

Effect of Season on *Myxosporean* Infections in *Oreochromis niloticus* Linnaeus, 1758 (Cichlidae) at MAPE Dam in Adamawa, Cameroon

Fonkwa Georges^{1,3*}, Lekeufack Folefack Guy Benoît², Tchuinkam Timoléon³, Ishtiyah Ahmad⁴ and Tchoumboue Joseph¹

¹Applied Hydrobiology and Ichthyology Research Unit, Department of Animal production, Faculty of Agronomy and Agricultural Science, University of Dschang, P.O. Box 222, Dschang-Cameroon

²Laboratory of General Biology, Faculty of Science, University of Yaoundé I, P. O. Box 812, Yaoundé-Cameroon

³Vector Borne Diseases Laboratory of the Applied Biology and Ecology Research Unit, Department of Animal Biology, Faculty of Science, University of Dschang P.O. Box 67, Dschang-Cameroon

⁴DST Sponsored Fish Nutrition Laboratory, Department of Zoology, University of Kashmir, Srinagar, J&K, India-190006

Abstract

In order to contribute to a better understanding of the effect of season on *Myxosporean* infections so as to elaborate prevention and control strategies, 350 *Oreochromis niloticus* specimens were sampled from May 2016 to May 2017 from the MAPE dam (Adamawa-Cameroon) and the prevalence of infection was determined after classical examination of fish. A total of 12 species of *Myxosporeans* belonging to the genus *Myxobolus* were identified. Irrespective of the parasite species, the prevalence was significantly higher in the dry season (52.94%) than the rainy season (39.59%). Four parasite species occurred mostly during the dry season (*Myxobolus brachysporus*, *M. kainijae*, *M. ellipsoides* and *M. pharyngeus*) and eight without seasonality. Male fish were significantly more infected in the dry season (57.78%) than the rainy season (39.53%). On the contrary, season did not significantly influence the prevalence in females. Fish of size 100 mm to 150 mm were significantly more infected in the dry season (68.10%) than the rainy season (44.44%). Parasite species were more prevalent in the organs during the dry season than the rainy season. Whether in the rainy season (47.70%) or in the dry season (29.44%), a significantly higher prevalence of parasites was recorded for the kidneys.

Keywords: *Myxosporeans*; Prevalence; Season; *Oreochromis niloticus*; MAPE dam; Cameroon

Introduction

Oreochromis niloticus is a delicious fish highly appreciated by many households in Cameroon and is involved in fish farming programs. Many pathogens among which *Myxosporeans* decrease the fish production [1]. *Myxosporeans* affect fish growth [2], their reproduction [3] and are involved in epizootics responsible for massive fish death [2,4]. Few studies were carried out in Cameroon particularly on the effect of season on *Myxosporean* infections apart from those of Tombi and Bilong Bilong [5], Lekeufack and Fomena [6], Nchoutpouen et al. [7]. This is problematic because the seasonal modification of the water physico-chemical characteristics can lead to fish's stress. Thus, water becomes more conducive to epizootics that can result in massive fish deaths and important economic losses. The study intended to assess the effect of season over the prevalence of *Myxosporean* infections in *Oreochromis niloticus* in order to boost its production, a major challenge for food security.

Materials and Methods

Study area

Fish were collected in the MAPE River (tributary of Mbam River), Bankim subdivision (6°00' - 6°20' NL and 11°20' - 11°40' EL, Mayo-Banyo Division, Adamawa- Cameroon Region, Central Africa). The average altitude is about 724m and the soil is a mixture of clay and sand. The climate is of tropical Sudano-guinean type with two seasons: A long rainy season running from March to November and a short dry season from November to March. The annual average temperature is about 23°C and the rainfall varies between 1500 and 2000 mm [8].

