatic EROD activity, genotoxicity biomarkers such as micronucleus formation or primary DNA damages have never been studied in *Sicydium* spp. No difference in erythrocyte micronucleus frequency was found in our study between the two sympatric species *S. plumieri* and *S. punctatum* living in the same river. Micronucleus frequency in *Sicydium* spp. from the low contaminated Grande Rivière de Vieux-Habitants was in the range of 0–0.3 ‰. This is in accordance with the baseline micronucleus frequency reported by many authors in fish to be in the average range of 0–1‰, even if a large interspecies variability has been described (Al-Sabti and Metcalfe, 1995; Bolognesi and Hayashi, 2011). A significant 4–5 fold increase in micronucleus frequency was shown in fish erythrocytes from the Rivière aux Herbes compared to that of fish from the Grande Rivière de Vieux-Habitants. After a 1 to 3 weeks exposure of fathead minnow (*Pimephales promelas*) to water samples from a river receiving petrochemical effluents, a 3–7 fold increase in micronuclei in peripheral erythrocytes was registred, depending both on the water sampling area and exposure duration time (Torres de Lemos et al., 2007). In the same way, De Flora et al. (1993) demonstrated a 2–3 fold increase in micronucleus frequency in peripheral erythrocytes of trout encaged for 1 to 3 weeks in the Po River contaminated by a variety of organic pollutants. Fish response was time-dependent as well, with the highest micronucleus increase observed after one week of exposure. Authors hypothesized a possible role of erythrocyte turn-over because damaged cells tend to be removed from the organism faster than undamaged cells. Micronucleus frequency in peripheral erythrocytes must be considered as the result of the balance between the formation of micronucleated cells and their elimination, direct or through apoptosis (Udroiu, 2006; Polard et al., 2011). Fish size used as a fish age proxy did not affect micronucleus formation in the present study, meaning that micronucleus formation was not influenced by fish age. Since in the present study *Sicydium* spp. was exposed continuously in the field to chemical contamination, although likely variable along the time, it could mean that a steady-state between erythrocyte turn-over and micronucleus formation was already reached in the smallest (youngest) fish having a 46 mm total body length. In order to improve assay sensitivity for further investigations it would be interesting to count both micronuclei and other nuclear abnormalities (ENAs) as recommended by Colin et al. (2016).

Increase in primary DNA damages in fish measured through the alkaline version of the Comet assay is considered as an ubiquitous biomarker in genotoxicity assessment, often pointed out as being more sensitive than micronucleus formation and responsive to a broader array of contaminants (Jha, 2008; Ali et al., 2009; Da Rocha, 2009; Polard et al., 2011). The level of DNA damage was shown to be higher only in *Sicydium* spp. from the upstream station of the Rivière aux Herbes compared to fish from the two stations of the less contaminated Grande Rivière de Vieux-Habitants. Moreover, it remained limited to a 1.3-fold increase. The high value of DNA damage measured in erythrocytes of fish from the Grande Rivière de Vieux-Habitants (around 12% tail intensity) argues for a chemically degraded state of this river detected by this sensitive biomarker. Indeed, as demonstrated in our previous studies when using the same comet assay protocol, basal level of DNA damage measured in erythrocytes of other fish species such as brown trout (*Salmo trutta fario*), European minnow (*Phoxinus phoxinus*), European perch (*Perca fluviatilis*) and white fish (*Coregonus lavaretus*) living in pristine areas, was around 5% tail intensity (Bony et al., 2008; Sotton et al., 2012a, 2012b). This could have accounted for the low difference in DNA damage level measured in erythrocytes of fish from the two rivers contrary to other studies reporting higher increase in fish erythrocyte DNA damage when comparing fish response from low and heavily contaminated rivers (Devaux et al., 1998; Winter et al., 2004; Deutschmann et al., 2016; Kolarević et al., 2016). As stated with the other genotoxicity endpoint, no difference regarding DNA damage level was observed between both sympatric *Sicydium* species. Fish size considered here as a fish age proxy did not influence the response of this biomarker as well. Effect of fish age on the extent of DNA damage remains controversial since it closely depends on many factors like fish species and characteristics of exposure in terms of intensity and duration (Akcha et al., 2004; Wirzinger et al., 2007; Santos et al., 2016). As highlighted for micronucleus formation, continuous exposure of *Sicydium* spp. in the river could explain that fish size (age) does not affect the biomarker response resulting from the balance between the genotoxicity pressure and DNA repair capacities of fish (Kienzler et al., 2013).

5. Conclusion

Results of the present work argue for the interest of using *Sicydium* spp. as sentinel species for environmental assessment in West Indies freshwater bodies. Because of their similar responses toward the environment, both sympatric species *Sicydium punctatum* and *Sicydium*

plumieri are good candidates for biomonitoring programs that implement biomarkers of exposure and effects. In complement to the induction of hepatic EROD activity and genotoxicity endpoints, assessment of oxidative stress through lipid peroxidation would be of great value as shown in our first tests (data not shown) but still requires some technical improvements in *Sicydium* spp. for a routine use.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.aquatox.2023.106623.

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