Journal of Research in Biology

An International Scientific Research Journal

ISSN No: Print: 2231 –6280; Online: 2231- 6299

Original Research

Seasonal patterns of *Myxobolus* (Myxozoa : Myxosporea) infections in *Barbus callipterus* Boulenger, 1907 (Cyprinidae) at Adamawa - Cameroon

Authors:
Fonkwa Georges[1,2], Efole Ewoukem Thomas[1], Tchuinkam Timoléon[2], Fomena Abraham[3] and Tchoumboue Joseph[1]

Institution:

1. Laboratory of Ichthyology and Applied Hydrobiology, Faculty of Agronomy and Agricultural Sciences, University of Dschang, P.O. Box 222, Dschang-Cameroon.

2. Laboratory of Biology and Applied Ecology, Vector Borne Parasitic and Infectious Diseases Unit, Faculty of Science, University of Dschang P.O. Box 67, Dschang-Cameroon.

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

Corresponding author:
Fonkwa Georges

ABSTRACT:

For a detail understanding of the seasonal patterns of *Myxobolus* infections so as to elaborate control strategies, 305 *Barbus callipterus* specimens were sampled from May 2016 to May 2017 at Mapé River (Adamawa-Cameroon). After classical examination, the prevalence of infection was determined. Ten *Myxobolus* species were identified. Irrespective of the parasite species, fishes were more infected in the dry season (74.93%) than the rainy season (69.72%) without significant difference. Seasons did not significantly influence on the prevalence of parasites in the sizes and sex of various fishes' class. However, *Myxobolus ngassami* was predominantly prevalent in male fishes during the dry season in contrast to the females. Regardless of the organ type and parasite species, the dry season was remarkably more favorable to the infection of organs. Mono and polyinfections occurred during the two seasons and whatever the season, the prevalence of polyinfection's categories dropped with the increasing number of associated parasite species.

Keywords:

Myxobolus, Prevalence, Season, *Barbus callipterus*, Mapé river, Cameroon.

Article Citation:

Fonkwa Georges, Efole Ewoukem Thomas, Tchuinkam Timoléon, Fomena Abraham and Tchoumboue Joseph

Seasonal patterns of *Myxobolus* (Myxozoa: Myxosporea) infections in *Barbus callipterus* Boulenger, 1907 (Cyprinidae) at Adamawa -Cameroon

Journal of Research in Biology (2018) 8(1): 2413-2424

Dates:

Received: 05 Jan 2018 **Accepted:** 01 Feb 2018 **Published:** 15 Feb 2018

Web Address:

http://jresearchbiology.com/documents/RA0659.pdf

INTRODUCTION

Myxosporeans are mainly fish parasites (Fomena *et al*., 2010; Eiras *et al*., 2010). Their pathogenic effects entail the depression of fish growth (Longshaw *et al*., 2010), reproduction (Obiekezie and Okaeme, 1990) and the massive fish deaths (Gbankoto *et al*., 2001; Feist and Longshaw, 2005). Lom and Diková (2006) reported that among about 2180 Myxosporeans species (62 genera) composing the world of Myxosporeans fauna, the genus *Myxobolus* Bütschli, 1882 was numerically the most abundant with 31% of species.

In Africa, researches emphasize more on Myxosporeans systematic than its seasonal dynamics (Folefack, 2010; Abakar, 2006). This is questionable because, the water physico-chemical characteristics variation resulting from the seasonal change may lead to the disruption of host/parasite equilibrium causing fish stress and deaths (Bilong and Jeannette, 2004). The objective of the study was to assess the seasonal patterns of *Myxobolus* infections in an economical and dietary fish namely *B. callipterus* in order to boost its production.

MATERIALS AND METHODS

Study Area

Fishes were captured at the MAPE River in a village named MGBADJI (6°00’- 6°20’NL and 11°20’- 11°40’ EL). The latter belongs to Bankim subdivision, Adamawa – Cameroon Region. The average altitude is about 724m above the sea level while the soil is a mixture of clay and sand. The climate is of tropical Soudano-guinean type with 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 whereas the rainfall ranges between 1500 and 2000 mm (Olivry, 1986).

Fish sampling and identification of Myxosporeans species

Fishes were harvested monthly from fishermen during the study period i.e. May 2016 to May 2017. They were captured both at the day and night using fish nets and fishing canes. On the field, specimens were immediately stored at 10% formalin solution and transported to the laboratory for examination. Overall 305 fish samples (159 males against 146 females) were caught. The standard lengths ranged from 52 to 110 mm with an average of 94.66 mm. Fishes were categorized into 3 class sizes of 20 mm amplitude each. The modal class i.e. [70 - 90] represented 64.92% of sampled fishes.

Amid the laboratory phase, identification and examination of fishes were done as per Stiassny *et al*. (2007) and Abakar (2006) respectively. Consequently, standard and total lengths were estimated to the nearest millimeter utilizing a slide caliper of stainless brand and the sex decided after dissection. External organs (fins, skin, scales and eyes) and internal organs (gills, spleen, kidneys, intestines, gall bladder, stomach and gonads) were analyzed with naked eyes and after that with Motic stereoscopic microscope at 10X to search for the macroscopic cysts. Three smears of kidneys, spleen and gonads were made (anterior, medium and posterior region) and were analyzed at a total magnification of 1000X with a light microscope keeping in mind the end goal to identify spores. Cysts were crushed amongst slide and cover glass in a drop of distilled water and their contents were identified with the light microscope at 1000X. After fixation of spores with methanol, they were stained with May-Grünwald-Giemsa and snapped with digital camera (Canon Ixus mark). Myxosporeans species were distinguished by Lom and Arthur (1989).