Fish sampling, identification of *Myxosporeans* and data collection

Fish were collected monthly from fishermen during the study

period i.e., May 2016 to May 2017. They were captured both at day and night using fish nets and fishing canes. In the field, specimens were immediately stored at 10% formalin solution and transported to the laboratory for examination. At total, 350 fish (219 males and 131 females) were captured i.e., 197 and 153 fish during the rainy and the dry seasons respectively. The standard lengths ranged from 51 mm to 240 mm with an average of 97.13 mm. Based on these sizes, fish were grouped into 3 classes of 50 mm amplitude each. The modal class which is [50-100] represented 65.43% of the sampled fish. In the laboratory, fish were identified according to Stiassny et al. [9] and examined as per Abakar [10]. Thus, standard and total lengths were measured to the nearest millimeter using a slide caliper and the sex was determined after dissection. External organs (fins, skin, scales and eyes) and internal organs (gills, spleen, kidneys, intestines, gall bladder, stomach and gonads) were examined macroscopically, and then with stereoscopic microscope (Motic brand) at 10X to look for cysts. As for kidneys, spleen and gonads, three smears were made per organ (anterior, medium and posterior regions) and examined at a total magnification of 1000X with a light microscope in order to look for spores. Cysts were crushed between a slide and cover glass in a drop of distilled water and their contents were

***Corresponding author:** Fonkwa Georges, Faculty of Agronomy and Agricultural Science, Applied Hydrobiology and Ichthyology Research Unit, University of Dschang, P.O. Box 222, Dschang-Cameroon Tel: (+237) 674 29 89 24; E-mail: fonkwageorges@gmail.com

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identified with the light microscope at 1000X. Spores were fixed using methanol, stained with May-Grünwald-Giemsa and recorded with digital camera (Canon Ixus). Species were identified according to Lom and Arthur [11].

Parasitological parameter studied

The prevalence (Pr) of infection expressed as a percentage was defined as the number of host species infected by a given parasite species divided by the number examined [12].

Statistical analysis

The Chi-square (X^2) test was used to compare prevalences. The error probability was $P < 0.05$ and the Graph Pad Prism 5 software helped for analysis.

Results

The results are illustrated in Table 1 and Figures 1-6.

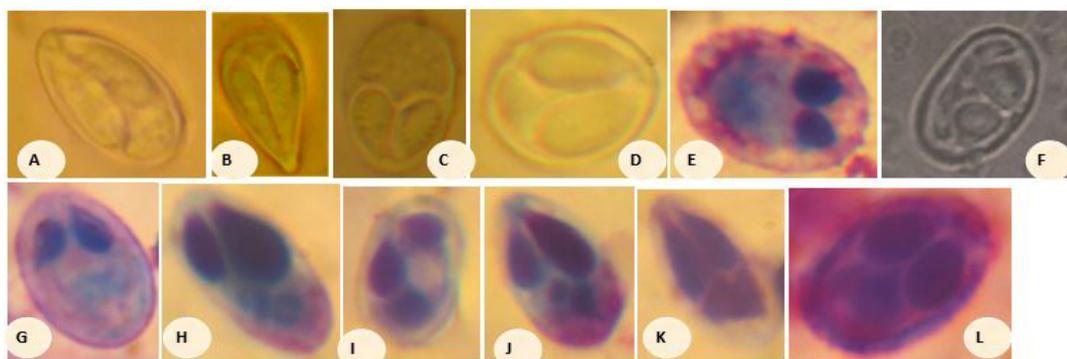
Effect of season on the prevalence of *Myxosporean* species

As shown in Figure 1, twelve species belonging to the genus *Myxobolus* were recorded. The effect of season on the prevalence of *Myxosporean* species as illustrated in Figure 2 reveals that fish were infected during both the dry and rainy seasons. Irrespective of the parasite species, the infection rate was significantly higher in the dry season than the rainy season ($X^2 = 6.19$; $P = 0.013$). Whatever the season, the prevalence of parasite varied considerably ($P < 0.001$). It fluctuated between 0 (*M. pharyngeus*) and 12.18% (*M. tilapiae*), then from 0.65 (*M. pseudodispar*) to 18.95% (*M. tilapiae*) during the rainy and the dry seasons respectively. All parasite species appeared during the two seasons except *M. pharyngeus* which was present only in the dry season ($X^2 = 3.90$; $P = 0.048$), *Myxobolus heterosporus* ($X^2 = 0.28$; $P = 0.599$) and *M. pseudodispar* ($X^2 = 2.51$; $P = 0.113$) occurred more in the rainy season than in the dry season.

