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Research Article

Some epidemiological aspects of Myxosporean infections in *Oreochromis niloticus* (Linnaeus, 1758) and *Hemichromis fasciatus* (Peters, 1857), two cultured Cichlid fishes in the West - Cameroon

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Abstract

In order to assess epidemiological aspects of myxosporean infection in cultured *Oreochromis niloticus* and *Hemichromis fasciatus* fishes in Cameroon to develop efficient prevention and control program, a total of 320 Cichlid fishes (189 *Oreochromis niloticus* and 131 *Hemichromis fasciatus*) were collected from June 2019 to April 2020 in the ponds located at the Ngoundoup Village, Koutaba Subdivision, Noun Division, Region of West-Cameroon. They were examined both macroscopically and microscopically for myxosporean infections. The prevalence of infection was determined as a function of fish species, sex, size, target organs, and seasons. Results showed that kidneys and ovaries were the only infected organs and harbored nine and three myxosporean species of the genus *Myxobolus* respectively. A total of 154 fish were infected (54.38%). Irrespective of the parasite species, *Oreochromis niloticus* (75.13%) was significantly more infected than *Hemichromis fasciatus* (24.27%). The prevalence of parasites was very low (<25%) whatever the fish species. The sex and fish size did not significantly influence the prevalence of parasite species. The prevalence of *Myxobolus tilapiae* was negatively and significantly correlated (r= -0.20; p= 0.02) with *Oreochromis niloticus* size. The overall prevalence was significantly higher during the dry season (88.76%) than during the rainy (75.31%) and the transitional (20.29%) seasons. *Oreochromis niloticus* was not infected during the transitional season while *Hemichromis fasciatus* was more infected (p<0.001) during the dry season (26.84%) followed by the rainy (15.80%) and the transitional (10.32%) seasons. The high prevalence of myxosporeans infection may decrease the fish farming yield. The epidemiological data recorded help develop prevention and control strategies to boost the production of *Oreochromis niloticus* and *Hemichromis fasciatus* in Cameroon.

Keywords: Fish; Myxosporean infections; Prevalence; Epidemiology; Cameroon

Introduction

Fish farming is an important socio-economic activity in a rural community, contributing to livelihoods, food security, and poverty alleviation [1]. This certainly explains why in Cameroon rural community is paying a lot of attention to fish farming, and investments in this sector are increasing in order to meet the high demand for animal proteins induced by population explosion. Many fish farms have been constructed among which the most important are the Ngoundoup fish ponds, in the Koutaba subdivision, Noun Division, West-Cameroon. In these ponds, two Cichlid fishes are reared and appreciated by households for consumption. These fishes include *Oreochromis niloticus* and *Hemichromis fasciatus* commonly called Nile tilapia and banded jewel fishes respectively.

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Having the full mastery of farming skills is not the sole prerequisite for success in fish farming. Epidemiology aspects of infections should be taken into account as well. In a natural environment, the balance established during the evolution in the host-parasite system results in the decrease of the pathogenic effect of parasites [2]. On the contrary, the anthropogenic activities in fish farming can modify the water physicochemical characteristics resulting in the disruption of the fish-parasite equilibrium. As a result, fish not only stress but water can become more conducible to epizootics leading to massive fish death and important economic losses [3]. The confinement of fish, the presence of a muddy vase, the weak oxygenation, and the low depth of ponds are also factors favoring the transmission of parasites [4-7].

Among fish parasites, Myxosporeans impedes fish growth [8], their reproduction [4] and are involved in epizootics responsible for massive fish deaths in farms and hatcheries [9- 10]. In Cameroon, studies on fish myxosporeans are essentially descriptive. Therefore, for the last decades, only a few epidemiological data are available comprising those by Lekeufack [11], Lekeufack and Fomena [12], Nchoutpouen, *et al.* [13], and Nchoutpouen [14]. Qualitative and quantitative data provided by epidemiology are essential in the implementation of prevention and control strategies against myxosporean infections. The goal of this study was to assess the prevalence and some epidemiological aspects of myxosporean infection in *Oreochromis niloticus* and *Hemichromis fasciatus* fishes from the Ngoundoup ponds in Koutaba Subdivision, West Cameroon in order to set up a database for their efficient prevention and control.

Materials and methods

Study area and geoclimatic characteristics

Fishes were collected from June 2019 to April 2020 in the ponds located at the village named Ngoundoup (Figure 1), Koutaba Subdivision (North Latitude: 5°37'- 5°52', East Longitude: 10°44'-10°54'), Noun Division, Region of West-Cameroon. The average altitude is about 1276m above sea level.