Parasitological parameter studied

The prevalence (Pr) of infection conveyed in percentage was the proportion between the quantity of fish's species infected by a given parasite species and the number examined (Bush *et al*., 1997). Parasites were qualified as principal (Pr > 50 %), intermediate (10 % ≤ Pr ≤ 50 %) and scarce (Pr < 10 %) (Valtonen *et al*., 1997).

Statistical analysis

The comparison of prevalences was performed by the Chi-square (X^2) test. The significance level was P < 0.05 and the Graph Pad Prism 5 software was used for analysis.

RESULTS

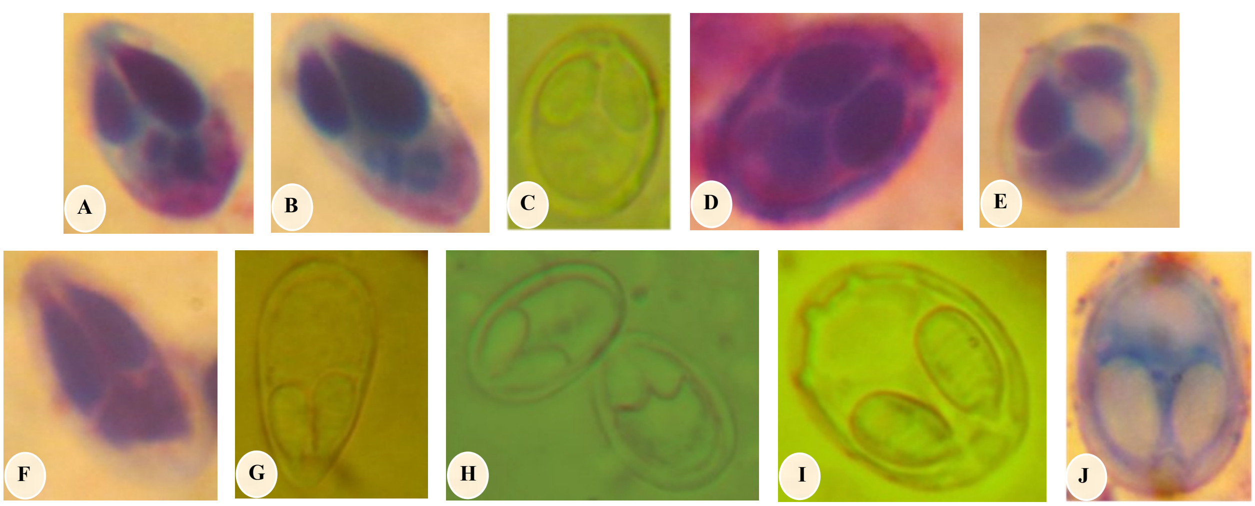
The results are illustrated on Table 1 and Figures 1 to 7.

Seasonal prevalence of Myxosporeans species

The myxosporeans fauna recorded (Figure 1) was composed of 10 *Myxobolus* species. The seasonal prevalence of Myxosporeans species (Figure 2) reveals that, irrespective of the parasite species, fishes were more infected in the dry season than in the rainy season, however without significant difference (X^2 = 0.77; P = 0.380). During the rainy season three parasite species were intermediate against five during the dry season. Whatever the season, no principal parasite was recorded. *Myxobolus pharyngeus* appeared only during a single season. On the one hand, during the rainy season, prevalences were significantly higher (X^2 = 58.14; P < 0.001) with *M. pseudodispar* and lower in *M. muelleri*. On the other hand, *M. ngassami* was the most prevalent during the dry season (X^2 = 68.46; P < 0.001) while *M. pharyngeus* was absent. The sole parasite species showing seasonal significant variation of prevalence was *Myxobolus mapei*. The latter being more prevalent (X^2 = 4.00; P < 0.05) in the dry season than in the rainy season.

Prevalence of infection as a function of class size and seasons

The prevalence of infection as a function of class size and seasons (Figure 3) shows that fishes were infected in all the three class sizes during the dry and rainy seasons. Whatever the class size, the prevalence did not differ between seasons (P > 0.05). Whether in the rainy season or the dry season, the higher prevalence was recorded in the class 90-110 mm, however without any significant difference (X^2 = 1.40; P = 0.237).



A : *Myxobolus tchadanayei* Abakar *et al*., 2006 (x 1500); B : *Myxobolus mapei* Fonkwa *et al*., 2017 (x 1200);
C : *Myxobolus muelleri* Bütschli, 1882 (x 1500); D : *Myxobolus ellipsoides* Thélohan, 1982 (x 1500);
E : *Myxobolus pseudodispar* Gorbunova, 1936 (x 1500); F : *Myxobolus pharyngeus* Parker *et al*., 1971 (x 1500);
G : *Myxobolus tchoumbouei* Fonkwa *et al*., 2017 (x 1000); H : *Myxobolus umidus* Carriero *et al*., 2013 (x 1500);
I : *Myxobolus sessabai* Lekeufack *et al*., 2017 (x 1500); J : *Myxobolus ngassami* Lekeufack *et al*., 2017 (x 1500)

