INNSPUB

Journal of Biodiversity and Environmental Sciences (JBES)
ISSN: 2220-6663 (Print) 2222-3045 (Online)
Vol. 23, No. 3, p. 124-138, 2023
http://www.innspub.net

RESEARCH PAPER

OPEN ACCESS

Fluctuating asymmetry in larvae of *Brachythemis* sp. (Odonate: Libellulidae) in relation to habitat conditions in four small Rivers in Tropical area (Côte d'Ivoire, West Africa)

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Article published on September 11, 2023

Key words: Côte d'Ivoire, Fluctuating asymmetry, *Brachythemis* sp. Streams, Water quality

Abstract

Random and subtle deviations from bilateral symmetry (fluctuating asymmetry) have long been of interest to biologists who wish to study the susceptibility of organisms to changes in environmental quality. We examined fluctuating asymmetry (FA) in larvae of the genus *Brachythemis* sp. (Odonata: Libellulidae) from four small, relatively disturbed rivers (Banco, Anguédédou, Dougodou and Bou) in Côte d'Ivoire as a water quality assessment tool. In situ measurements of water pH, temperature, conductivity and dissolved oxygen were made at two sampling points along each river. Water was sampled and analyzed in the laboratory for nitrate, nitrite, phosphate, manganese, and ammonium concentrations. The left and right sides of 11 segments were photographed separately using a portable digital microscope (Celestron 2.0). Thus, the perimeter, the surface area of both sides of the labial palp, the ocular diameter, and the length of the different appendages (femur, tibia, and tarsus) of the 3 legs were measured using the imageJ measurement software. This study revealed that the water quality of each river affects the developmental stability of *Brachythemis* sp. We concluded that the length of the first femur (LF1) and the length of the third tibia (LT3) are useful bioindicators for this taxon because the impact of fluctuating asymmetry on these traits was strongly associated with five physicochemical parameters of each River.

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Introduction

The order Odonata, commonly known as dragonflies and damselflies, form a group of about 7000 species (Kalkman *et al.*, 2008). They have long aroused the interest of scientists, because of the importance of their roles in aquatic and terrestrial ecosystems (Corbet, 1999). At the larval stage, they can be associated with aquatic plants or the bottom substrates of watercourses (Corbet, 1999; Juen *et al.*, 2007). Numerous studies have indicated that odonates are sensitive to physical disturbance or habitat change, but tolerate a wide range of water quality conditions (Dolný *et al.*, 2012; Júnior *et al.*, 2015). Thus, morphological analysis plays an important role in many such studies, as it can be used to answer various research questions (Bell & Foster, 1994). A promising morphological analysis is fluctuating asymmetry (FA), which measures deviations from perfect morphological symmetry. Indeed, FA can offer an early warning tool to determine the effects of environmental stresses on organisms before critical changes in population and community structures (Clarke, 1993). Also, the developmental stability of any organism is indicated by its ability to maintain a normal form under the effects of certain conditions (Kozlov *et al.,* 2002). Therefore, any deviation in the shape and/or size of any morphological trait is considered a disruption of developmental mechanisms due to genetic (Clarke, 1993) or environmental (Al-Shami *et al.,* 2011) factors.

In Côte d'Ivoire, water pollution caused by anthropogenic chemical residues has become a concern in recent years. Water quality studies generally detect polluting particles in water resources (Koffi *et al.*, 2020; Ouattara *et al.*, 2021) although they are generally in low quantities. Little is known about the effect of lightly polluted water on aquatic organisms. There are many methods to assess the level of pollutant in freshwater ecosystems and its effects on aquatic organisms (Bahroun *et al.*, 2022; Charvet *et al.*, 1998; Knoben *et al.*, 1995; Ouattara *et al.*, 2021) such as biological, physical and chemical indices. Despite the fact that chemical indices are widely used, biological indices have extraordinary advantages over chemical indices (Marneffe *et al.*, 1996; Wallace *et al.*, 1996)

Although several studies focus on the impact of human activities related to aquatic organisms in the various rivers of the Ivory Coast (Camara *et al.*, 2014; Edia *et al.*, 2013; Jean-renaud *et al.*, 2022; Stevens *et al.*, 2022), none has been devoted to the use of fluctuating asymmetry in these organisms as a bioindicator of environmental stress.

Thus, the present study aims to study the degree of AF in *Brachythemis* sp. (Odonata), relate them to water quality indices in four relatively disturbed small rivers in Côte d'Ivoire with the aim of confirming their relevance as a tool for monitoring water quality.

Materials and methods

Sampling site

The data were collected in four rivers; namely Banco, Anguédédou, Dougoudou and Bou. Banco stream is located in the Banco National Park (5°23′40″ N and 4°03′07″ W) in the city of Abidjan (Fig.1). This stream receives wastewater discharges from the municipality of Abobo. The Anguédédou (5°23'13" N and 4°8'52" W) is located on the northwestern outskirts of Abidjan city. In addition to runoff water from surrounding municipalities and agricultural land, it also receives wastewater from a brewery. The Dougoudou River is located in Lauzoua area (5°19' W and 5°12' N) in the department of Guitry (Lôh Djiboua region). This river crosses an industrial manganese mining area (Nangah *et al.*, 2012). Bou is a tributary of Bandama River that crosses a diamondiferous area located in Tortiya (Department of Katiola, Hambol region). The geographic coordinates of this locality are 8°46'0" N and 5°40'60" W.

Two stations were selected on each of these hydrosystems (Fig. 1). For the Banco stream, station BAN1, close to the forestry school, receives runoff from the municipality of Abobo and station BAN2, located downstream just outside the park. The latter receives a significant amount of runoff from Abobo and the forestry school. As for the Anguédédou, ANG1 is located near a road, and bordered by a cassava field, and ANG2 which does not receive any domestic discharge. At the Dougoudou River, the both stations (DOU1 and DOU2) were chosen downstream manganese mining and bridges. In addition, DOU1 was closed to a village, cocoa plantation and rice fields while DOU2 was surrounded by fallow and cocoa plantation. On Bou River, BOU1 (upstream station) was located 5 km from the town of Tortiya. The main anthropogenic activities at this site are cashew plantation, fishing and gold panning. BOU2 (downstream station) was chosen near Tortiya, where Diamond mining, fishing, washing cars, washing and watering of oxen were observed.