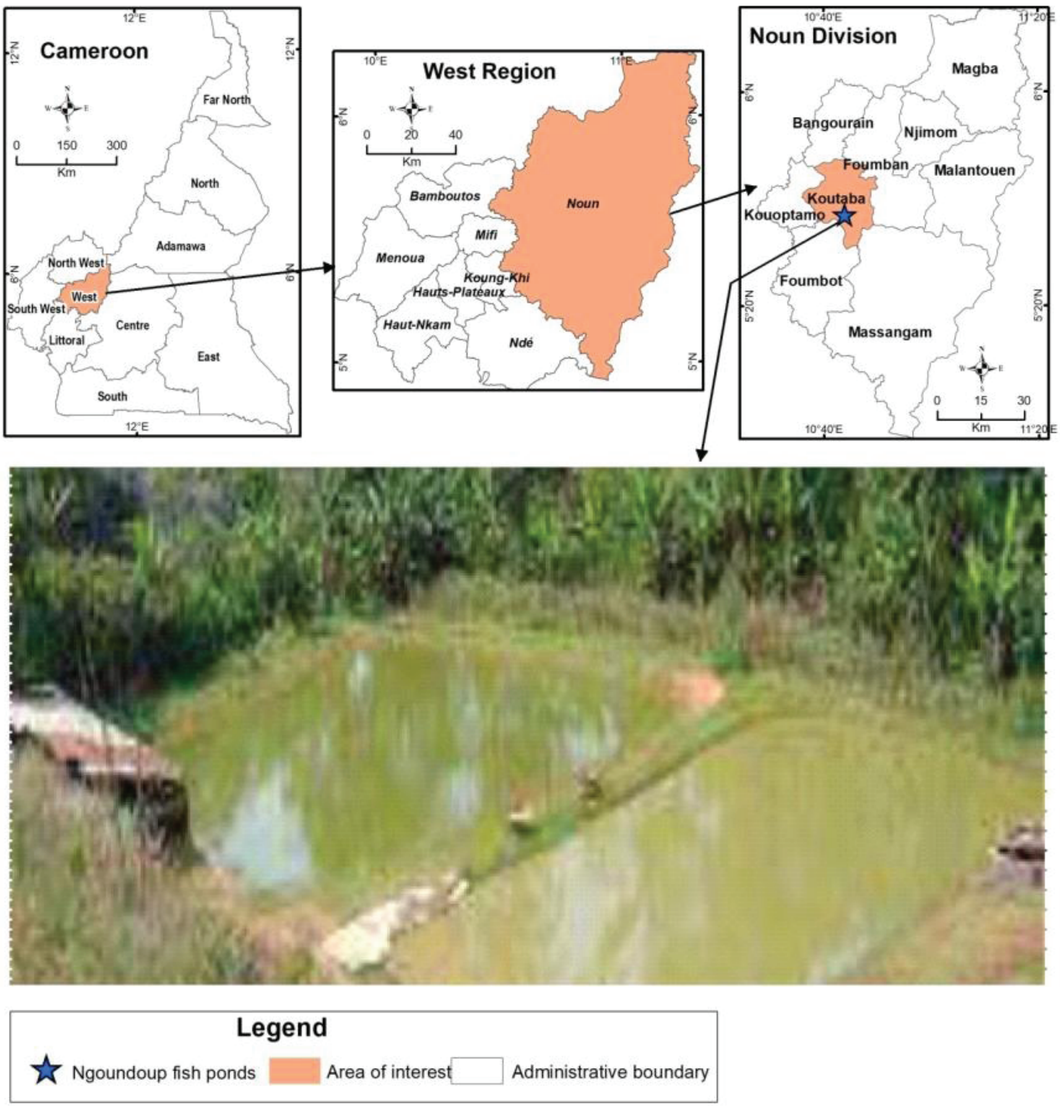

Figure 1: Map showing the study area and collection site.

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Climate is of the tropical mountain subset with two seasons: a long rainy season running from March to November and a short dry season from November to March. There are two transitional seasons i.e. from mid - October to mid - November and from mid-March to mid-April. The annual average temperature ranges from 19.80°C to 22.00°C while the rainfall varies between 1313.72 and 1988.60mm. The soil is of ferritic type and rich in organic matter. The sub-highland forest is often degraded by coffee plantations and other food crops [15].

Collection, examination of fishes and Myxosporeans identification

Fishes were captured at day using fish nets and were immediately stored in a vial containing 10% formalin solution and transported to the laboratory for examination. Fishes were identified as described by Stiassny, *et al.* [16] and examined for the presence of myxosporeans as per Abakar [17]. Thus, standard and total lengths were measured to the nearest millimeter using a slide caliper. Fishes were sex- determined after dissection.

The community structure of fishes (Table 1) reveals that a total of 320 specimens (Figure 2) comprising 189 *Oreochromis niloticus* and 131 *Hemichromis fasciatus* were collected. Various organs (skin, eye, kidneys, livers, gonads, spleens, gills, fins, buccal cavity, brain, digestive tract, gall bladder, brain) were examined with naked eyes, then with a stereoscopic microscope using the 10X lens to look for the cysts. Smears of the kidneys, spleen, and gonads were made and examined at a total magnification of 1000X with a light microscope to search for myxospores. Cysts were crushed between a slide and a cover glass in a drop of distilled water and their contents were identified with the light microscope using the 100X lens. Spores were fixed and stained with methanol and May-Grünwald-Giemsa respectively and photographed with a digital camera (Canon Ixus brand).

