Original Research Article

Assessing the diversity of metazoan parasites of *Sarotherodon melanotheron* (Cichlidae) from inlands waters in Southern Benin (west Africa)

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ABSTRACT

Aims: This paper presents a snapshot of the metazoan parasite fauna of *Sarotherodon melanotheron* collected from two stations across lake Nokoué.
Study design: Fish samplings were done at the landing stage of Lake Nokoué (station 1) and Tota (station 2) from october 2016 to march 2017. Water physicochemical parameters such as pH, salinity, temperature, and conductivity were measured monthly.
Methodology: A total of 246 specimens of *Sarotherodon melanotheron* comprising 149 males and 97 females were investigated. Size and weight were measured. Then,fish were dissected and parasites were checked in several organs. Collected parasite were identified using adequate keys of identification. The chi square test was used to compare the prevalence of different parasite groups whereas Student T-test was applied to assess difference between parasite mean abundance within the two sampling stations. The pearson correlation coefficient was used to appraise possible influence of water parameters on parasite prevalence.
Results: The total prevalence was 86.58%. Eight parasite species were collected belonging to Myxosporidia, Monogena, Digena, Cestoda, Acanthocephala and Copepoda. Epidemiological parameters were calculated in order to appreciate the diversity of the parasitofauna according to sampling site, sex, and fish size. Females were significantly more infected than males ($\chi 2$ = 10.093; $p < .05$). Among the parasites, only monogenean prevalence (41.46%) was significantly different ($\chi 2$ = 149.645; $p < .05$). Correlation between physicochemical parameters and the prevalence of parasite groups showed that monogenea and cestoda were the most abundant in rainy season in opposite to the Digena that are mainly recorded in the dry season.

Keywords: Cichlid– Parasite fauna – Prevalence – Monogenea – Abundance – Lake Nokoué.

1. INTRODUCTION

Freshwater aquatic organisms are posing health challenges. Fish in their several specifities, could serve as host of parasites that are harmful to human and animals. In Benin, some parasitological investigations were made on Cichlids [1, 2, 3, 4, 5, 6]; Silurids [2,5, 7, 8] and Channids [2, 9, 10]. Concerning the Cichlids, the main research focused on the genus *Oreochromis* [11, 12, 13, 14, 15] within the continent. Indeed, it appears that *Sarotherodon melanotheron* received little attention despite of its capacity of adaptating to environmental stress [16]. Only the studies proposed by [1] and [4] presented Myxosporean parasites occurring in this species from Benin. As a fish living either in freshwater or in brackish water fish, there is a need to focus on its parasite fauna found in its different habitats. Hence, the present study was conducted to provide the first snapshot on the identity and infestation level of parasites and to evaluate the relationship between the infection and the sex or size of *S. melanotheron*. The physicochemical parameters were also collected in order to appreciate the possible influence on the parasite prevalence.

2. MATERIAL AND METHODS

2.1. Study area and sampling

A survey of parsasite fauna in a Cichlid fish was carried in the continum river Sô and Lake Nokoué in southern Benin from October 2016 to March 2017. As it's shown in figure 1, River Sô situated between 6°24' N and 2°30 E and Lake Nokoué is located between 6°26' N and 2°27' E. It joined Porto-Novo lagoon on the East by the channel of Totchê and communicated southerly with Atlantic Ocean (figure 1).

Sampling a total of 246 individuals of *Sarotherodon melanotheron* collected from two sampling sites (ST) : the landing stage of Lake Nokoué (169 specimens) and Tota (77 specimens). Fish were collected twice a month in the morning between 7h and 10h, kept alive and transported to the Laboratoire de Parasitologie et Ecologie Parasitaire in University of Abomey-Calavi. The physicochemical parameters such as dissolved oxygen, temperature, pH, salinity and conductivity were measured *in situ* using a multiparameter of type pH/Oxi340i/SET.