Parasite Species	Host Sex						X^2	P
	Males		X^2	P	Females			
	Seasons				Seasons			
	Rainy 129*	Dry 90*			Rainy 68*	Dry 63*		
<i>M. camerounensis</i>	10.85	12.22	0.10	0.754	7.35	9.52	0.20	0.654
<i>M. israelensis</i>	8.53	14.44	1.90	0.168	8.82	9.52	0.02	0.890
<i>M. heterosporus</i>	7.75	12.22	1.22	0.269	14.71	3.17	5.23	<0.05
<i>M. agolus</i>	4.65	8.89	1.59	0.207	4.41	4.76	0.01	0.924
<i>M. tilapiae</i>	13.18	23.33	3.81	0.05	10.29	12.70	0.19	0.666
<i>M. brachysporus</i>	6.98	16.67	5.10	<0.05	10.29	19.05	2.02	0.155
<i>M. kainjiae</i>	0.00	0.00	--	--	4.41	19.05	6.91	<0.01
<i>M. tchadanayei</i>	3.88	1.11	1.52	0.218	2.94	11.11	3.41	0.065
<i>M. mapei</i>	2.33	5.56	1.57	0.210	2.94	3.17	0.01	0.938
<i>M. ellipsoides</i>	0.78	11.11	11.87	<0.001	2.94	6.35	0.87	0.351
<i>M. pseudodispar</i>	2.33	1.11	0.44	0.509	4.41	0.00	2.85	0.092
<i>M. pharyngeus</i>	0.00	1.11	1.44	0.230	0.00	3.17	2.19	0.139
Total	39.53	57.78	7.08	<0.01	39.71	46.03	0.53	0.645
X^2	44.18	52.00	--	--	22.92	34.55	--	--
P	<0.001	<0.001	--	--	<0.05	<0.001	--	--

*: Number of examined fish; -: No value; P: Error probability

Table 1: Prevalence of parasite species as a function of host sex and season.



A: *Myxobolus israelensis* (1600x); B: *Myxobolus heterosporus* (1600x); C: *Myxobolus camerounensis* (1500x); D: *Myxobolus agolus* (1600x); E: *Myxobolus kainjiae* (1800x); F: *Myxobolus brachysporus* (1500x); G: *Myxobolus tilapiae* (1500x); H: *Myxobolus mapei* (1200x); I: *Myxobolus pseudodispar* (1200x); J: *Myxobolus tchadanayei* (1600x); K: *Myxobolus pharyngeus* (1600x); L: *Myxobolus ellipsoides* (1600x)

Figure 1: Micrographs of *Myxosporean* spores.