Figure 1. Spores micrographs of *Myxobolus* species studied

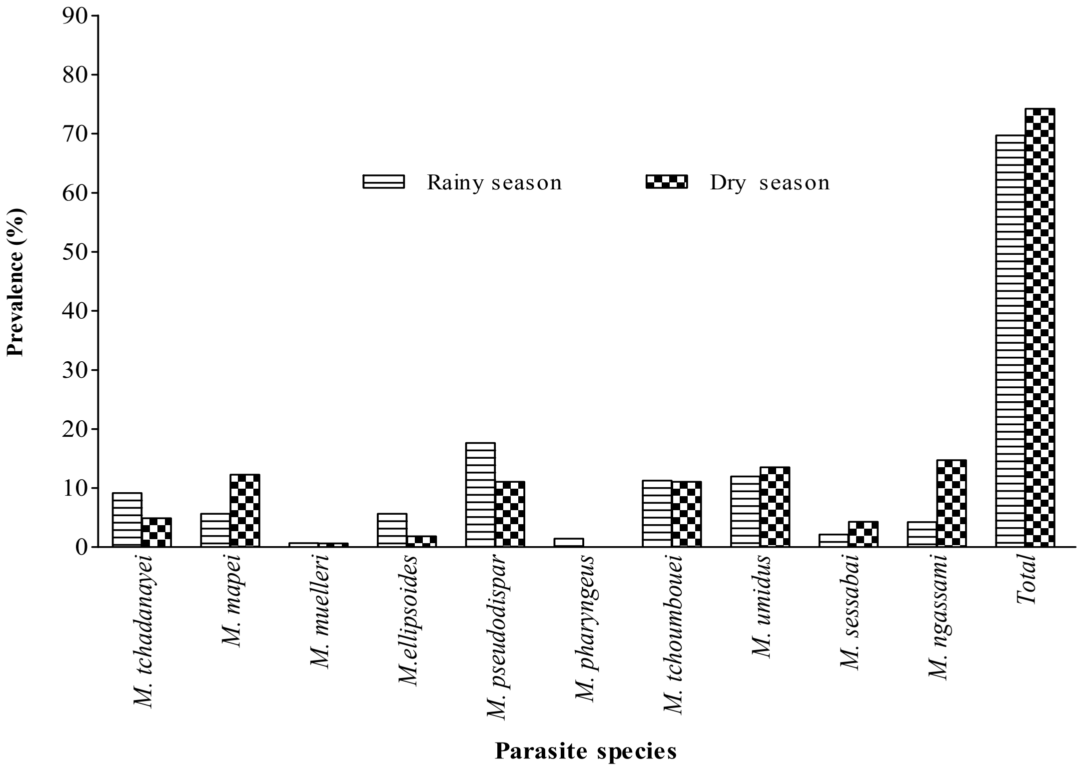


Figure 2. Seasonal prevalence of Myxosporeans species

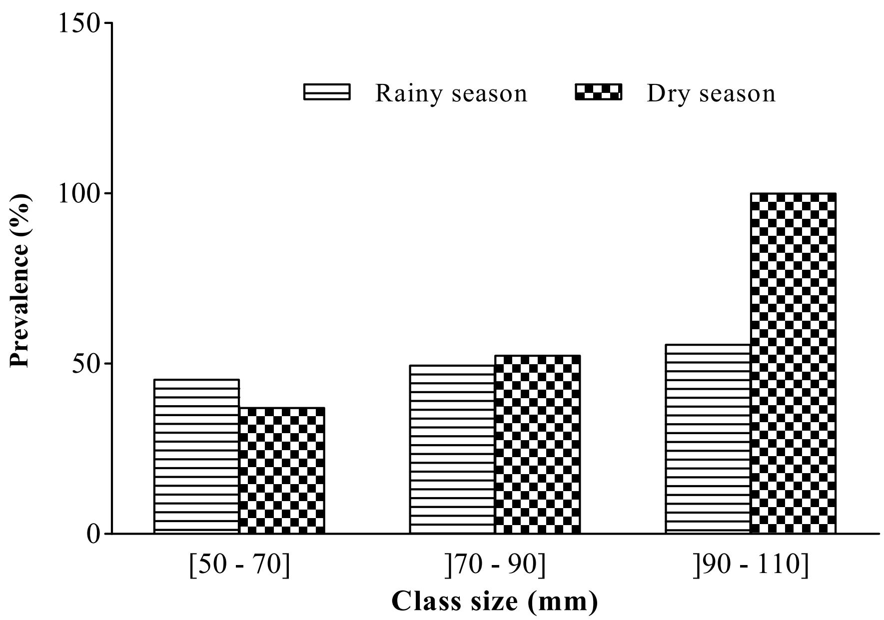


Figure 3. Prevalence of infection as a function of class size and seasons

Seasonal prevalence of parasite species as a function of class size

The seasonal prevalence of parasite species as a function of class size is illustrated on Figure 4. It appears that, in term of species number, whatever the class size, parasite species occurred more in the rainy season apart from the class size 50-70 mm, where 8 species were recorded both in the dry and the rainy seasons. During the dry season, the higher prevalence ($P < 0.05$) was noticed for *Myxobolus ngassami* in the class [90 - 110] while in the rainy season the higher ($P < 0.05$) prevalence (*M. pseudodispar*) was observed in the class 70 - 90 mm. In this latter class, *Myxobolus ngassami* was the sole parasite exhibiting a very significant ($X^2 = 8.55$; $P < 0.01$) variation of prevalence between the two seasons. Moreover, it was more prevalent in the dry season than in the rainy season.