2.2. Parasitic investigation

Then, size and weight were measured for each fish specimen while the sex was determined after dissection. Parasite were checked with necked eye and by microscopic observation. The collected parasites were preserved in alcohol 70%. Some smears were instantaneously made with May Grunwald Giemsa, and Canada basalm. Parasite were identified with adequate keys of identification. For the parasite community structure, the common measures were evaluated using [17]

2.3. Statistical analysis

The chi square test was used to compare the prevalence according to the different parasite groups and the sampling stations whereas Student T-test was used to compare the parasite mean abundance within the stations. All test were performed with the statistical program MINITAB 16. All results were considered to be significant at 95% (P < .05).

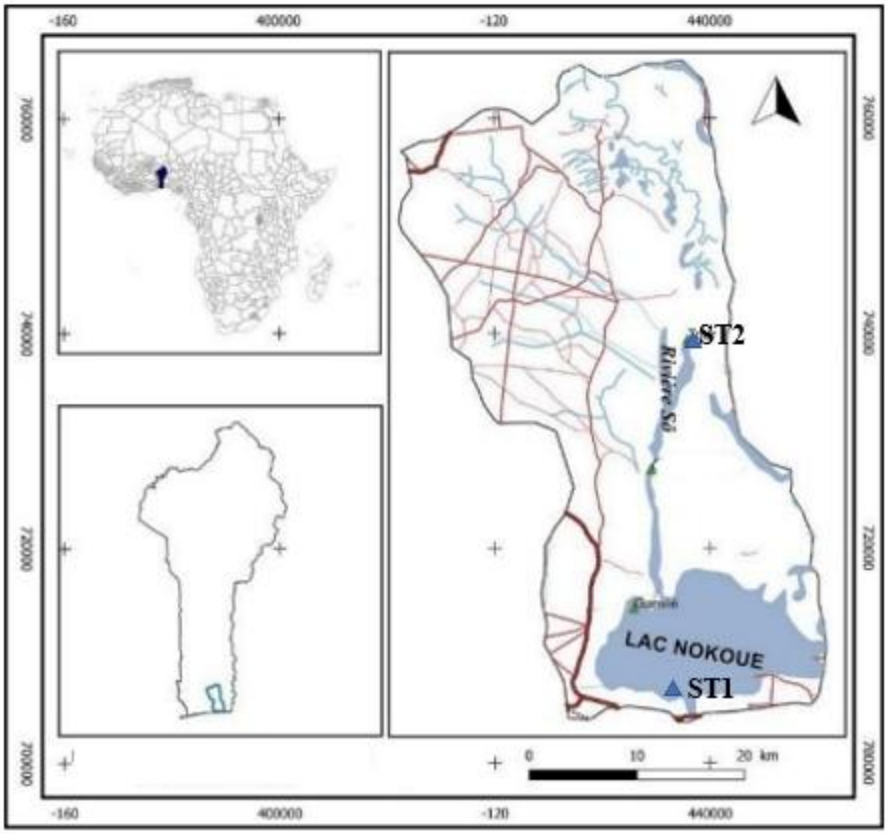

Figure 1 : Map of the complex river Sô and Lake Nokoué showing fish sampling sites ST1 (Lake Nokoué) and ST2 (Tota)

3. RESULTS AND DISCUSSION

3.1. Results

Out of 246 specimens of *Sarotherodon melanotheron* investigated, 213 were infected by at least one parasite species. The overall prevalence of infestation was 86.58%. Organs like skin, gills, lamellae, opercula, and intestine were infected (Table 1). The parasite community is composed of 8 species of metazoans distributed into six (6) parasite groups. They are Myxosporidia, Monogenea, Digena, Cestoda, Acanthocephala and Copepoda. Prevalence by parasite species in fish varies from 1.63 to 41.46% (Table 1).

Table 1. Prevalence of parasites species and their location in the host

Parasites groups	Parasites species	Location	Prevalence (%)
Myxosporidia	*Myxobolus fotoi*	Gills	20.32
	M. heterospora	Kidney	15.04
Monogena	*Cichlidogyrustilapiae*	Gills	41.46
Digena	*Clinostomum*sp.	Gills Opercula	1.63
Cestoda (Gryporhynchidae)	Undeterminated	Intestine	23.98

Acanthocephala	*Acanthogyrus* sp.		22.36
Copepoda	*Ergasilus* sp.	Gills	4.90
	Lernaea sp.	Gills	6.89