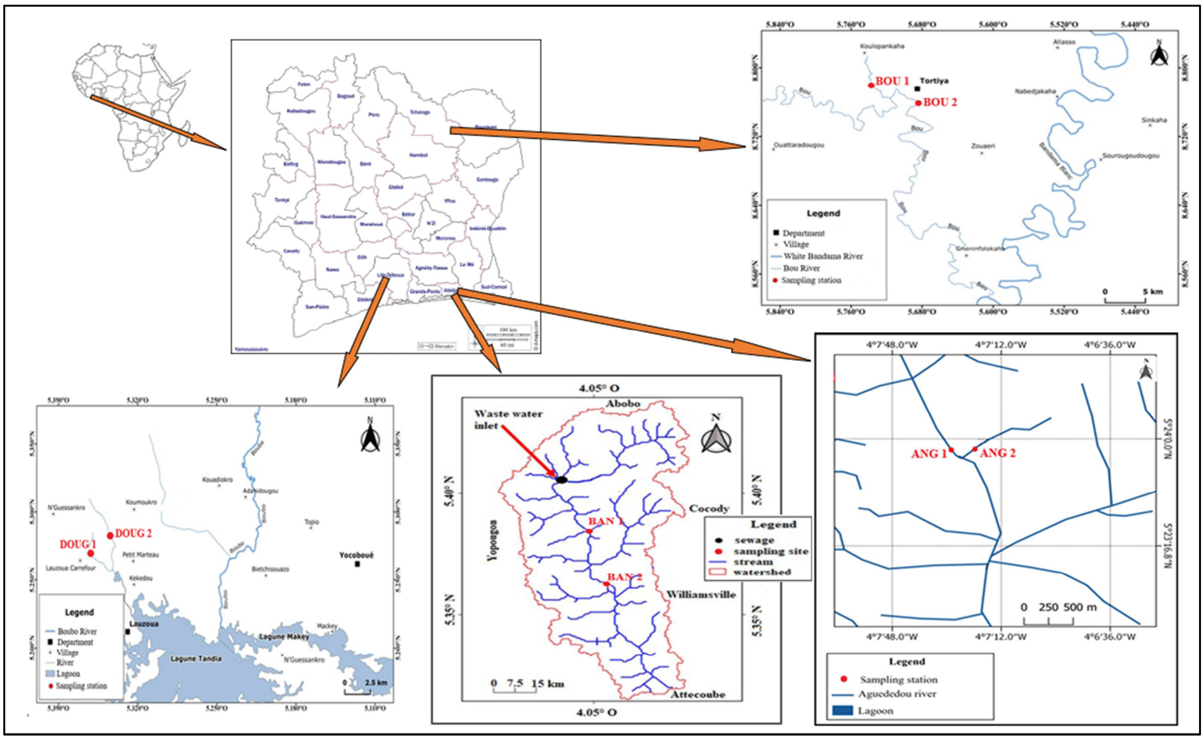

Fig. 1. Map of the study area.

Water sampling and Analysis methods

Sampling was carried out monthly from July 2020 to June 2021, with the exception of October 2020 on the Banco and Anguédédou streams. Parameters such as temperature, pH, electrical conductivity (EC) and dissolved oxygen (DO) were measured in situ using a WTW3110 multiparameter. The chemical analyses carried out in the laboratory concerned the NH_4^+, PO_4^{3-}, SO_4^{2-} and NO_3^- ions using the analysis methods recommended by the (AFNOR, 1997) standards. As for the mining sites (Bou and Dougoudou rivers), these data come from a mining project carried out in 2015 entitled 'Mapping and Assessing the Environmental and Health Impacts of Abandoned Mines in Sub-Saharan African Countries' (Ani *et al.*, 2020; Séniva *et al.*, 2022).

Odonata larvae sampling and morphometric measurement

Odonata larvae were collected from all eight stations with the kick net using the kick sampling technique (Merritt & Cummins, 1996). The contents of the fillet are then preserved in labelled plastic jars and fixed with alcohol at 76°. In the laboratory, Odonata larvae were sorted and preserved in 76° ethanol for taxonomic identification and subsequent measurement.

All Odonata specimens were identified using the taxonomic keys of (Stals, 2007; Tachet *et al.*, 2010). In the present study, the anisopteran *Brachythemis* sp. (Libellulidae) was found to be the most dominant across all study sites, so it was selected for fluctuating asymmetry investigation.

Late instar larvae were selected in this study. A total of 11 bilateral morphological traits (Table 1, Fig. 2) were selected to test the presence of FA and to relate it to environmental variables. The pairs of legs and the labial palps were dissected in a Petri dish containing glycerine to facilitate the correct orientation of each piece to be photographed. The left and right sides of each segment were photographed separately, using a portable digital microscope (Celestron 2.0) equipped with the imaging system. For all the photos, the microscope objective remained fixed and located 2 cm above each preparation. The perimeter, the area on both sides of the labial palp, the ocular diameter and the length of the different appendages (femur, tibia and tarsi) of the 3 legs were measured using the image measurement software. For all traits, measurements were taken twice following general procedures as recommended by (Palmer, 1994) and (Palmer & Strobeck, 2003).

Table 1. Different traits measured on *Brachythemis* sp. collected in four Rivers of Côte d'Ivoire.

Character measured on one side	Abbreviation
First femur length	LF1
First tibia length	LT1
First tarsus length	Lta1
Second femur length	LF2
Second tibia length	LT2
Second tarsus length	Lta2
Third femur length	LF3
Third tibia length	LT3
Third tarsus length	Lta3
Perimeter of the labial palp	PLP
Area of the labial palp	ALP

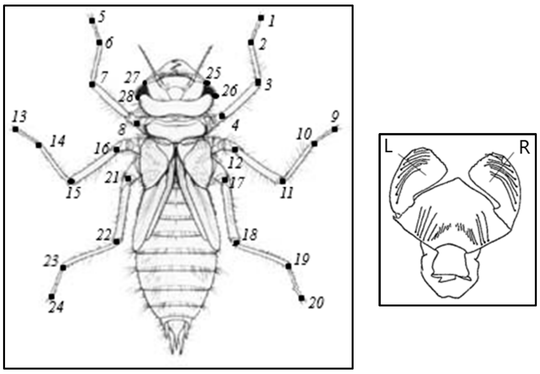

Fig. 2. Morphological characteristics measured on dragonfly larvae (WfHC, 2004): Tarsal length (1-2, 5-6, 9-10, 13-14, 19-20, 23-24); Tibial length (2-3, 6-7, 10-11, 14-15, 18-19, 22-23); Femoral length; (3-4, 7-8, 11-12, 15-16, 17-18, 21-22); Eyepiece diameter (25-26, 27-28); R: Right labial palp; L: Left labial palp.