Prevalence of parasite species as a function of host's sex and seasons

The prevalence of parasite species as a function of host's sex and seasons (Table 1) shows that independently of the parasite species, male fishes were more infected in the rainy season than the dry season ($X^2 = 0.06$; $P = 0.812$) contrary to females mostly infected during the dry season ($X^2 = 0.01$; $P = 0.909$). Whether in the rainy season ($X^2 = 0.30$; $P = 0.586$) or dry season ($X^2 = 0.05$; $P = 0.822$), the prevalence did not vary significantly between males and females.

When parasite species are taken into account, it appears that in both males and females, *M. ngassami* was the only parasite exhibiting a significant difference ($P < 0.05$) in prevalence between the two seasons. Moreover, it was more present in male fishes during the dry season contrary to the females. No matter the season and the fish's sex, the prevalence fluctuated very significantly ($P < 0.001$) between parasite species.

Percentage of infected organs as a function of seasons

The percentage of infected organs as a function of seasons (Figure 5) illustrates that, organs were infected during both the dry and the rainy seasons. According to the seasonality of infections, organs are grouped into three categories: those infected only in the rainy season (gills), the dry season (fins) and those infected during the two seasons (operculum, skin, kidney and liver). Regardless of the organ type, the dry season was remarkably ($P < 0.05$) more favorable for organ infection. Although kidneys were the most infected organs during both the rainy ($X^2 = 308.60$; $P < 0.001$) and the dry seasons ($X^2 = 392.20$; $P < 0.001$), no significant difference of prevalence was observed.

Prevalence of parasite species as a function of organs and seasons

As shown on Figure 6, the prevalence of parasite species fluctuated according to seasons and organs. Fins and gills were infected only during a single season by one parasite species. Kidneys were the only organs where intermediate species were found contrary to other organs harboring scarce parasite species. In the operculum, the two seasons were not only favorable to the occurrence of *Myxobolus mapei* but also, its prevalences were the highest ($P < 0.05$) compared to those of other parasite species. In the kidneys, apart from *M. pharyngeus*, all parasite species appeared during both the dry and rainy seasons. The prevalence of *Myxobolus ngassami* in the kidneys was about twice higher in the dry season than in the rainy season ($X^2 = 7.82$; $P < 0.01$). Meanwhile, *M. pseudodispar* exhibited the higher prevalence ($P > 0.05$) during the rainy season.

Seasonal prevalence of mono and polyinfections

The seasonal prevalence of mono and polyinfections is summarized on Figure 7. It appears that during both the dry and the rainy seasons, fishes were infected by a single parasite species (monoinfection) and more than one species (polyinfection). The monoinfections were more frequent during the rainy season than the dry season ($X^2 = 0.79$;

