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Myxobolus (Myxosporea: Myxobolidae) polyinfection patterns in *Oreochromis niloticus* in Adamawa-Cameroon

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Abstract
Fish are prone to several parasitic diseases among which are the myxosporean infections. The goal of this study was to assess the prevalence and patterns of *Myxobolus* infection in *O. niloticus* in the Adamawa region of Cameroon. Specimens of *Oreochromis niloticus* were collected monthly in Mape dam from May 2016 to May 2017, and examined for Myxosporean infections. The overall prevalence was 45.43%. *Myxobolus tilapiae* significantly exhibited the highest prevalence (15.14%) followed by *Myxobolus brachiosaurus* (12.29%). *Myxobolus pharyngeus* (0.86%) was the less prevalent parasite. Out of 350 examined fish, 23.14% were poly infected. Five categories of polyinfections were observed i.e. bi (15.71%), tri (4.00%), tetra (2.29%), penta (1.14%) and hexa specific (0.57%) corresponding to two to six parasite species combinations respectively. Their prevalence dropped significantly with the number of coexisting species whatever the fish sex, class size, infection site and season. Kidneys harbored up to four categories of poly infections, and *Myxobolus tilapiae* was the most associated contrary to *Myxobolus agolus* which appeared only in one combination of the tetra specific infection. Fish were significantly more poly infected during the dry season (28.76%) than the rainy season (18.78%). The poly infection of *O. niloticus* with *Myxobolus* species is an alert calling for urgent control strategies.

Keywords: *Myxobolus*, polyinfection, prevalence**,** *Oreochromis niloticus,* Cameroon

1. Introduction
Tilapia species, especially *Oreochromis niloticus* commonly known as Nile tilapia is highly appreciated in Aquaculture because of its fast growth and ability to grow under wide range of environmental conditions (Mekkawy *et al*., 2017) [18]. Due to the high demand in fish proteins, attention is increasingly paid on parasites affecting *O. niloticus* growth, health and survival since diseases are recognized as a major constraint to fish production (Nounagnon *et al*., 2016) [19]. Early diagnosis of fish parasites is a prerequisite for reducing the outbreaks of disease.

Myxosporeans are among fish parasites causing harm in fish production. They affect the fish growth (Longshaw *et al*., 2010) [17], their reproduction (Obiekezie and Okaeme, 1990) [20] and induce epizootics leading to massive fish deaths in farms and hatcheries (Gbankoto *et al*., 2001; Feist and Longshaw, 2005) [9, 7]. The health of immuno depressed persons can be affected by Myxosporeans when they consume infected fish (Hessen and Zamzame, 2004) [10]. Lom and Diková (2006) [14] revealed that among the 62 genera (2180 species) characterizing the world of Myxosporeans fauna, the genus *Myxobolus* Bütschli, 1882 was numerically the most important with about 34% of species. The pathogenic effects being scarcely caused by a single parasite species (Combes, 1995) [3], polyinfection and interactions between *Myxobolus* species can increase the pathogenicity of parasites by synergistic actions. This is likely to complicate the control strategies since effective broad spectrum drugs against myxosporidiosis are unavailable. This study intended to assess the patterns and prevalence of *Myxobolus* polyinfection in *O. niloticus* in the Adamawa region of Cameroon to provide baseline data for the development of control methods.

2. Materials and Methods
2.1 Study Area
Fish were collected from MAPE dam (Fig. 1) at Bankim subdivision (6°00’- 6°20’NL/

11°20'-11°40' EL, Mayo-Banyo Division, Adamawa Region of Cameroon). The average altitude is about 724m and the soil is composed of clay and sand. Climate is of the 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 ranges from1500 to 2000 mm (Olivry, 1986) [21].