Data analysis

The Kruskal-Wallis test is a non-parametric alternative to the one-way ANOVA. It was used to test the significance of differences between the medians of the abiotic variables of the stream studied. Where the analysis revealed significant differences, the Mann-Whitney U test was used to test the significance of pairwise differences.

For Calculation of the Water Quality Index (WQI), nine important parameters (pH, Dissolved Oxygen, Water Conductivity, Temperature, sulphate ion, phosphate ion, ammonium ion and nitrate ion were selected. This index is a water quality classification technique based on the comparison of water quality parameters with international or national Ivorian standards as part of this study. In this study, the WQI index is applied to estimate the influence of natural and anthropogenic factors on the basis of several key parameters of the surface waters of Côte d'Ivoire. This index will be calculated using the weighted arithmetic index method (Yidana & Yidana, 2010; Tyagi *et al.*, 2013; Talhaoui *et al.*, 2020). In this approach, a numerical value called relative weight (Wi), specific to each physico-chemical parameter, will be calculated according to the following formula:

$$Wi = \frac{k}{Si} \qquad (1)$$

Where:

k: constant of proportionality and can also be calculated using the following equation:

$$k = \frac{1}{\sum_{i=1}^{n}(1/Si)} \qquad (2)$$

n: number of parameters

Si: maximum value of the WHO surface water standard (WHO, 2011) of each parameter. Then, a quality assessment scale (Qi) is calculated for each parameter according to the following formula:

$$Q_i = \left(\frac{Ci}{Si}\right) \text{x } 100 \qquad (3)$$

Qi: quality assessment scale for each parameter.

Ci: the concentration of each parameter in mg/l

Finally, the overall water quality index is calculated by the following equation:

$$\mathrm{WQI} = \frac{\sum_{i=1}^{n} Qi \times Wi}{\sum_{i=1}^{n} Wi} \qquad (4)$$

The analysis of the FA assessment must meet precise statistical assumptions, the departure of which could weaken the interpretation of the results. Before comparing the samples by FA, it will be worth first identifying the outliers of the differences between the two sides of the body (R-L) by visual inspection of the point clouds. If one or more values were suspected to be extreme, the use of Grubb's test as recommended by (Palmer & Strobeck, 2003) will reduce subjectivity. Next, the two-way ANOVA test used to test measurement error. A Kolmogorov-Smirnov test was used to test the normality of the values (R-L) in order to show the absence of anti-symmetry. The absence of directional asymmetry was tested with a t-test comparing the mean (R-L) to zero (Palmer, 1994; Palmer & Strobeck, 1986). Biased FA level results could be found when these levels depend on the size of traits within and between samples (Palmer, 1994). Finally, the Pearson correlation was performed between FA [| R - L |] and character size [(R + L) / 2] for each sample.

In this study, FA levels were calculated as the average of the difference in absolute value between the measurements of the right and left sides |R-L| for each trait (FA1=mean |R-L|) (Palmer, 1994).

To test for differences in FA between samples, multivariate tests were performed using one-way ANOVA and non-parametric one-way ANOVA (Almeida *et al.*, 2008; Chang *et al.*, 2009; Fessehaye *et al.*, 2007). The results were corrected using the Bonferroni adjustment (Chang *et al.*, 2009). To verify the extent of the influence of disturbance on the asymmetry, a linear regression between the AF1 index and the chemical variables was carried out. All these statistical analyses were performed using R software (RStudio Team, 2020) with a level of significance p = 5%.

Results

Physico-chemical analyzes

The Kruskal-Wallis test makes it possible to differentiate the physico-chemical variables of the eight sites. Thus, all the parameters vary significantly from one station to another with the exception of dissolved oxygen and phosphate (Table 2).

Table 2. Values of physico-chemical parameters (median ± SD) at the eight sampling sites (median values ± standard deviation. Different letters in the same line indicate significant differences (p < 0.05, according to the Kruskal-Wallis test)).

Parameters	DOU1	DOU2	BOU1	BOU2	ANG1	ANG2	BAN1	BAN2
	median ± SD	median ± SD	median ± SD	median ± SD	median ± SD	median ± SD	median ± SD	median ± SD
T (°C)	27.20ab ± 1.17	26.9 ab ± 2.00	30.50a ± 2.60	28.20 ab ± 2.16	26.16 ab ± 0.72	26.2 ab ± 0.93	25.75b ± 0.55	25.65b ± 0.45
CND (µS/cm)	192.10a ± 113.10	176.60a ± 88.90	150.02ac ± 34.10	107.3ac ± 113.65	25.26b ± 2.90	26.25b ± 6.64	41.00bc ± 26.74	40.00bc ± 16.24
pH	6.93a ± 0.47	6.84a ± 0.54	6.82a ± 0.30	6.68ac ± 0.34	5.47b ± 0.65	5.63bc ± 0.54	5.86bc ± 0.41	5.76b ± 0.29
DO (mg/L)	4.20a ± 2.24	4.31a ± 2.31	4.84a ± 2.30	4.26a ± 1.98	2.9a ± 1.49	2.83a ± 1.80	4.21a ± 1.75	3.40a ± 1.86
Turb (NTU)	39.35acd ± 23.09	10.55a ± 6.25	51.31ab ± 20.2	22.21abc ± 38.25	4.68d ± 5.51	8.04abcd ± 27.96	58.60c ± 27.05	42.60ac ± 23.39
TDS	95.97a ± 57.46	64.35a ± 22.88	71.00a ± 16.90	71.15a ± 54.54	7.66b ± 5.46	13.25b ± 3.03	16.00b ± 14.17	16.70b ± 8.04
Nitra (mg/L)	3.5a ± 13.02	6.85 ± 11.48	4.25 ± 7.60	5.05 ± 16.34	5.00 ± 1.96	7.00 ± 3.11	8.20 ± 2.57	12.10b ± 3.36
Nitri (mg/L)	0.03a ± 0.02	0.02 ± 0.05	0.02 ± 0.00	0.02 ± 0.05	0.01b ± 0.01	0.01b ± 0.01	0.02 ± 0.00	0.02 ± 0.01
Am (mg/L)	0.54a ± 0.24	0.42a ± 0.25	0.41a ± 2.10	1.18a ± 2.25	0.07b ± 1.61	0.07b± 0.16	0.11 ± 0.53	0.17 ± 0.56
Phos (mg/L)	0.30 ± 0.87	0.22 ± 0.29	0.21 ± 1.20	0.30 ± 0.64	0.05 ± 0.18	0.14 ± 0.13	0.09 ± 0.10	0.10 ± 1.10