Myxospores were measured with a calibrated ocular micrometer as recommended by Lom and Arthur [18] and were morphologically identified using the key provided by Lom and Dyková [19,20], Eiras, *et al.* [21-22], Fomena and Bouix [23].

Epidemiological parameter studied and statistical analysis

The epidemiological parameter studied was the prevalence (Pr) of infection expressed in percentage and defined as the number of fish species infected by a given parasite species divided by the number of fish examined [24]. The prevalence was classified as very low (Pr<25%), low (25% ≤ Pr <50%), high (50% ≤ Pr <75%) and very high (75% ≤ Pr ≤ 100%).

The comparison of prevalence was performed using the Chi-square (X^2) test. The Spearman correlation coefficient "r" was calculated to determine a probable relationship between the prevalence of parasite species and the fish size. The significance level of the probability was $p < 0.05$ and the Graph Pad Prism 5 software was helpful for analysis.

Results

Results are illustrated in Figures 3-7 and Tables 2-3.

Myxosporean fauna of fishes and prevalence of fish species

The myxosporean fauna of fishes shown in Table 2 was composed of 8 species of the genus *Myxobolus*. The prevalence of myxosporeans species in relation to fish species illustrated in Figure 3 reveals a high (54.38%) overall prevalence of infection in the ponds. Irrespective of the parasite species, both fish species were parasitized. In addition, *Oreochromis niloticus* (75.13%) was significantly (X^2= 25.92; p= 0.001) more infected than *Hemichromis fasciatus* (24.27%). The prevalence of parasites was very low (<25%) whatever the fish species.

Oreochromis niloticus and *Hemichromis fasciatus* harbored 8 and 3 myxosporean species respectively. Moreover, 5 parasite species (*M.tchadanayei*, *M. agolus*, *M. heterosporus*, *M.tilapiae* and *M. kainjiae*) were specific to *Oreochromis niloticus* while 3 (*M. camerounensis*, *M. israelensis* and *M. brachysporus*) were common to both fish species. In *Hemichromis fasciatus*, the prevalence varied significantly (X^2= 17.41; p<0.001) from 5.30 (*M. brachysporus*) to 10.30% (*M. camerounensis*) while in *Oreochromis niloticus*, *Myxobolus tilapiae* and *M. tchadanayei* exhibited the highest (24.50%) and the lowest (0.92%) prevalence respectively (p<0.001).

Prevalence of Myxosporean species as a function of fish sex

The prevalence of myxosporean species as a function of fish sex exhibited in Figure 4 reveals that, both males and females were infected. Regardless of the fish and parasite species, females (67.01%) were insignificantly (X^2= 1.63; p= 0.201)

Table 1: Community structure of *Oreochromis niloticus* and *Hemichromis fasciatus* in Ngoundoup ponds, Koutaba Subdivision, West-Cameroon.

Fish species	Sex	Class size (mm)			Total	Seasons			MSL (mm)
		[25 - 75]	[75 - 125]	>125		Rainy	Transitional season	Dry	
Oreochromis niloticus	♂	23	46	42	111	35	31	45	46.35 (25-200)
	♀	15	30	33	78	27	17	34	46.83 (26-210)
	♂+♀	38	76	75	189	62	48	79	46.55 (25-210)
Hemichromis fasciatus	♂	12	35	35	82	28	26	28	46.89 (25-140)
	♀	9	26	14	49	17	16	16	45.13 (27-135)
	♂+♀	21	61	49	131	45	42	44	46.26 (25-140)
Total	♂	35	81	77	193	63	57	73	46.60 (25-200)
	♀	24	56	47	127	44	33	50	46.14 (26-210)
	♂+♀	59	137	124	320	107	90	123	46.42 (25-210)
	(%)	18.44	42.81	38.75	100	33.44	28.13	38.44	

Mean Standard Length (MSL) is followed in the bracket by minimum-maximum values; ♂: male; ♀: female

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Table 2: Myxosporean fauna found in fishes in Ngoundoup ponds, West-Cameroon.

N°	Myxosporean species	References
1	*Myxobolus camerounensis*	Fomena, *et al*.1993
2	*Myxobolus israelensis*	Landsberg, 1985
3	*Myxobolus brachysporus*	Baker, 1963
4	*Myxobolus tchadanayei*	Abakar, et *al*. 2006
5	*Myxobolus agolus*	Landsberg, 1985
6	*Myxobolus heterosporus*	Baker, 1963
7	*Myxobolus tilapiae*	Abolarin, 1974
8	*Myxobolus kainjiae*	Obiekezie and Okaeme 1990

Table 3: Correlations between the prevalence of Myxosporean species and the fish size.

Myxosporean species	Fish species			
	Oreochromis niloticus		*Hemichromis fasciatus*	
	r	p	r	p
Myxobolus camerounensis	-0.09	0.280	+0.05	0.613
Myxobolus israelensis	-0.03	0.778	+0.04	0.708
Myxobolus brachysporus	-0.01	0.948	-0.03	0.749
Myxobolus tchadanayei	+0.06	0.498	-	-
Myxobolus agolus	-0.03	0.728	-	-
Myxobolus heterosporus	0.00	0.971	-	-
Myxobolus tilapiae	-0.20	0.02	-	-
Myxobolus kainjiae	+0.09	0.294	-	-

r: correlation coefficient; p: error probability; -: no value

more infected than males (58.94%). Whether in *Oreochromis niloticus* (X^2= 2.12; p= 0.145) or *Hemichromis fasciatus* (X^2= 0.08; p= 0.782), the prevalence of parasite species did not differ between males and females.