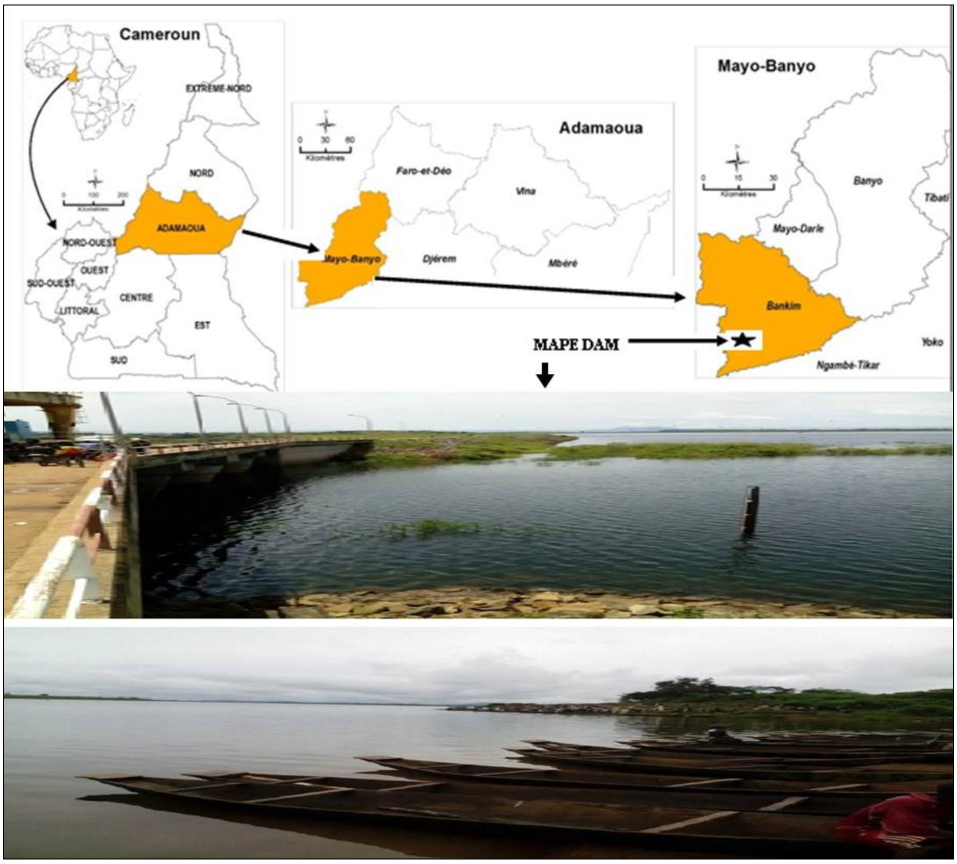


Fig 1: Cameroon map showing the study area

2.2 Fish sampling and data collection

A total of 350 fish (Table 1) specimens were collected monthly from fishermen from May 2016 to May 2017. They were captured using fishing nets and fishing canes. After immediate storing into 10% formalin solution, they were transported to the laboratory for identification (Stiassny *et al.*, 2007) [22] and examination for the presence of myxosporeans as per Abakar (2006) [1]. Standard and total lengths of fish were measured to the closest millimeter using a slide caliper. After dissection, fish were sex determined.

External organs (fins, skin, scales and eyes) and internal organs (gills, spleen, kidneys, intestines, gall bladder, stomach and gonads) were examined macroscopically, then with a stereoscopic microscope at 10X lens to look for cysts. Three smears of the kidneys, spleen and gonads were made per organ (anterior, medium and posterior regions) and examined at a total magnification of 1000X with a light microscope for the presence of spores.

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 at 100X lens. May-Grünwald-Giemsa was used for spores staining after fixation with methanol. A digital camera (Canon Ixus brand) helped to realize micrographs. The recommendations from Lom and Arthur (1989) [15] were applied for myxosporean species identification. The identification keys provided by Fomena and Bouix (1997) [8], Lom and Dyková (1992, 2006) [16, 14], Eiras *et al.* (2005, 2014) [4, 5] were equally used.

Table 1: Structure of *Oreochromis niloticus* population from MAPE dam

Fish sex	Fish class size (mm)			Total	Season		MSL (mm)
	[50 - 100]	[100 - 150]	> 150		Rainy	Dry	
Male	134	76	9	219	129	90	99.52 (52-240)
Female	95	34	2	131	68	63	92.58 (51-173)
Total (%)	229 (65.43)	110 (31.13)	11 (3.14)	350 (100)	197 (56.29)	153 (43.71)	97.13 (51-240)