Indeed, the temperature varies from 25.65 ± 0.45 (BAN2) to 30.50 ± 2.60 (BOU1). This temperature changes significantly between BOU1 and the both sites of Banco stream. Turbidity varies significantly between ANG1 (4.68 ± 5.51) and BAN1 (58.60 ± 27.05). Nitrate levels are low in DOU1 (3.5 ± 13.02) and high levels are recorded in BAN2 (12.10 ± 3.36). Nitrite varies significantly between 0.01 ± 0.01 (ANG1, ANG2) and 0.03 ± 0.02 (DOU1). For conductivity, TDS, ammonium, iron and manganese, the results of the statistical tests indicate a significant difference between the stations in the mining areas (BOU1, BOU2, DOU1, DOU2) and the stations located in the peri-urban areas of Abidjan (BAN1, BAN2, ANG1, ANG2).

Water Quality Index (WQI)

After calculating the overall quality index of the IQE using the results of physico-chemical analyzes and the standard values of the WHO standard for drinking water. The water quality class is determined for the 8 sampling stations. Thus, the good quality class identifies the station Anguédédou2 (ANG2); the poor-quality class for the Dougoudou2 (DOU2), Angueledou1 (ANG1), Banco1 (BAN1) and 2 (BAN1) stations; very poor quality and undrinkable classes are recorded respectively at the Dougoudou1 (DOU1), Bou1 and Bou2 stations (Table 3).

Table 3. Results of the WQI index and water quality class of four different rivers in Côte d'Ivoire.

Station	WQI	Quality classes
DOU1	87.50	Very poor quality
DOU2	70.67	poor quality
BOU1	184.66	Non-drinking water
BOU2	279.93	Non-drinking water
BAN1	61.09	poor quality
BAN2	62.67	poor quality
ANG1	57.11	poor quality
ANG2	27.07	Good quality

Asymmetry and measurement error models

A total of 374 specimens of Odonate larvae are examined for the presence of fluctuating asymmetry. Of the data set, 8.51% are outliers. After removing outliers, the effect of measurement errors on asymmetry was assessed for each trait and for each site using a two-way ANOVA. The sides*individuals interaction revealed that there was no significant difference (p > 0.05) between the sides for each trait, indicating an absence of directional asymmetry (DA) (Table 4).

Table 4. The results of the two-way (error) ANOVA performed for each selected trait on larvae of the genus *Brachythemis* sp. collected in four rivers of Côte d'Ivoire.

Trait	BOU1		BOU2		DOU1		DOU2	
	F	p	F	p	F	p	F	p
LF1	0.002	0.967	0.023	0.880	0.003	0.959	0.001	0.973
LT1	0.011	0.916	0.001	0.970	0.007	0.934	0.005	0.945
Lta1	0.003	0.955	0.012	0.912	0.095	0.757	0.036	0.848
LF2	0.000	0.996	0.003	0.957	0.092	0.762	0.050	0.823
LT2	0.002	0.968	0.008	0.931	0.001	0.973	0.016	0.899
Lta2	0.072	0.787	0.076	0.783	0.018	0.893	0.055	0.814
LF3	0.002	0.961	0.000	0.993	0.007	0.933	0.030	0.862
LT3	0.004	0.949	0.011	0.918	0.060	0.806	0.000	0.994
Lta3	0.000	0.995	0.005	0.943	0.006	0.936	0.012	0.914
DO	0.003	0.952	0.001	0.977	0.204	0.652	0.000	0.991
PLP	0.011	0.918	0.002	0.966	0.019	0.891	0.000	0.992
ALP	0.000	0.988	0.001	0.978	0.001	0.971	0.001	0.977
	ANG1		ANG2		BAN1		BAN2	
	F	p	F	p	F	p	F	p
LF1	0.002	0.968	0.078	0.780	0.000	0.991	0.001	0.973
LT1	0.008	0.927	0.009	0.924	0.001	0.973	0.001	0.980
Lta1	0.000	0.987	0.010	0.919	0.958	0.958	0.051	0.821
LF2	0.012	0.914	0.004	0.945	0.000	0.978	0.003	0.955
LT2	0.004	0.95	0.004	0.948	0.040	0.841	0.002	0.959
Lta2	0.022	0.881	0.008	0.927	0.002	0.964	0.000	0.993
LF3	0.001	0.972	0.009	0.921	0.002	0.964	0.001	0.976
LT3	0.000	0.986	0.003	0.951	0.037	0.848	0.000	0.989
Lta3	0.002	0.965	0.225	0.635	0.039	0.842	0.001	0.979
DO	0.000	0.996	0.004	0.945	0.005	0.940	0.000	0.981
PLP	0.040	0.841	0.031	0.861	0.000	0.985	0.000	0.986
ALP	0.010	0.919	0.052	0.820	0.000	0.998	0.000	0.990

For each trait and for each station, all the measurements show a normal distribution and a zero mean, with the exception of LT1, Lta2 and DO (BOU1); Lta3 and DO (DOU1); LT1, LF3 and DO (DOU2); LT1, Lta1, Lta2, LF3, and DO (ANG1); Lta1; Lta2, LF3, Lta3 and DO (ANG2); LT1, Lta3 and DO (BAN1) and finally Lta1, Lta2, Lta3 and DO (BAN2). The assumption of normality being satisfactory for these characters, so that there is no evidence of antisymmetry and directional asymmetry (Table 5). Therefore, we can assume that these traits exhibit fluctuating asymmetry. Of these characters, six (LF1, LF2, LT2, LT3 PLP and ALP) exhibit FA at all stations. These characters will be used for the determination of FA in this study.

Table 5. Distribution of normality using Kolmogorov-Smirnov d-test and zero-mean tests of R-L values using one-sample t-test for all variables (p = p value ; K–S: Kolmogorov-Smirnov test).