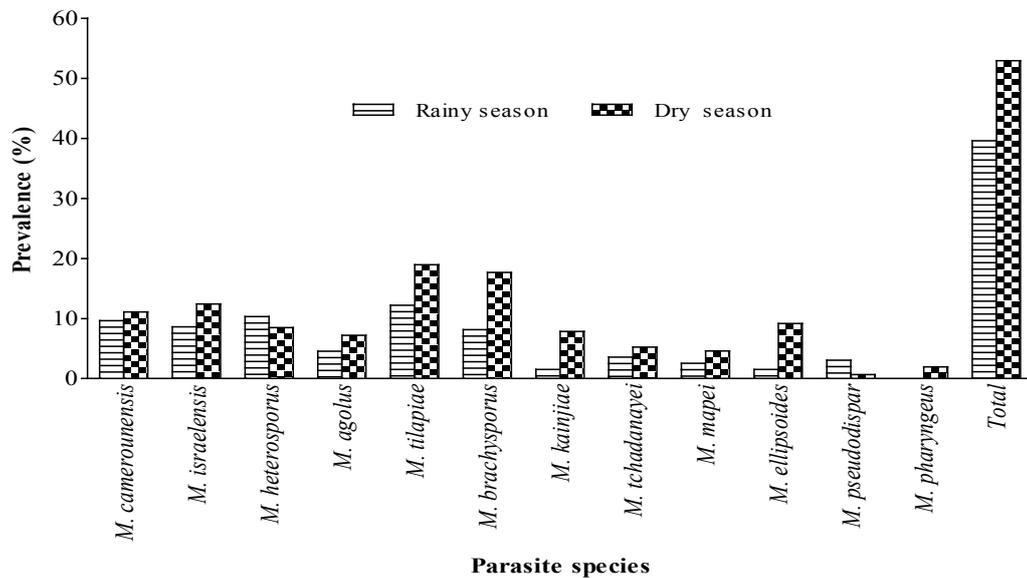


Figure 2: Effect of season on the prevalence of Myxosporean species.

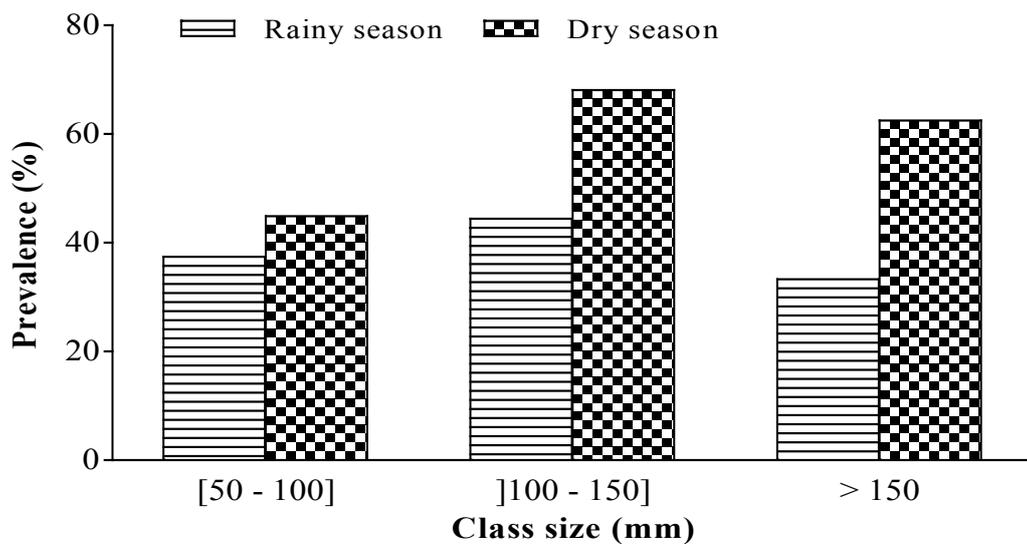


Figure 3: Prevalence of infection as a function of class size and season.

Prevalence of parasite species as a function of host sex and season

The prevalence of parasite species as a function of host sex and season (Table 1) shows that independently of the parasite species, male was significantly more infected in the dry season than the rainy season ($X^2 = 6.07$; $P < 0.05$). On the contrary, in females, the season did not significantly influence the infection rate ($X^2 = 0.53$; $P = 0.645$). Whether in the rainy season ($X^2 = 0.00$; $P = 0.981$) or dry season ($X^2 = 2.05$; $P = 0.152$), the prevalence did not vary significantly between males and females. When parasite species are taken into account, it appears that in male fish, *M. brachysporus* ($X^2 = 5.10$; $P < 0.05$) and *M. ellipsoides* ($X^2 = 11.87$; $P < 0.001$) were more prevalent during the dry season than the rainy season. In females, *M. heterosporus* occurred more significantly ($X^2 = 5.23$; $P < 0.05$) in the rainy season than in the

dry season. On the contrary, *M. kainjiae* infected females mostly in the dry season ($X^2 = 6.91$; $P < 0.01$). No matter the season and the fish sex, prevalence varied significantly ($P < 0.001$) between parasite species.

Prevalence of infection as a function of class size and season

As illustrated in Figure 3, fish were infected in all the three class sizes during the dry and rainy seasons. Whatever the class size, although the prevalence of infection was higher in the dry season than the rainy season, the only significant difference ($X^2 = 6.07$; $P < 0.05$) was noticed for the class [100 - 150]. In the rainy season, the infection rate did not differ ($X^2 = 0.93$; $P = 0.628$) between the class sizes. Meanwhile in the dry season, the class [100 - 150] recorded the higher prevalence ($X^2 = 7.17$, $P < 0.05$; $Pr = 68.10\%$).