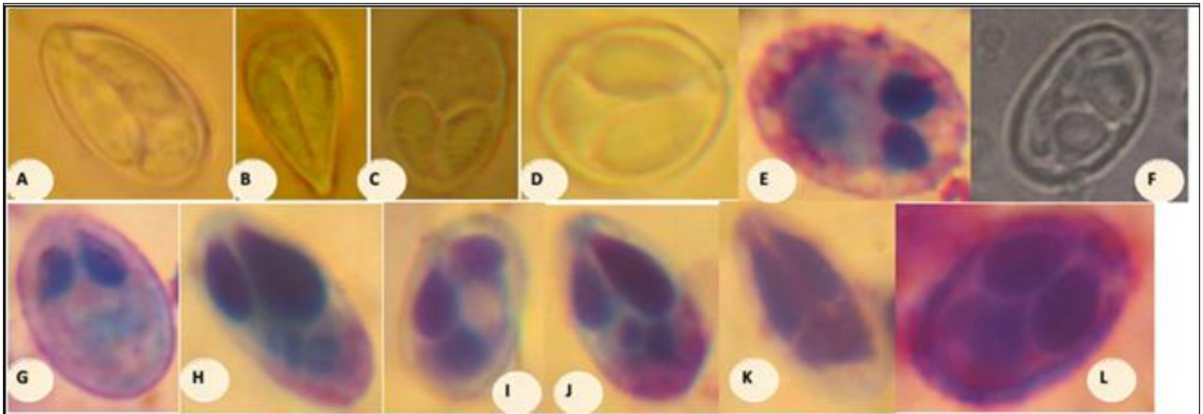
The Mean Standard Length (MLD) is followed in brackets by minimum-maximum values

2.3 Parasitological parameter and statistical analysis

The definition of the prevalence of infection was modified from Bush *et al*. (1997) [2]. Thus, the prevalence (Pr) of infection expressed as a percentage was defined as the number of fish infected by a single or a combination of parasite species divided by the number of fish examined. The comparison of prevalence among groups was performed using the Chi-square (X^2) test. The significance level of probability was P<0.05. The Graph Pad Prism 5 software was used for analysis.

3. Results
Results are illustrated in Figures 2 -8 and Tables 2- 3.

3.1 Types of infections prevalence
Figure 2 reveals that *Oreochromis niloticus* was infected with twelve *Myxobolus* species. The overall prevalence was 45.43% (Figure 3). In addition, *Myxobolus tilapiae* (15.14%) followed with *Myxobolus brachiosaurus* (12.29%) exhibited the highest prevalence (X^2= 140.40; p = 0.001) while *Myxobolus pharyngeus* (0.86%) was the less prevalent parasite. As shown in Figure 4, two types of infections namely mono infection and poly infection were recorded. Polyinfections are more prevalent than mono infections but without any significant difference (X^2= 0.07; p = 0.787). Moreover 5 categories of polyinfections were observed comprising bi, tri, tetra, penta and hexa specific infections. Their prevalence dropped significantly (X^2= 140.40; p = 0.001) of about 30% with the increasing number of combined species.



A:*Myxobolus israelensis (x1600)* ; **B:** *Myxobolus heterosporus (x1600)* ; **C**: *Myxobolus camerounensis (x 1500)* ; **D:** *Myxobolus agolus (x1600)* ; *E: Myxobolus kainjiae (x1800)* ; **F**: *Myxobolus brachysporus (x1500)* ; *G : Myxobolus tilapiae (x 1500)* ; **H** : *Myxobolus mapei (x 1200)* ; **I** : *Myxobolus pseudodispar (x1200)* ; **J**:*Myxobolus tchadanayei (x1600)* ; **K :** *Myxobolus pharyngeus (x1600)* ; **L** :*Myxobolus ellipsoides (x1600)*

Fig 2: Spore micrographs of *Myxobolus* species found in *Oreochromis niloticus* in MAPE dam, Adamawa, Cameroon