Traits	BOU1				BOU2				DOU1				DOU2			
	Test for mean = 0		Test for normality		Test for mean = 0		Test for normality		Test for mean = 0		Test for normality		Test for mean = 0		Test for normality	
	t test	p	d (K–S)	p	t test	p	d (K–S)	p	t test	p	d (K–S)	p	t test	p	d (K–S)	p
LF1	-0.680	0.501	0.175	0.280	1.544	0.133	0.178	0.292	-0.802	0.426	0.143	0.263	-0.235	0.815	0.161	0.353
LT1	2.040	0.049	0.202	0.143	-0.604	0.550	0.209	0.143	-0.188	0.851	0.145	0.253	1.073	0.291	0.413	3.5e-5
Lta1	0.462	0.647	0.181	0.258	0.864	0.394	0.175	0.331	-0.100	0.920	0.183	0.074	-1.200	0.238	0.158	0.378
LF2	-1.508	0.141	0.189	0.199	0.515	0.610	0.255	0.051	-0.905	0.369	0.167	0.126	-0.383	0.704	0.166	0.337
LT2	-1.071	0.292	0.171	0.300	-1.948	0.061	0.158	0.460	-0.343	0.733	0.194	0.057	0.250	0.803	0.138	0.570
Lta2	0.000	1.000	0.273	0.016	0.509	0.613	0.203	0.165	-0.589	0.558	0.173	0.099	-1.646	0.109	0.239	0.051
LF3	0.304	0.763	0.166	0.353	-0.787	0.437	0.198	0.190	1.069	0.290	0.172	0.106	-2.200	0.034	0.187	0.182
LT3	1.161	0.255	0.183	0.261	-1.201	0.239	0.179	0.286	0.475	0.636	0.150	0.209	0.436	0.665	0.173	0.259
Lta3	-0.229	0.820	0.214	0.105	-0.586	0.562	0.218	0.127	-0.368	0.714	0.254	0.003	0.155	0.877	0.148	0.477
DO	1.853	0.073	0.267	0.017	0.319	0.751	0.213	0.142	-0.274	0.784	0.250	0.004	-1.559	0.128	0.260	0.019
PLP	0.656	0.516	0.117	0.787	0.462	0.647	0.132	0.684	-0.985	0.329	0.117	0.522	0.468	0.642	0.124	0.682
ALP	-1.861	0.072	0.113	0.836	0.230	0.819	0.109	0.867	0.035	0.971	0.092	0.806	0.347	0.730	0.074	0.991

Traits	ANG1				ANG2				BAN1				BAN2			
	Test for mean = 0		Test for normality		Test for mean = 0		Test for normality		Test for mean = 0		Test for normality		Test for mean = 0		Test for normality	
	t test	p	d (K–S)	p	t test	p	d (K–S)	p	t test	p	d (K–S)	p	t test	p	d (K–S)	p
LF1	-0.188	0.851	0.136	0.441	-0.102	0.918	0.116	0.305	0.558	0.578	0.122	0.234	0.796	0.430	0.130	0.538
LT1	0.297	0.767	0.229	0.036	1.498	0.138	0.150	0.080	0.289	0.772	0.194	0.008	-0.466	0.643	0.214	0.060
Lta1	0.725	0.472	0.224	0.035	1.435	0.155	0.207	0.004	1.551	0.125	0.156	0.058	-2.255	0.030	0.186	0.143
LF2	-1.5e-^{9}	1.000	0.169	0.214	-1.652	1.103	0.144	0.112	-0.144	0.885	0.144	0.111	-1.732	0.091	0.168	0.257
LT2	0.000	1.000	0.173	0.191	0.499	0.618	0.157	0.066	-0.437	0.663	0.145	0.094	-1.480	0.147	0.176	0.197
Lta2	-1.865	0.069	0.263	0.008	0.814	0.417	0.269	5.7e-5	-0.092	0.926	0.159	0.060	2.537	0.015	0.152	0.342
LF3	2.614	0.012	0.229	0.032	-0.608	0.544	0.134	0.153	0.279	0.781	0.133	0.157	1.957	0.057	0.161	0.261
LT3	1.138	0.262	0.162	0.257	-1.354	0.179	0.168	0.303	-0.959	0.340	0.120	0.267	1.362	0.181	0.141	0.452
Lta3	1.462	0.152	0.198	0.099	-0.242	0.809	0.193	0.009	-2.720	0.008	0.218	0.002	3.450	0.0014	0.192	0.139
DO	0.190	0.850	0.229	0.029	-2.837	0.005	0.248	0.0002	-4.330	4.8e-05	0.251	0.0002	-2.434	0.019	0.275	0.005
PLP	-0.396	0.694	0.134	0.528	-0.510	0.611	0.075	0.813	-1.951	0.055	0.050	0.995	0.600	0.552	0.114	0.699
ALP	0.587	0.560	0.114	0.666	1.172	0.245	0.051	0.991	-1.887	0.063	0.083	0.691	1.057	0.297	0.152	0.353

AF Size dependency

The results of the Pearson correlations of | R–L| (the absolute difference between the right and left measurements of a specific character) versus (R+L)/2 (an indicator of character size) are shown in Table 6. These results indicated that the asymmetry in APL at BOU1 (r = 0.373, p < 0.05), BOU2 (r=0.476, p<0.001), DOU1 (r=0.306, p<0.05), ANG1 (r=0.372, p<0.05), ANG2 (r=0.439, p<0.001), BAN1 (r=0.384, p<0.001), BAN2 (r=0.637, p<0.001), in PPL at BOU2 (r = 0.591, p < 0.001), ANG2 (r=0.241, p<0.05), in LT3 at the BAN1 level (r = 0.241, p < 0.05), BAN2 (r = 0.355, p < 0.05) and in LT2 at ANG1 (r = 0.362, p < 0.05) indicated significantly positive correlations of asymmetry with feature size.

The fluctuating asymmetry index

The results of the various fluctuating asymmetry indices, namely AF1 = mean |R-L| are given in Table 7. These results show that the indices of the different characters studied vary from one station to another.

For the length of femur1 (LF1), the AF1 index varies significantly (p<0.05) from 0.051 ± 0.041 (BOU1) to 0.105 ± 0.079 (BAN1). At the level of the length of the femur2 (LF2), this index varies significantly (p<0.05) from 0.053 ± 0.043 (BOU1) to 0.097 ± 0.061 (BAN1).

In addition, for the perimeter of the labial palp (PPL) and the surface of the labial palp (APL), they vary very significantly (p<0.001) respectively from 0.070 ± 0.051 (BAN1) to 0.267 ± 0.220 (ANG2) and 0.048 ± 0.044 (BAN2) to 0.103 ± 0.090 (BAN1). However, for tibia length2 (LT2) and tibia length3 (LT3), this index does not indicate a significant variation (p>0.05) between the different stations.