Table 2 shows the different parasite prevalence obtained according to the fish sex and the study stations (sampling site 1 or Lake Nokoué and sampling site 2 or Tota). For all the fish examined, parasites were found in 122 /149 males, i.e. a prevalence of 81.88%, compared with 93.81% for females (91 /97 individuals). Females were significantly more parasitized than males ($\chi2 = 7.205$; $p < .05$). Taking stations into account, a prevalence of 78% was recorded for males (78/100) from sampling site 1, compared with 95.65% (66/69) for females meaning that females were significantly more parasitized than males ($\chi2 = 10.093$; $p < .05$). At sampling site 2, males appear to be more parasitized (89.79% prevalence) than females (89.28%); but this difference remained non-significant ($\chi2 = 0.005$; $p > .05$). The calculated sex ratio indicates that the populations at the two study stations do not appear to be in equilibrium, with males almost doubling the number of available, breeding females.

Table 2. Parasite prevalence by sex of *S. melanotheron*

	Samples			**sampling site 1**			**sampling site 2**		
	Female	Male	Total	Female	Male	Total	Female	Male	Total
Examined	97	149	246	69	100	169	28	49	77
Infected	91	122	213	66	78	144	25	44	69
Prevalence (%)	93.81	81.88	86.58	95.65	78	85.2	89.28	89.79	89.61
Sex ratio(F/M)	0.65			0.69			0.57		
Chi square (p)	7.205 (0.007)			10.093 (0.001)			0.005 (0.944)		

Figure 2 shows the general prevalence of infestation of *S. melanotheron* individuals according to size, depending on the sampling site. In general, large sized (14-18 cm) individuals are more parasitized (91.67%) than medium-sized (10-14 cm) individuals (89.04%), which in turn are more parasitized than small sized (6-10 cm) individuals (81.81%). Nevertheless, the differences between these prevalences are not significant ($\chi2 = 2.747$; $p > .05$). At sampling site 1, medium-sized individuals are the most parasitized (88.68%), while at sampling site 2, large-sized individuals are the most parasitized (100%). For both stations, small-sized individuals show the lowest prevalence. There was no significant difference between the prevalence of the different size classes of *S. melanotheron* at either station1 ($\chi2 = 3.201$; $p > .05$) or station 2 ($\chi2 = 0.083$; $p > .05$). Infestation is not distributed according to the size class of the *S. melanotheron* individuals examined.

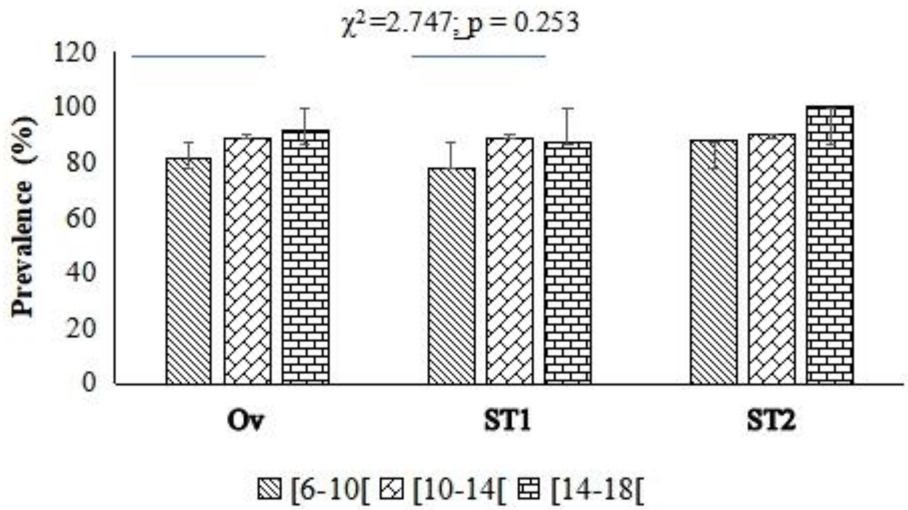

Figure 2: Prevalence of *S. melanotheron*parasitic infestation according to size (cm). Ov = total samples, ST1 = sampling site 1 and ST2 = sampling site 2.