Prevalence of Myxosporean species in relation to fish size class

As illustrated in Figure 5, fish of all size classes were infected. Whatever the fish species, the prevalence of myxosporean species did not significantly differ (p>0.05) between size classes. The prevalence of *Myxobolus tilapiae* (Table 3) was negatively and significantly correlated (r= -0.20; p= 0.02) with *Oreochromis niloticus* size.

Prevalence of Myxosporean species as a function of the infection sites and fish species

The prevalence of myxosporean species as a function of infection sites and fish species as illustrated in Figure 6 reveals that irrespective of the fish species, only two organs were infected namely kidneys and ovaries. In both fish species, kidneys were infected while ovaries were the only parasitized organs in *Oreochromis niloticus.* Whether between or within fish species, the prevalence was higher for the kidneys. The comparison of the infection sites in terms of parasites richness shows that all the 8 myxosporean species were encountered in the kidneys while *Myxobolus kainjiae* was specific to *O. niloticus* ovaries.

Seasonal prevalence of Myxosporean species

The seasonal prevalence of myxosporean species (Figure 7) shows that irrespective of the fish and parasite species, all the considered seasons were favorable to fish infections. Moreover, the overall prevalence was significantly (X^2= 85.84; p<0.001) higher during the dry season (88.76%) than the rainy (75.31%) and transitional (20.29%) seasons. *Myxobolus tchadanayei*, *M. heterosporus* and *M. tilapiae* were the sole parasites exhibiting no significant (p>0.05) seasonal variation of the prevalence. The comparison of the seasonal prevalence of parasites between fish species reveals that *Oreochromis niloticus* was not infected during the transitional season. On the contrary, *Hemichromis fasciatus* was significantly more parasitized by *Myxobolus israelensis* (24.32%) during the transitional season compared to other seasons. In *Oreochromis niloticus*, the prevalence of parasites did not show a significant seasonal variation (X^2= 0.47; p= 0.92) while in *Hemichromis fasciatus*, parasites were more prevalent (X^2= 14.254; p<0.001) during the dry season (28.84%) followed by the rainy (15.80%) and the transitional seasons (10.32%).

Figure 2: Photographs of fish specimens.
a: *Oreochromis niloticus* Linnaeus, 1758 (bar: 13 cm);
b: *Hemichromis fasciatus* Peters, 1857 (bar: 4.5 cm)

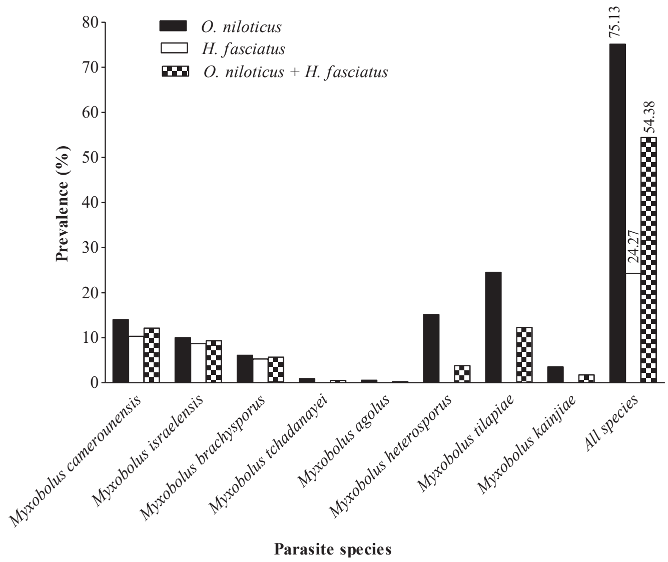

Figure 3: Prevalence of Myxosporean species in relation to fish species.