Table 6. Descriptive statistics of selected traits of *Brachythemis* sp. larvae collected in different rivers of Côte d'Ivoire for fluctuating asymmetry.

	(R+L)/2			Pearson's r value	R-L				
Station	Trait	N	Mean ± SD		Mean ± SD	Skew ness		Kurtosis	
						T	p	T	p
BOU1	LF1	32	3.251 ± 1.500	0.165	-0.007 ± 0.065	-0.098	0.803	2.200	0.207
	LF2	32	4.203 ± 2.061	0.327	-0.018 ± 0.066	0.078	0.832	2.251	0.269
	LT2	32	3.639 ± 1.680	0.048	-0.016 ±0.083	-0.213	0.582	2.070	0.158
	LT3	30	5.321 ± 2.474	0.100	0.017 ± 0.079	0.330	0.401	2.171	0.230
	PLP	31	7.119 ± 3.054	0.121	0.022 ± 0.183	0.529	0.185	3.198	0.821
	ALP	30	3.058 ± 2.553	0.373^{*}	0.029 ± 0.082	0.705	0.070	2.533	0.542
BOU2	LF1	30	3.252 ± 1.501	0.191	0.030 ± 0.104	0.105	0.773	2.352	0.362
	LF2	30	4.204± 2.061	-0.147	-0.002 ± 0.075	-0.436	0.252	1.965	0.112
	LT2	30	3.639 ± 1.688	0.005	-0.032 ± 0.089	0.037	0.924	2.893	0.906
	LT3	30	5.321 ± 2.474	0.174	-0.019 ± 0.087	0.310	0.424	2.875	0.882
	PLP	30	7.119 ± 3.054	0.591^{***}	0.009 ± 0.137	-0.241	0.521	2.714	0.712
	ALP	31	3.058 ± 2.553	0.476^{**}	0.001 ± 0.073	0.625	0.101	3.452	0.542
DOU1	LF1	49	3.467 ± 0.721	0.257	-0.012 ± 0.101	0.088	0.778	2.102	0.092
	LF2	49	4.198 ± 0.805	0.170	-0.008 ± 0.104	-0.259	0.408	2.450	0.353
	LT2	50	3.796 ± 0.669	0.196	0.000 ± 0.091	0.247	0.426	2.681	0.626
	LT3	50	5.222 ± 0.985	-0.076	0.006 ± 0.094	0.090	0.771	2.602	0.503
	PLP	48	5.630 ± 1.376	0.195	-0.025 ± 0.177	0.199	0.524	3.031	0.963
	ALP	48	1.909 ± 1.150	0.306^{*}	0.000 ± 0.069	0.612	0.063	3.530	0.388
DOU2	LF1	33	2.769 ± 0.622	-0.013	-0.004 ± 0.094	0.105	0.762	2.750	0.745
	LF2	32	3.319 ± 0.810	0.244	-0.008 ± 0.117	-0.127	0.731	2.104	0.152
	LT2	32	2.960 ± 0.602	0.232	0.006 ± 0.134	-0.394	0.29	2.824	0.817
	LT3	34	4.190 ± 0.989	0.113	-0.019 ± 0.102	0.088	0.794	2.131	0.158
	PLP	33	6.360 ± 1.935	0.087	0.012 ± 0.143	0.109	0.752	2.000	0.118
	ALP	34	2.478 ± 1.809	-0.102	0.004 ± 0.070	0.119	0.744	2.715	0.700
ANG1	LF1	40	3.070 ± 0.664	0.198	0.033 ± 0.101	-0.482	0.170	2.476	0.426
	LF2	39	3.718 ± 0.853	-0.068	0.000 ± 0.102	1.9e-^{9}	1.000	1.957	0.073
	LT2	39	3.35 ± 0.765	0.362^{*}	0.000 ± 0.100	0.082	0.806	2.215	0.199
	LT3	39	4.51 ± 1.113	0.104	0.019 ± 0.107	-0.128	0.689	2.455	0.410
	PLP	36	5.066 ± 1.120	0.001	-0.011 ± 0.174	0.240	0.516	2.512	0.483
	ALP	37	1.572 ± 0.684	0.372^{*}	0.010 ± 0.068	0.195	0.591	2.225	0.224
ANG2	LF1	69	3.667 ± 0.719	0.212	-0.002 ± 0.126	-0.073	0.777	2.273	0.143
	LF2	69	4.349 ± 0.924	0.189	-0.015 ± 0.092	-0.294	0.308	2.276	0.124
	LT2	71	3.898 ± 0.840	0.158	-0.026 ± 0.115	0.127	0.636	2.627	0.461

	LT3	68	5.519 ± 1.350	0.122	-0.049 ± 0.101	0.119	0.656	2.048	0.063
	PLP	70	6.073 ± 1.174	0.241	-0.022 ± 0.363	0.103	0.716	3.018	0.979
	ALP	70	2.137 ± 0.822	0.439***	0.018 ± 0.127	-0.114	0.679	3.179	0.737
BAN1	LF1	72	3.859 ± 0.493	0.040	0.008 ± 0.120	-0.230	0.385	2.534	0.371
	LF2	69	4.783 ± 0.709	0.120	-0.002 ± 0.105	-0.243	0.393	3.090	0.865
	LT2	72	4.355 ± 0.640	0.053	-0.006 ± 0.119	-0.100	0.697	2.949	0.927
	LT3	69	6.520 ± 1.099	0.241	-0.015 ± 0.127	0.387	0.171	3.215	0.685
	PLP	67	6.888 ± 0.992	-0.185	-0.020 ± 0.085	0.234	0.405	2.545	0.382
	ALP	72	2.913 ± 0.740	0.384***	-0.030 ± 0.134	-0.004	0.999	3.919	0.056
BAN2	LF1	38	4.770 ± 1.209	0.104	0.017 ± 0.129	0.065	0.842	2.944	0.942
	LF2	37	5.523 ± 1.443	-0.079	-0.039 ± 0.109	0.060	0.843	2.817	0.814
	LT2	37	5.228 ± 1.433	-0.008	-0.019 ± 0.077	-0.063	0.859	2.298	0.268
	LT3	37	6.839 ± 1.781	0.355*	0.024 ± 0.107	0.195	0.579	2.709	0.660
	PLP	38	6.788 ± 1.894	0.171	0.015 ± 0.105	-0.210	0.563	2.635	0.625
	ALP	37	2.649 ± 1.370	0.637***	0.011 ± 0.065	0.338	0.358	2.817	0.812