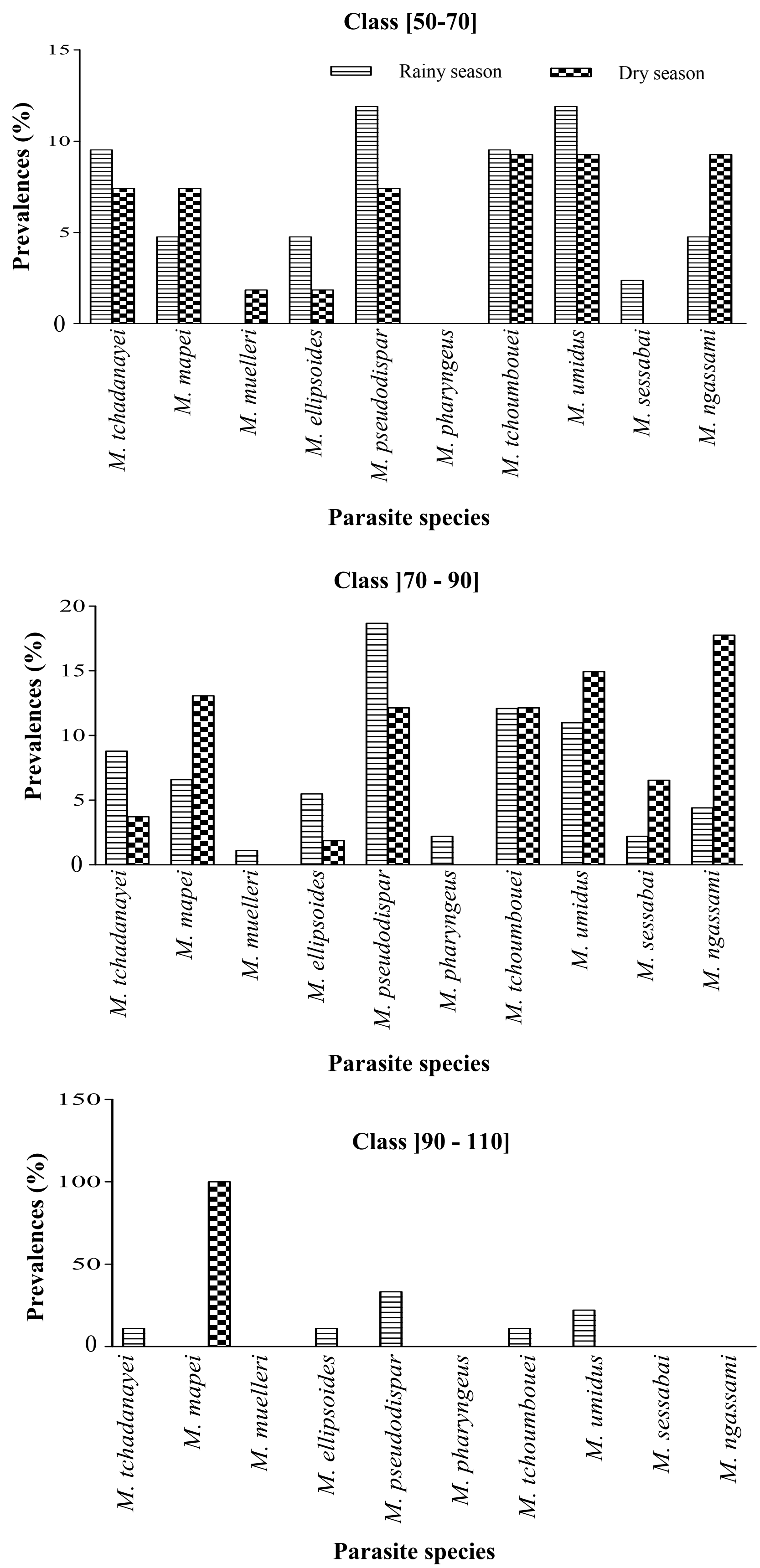


Figure 4. Seasonal prevalence of parasite species as a function of class size (mm)

Table 1. Prevalence of parasite species as a function of host's sex and seasons

S. No	Parasite species	Males				Females			
		Seasons		X^2	P	Seasons		X^2	P
		Rainy 79*	Dry 80*			Rainy 63*	Dry 83*		
1	*M. tchadanayei*	8.86	2. 50	3.01	0.083	9.52	7.23	0.25	0.617
2	*M. mapei*	6.33	11.25	1.20	0.274	4.76	13.25	2.98	0.084
3	*M. muelleri*	0.00	0. 00	-	-	1.59	1.20	0.04	0.844
4	*M. ellipsoides*	5.06	2. 50	0.72	0.396	6.35	1.20	2.87	0.091
5	*M. pseudodispar*	20.25	10.00	2.38	0.123	14.29	12.05	0.16	0.691
6	*M. pharyngeus*	2.53	0. 00	2.05	0.152	0.00	0.00	-	-
7	*M. tchoumbouei*	12.66	12.50	0.00	0.980	9.52	9.64	0.00	0.981
8	*M. umidus*	11.39	15.00	0.45	0.502	12.70	12.05	0.01	0.906
9	*M. sessabai*	2.53	3. 75	0.19	0.660	1.59	4.82	1.13	0.288
10	*M. ngassami*	5.06	16.25	5.21	< 0.05	3.17	13.25	4.49	< 0.05
	Total	50.63	48.78	0.06	0.812	46.03	46.99	0.01	0.909
	X^2	37.17	41.52			23.49	30.61		
	P	< 0.001	< 0.001			< 0.001	< 0. 001		

*: number of examined fishes; -: no value; P : error probability

P = 0.374) contrary to polyinfections (X^2 = 0.72; P = 0.397). Whatever the season, four categories of polyinfections were recorded namely bi, tri, tetra and pentaspecific i.e. respectively 2 to 5 parasite species combinations. No matter the season, their frequencies dropped very significantly (P < 0.001) with the increasing number of combined parasite species.