S. melanotheron specimens collected at station 1 (ST1) are more parasitized by Monogeneans, Cestodes, Acanthocephalans and Copepods than specimens collected at station 2 (ST2). This trend is significant only for Cestodes ($\chi 2 = 4.337$; $p < .05$). *S. melanotheron* specimens collected at station 2 (ST2) were more infected with Myxosporidia and Digena (fig 3). This trend was significant only for Myxosporidia ($\chi 2 = 65.561$; $p < .001$).

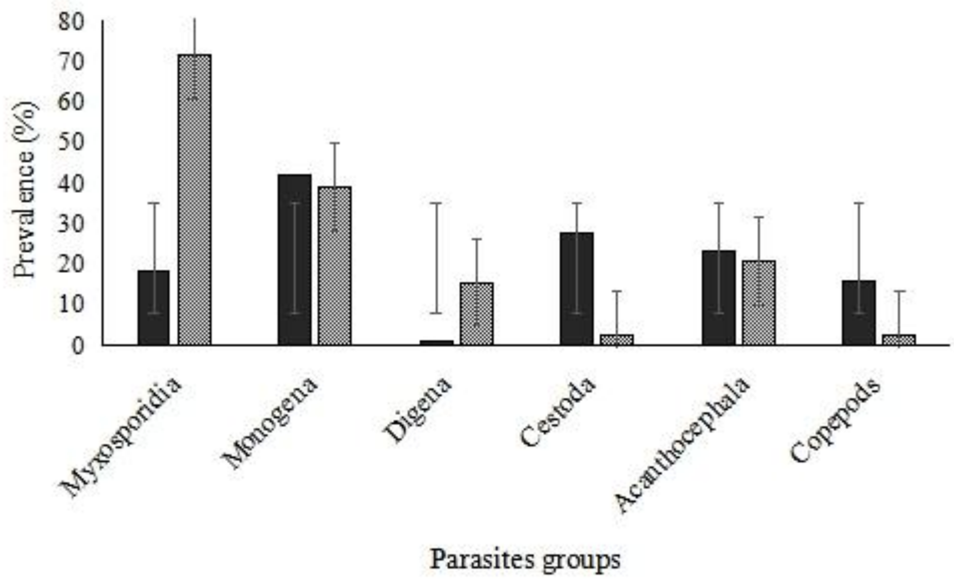

Figure 3. Prevalence of major parasitic groups according to the stations. ST1 in black band and ST2 in gray band.

Depending on the sampling station, fish sex and size, the prevalence of the parasite groups indicate that the *S. melanotheron* specimens collected at sampling station 2 are significantly more infected with Myxosporidia than those at sampling station 1. On the other hand, Cestodes and Copepods infested *S. melanotheron* specimens from sampling sampling station 2 significantly more than those from Station 1, and infestation by Cestodes and Copepods was evenly distributed according to sex. Depending on the size, Copepods infested more the large fish while Cestoda were more encountered in small and medium-sized fish. There was no cestoda infection in large fish.

Table 3. Prevalence (%) of major parasite groups of *S. melanotheron* by sampling station, sex and size

	Myxosporidia	**Monogena**	**Digena**	**Cestoda**	**Acanthocephala**	**Copepods**
			Stations			
ST1	18.34	42.01	1.18	27.81	23.08	15.98
ST2	71.43	38.96	2.6	15.58	20.78	2.67
Khi -2 (*P*)	65.561 (.00)	0.289 (.59)	0.661 (.41)	4.337 (.03)	0.161 (0.68)	9.105 (.003)
			Fish sex			
Male	43.27	40.26	1.34	23.49	18.12	12.08
Female	42.27	43.3	2.06	24.74	28.86	11.34
Khi -2 (*P*)	3.762 (.05)	0.222 (.63)	0.19 (.66)	0.051 (.82)	3.908 (.04)	0.031 (.86)
			Fish size (cm)			
[6-10[	39.77	38.64	1.14	23.86	13.64	4.54
[10-14[	38.88	42.46	1.37	26.03	26.71	15.75
[14-18[	25	50	8.33	0	33.33	20
Khi -2 (*P*)	1.699 (.42)	0.711 (.70)	3.567 (.09)	4.121 (.12)	6.284 (.04	6.921 (.03)