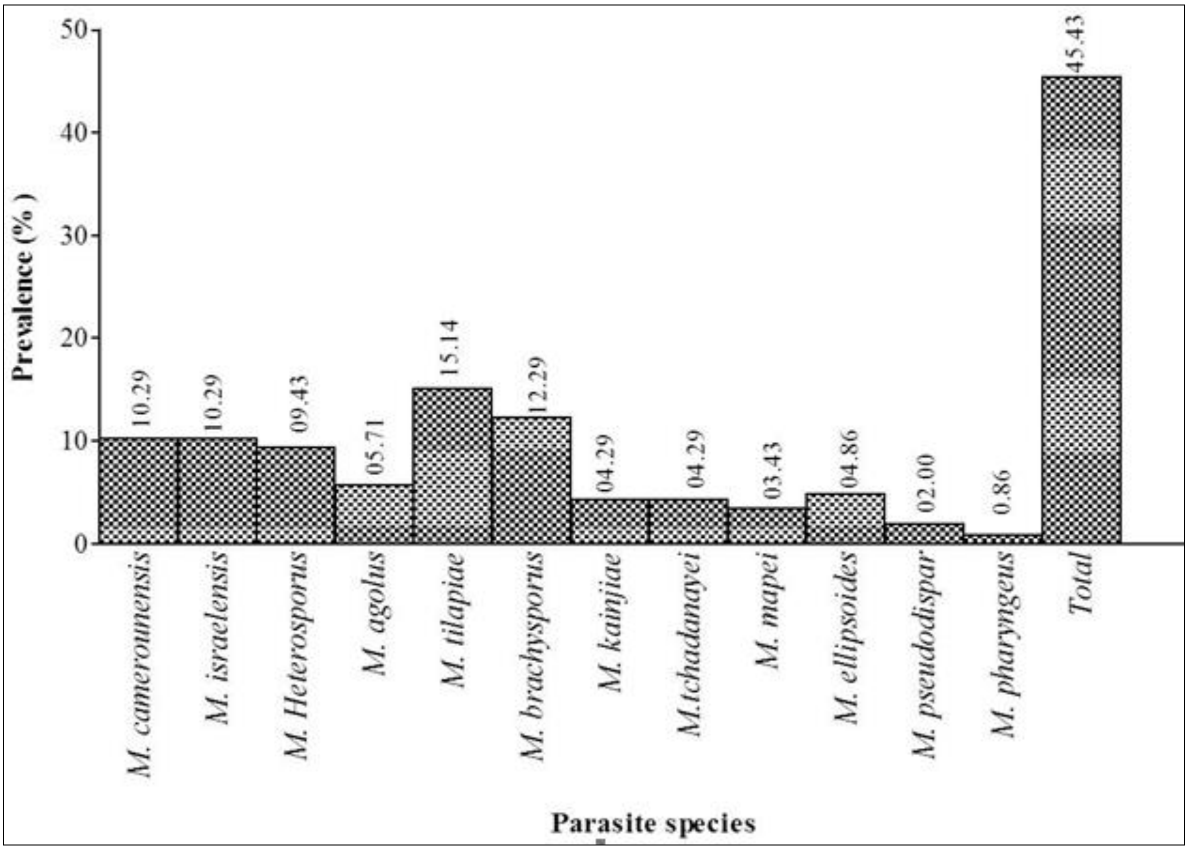


Fig 3: Prevalence of myxosporean species

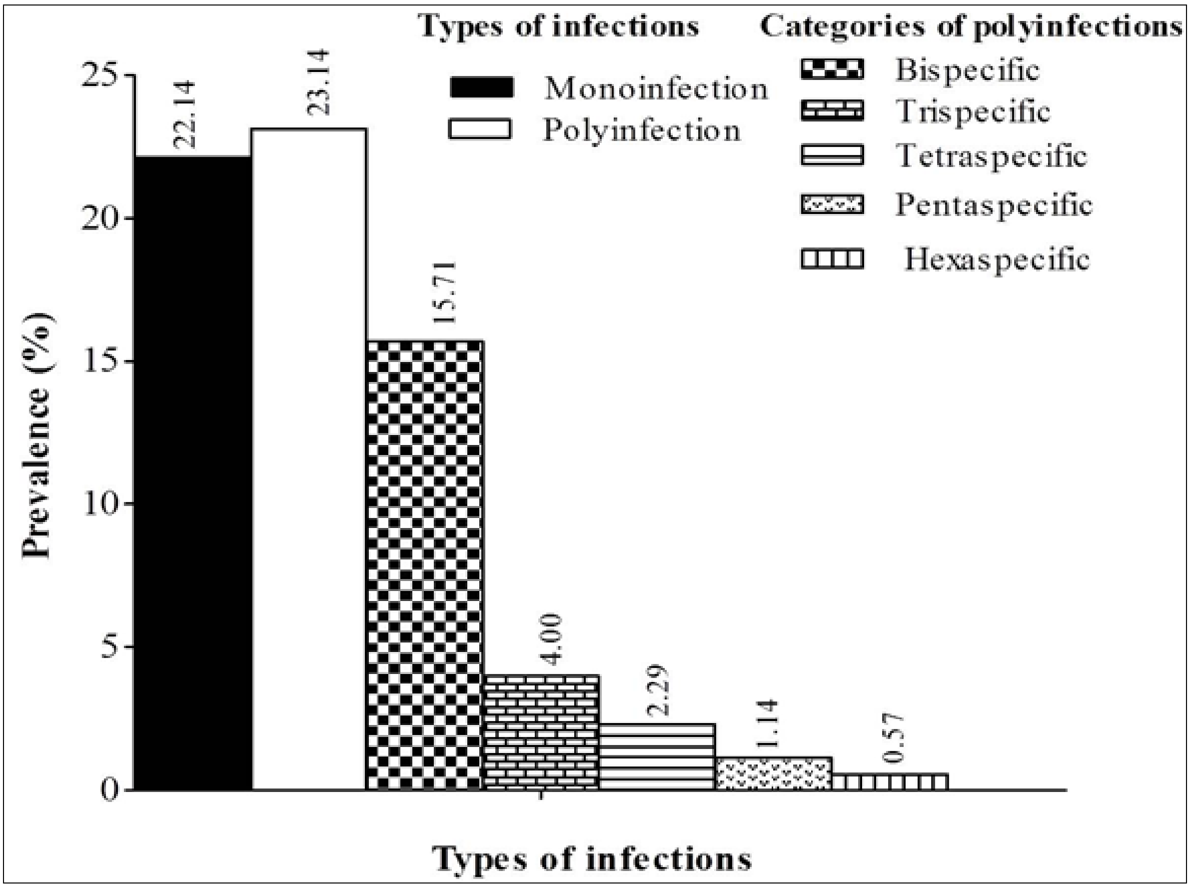


Fig 4: Prevalence of mono and polyinfection with *Myxobolus* spp in *Oreochromis niloticus* in MAPE dam, Adamawa-Cameroon

3.2 Prevalence of mono and polyinfection in relation to *O. niloticus* sex

The prevalence of mono and polyinfection in relation to *O. niloticus* sex are shown in Figures 5A and 5B. As illustrated in Figure 5A, both males and females were mono and poly infected. Whatever the fish sex, the prevalence of mono and polyinfection were all beyond 20% but did not significantly differ ($p > 0.05$) between fish sex. Figure 5B indicates that female fish harbored all the 5 categories of polyinfections contrary to males in which the hexa specific parasite combination was lacking. As a whole, the prevalence of polyinfection categories decreased considerably ($p<0.001$) with the number of associated parasite species. Female's fish exhibited two hexa specific combinations i.e. *M. israelensis* + *M. tilapiae* + *M. brachiosaurus* + *M. kainjiae* + *M. mapei* + *M. pseudodispar* and *M. israelensis* + *M. heterosporous* + *M. tilapiae* + *M. brachiosaurus* + *M. kainjiae* + *M. ellipsoid*. In addition, four parasite species were common to both combinations while only two species were specific to each combination.