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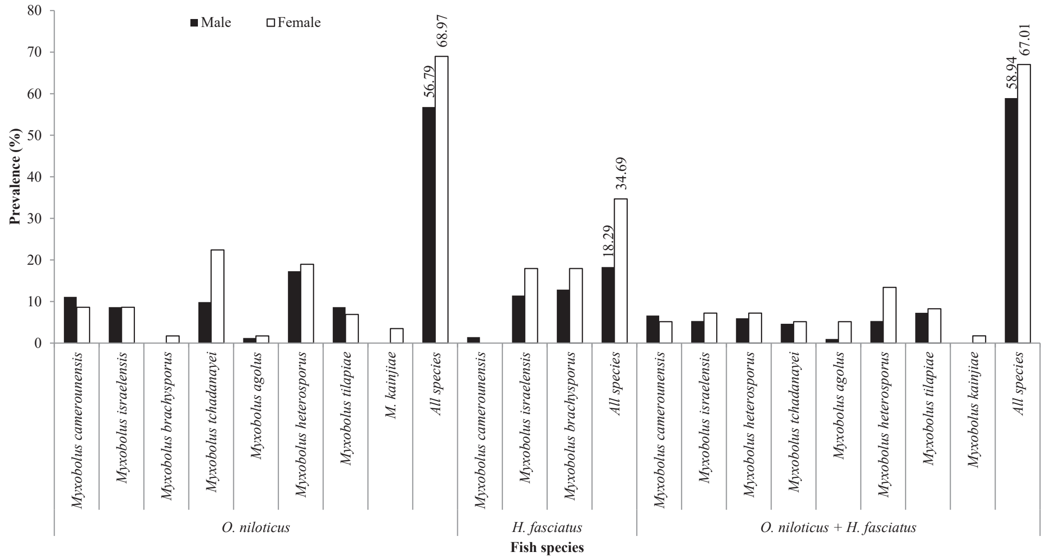

Figure 4: Prevalence of Myxosporean species as a function of *Oreochromis niloticus* and *Hemichromis fasciatus* sex.

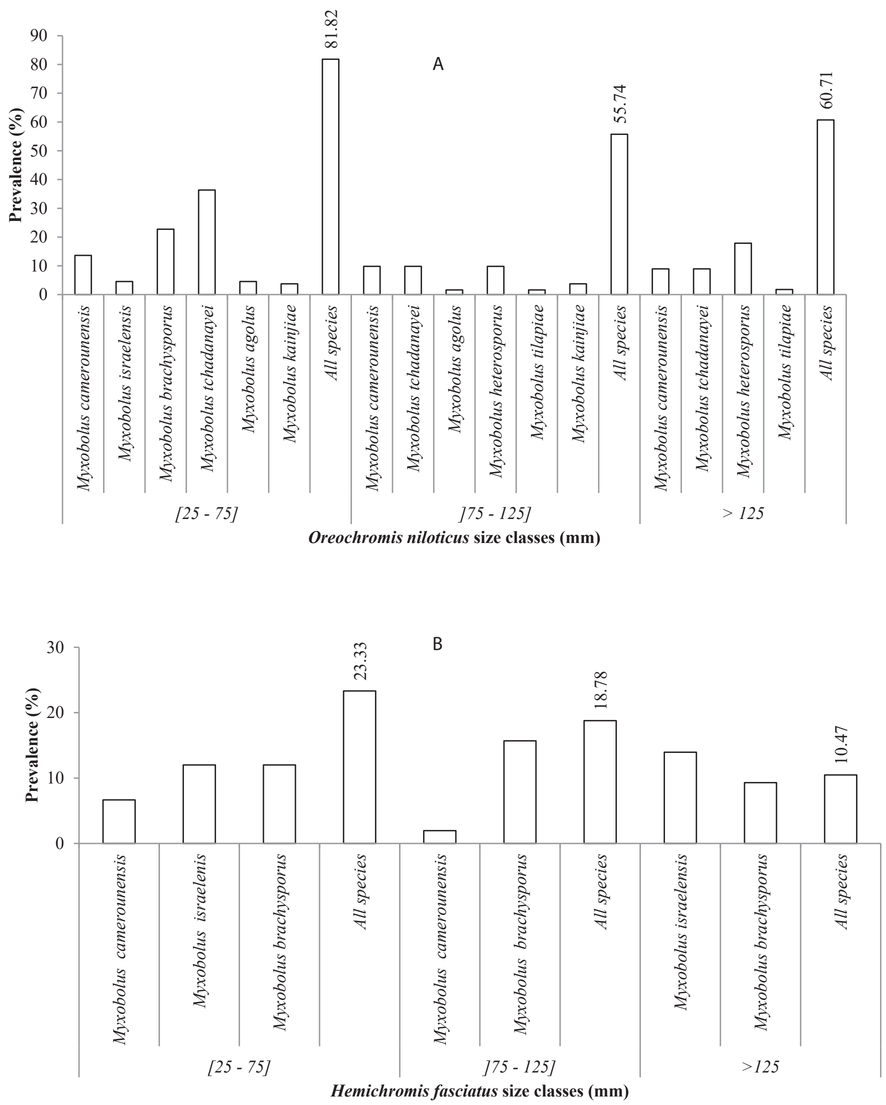

Figure 5: Prevalence of Myxosporean species to *Oreochromis niloticus* (**A**) and *Hemichromis fasciatus* (**B**) size classes.