N: sample size; (D+G)/2: average character length (mm); D-G: difference between the right and left sides of the character considered (mm); SD: standard deviation; * p < 0.05; ** p < 0.01; *** p < 0.001

Relationship between AF1 indices and chemical parameters

The results of the linear regression test between the AF1 index and the environmental parameters and the water quality index (WQI) are given in Table 8. These results reveal that out of the six (6) trait studied, two (2) are influenced by the physico-chemical parameters. These are the length of the first femur (LF1) and the length of the third tibia (LT3). Indeed, the length of the first femur (LF1) is influenced by temperature (84%), pH (58%), dissolved oxygen (62%), phosphorus (56%) and iron at 57%. For the length of the third tibia (LT3), in addition to the water quality index (76%), six (6) physico-chemical parameters have an influence on this character, namely, temperature (74%), pH (68%), dissolved oxygen (53%), ammonium (67%), phosphorus (67%) and iron (87%).

Table 7. Fluctuating asymmetry indices for each trait (median ±SD) of *Brachythemis* sp. collected in four different rivers in Côte d'Ivoire.

Station	LF1	LF2	LT2	LT3	PPL	APL
	median ± SD	median ± SD	median ± SD	median ± SD	median ± SD	median ± SD
BOU1	0.051^{b} ± 0.041	0.053^{b} ± 0.043	0.067 ± 0.050	0.063 ± 0.049	0.135^{a} ± 0.124	0.064^{a} ± 0.057
BOU2	0.089^{ab} ± 0.061	0.061^{ab} ± 0.042	0.069 ± 0.063	0.065 ± 0.058	0.105^{a} ± 0.086	0.053^{a} ± 0.048
DOU1	0.084^{ab} ± 0.057	0.097^{a} ± 0.061	0.070 ± 0.056	0.074 ± 0.056	0.141^{a} ± 0.107	0.052^{a} ± 0.045
DOU2	0.073^{ab} ± 0.057	0.096^{a} ± 0.057	0.100 ± 0.086	0.085 ± 0.055	0.120^{a} ± 0.075	0.053^{a} ± 0.043
ANG1	0.101^{a} ± 0.060	0.084^{ab} ± 0.055	0.081 ± 0.056	0.081 ± 0.071	0.137^{a} ± 0.104	0.055^{a} ± 0.041
ANG2	0.099^{a} ± 0.070	0.075^{ab} ± 0.056	0.057 ± 0.049	0.092 ± 0.064	0.267^{b} ± 0.220	0.101^{b} ± 0.079
BAN1	0.105^{a} ± 0.079	0.079^{ab} ± 0.069	0.088 ± 0.079	0.097 ± 0.082	0.070^{c} ± 0.051	0.103^{b} ± 0.090
BAN2	0.097^{ab} ± 0.085	0.087^{ab} ± 0.075	0.063 ± 0.047	0.082 ± 0.071	0.116^{a} ± 0.094	0.048^{a} ± 0.044
p	0.018	0.022	0.297	0.486	< 0.001	< 0.001

median values ± standard deviation. Different letters in the same column indicate significant differences (P <0.05, according to the Kruskal-Wallis test). SD : Standard Deviation

Table 8. Results of the linear regression between the AF1 index and the water quality index (WQI) as well as the physico-chemical parameters.

Parameters	LF1		LF2		LT2		LT3		PPL		APL	
	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2	p	R^2
IQE	0.273	0.194	0.059	0.473	0.673	0.031	0.004	0.764	0.414	0.113	0.397	0.121
T	0.001	0.847	0.089	0.405	0.638	0.039	0.005	0.746	0.926	0.001	0.540	0.065
CND	0.070	0.446	0.775	0.014	0.301	0.175	0.074	0.436	0.587	0.051	0.199	0.257
pH	0.027	0.581	0.792	0.0125	0.792	0.012	0.012	0.680	0.578	0.054	0.304	0.173
DO	0.019	0.626	0.820	0.009	0.376	0.132	0.039	0.532	0.087	0.409	0.359	0.140
Turb	0.600	0.046	0.515	0.073	0.710	0.024	0.455	0.096	0.334	0.155	0.487	0.083
Nitra	0.783	0.013	0.972	0.009	0.733	0.020	0.183	0.273	0.273	0.194	0.300	0.175
Nitri	0.336	0.153	0.740	0.019	0.392	0.123	0.108	0.372	0.496	0.080	0.256	0.207

Am	0.341	0.150	0.054	0.485	0.675	0.031	0.011	0.679	0.454	0.096	0.383	0.128
Phos	0.031	0.560	0.450	0.098	0.832	0.008	0.012	0.674	0.786	0.013	0.336	0.154
Fe	0.028	0.577	0.266	0.200	0.96	0.000	0.001	0.875	0.616	0.044	0.275	0.193
Mn	0.407	0.116	0.182	0.274	0.114	0.362	0.516	0.073	0.778	0.014	0.254	0.209

R^2: coefficient of determination, p: p-value

Discussion

The results obtained during this study showed that the water temperature varies from 25.65°C to 30.50°C with an average value of 27.07°C. This range of variation is in agreement with the work of Ahoussi (2010) and Yao (2018) in the waters of the Greater Abidjan region (25.5 to 30.4°C). These results are similar to other work on surface waters such as Eblin *et al.* (2014) who report, that in the humid tropics, the average water temperature is about 30°C. According to the work of Dudka & Adriano (1997), when in a mining environment the average water temperature is below 30°C, this could influence Acid Mine Drainage (AMD), which is the most important environmental problem of the extractive industry. Indeed, it could accelerate the oxidation reactions of sulfide minerals releasing the metals present in these minerals. These results are in agreement with the work of Yao (2018) who conducted a study on water resources in the vicinity of the Afema mining site (30°C). The pH of these waters range from 5.47 to 6.93 with an average of 6.25. In fact, this average pH shows that the waters studied are acidic as a whole. On the other hand, it should be noted that the stations located in the peri-urban area of the city of Abidjan (Banco and Anguédédou) record relatively low pH values (5.47 to 5.86). The acidity of this area could be attributed to the pH of the substrate because according to Bourlière (1972), the soil in the Banco and Anguédédou is acidic, the physico-chemical characteristics of a river being closely linked to the nature of the soil in its watershed (Arienzo *et al.,* 2001). Electrical conductivity varies from 25.26µS/cm to 192.10µS/cm, with an average of 94.82µS/cm. This shows that the waters are largely moderately mineralized and therefore do not contain sufficient dissolved mineral salts. However, the waters in the mining areas have a high mineralization well above the raw water values. This is the case for stations BOU1 (150.02µS/cm), BOU2 (107.3µS/cm), DOU1 (192.10µS/cm) and DOU2 (176.60µS/cm). This high mineralization in these streams is related to intense anthropogenic activities upstream of the area (mining activities) Eblin *et al.* (2014) report that anthropogenic activity is an intervening phenomenon in mineralization by surface inputs.