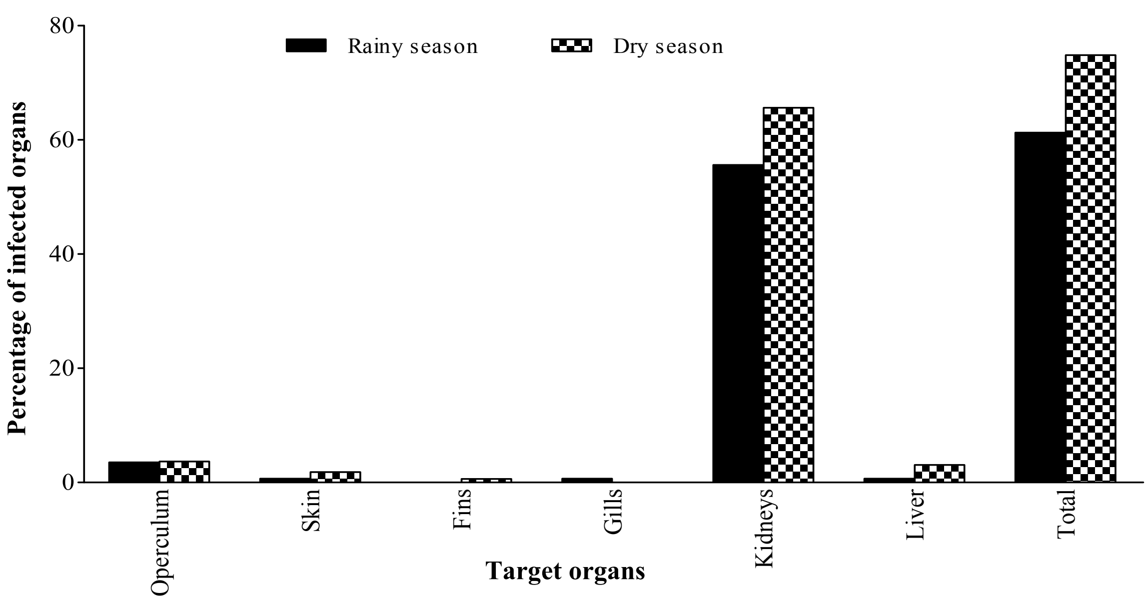


Figure 5. Percentage of infected organs as a function of seasons

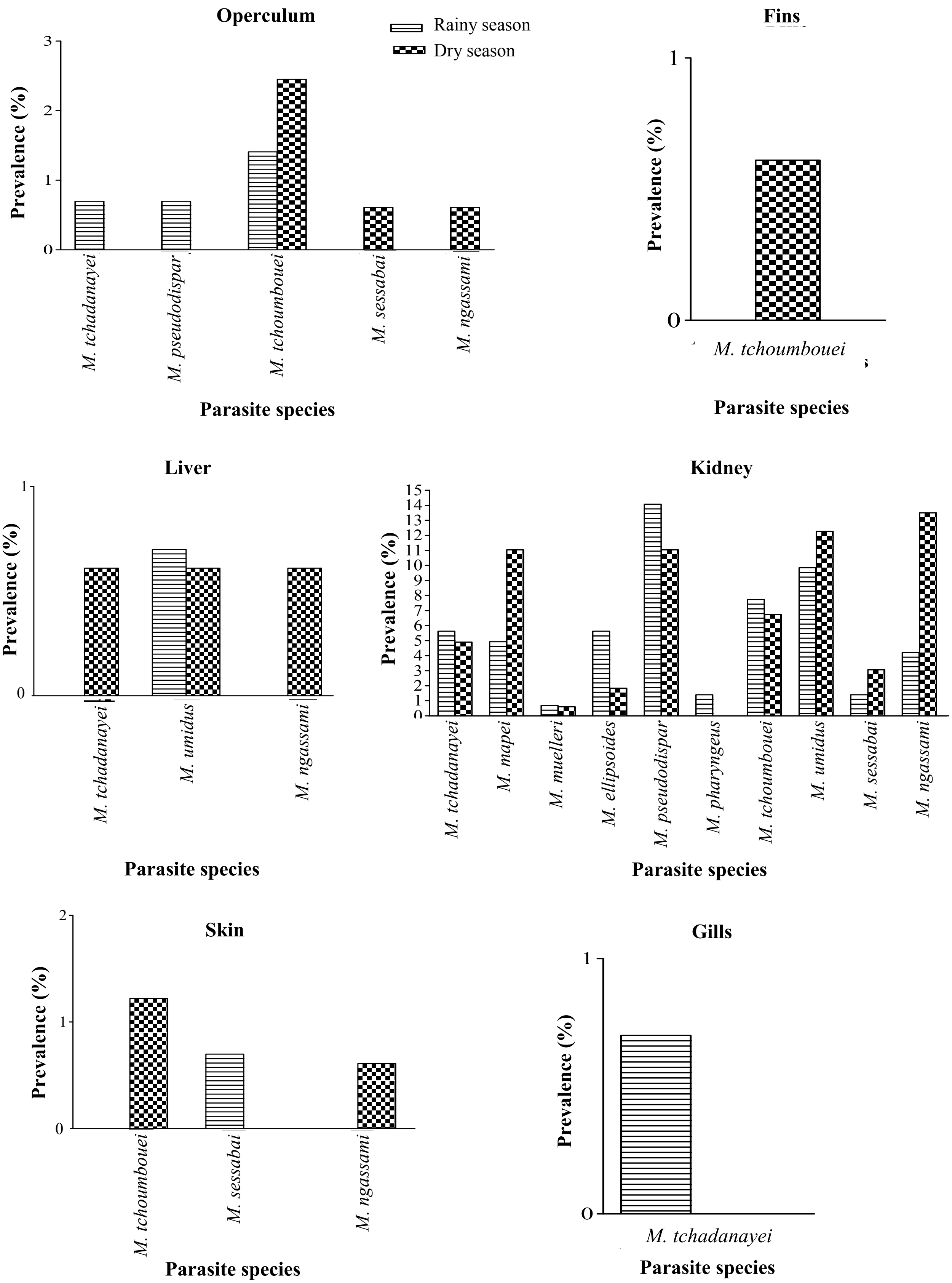


Figure 6. Prevalence of parasite species as a function of organs and seasons

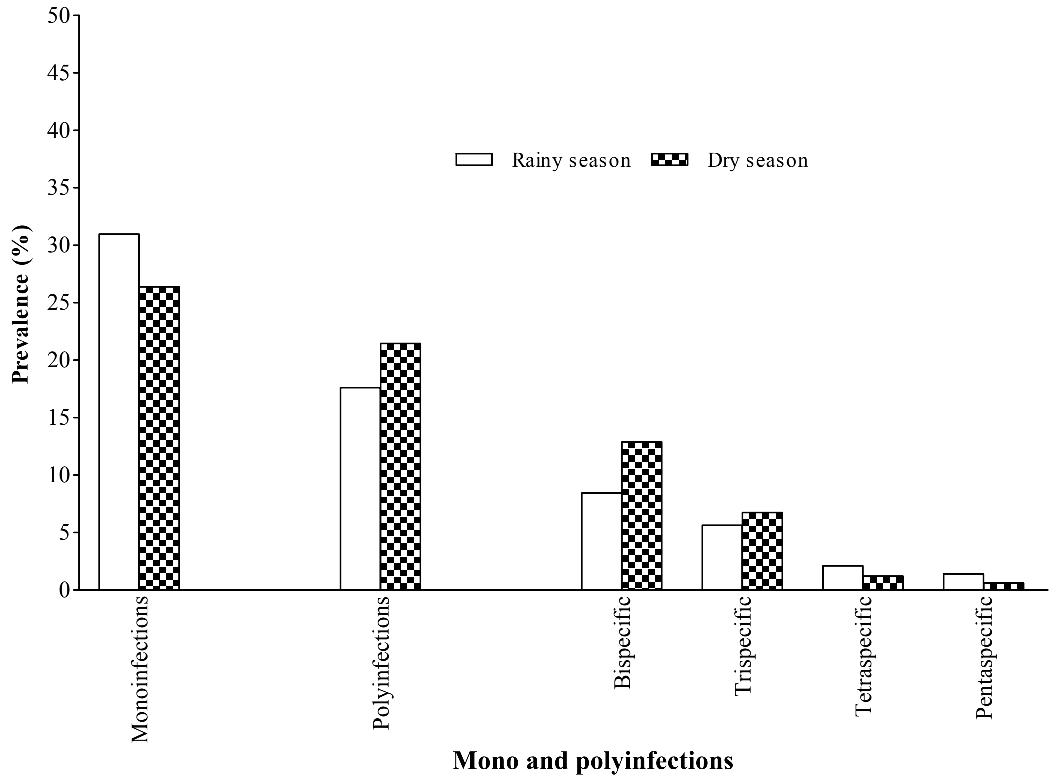


Figure 7. Seasonal prevalence of mono and polyinfections

DISCUSSION

Irrespective of the parasite species, the prevalence of infection did not differ between seasons. This may be due to the insignificant variation of water physico-chemical characteristics with the seasons. Based on season, parasites were grouped into three categories: those occurring mostly during the dry season (category A), the rainy season (category B) and those occurring without seasonality (category C). The results of category (A) parasites were explained by Obiekezie and Okaeme (1990). In fact, those authors thought that during the dry season, the high temperature of water and mud can encourage the infection with myxosporeans. According to Uspenskaya (1995), when myxospores sink in water, they undergo aging and maturation in the mud or sludge in order to parasitize new hosts. Gbankoto *et al*. (2001) indicated that the prevalence of *Myxobolus* sp. and *Myxobolus zillii* which are gills parasites for *Tilapia zillii* and *Sarotherodon melanotheron* in Bénin was higher in the dry season. In the fishes *Ctenopoma petherici*, *Clarias pachynema* and *Hepsetus odoe* from the river Sangé (Cameroon), Folefack and Fomena (2013) showed that *Myxobolus* sp2 occurred mostly during the dry season whereas *M. petrocephali*, *M. nkamensis* and *M. gariepinus* were more frequent in the rainy season. Abakar (2006) asserted that, in the river Chari (Chad), *Myxobolus brachysporus* and *M. camerounensis* were more present in *Oreochromis niloticus* during the dry season than the rainy season. The explanation being the pollution of Chari river by factories installed in Moundou and Sarh (Chad towns). The pollution was significant during the dry season because of the intensive water evaporation and this could negatively affect the actinospores. This pollution is also noticed in Mapé river because neighboring population dump refuse in the water and use nitrated fertilizers for agriculture. As for the category B parasites, Abakar (2006) claimed that