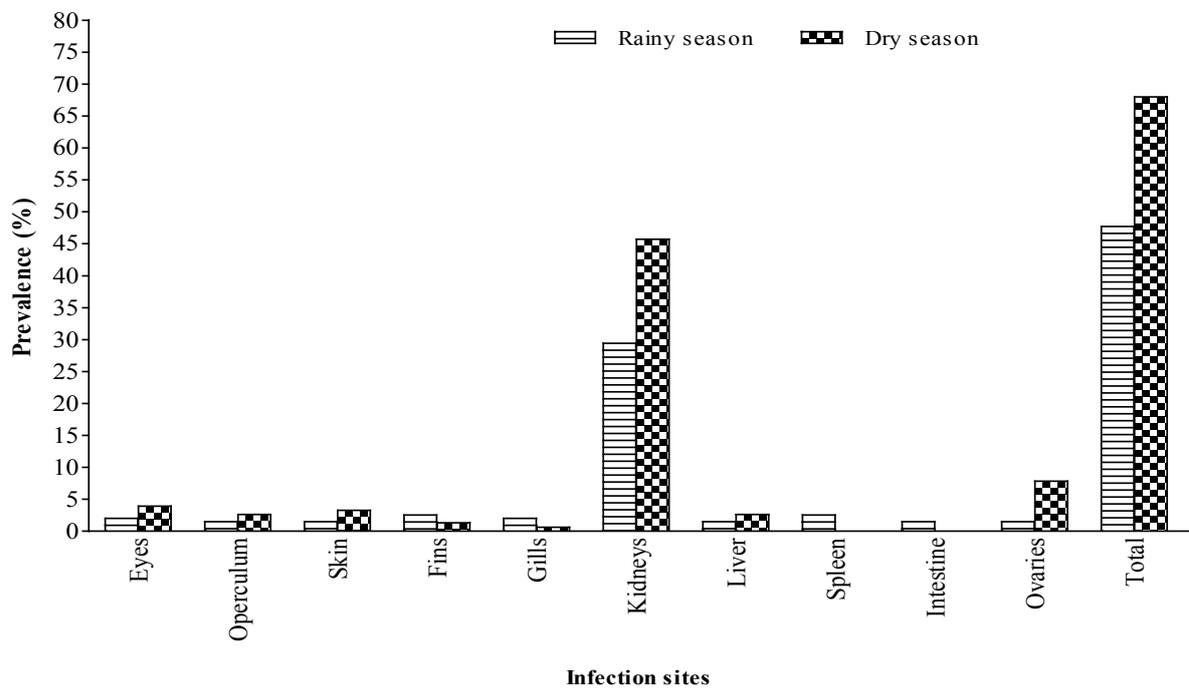


Figure 4: Prevalence in relation to infection sites and season.