Among the main parasites groups, Monogenea were the most abundant (1.6), while Digena were the relative least abundant (0.05) (Table 4). In terms of sampling site, the abundance of the various parasite groups was higher at ST 1 than at ST 2. The abundance of the different parasite groups was also higher in female than in male. The abundance of Cestoda, Acanthocephala and Copepod is higher in medium-sized individuals, while large individuals have a higher rate of Monogenea and Digena. It should be noted, however, that the different trends observed with regard to station, sex and size are only significant for Monogeneans. Also, the mean intensity of Digena is higher in the largest size class (14-18 cm), whereas the medium-sized individuals (10-14 cm) showed a high level of mean intensity compared to the two others size classes considering all the parasite groups.

Table 4. Mean abundance (Ab) of the parasite groups according to sampling site (ST), fish sex and fish size

Parameters	Monogena	Digena	Cestoda	Acanthocephala	Copepods
			Sample		
NP	394	12	299	102	48
Ab	1.6	0.05	1.22	0.41	0.20
			Sampling site		
ST 1	1.63	0.06	1.63	0.45	0.27
ST 2	1.53	0.03	0.31	0.34	0.03
Student T-test of (*P*)	31.60 (.02)	3.00 (.205)	1.47 (.380)	7.18 (.088)	1.25 (.430)
			Fish Sex		
Male	1.54	0.05	1.10	0.36	0.17
Female	1.70	0.05	1.39	0.51	0.23
T-test of Student (*P*)	20.25 (.031)	-	8.59 (.074)	5.80 (.109)	6.67 (.095)
			Fish Size (cm)		
[6-10[	1.27	0.01	0.91	0.22	0.07
[10-14[	1.78	0.05	1.50	0.53	0.27
[14-18[	1.83	0.33	0.00	0.50	0.17
T-test of Student (*P*)	9.09 (.012)	1.29 (.326)	1.84 (.207)	4.22 (.052)	2.94 (.099)

NP: Number of parasites collected for each group; Ab = Mean abundance

Overall, Cestoda presented the highest mean intensity and the lowest MI was recorded with Copepods (Table 5). The average intensity of the parasite groups is higher at station 1 than at station 2. This trend is significant for Monogenea ($p < 0.001$) only. Mean intensity of Monogenea, Cestoda is higher in females than in males with a significant difference, while the contrary is observed with Digena and Acanthocephala, even it revealed a significant difference with Acanthocephala.

Table 5. Mean intensity (MI) of the parasite groups according to sampling site (ST), fish sex and fish size

	Monogenea	Digena	Cestoda	Acanthocephala	Copepoda
			Overall		
NP	394	12	299	102	48
MI	3.94	3	4.98	1.85	1.66
			Station		
ST 1	3.94	5.00	5.73	1.95	1.70
ST 2	3.93	1.00	2.00	1.63	1.00
T-test of Student (*P*)	787.00 (.001)	1.50 (.374)	2.07 (.286)	11.19 (.057)	3.86 (.161)
			Fish sex		
Male	3.88	3.50	4.56	1.96	1.44
Female	4.02	2.50	5.63	1.75	2.00

T-test of Student (*P*)	56.43 (.011)	6.00 (.105)	9.52 (.067)	17.67 (.036)	6.14 (.103)
			Size (cm)		
[6-10[	3.29	1.00	3.64	1.58	1.50
[10-14[	4.33	3.50	5.76	1.97	1.74
[14-18[	3.67	4.00	0.00	1.50	1.00
T-test of Student (P)	12.39 (.006)	3.05 (.093)	1.86 (.204)	11.59 (.007)	6.48 (.023)

NP: Number of parasites collected for each group; MI = Mean Intensity

The correlation between physico-chemical parameters and the prevalence of parasite groups in *S. melanotheron* (Table 6) showed that only salinity has an influence on parasite distribution. Of the six (06) parasite groups collected in *S. melanotheron*. Myxosporidia and Cestoda were negatively correlated with salinity with a significant difference. On the other hand, Digena were positively correlated with salinity (p = .03).