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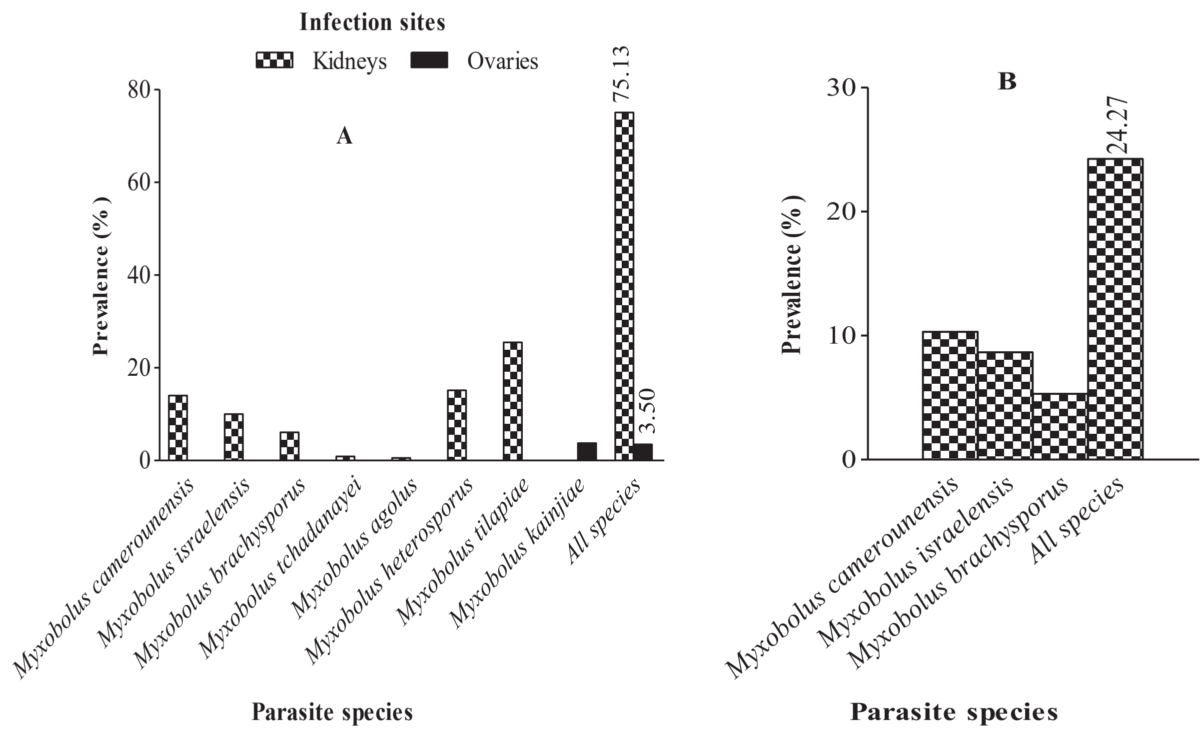

Figure 6: Prevalence of Myxosporean species as a function of infection sites in *Oreochromis niloticus* (**A**) and *Hemichromis fasciatus* (**B**).