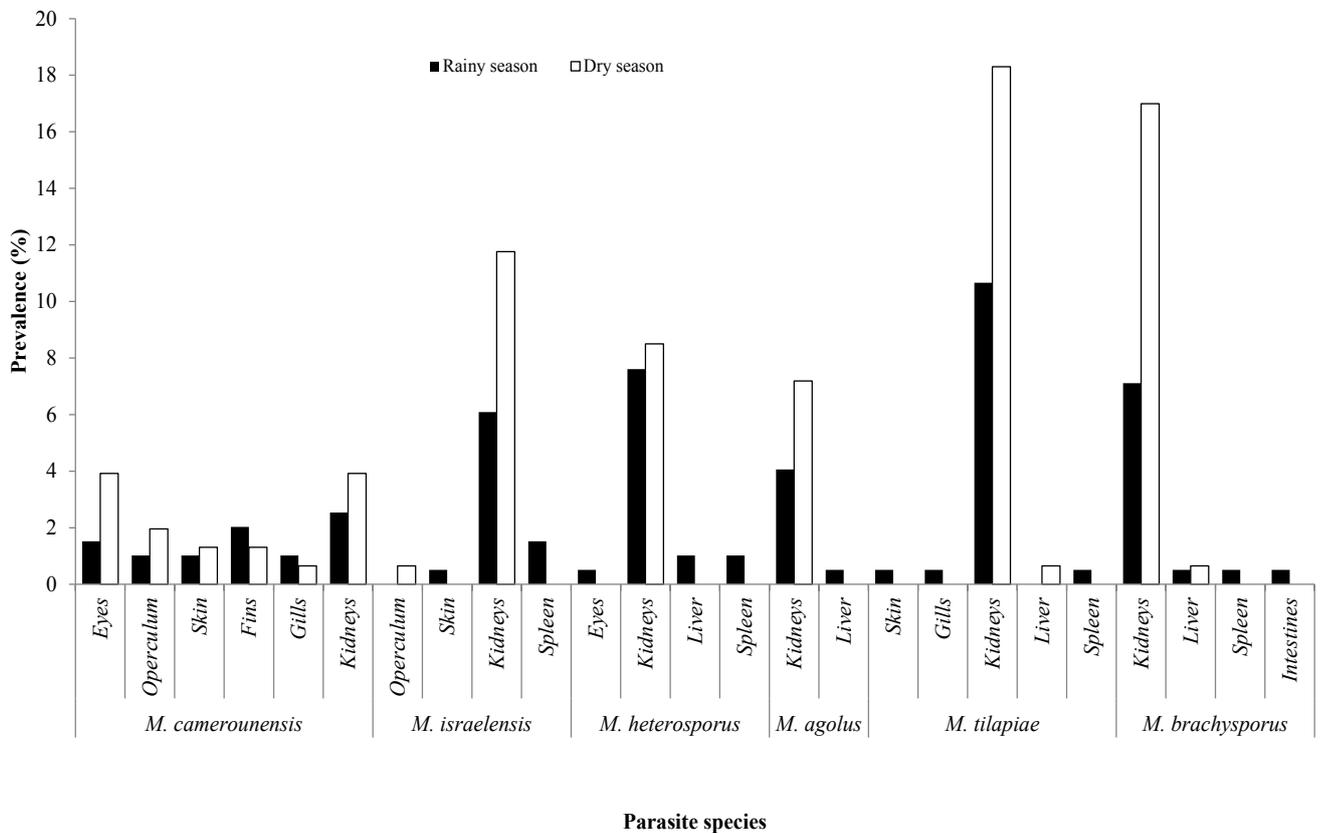


Figure 5A: Prevalence of parasite species as a function of infection sites and season

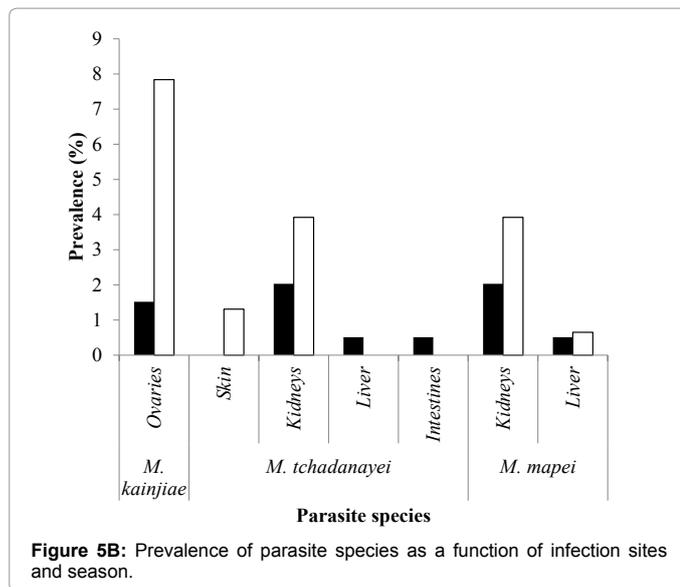


Figure 5B: Prevalence of parasite species as a function of infection sites and season.