Table 6. Correlation between physico-chemical parameters and the prevalence of the parasite groups of *S. melanotheron*

Parameters		**Prevalence**	**pH**	**Temperature**	**Salinity**	**Conductivity**	**Dissolved Oxygen**
Myxosporidia	r	39.96	-0.657	-0.970	**-0.999**	-0.963	-0.570
	P		0.544	0.157	**0.024**	0.174	0.351
Monogena	r	41.46	0.859	0.846	0.938	0.831	0.711
	P		0.342	0.358	0.226	0.376	0.657
Digena	r	1.63	0.663	0.968	**0.999**	0.961	-0.216
	P		0.539	0.162	**0.030**	0.179	0.074
Cestoda	r	23.98	-0.582	-0.989	**-0.998**	-0.984	0.929
	P		0.604	0.096	**0.036**	0.113	0.060
Acanthocephala	r	22.36	0.987	0.592	0.746	0.570	0.657
	P		0.104	0.596	0.464	0.614	0.238
Copepoda	r	5.69	0.995	0.537	0.699	0.514	-0.186
	P		0.061	0.639	0.507	0.657	0.992

Values in bold are different from 0 at a significant level equal to 0.05; r = coefficient of correlation, p = probability.

Variation in physico-chemical parameters on the prevalence *S. melanotheron.*

Among all of the physico-chemical parameters, only salinity showed significant differences ($P < 0.05$) between the sampling sites. Nevertheless, the parameters did fluctuate depending on the sampling site (figure 4). Thus, they exist great amplitude between minimum (6.34) and maximum (7.95) of pH in ST 2 than in ST 1 where pH ranged from 7.33 to 7.63. The average temperature at ST 2 was 29.13°C and 28.68°C at ST 1. The maximum (31.3°C) and minimum (27.47°C) values of temperature were recorded at ST 2 and ST 1 respectively.

Salinity values at ST 1 (6.88 g/L) were higher than at ST 2 (2.12 g/L). The amplitude of salinity variation was trending as the same. The minimum salinity value (1.26 g/L) was observed at ST 2 whereas the maximum (13.69 g/L) was recorded at ST 1. As for conductivity, the mean value recorded at ST 2 (93.07 µS/cm) is significantly higher ($p < 0.008$) than that observed at ST 1 (13.40 µS/cm). This trend is confirmed by the amplitude of variation where the lowest conductivity values were grouped together at ST 1 (5.93-26.03 µS/cm) and the highest in the second site 2 (76.2-117 µS/cm). Whereas, Dissolved oxygen in the water varies widely at each station. The average dissolved oxygen value observed at ST 1 (3.04) is slightly higher than at ST 2 (2.86) without being significantly different (= 0.068).