The WQI water quality index indicates that only station ANG2 has good water quality (25 <WQI ≤ 50), or 12.5% of the stations. Half of the stations (BAN1, BAN2, ANG1 and DOU2), have poor water quality (50 <WQI ≤75). The remaining three stations, representing 37.5% and located in mining areas have very poor water quality (75 <EQI ≤100) for station DOU1 and non-potable water (EQI> 100) for stations BOU1 and BOU2. The increasing degree of water degradation in all these stations except ANG1 is related to anthropogenic activities in the mining areas as well as the increasing demographic and urbanistic impact in the periurban area of the city of Abidjan. According to (Renaud-Hellier, 2006), urban development makes a significant contribution to the anthropogenic pressure on water resources. Cities are variously held responsible for the state of water resources both above and below ground, especially for pollutants from industrial and agricultural activities (Lanmandjekpogni *et al.*, 2019).

The outliers (8.51%) far exceed those observed in similar studies by Bechshoft *et al.* (2008) who identified only 2.1% while Palmer & Strobeck (2003) reported that only 5.7% of their data as outliers. However, this percentage is lower than the outliers recorded by Al-Shami *et al.* (2014) on Odonata larvae (9.33%) collected in a Malaysian river. It has been suggested that a high percentage of outliers is related to the appearance of malformations (Servia *et al.*, 2004). In this case, the lower percentage of outliers in this study could indicate a lower occurrence of deformations in Odonata larvae inhabiting these different (moderately polluted) Rivers.

In general, the direct relationship between FA, pollution and environmental stresses remains unclear, as it has always been difficult to prove which pollutant is responsible for developmental instability in aquatic organisms. Despite this, some studies have proven that heavy metal contaminations as well as organic pollutants are the main factors influencing the normal development of aquatic insects (Al-Shami *et al.,* 2011).

In our study, although odonata are able to survive relatively high levels of pollution, for all measured traits, the length of the first femur and the length of the third tibia (LT3) seem to be influenced by physico-chemical parameters. Thus these two traits are influenced by temperature, pH, dissolved oxygen, phosphate and iron. The large number of morphological traits and environmental variables measured in this study supports the suggestion made by several authors that in order to determine the relationship between asymmetry and pollution, a large set of variables is needed, as AF cannot be detected by all environmental stressors individually (Clarke, 1995; Hogg *et al.*, 2001; Leary & Allendorf, 1989). In this sense, Clarke *et al.* (1995) emphasized that AF is the result of the combined effect of several environmental variables rather than single variables. Indeed, the present results showed that the AF1 index of both traits (LF1 and LT3) of *Brachythemis* sp. larvae (Odonata) was strongly associated with phosphate. Thus, this implies that the fluctuating asymmetry in these traits was strongly induced by organic pollution. This trend is similar to various studies reported on chironomid larvae (Al-Shami *et al.,* 2011) and odonata larvae (Al-Shami *et al.*, 2014). In addition, numerous reports in the literature have documented the effects of several organic and inorganic substances, including heavy metals, alone or in combination, on the morphological alteration of insect larvae (Bhattacharyay *et al.,* 2005; MacDonald & Taylor, 2006). As a result, the length of the first femur and the length of the third tibia can be considered the most sensitive structures in *Brachythemis* sp. larvae and their development is easily affected by pollution.

Conclusion

The present study made it possible to highlight the physico-chemical characteristics, the water quality index (WQI) and the fluctuating asymmetry indices (FA) of eleven bilateral traits measured in the larvae of B*rachythemis* sp. (Odonata) from four streams in Côte d'Ivoire. These are the Anguédédou, Banco, Dougoudou and Bou streams. The results show that the waters of these rivers are acidic. These waters are poorly mineralised with a conductivity of between 25.26 and 192.10µS/cm. The waters located in the mining areas are the most mineralised. The stations with high mineralization are BOU1, BOU2, DOU1 and DOU2. Furthermore, conductivity, TDS, ammonium, iron and manganese show a significant difference between the stations in the mining areas (BOU1, BOU2, DOU1, DOU2) and the stations located in the peri-urban areas of Abidjan (BAN1, BAN2, ANG1, ANG2). This result is in line with the water quality index, which indicates that the stations in the mining areas have a higher level of water quality. However, the stations located in the Abidjan area, with the exception of ANG2, show a deterioration of their water quality. Our study shows a relationship between the degradation of water quality and the increase of FA in the larval population of Odonata. Thus, the length of the first femur (LF1) and the length of the third tibia (LT3) are influenced by temperature, pH, dissolved oxygen, phosphate and iron. Indeed, measuring FA in *Brachythemis* sp. from different stressed rivers would be a useful technique to include in the overall bioassessment methods. However, the correct application of the linear regression method between environmental parameters and the asymmetry index, such as the FA1 index, allows the deduction of environmental stressors in the morphological alterations of Odonata larvae. In this context, the selection of appropriate traits and robust statistical analyses will improve the reliability of FA as a practical bioindicator of aquatic ecosystem health. This would facilitate the identification of the effects of pollution on the developmental instability of aquatic organisms.

Acknowledgments

Data collection was funded by the United Nations Educational, Scientific and Cultural Organization / SIDA (UNESCO-SIDA) as part of a project entitled "Ecological quality of the Rivers of the mining basins of TORTIYA (Zone Diamantaire), LAUZOUA (Manganese Zone) and HIRE (Auriferous Zone) (Ivory Coast)" and the PASRES (Strategic Support Program for Scientific Research) (Ivory Coast) within the framework of a scholarship granted to the best master's students. Thanks to all anonymous reviewers and the editor for their constructive comments and suggestions which have considerably improved the article.

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