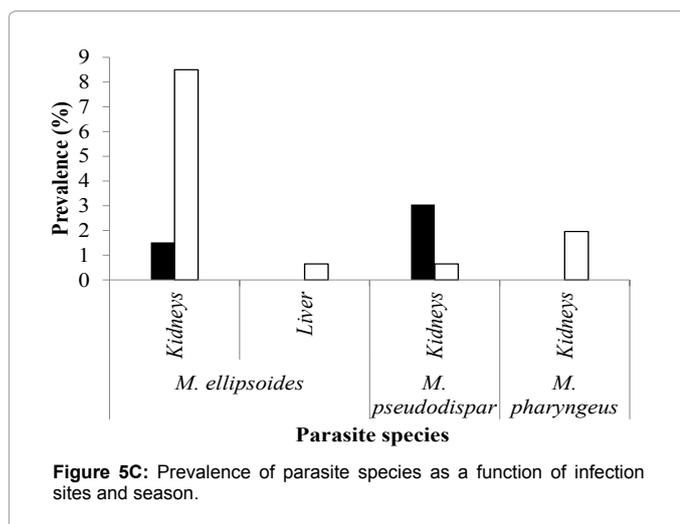


Figure 5C: Prevalence of parasite species as a function of infection sites and season.

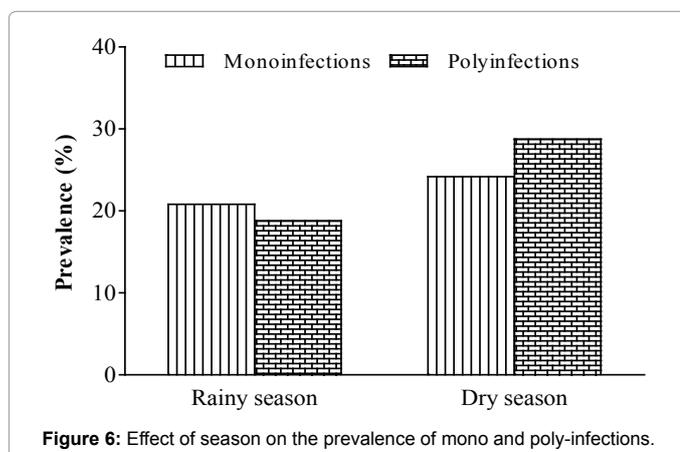


Figure 6: Effect of season on the prevalence of mono and poly-infections.

Prevalence in relation to infection sites and season

The prevalence in relation to infection sites and season (Figure 4) illustrates that, organs were infected during both the dry and the rainy seasons. According to the seasonality of infection, organs were sorted

into two categories: those infected only in the rainy season (spleen and intestine) and those infected during the two seasons (8 organs). Regardless of the organ, the dry season was remarkably ($P < 0.001$) more favorable to the infection of organs. Whether the season was rainy ($X^2 = 308.60$; $P < 0.001$; $Pr = 29.44\%$) or dry ($X^2 = 392.20$; $P < 0.001$; $Pr = 45.70\%$), the higher and significant prevalence was recorded for kidneys. Furthermore, kidneys ($X^2 = 9.88$; $P < 0.01$) and spleen ($X^2 = 3.94$; $P < 0.05$) were the only organs exhibiting significant infection rate between the two seasons. Moreover, kidneys were more infected in the dry season contrary to the spleen.

Prevalence of parasite species as a function of infection sites and season

As shown in Figures 5A-5C, parasite species had a higher prevalence ($P < 0.05$) in organs during the dry season than the rainy season. *Myxobolus heterosporus* (spleen, liver), *M. agolus* (liver, spleen and gills), *M. brachysporus* (spleen and intestine), *M. tchadanayei* (liver and intestine) and *M. pseudodispar* (kidneys) appeared more commonly in the organs in the rainy season than the dry season ($P > 0.05$). Whatever the season, *M. tilapiae* and *M. brachysporus* were significantly more prevalent ($P < 0.05$) in the kidneys compared to other organs and parasite species.