Sarotherodon galilaeus and *O. niloticus* were more infected during the rainy season in Chad. His explanation was based on Lausanne (1988) literature. So, during the rainy season, flood water dissolves soil minerals and organic matters accumulated during the dry season. This will encourage the growth of planktons and algae which serve as food for fishes. Actinospores being stuck on this food, fishes get infected by consuming them. The case of category (C) is similar to that noticed by Bilong and Jeannette (2004) and Abakar (2006). According to them, some myxosporeans species are present in the fishes throughout the year; others appear once in a year whereas some exhibit two or three peaks of infection each year (Blazer *et al*., 2003). Oligochaetes being intermediate hosts in myxosporeans life cycle (Markiw and Wolf, 1983) the seasonal variation of parasitism by myxosporeans could be due to the seasonal supplying of actinospores by oligochaetes (Özer *et al*., 2002).

Seasons did not significantly influence the prevalence of parasites in various fish's class sizes and sex. However, *Myxobolus ngassami* was more present in male fishes during the dry season contrary to females. Probably during the dry season, males lose huge amount of energy for testosterone synthesis thus weakening the efficiency of their immune system (Poulin, 2006).

Regardless of the organ type and parasite species, the dry season was remarkably more favorable to organ infections. The effect of seasons on the organs depended on the parasite species. Hence, the prevalence of *Myxobolus ngassami* in the kidneys was about twice higher in the dry season than in the rainy season whereas *M. pseudodispar* exhibited the higher prevalence during the rainy season. The organs' physiology may be influenced by the physico-chemical characteristics of water which in turn depend on the seasons too. So, any modification of the physiology of an organ within a given season will encourage or discourage its infection by a given parasite species. The two seasons were favorable to the mono and polyinfections and whatever the season, the prevalences of polyinfection categories dropped with the increasing number of associated parasite species. This observation may be due to the interspecific competition between parasite species. The higher the number of associated species, the higher the intensity of interspecific competition due to resources shortage and thus lower the prevalence.