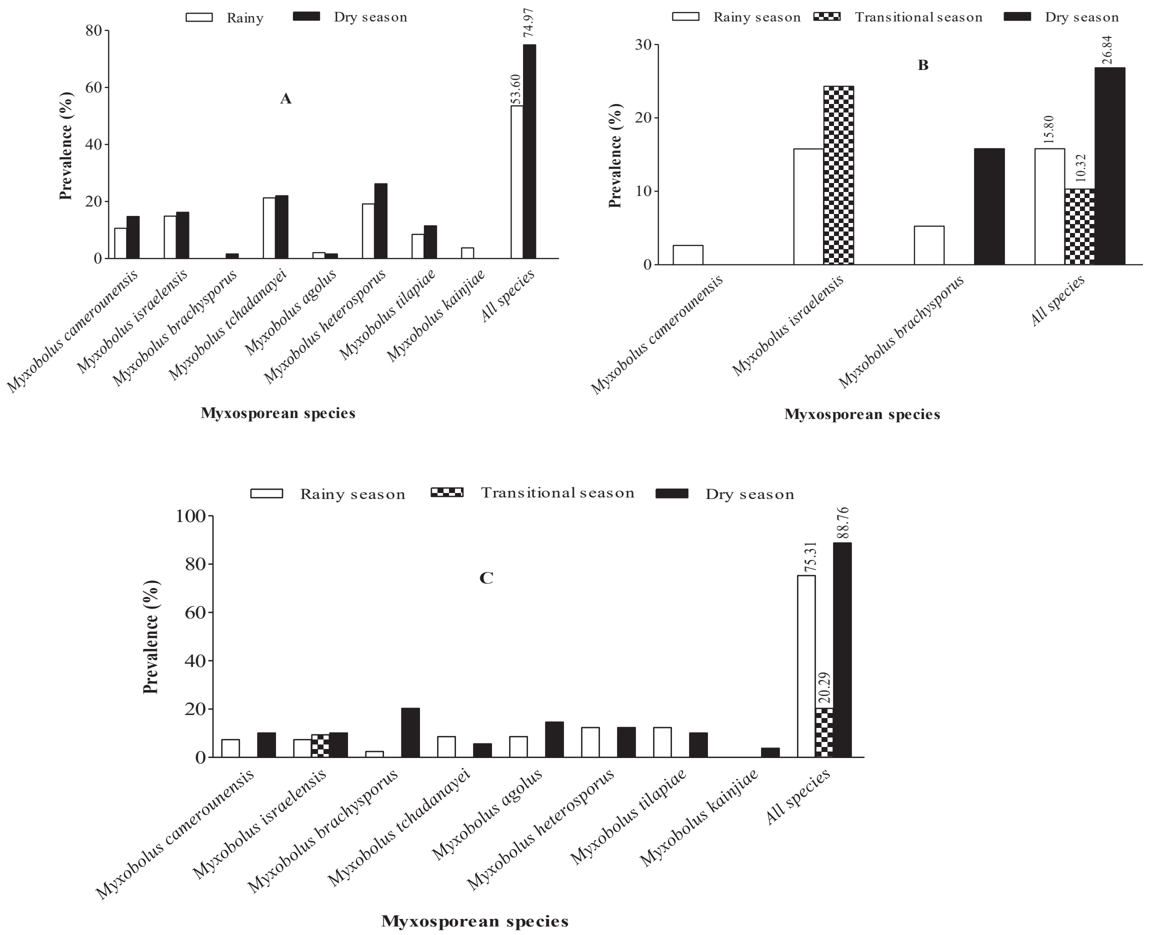

Figure 7: Seasonal prevalence of Myxosporean species in *Oreochromis niloticus* (**A**), *Hemichromis fasciatus* (**B**), and *O. niloticus* + *H. fasciatus* (**C**).

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Discussion

The predominance of myxosporeans of the genus *Myxobolus* (8 species over 8) in the study site is well documented. The world myxosporeans fauna is composed of about 2180 species (62 genera) among which the genus is numerically the most abundant with about 35% of species [20]. Kaur [25] reported 55 myxosporean species grouped in 6 genera in various organs of wetlands and cultured carps in Punjab out of which, the genus *Myxobolus* was the most represented (28 species) compared to other genera namely *Thelohanellus* (18 species), *Henneguya* (6 species), *Triangula* (2 species), *Neothelohanellus* and *Unicauda* (1 species each). The same observation was made by Anu [26] who recorded up to 64% of *Myxobolus* species infecting cultured native and exotic carps in Punjab. Abakar [17] reported 60% of *Myxobolus* species in Myxosporeans fauna of Chad freshwater fishes. Meanwhile, in Cameroon, Lekeufack and Fomena [12] collected 54.55% of the myxosporeans of the genus *Myxobolus* in the River Sangé infecting several hosts. Nchoutpouen, *et al.* [13] reported 100% *Myxobolus* species (10 species over 10) in Foumban fish ponds. The broad spectrum of hosts experienced by the genus *Myxobolus*, its preponderance, wide geographic distribution, and ecological plasticity might be due to the versatility of its metabolic pathway and the genetic background enabling it to get adapted to various biotopes.

Myxobolus camerounensis, *M. israelensis* and *M. brachysporus* could be of stenoxenous specificity since they commonly infected *Oreochromis niloticus* and *Hemichromis fasciatus* which belong to the same family (Cichlidae). Myxosporean species that were specific to *O. niloticus* probably might be oïoxenous (narrow specificity). The fish/parasite specificity can be explained by the fact that different hosts constitute different habitat options (ecological niches) for parasites. Each parasite is thus adapted to the host which provides a maximum resource for the parasite survival.

The high overall prevalence of infection (54.38%) in the ponds is in agreement with Nchoutpouen, *et al.* [13] who reported a high prevalence (64.80%) in *Oreochromis niloticus* (Nile tilapia) in Foumban fish ponds (West-Cameroon). Moreover, the latter authors also observed a prevalence of 61.10% in *O. niloticus* from the natural environment (Noun River). In Punjab, 34.71% of fishes were infected in three wetlands (Harike, Kanjali, and Ropar) and 26.28% in aquaculture [25]. Anu [26] reported 44.20% of infected native Indian carps in aquaculture. In Egypt, Mohammed, *et al.* [27] recorded in the Nile River the prevalence of 25.00% and 24.20% in *O. niloticus* and *Tilapia zilli* respectively. In the natural environment, the prevalence of infection is generally low (compared to the farming situation) because the balance established during the evolution of host/parasite system reduces the pathogenic effects of parasites [2]. The prevalence of the parasite species vary geographically [28] as per the host species [29]. In aquaculture, the anthropogenic activities, the confinement of fish, the presence of muddy vase, the low oxygenation and lo water depth are factors increasing the prevalence of parasites [4-7].

In ponds, the low water flow may result in heavy myxosporean infections. In fact, Ray, *et al.* [30], Ray and Bartholomew [31] claimed that the water flow was the most important abiotic factor after the water temperature influencing the transmission of myxosporeans. High flows may scour and remove preferred oligochaete habitat, dilute infectious stages and decrease transmission of actinospores to fish [32]. On the other hand, lower flows encourage higher retention of spores and transmission of myxospores to oligochaete hosts [33] resulting in a higher prevalence of infection in both oligochaete and fish hosts, as well as higher infection severity in fish [32]. Actinospores transmission as well as the prevalence of myxosporean infections are greatly reduced above a velocity threshold of about 0.2–0.3m/s [34- 35]. If the soil of our study area was clayey or silty, one might think that it would favor the myxosporean infections. Indeed, silt and clay harbor more infected oligochaetes than other substrates [36]. At the same water flow, oligochaetes inhabiting silt or mud produce more actinospores than those in the sand [37-38]. However, Neudecker, *et al.* [39] found no significant association between fine sediment abundance and infection severity.