Effect of season on the prevalence of mono and polyinfections

The effect of season on the prevalence of mono and polyinfections is summarized in Figure 6. It reveals that fish were mono-infected (single parasite species) and poly-infected (several parasite species) during both the dry and the rainy seasons. During the dry season ($X^2 = 0.82$; $P = 0.364$), the polyinfections (28.76%) were more frequent than mono-infections (24.18%) contrary to the rainy season ($X^2 = 0.26$; $P = 0.613$). Although mono-infections were more frequent in the dry season than the rainy season, no significant difference was found ($X^2 = 0.56$; $P = 0.452$). Fish were significantly more poly-infected during the dry season than the rainy season ($X^2 = 4.82$; $P < 0.05$).

Discussion

Irrespective of the parasite species, the prevalence was higher in the dry season than the rainy season. Obiekezie and Okaeme [3] thought that during the dry season, the higher temperature of water and mud can encourage infection with *Myxosporeans*. Uspenkaya [13] reported that, myxospores sink in water where they get aging and become mature in mud or sludge in preparation to infect the new host. Based on season, parasites were grouped into two categories: those occurring mostly during the dry season (category A) and those occurring without seasonality (category B). The results of category (A) parasites (*M. brachysporus*, *M. kainjia*, *M. ellipsoides* and *M. pharyngeus*) are explained previously by Obiekezie and Okaeme [3]. In the fishes *Ctenopoma petherici*, *Clarias pachynema* and *Hepsetus odoe* from the river Sangé (Cameroon), Lekeufack and Fomena [6] showed that *Myxobolus* sp₂ occurred mostly during the dry season whereas *M. petrocephali*, *M. nkamensis* and *M. gariepini* were more frequent in the rainy season. Abakar [10] asserted that, in the River Chari (Chad), *Myxobolus brachysporus* and *M. camerounensis* were more prevalent in *Oreochromis niloticus* during the dry season than the rainy season. The pollution of Chari River by factories built in Moundou and Sarh (Chad towns) being the cause. In fact, the pollution was accentuated during the dry season because of the intensive water evaporation and this could negatively affect the actinospores. This pollution was also noticed in MAPE dam because neighboring population dump refuse in the water

and use nitrated fertilizers for agriculture. Gbankoto et al. [4] indicated that the prevalence of *Myxobolus* sp and *Myxobolus zillii* which are gill's parasites for *Tilapia zillii* and *Sarotherodon melanotheron melanotheron* in Bénin was higher in the dry season. The case of category (B) is similar to that noticed by Bilong Bilong and Tombi [14] and Abakar [10]. Thus, some *Myxosporeans* species are present in the fishes throughout the year; others appear once a year whereas some exhibit two or three peaks of infection each year [15]. Oligochaetes being definitive hosts in *Myxosporeans* life cycle [16], the seasonal variation of parasitism by *Myxosporeans* could be due to the seasonal supply of actinospores by oligochaetes [17].

Regardless of the parasite species, male fish were significantly more infected in the dry season than the rainy season. On the contrary, the season did not significantly influence the infection rate in females. Probably during the dry season, males lose huge amounts of energy for testosterone synthesis thus weakening the efficiency of their immune system [18]. The higher prevalence in the class [100 - 150] during the dry season may suggest that the dry season weakens the immune system of the fish belonging to that class size.

Parasite species were more prevalent in organs during the dry than the rainy seasons. Probably, the high evaporation of water during the dry season increases salt concentration which renders fish more susceptible to infection. Whether in the rainy season or the dry season, the highest prevalence was recorded for the kidneys which also were more infected in the dry season contrary to the spleen. The physiology of the organ may be influenced by the physico-chemical characteristics of the water which in turn depend on the season too. Therefore, any modification of the physiology of an organ within a given season will encourage or discourage its infection by a given parasite species. The fact that kidneys were the most infected organs in both dry and rainy seasons may be because they filter blood and secrete many solutes [19] that encourage parasites to converge there for needed metabolites.

Conclusion

Irrespective of the parasite species, infection rate was significantly higher in the dry season than the rainy season. The seasons influenced the occurrence of parasites at the levels of organs, host sex and class size. The available data are useful for developing prevention and control strategies against *myxosporidiosis*.

Conflicts of Interest

The authors have no conflicts of interest to declare.

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