CONCLUSION

The seasonal patterns of *Myxobolus* infections in *Barbus callipterus* were affected by the parasite species, the host's sex, class size and the target organ. The recorded data are useful for elaborating control strategies.

REFERENCES

Abakar O. 2006. Les myxosporidies (Myxozoa : Myxosporea) parasites des poissons d'eau douce du Tchad : Faunistique et biologie des espèces inféodées à *Oreochromis niloticus* (Linné, 1758) et *Sarotherodon gallilaeus* (Linné, 1758) cichlidae. Thèse de Doctorat d'Etat. Université de Yaoundé I. 163 p.

Bilong Bilong CF and Tombi Jeannette 2004. Hétérogénéité du système branchial de *Barbus martorelli* Roman, 1971 (poisson Cyprinidae) et modèle de croissance. Revue de l'académie des Sciences du Cameroun. 43: 211-218.

Blazer VS, Waldrop TB, Schill WB, Ensmore CL and Smith D. 2003. Effects of water temperature and substrate type on spore production and release in Eastern *Tubifex tubifex* worms infected with *Myxobolus cerebralis*. *Journal of Parasitology,* 89(1): 21 -26.

Bush AO, Lafferty KD, Lotz JM and Shostak AW. 1997. Parasitology meets ecology on its own terms. *Journal of Parasitology*, 83(4): 575-583.

Eiras JC, Monteiro CM and Brasil-Sato MC. 2010. *Myxobolus franciscoi* sp. Nov. (Myxozoa: Myxosporea), a parasite of *Prochilodus argenteus* (Actinopterygii: Prochilodontidae) from the Upper Sao Francisco River, Brazil, with a revision of *Myxobolus* spp. from South America. *Zoologia,* 27(1): 131-137.

Feist SW and Longshaw M. 2005. Myxozoan diseases of fish and effects on host population. *Acta Zoologica Sinica*, 51(4): 758 – 760.

Fomena A, Lekeufack Folefack GB and Bouix G. 2010. Deux espèces nouvelles de *Myxidium* (Myxosporea : Myxidiidae) parasites des poissons d'eau douce du Cameroun. *Parasite,* 17(1) : 9-16.

Gbankoto A, Pampoulie C, Marques A and Sakiti GN. 2001. Occurrence of Myxosporean parasites in the gills of two *Tilapia* species from lake Nokoué (Benin, West Africa). Effect of host size and sex, and seasonal pattern of infection. *Disease Aquatic Organism,* 44(3): 217-222.

Lauzanne L. 1988. Les habitudes alimentaires des poissons d'eau douce africains. In, Biologie et écologie des poissons d'eau douce africains (Levêque, M.N, Barton et Sentongo. Eds). ORSTOM, 165-186.

Lekeufack Folefack GB. 2010. Faunistique et Biologie des Myxosporidies (Myxozoa:Myxosporea) parasites de quelques poissons téléostéens dans la rivière Sangé (afluent du Wouri). Thèse de Doctorat/Ph.D, université de Yaoundé I. 181 p.

Lekeufack Folefack GB and Fomena A. 2013. Structure et dynamique des infracommunautés de myxosporidies parasites de *Ctenopoma petherici* Günther, 1864 (Anabantidae), *Clarias pachynema* Boulenger, 1903 (Clariidae) et Hepsetus odoe (Bloch, 1794) (Hepsetidae) dans la rivière Sangé au Cameroun. *International Journal of Biological and Chemical Sciences,* 7(6): 2301-2316.

Lom J and Arthur JR. 1989. A guideline for the preparation of species description in Myxosporea. *Journal of Fish Diseases*, 12(2): 151 – 156.

Lom J and Diková I. 2006. Myxozoan genera: definition and notes on taxonomy, life-cycle terminology and pathogenic species. *Folia Parasitologica,* 53(1): 1 -36.

Markiw ME and Wolf K. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of Salmonid whirling disease requires tubificid worms (Annelida: Oligochaetea) in its life cycle. *The Journal of Protozoology,* 30(3): 561-564.

Obiekezie AI and Okaeme AN. 1990. Myxosporea (Protozoa) infections of cultured tilapias in Nigeria. *Journal of African Zoology,* 104: 77- 91.

Olivry JC. 1986. Fleuves et rivières du Cameroun. ORSTOM, 733 p.

Özer A, Wootten R and Shinn AP. 2002. Survey on actinosporean types (Myxozoa) belonging to seven collective groups found in a freshwater salmond in Northern Scotland. *Folia parasitologica,* 49(3): 189 - 210.

Poulin R. 2006. Variation in infection parameters among populations within parasite species: Intrinsic properties versus local factors. *International Journal of Parasitology,* 36(8) : 877-885.

Stiassny MLG, Teugels GG and Hopkins CD. 2007. Poissons d'eaux douces et saumâtres de la Basse Guinée, Ouest de l'Afrique Centrale. Collection faune et flore tropicales, IRD (éd.), Paris I : 797 p.

Uspenskaya AV. 1995. Alternation of actinosporean and myxosporean phases in the life cycle of *Zschokklella nova* (Myxozoa). *Journal of Eukaryotic Microbiology,* 42(6): 665 – 668.

Valtonen ET, Holmes JC and Koskivaara M. 1997. Eutrophisation, pollution and fragmentation : effects on parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in Central Finland. *Canadian Journal of Fisheries and Aquatic Sciences*, 54 (3): 572-585.