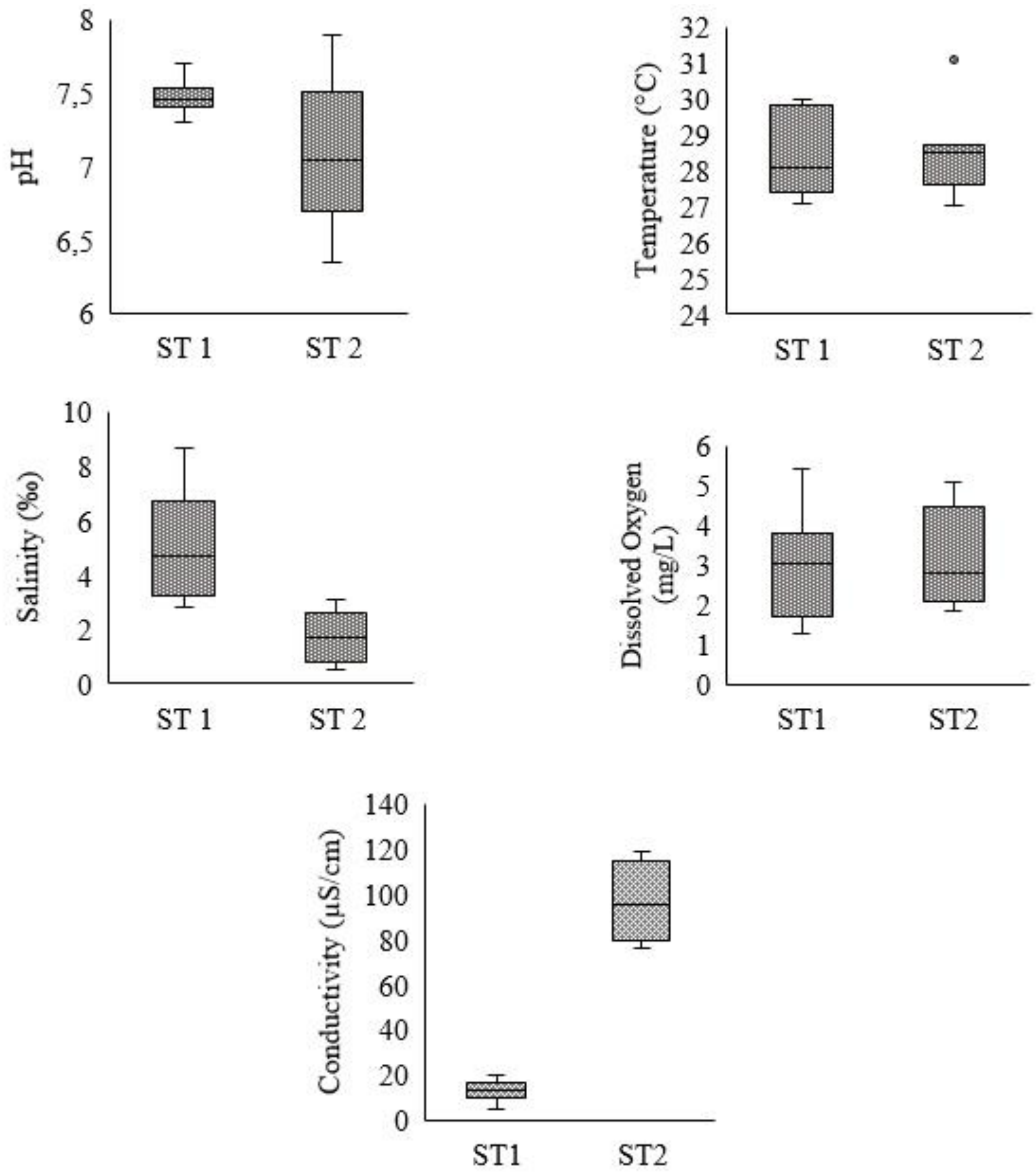

Figure 4: Variation in water physico-chemical parameters in the two sampling station of *S. melanotheron*. ST = station

3.2. Discussion

The present study reports a species of myxosporidia never before recorded on any fish species in Benin. With regards to the myxosporidian parasite checklist, *Myxobolus fotoi* is recorded for the first time in this area. Described in Cameroon by [18] in the fatty tissues of gill arch, kidney and spleen of *Oreochromis niloticus*, it was reported by [19] and never in *Sarotherodon melanotheron*, nor in other Cichlids species. Whereas *Myxobolus heterospora* in the viscera of *Coptodon esculenta*, C. *variabilis, O. niloticus* and in the spleen of *Haplochromi*s sp. [20] and either reported by [19; 21] in the kidney of *Oreochromis niloticus*.

Myxosporean kidney infection (34.96%) obtained in this study is much lower than that obtained by [19]. Authors justify this high prevalence by a decrease in the defense capacity of the hematopoietic organs of the fish. They agree with [18] and [22] who suggested that spores could be transported and accumulate in the host's macrophage cells [23]. Indeed, large groups of spores accumulated in the melanomacrophage cells of the kidney, resulting in a high prevalence of infection in fish kidneys at that precise moment. Conversely, taking into account the role of melanomacrophages in host defense reactions, these cells are capable in some cases of attacking small cysts and large mature spores where the parasites are isolated and progressively destroyed [24] melanomacrophage centers of the hematopoietic organs. Due to the defense mechanism of melanomacrophages, high infestation by myxospores accumulation in the kidneys could be reduced justifying the prevalence of 34.96% observed in this study. Several studies have been carried out on monogenea of inland fish in Africa [25, 26, 27, 28, 29]. In Benin, *Cichlidogyrus tilapiae* was described by [26] in *Chromidotilapia guentheri* and by [28] in farmed *Coptodon zillii*. In 2014, [31] attested the presence of *C. tilapiae* in the gill filaments of *Oreochromis niloticus* from fish farms in Brazil. In contrast, [30] revealed the presence of *Cichlidogyrus acerbus* and *C. halli*in *S. melanotheron*. Despite multiple studies on monogenean, only few results displayed the presence of *Cichlidogyrus tilapiae*. Out of the six (06) helminth species collected, Monogenea are highly prevalent. This high prevalence of Monogenea may be due to their short developmental cycle. Indeed, monogenea are very contagiousness, representing a major threat for fish [32]. The mechanical action exerted by the opisthohaptor could induce extensive damage and exposed the host to secondary infections by bacteria, fungi and viruses [33]although Monogenea are considered one of the important parasites sensitive to any change in water quality [34].