The infection rate of *Oreochromis niloticus* (75.13%) was significantly higher than that of *Hemichromis fasciatus* (24.27%). This may be due to the difference in fish genetic background. If we assume that the genetic background has nothing to do with the fish's susceptibility to myxosporean infections, then the infection rate will not vary between fishes sharing the same confined biotope.

The fish sex did not significantly influence the prevalence of parasite species. The same observation was made by Abakar [17], Milanin, *et al.* [40], Lekeufack and Fomena [12]. Fomena [5] did not observe any significant difference between the prevalence of myxosporean in male and female *Oreochromis niloticus* at Mélen fish ponds in Cameroon. Viozzi and Flores [41] also claimed that the prevalence of *Myxobolus biliare* in *Galaxias maculates* was not sex-related. They further opined that it is a general situation with myxosporean infection. Anu [26] instead reported that female carps were more infected (38.25%) compared to males (26.21%). Gbankoto, *et al.* [42] thought that the prevalence of myxosporidiosis is often higher in males than in females probably due to the fact that males lose a huge amount of energy for testosterone synthesis, resulting in a weaker immune system [43]. The effect of fish sex on the prevalence of myxosporean infections is still to be thoroughly investigated [44].

Fishes of all size classes were infected without any significant difference in the prevalence among size classes. Obiekezie and Okaeme [4] made the same remark. According to Viozzi and Flores [41], Tombi and Bilong Bilong [7], and Abakar [17], young fishes are more vulnerable to myxosporean infections than older ones. On the contrary, Nchoutpouen, *et al.* [13] outlined that in ponds, older *Oreochromis niloticus* were more infected than the younger ones. As *Oreochromis niloticus* grows, the prevalence of *Myxobolus tilapiae* decreased (r= -0.20; p= 0.02). A similar phenomenon was observed in Finland where the prevalence of infection of *Rutilus rutilus* by *Myxobolus rhodei* and *M. pseudodispar* decreased with the fish size; this might be due to the increase of the immune system response with the fish size [29].

The infections of the kidneys with all the 8 myxosporean species suggest that those organs offer suitable micro biotopes with optimal life conditions for parasites. Since kidneys filter

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blood and secrete many solutes [45], parasites converge there for the metabolites they need, this may be the reason why they harbored more parasite species than ovaries. The specificity of *Myxobolus kainjiae* to *O. niloticus* ovaries probably is because ovaries provide a suitable environment for the *M. kainjiae* survive.

The prevalence was higher in the dry season than in the rainy and transitional seasons. In Foumban ponds (Cameroon), Nchoutpouen, *et al.* [13] noticed that the infection rates were higher in the rainy season and low in the dry season. Moreover, Sitjà and Alvarez [46] observed that in Spain, the prevalence of *Sphaeropora dicentrarchi,* in *Dicentrarchus labrax* varied with seasons in the fish ponds higher, being during the summer than the autumn. Also, the prevalence of *Myxidium biliaire* was reported to be higher in summer than in winter [41]. Thus there appear to be contradictory results regarding the season of higher infection rate suggesting that other factors may be intrinsic (parasite and host) or extrinsic (water Physico-chemical characteristics, general management of ponds) regulate parasite prevalence. For instance, during the dry season, the increase in water temperature and the presence of a muddy vase raise the prevalence of the myxosporean and the oligochaetes (definitive host). Özer, *et al.* [47] revealed that mud subtracts favors rapid growth and multiplication of oligochaetes. Consequently, during the dry season, the definitive hosts are very abundant and their infecting stages (actinospores) multiply rapidly. This situation is favorable to fish infection [48].

Conclusion

The overall prevalence of myxosporean infection in the ponds was high. The main risk factors of myxosporean infection were the seasons and fish species. Kidneys and ovaries were the only infected organs. Fish kidneys being mixed organs with hematopoietic, reticuloendothelial, endocrine, and excretory functions, its infection can lead to fish death. Also, the infection of the ovaries may result in the barrenness of fishes and the decrease of their productivity. The epidemiological data recorded help develop control strategies in order to increase the production of *Oreochromis niloticus* and *Hemichromis fasciatus* in the ponds.

We do not advise the exportation of *O. niloticus* and *H. fasciatus* fingerlings of our ponds for any aquaculture purpose without implementing a control program. Since the prevalence of myxosporean species significantly decreased from the dry, rainy to transitional seasons, it is better to rear those Cichlids during the transitional season.

Ethical approval and consent to participate

Fishes used followed a protocol approved and authorized by the Institutional Animal Care and Use Committee at the Department of Animal Production, Faculty of Agronomy and Agricultural Science, University of Dschang, Cameroon. Fish farmers agreed to the study.

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Availability of data and material

The raw data used to support the findings are available from the corresponding author upon reasonable request.

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