The present work reported Gryporhynchidae metacercariae. Information based on taxonomy, development cycle, ecology and pathology are very scarce or non-existent on this helminth [35]. Identifying the Gryporhynchidae down to genus and species level according to morphological characteristics of the larvae is very difficult as the adult parasites is located in piscivorous birds [36]. Gryporhynchidae are parasitic on several groups of fish [35], but studies on the damage caused by Gryporhynchidae on their hosts are very rare [36]. Fish infested with metacestodes showed ungrowth compared to the uninfested or low infested fish. Several species of the *Acanthogyrus* (*Acanthocephalus*) genus have been described in African Cichlids particularly in Tanzania [37], Congo [38] and Madagascar [39]. [40] investigated the organic preferences of Acanthocephalans. For these authors, differences in the physical environment of the gut and food supply availability and quantity are the factors that limit the distribution of parasites in different sections of the alimentary tract. It is reported that *A. tilapiae* is better adapted to some cichlid hosts than others [41]. There no special studies focused on Acanthocephalans and copepoda, but several authors have reported serious economic losses caused these parasites [42, 43, 44,]. According to some authors, gill infestations by Ergasilidae cause depletion of fish populations and weight loss.

According to [45], life traits, size, habitat and feeding behavior could directly or not influence the parasite richness. Whereas prevalence variation could depend on the genetics, proximity living environment, host age, energy expenditure to potential hosts or the presence of other parasites [46]. According to [47], euryhaline fish cross various endemiotopes and could tolerated great variations in salinity. Indeed. the euryhaline parasitofauna would be affected by their displacement. On the other hand, fish that migrate without changing environmental conditions would be less affected as physicochemical parameters are of very low amplitude as reported by [9]

Changes in diet and habitat requirements at different developmental stages of a species can strongly affect the composition, abundance and richness of the parasitic community. For [48], tropical fish whose diets are based on macroinvertebrates have helminth-rich digestive tracts. [49] attributes this richness of gastrointestinal helminths to physio-reproductive and feeding behavior factors.

Considering the pollution criteria, [6] admitted that the distribution of Monogena and Digena was limited to unpolluted and moderately polluted lakes. [50] revealed that fish parasite community is known to respond directly to the abiotic environment of the host. The correlation between physico-chemical parameters and the prevalence of the main groups of parasites of *S. melanotheron* in the present work showed that only salinity does influence parasite distribution.

According to the present study, fish with great size were more parasitized than smaller ones. This finding is agreed with [51] and [52] who noted that parasite richness is positively correlated with host size. Indeed [50] and [53] assured that longsized fish carry more parasite species or a great abundance of parasites. However, we can conclued with [54] that the significant difference between prevalence and intensity does not necessarily indicate that larger hosts have a higher parasite load than the smaller one. Like [55], the prevalence in *S. melanotheron* according to sex reveal that females are more parasitized than males. Sex-related differences in parasite infestation rates may be attributed to the host immune response due to differences in the endocrine gland activities of host fish. [56] [57] and [58] suggested that failure in resistance to parasitic infection in female fish may be due to investment in gonadal development.

4. CONCLUSION

The Cichlid *Sarotherodon melanotheron* hosted a great diversity of parasites from many organs. Parasite prevalence was dominated by monogeneans. Salinity remained a chemical determinant that either control the life of this fish or influence its prevalence in parasites.

ETHICAL APPROVAL

Non applicable.

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