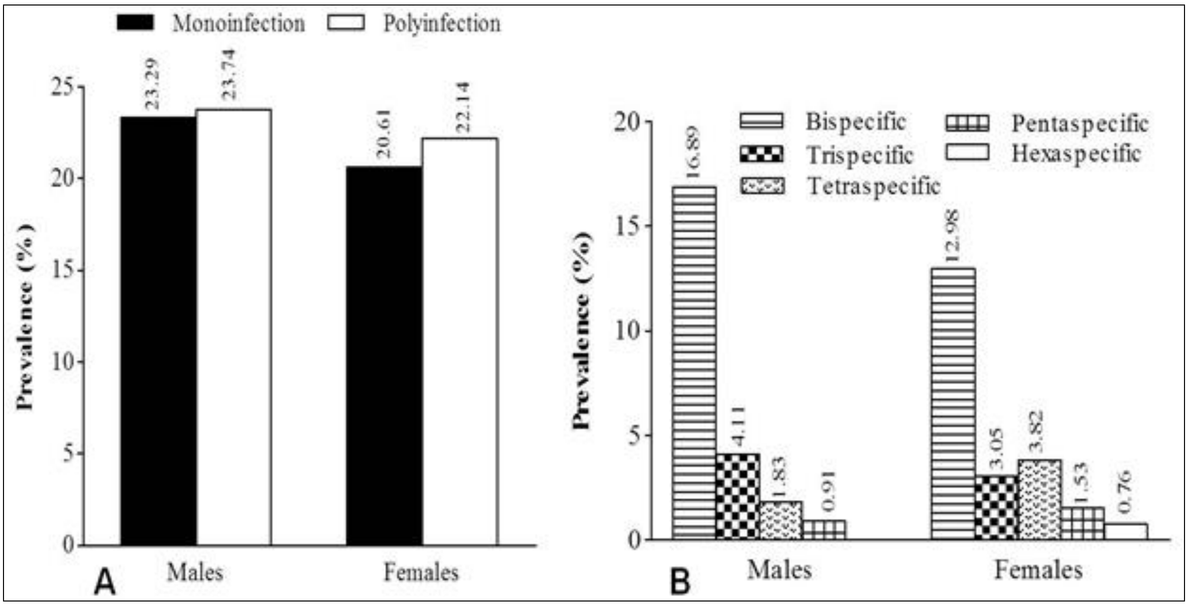


Fig 5: Prevalence in relation to *Oreochromis niloticus* sex; A: mono and poly infection; B: poly infection categories

3.3 Prevalence of mono and polyinfection in relation to body class size

The prevalence of mono and polyinfection in relation to *O. niloticus* body class size (Figure 6A) shows the occurrence of two types of infections in all class sizes. Mono infection were more prevalent (p = 0.01) in class [50 - 100] compared to other class sizes. When polyinfection categories are considered (Figure 6B), it appears that the number of associated parasite species fluctuated according to fish class size. The higher was the number of combined species, the lower was their prevalence. The prevalence fluctuation between different categories of polyinfection was not significantly different ($p>0.05$), whatever the combination considered.

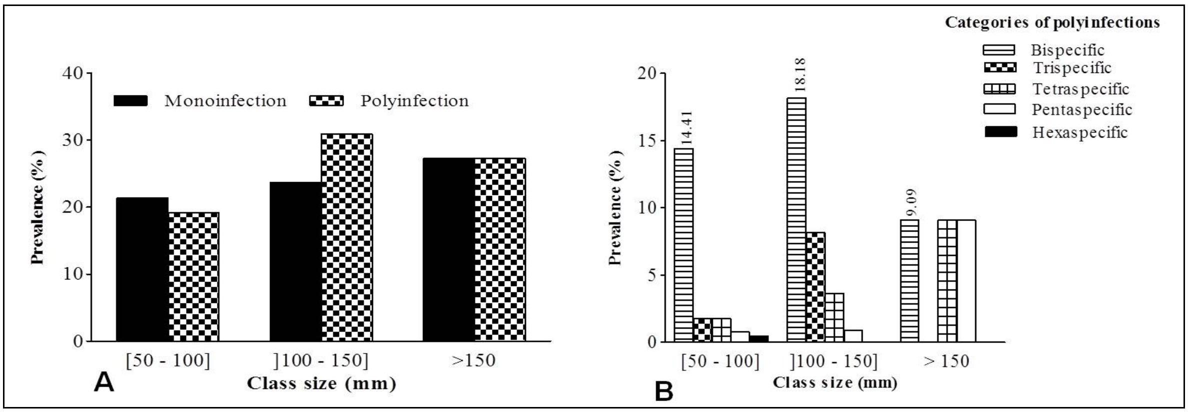


Fig 6: Prevalence in relation to *Oreochromis niloticus* body class size; **A:** mono and polyinfection; **B:** polyinfection categories

3.4 Prevalence of types of infections in relation to infection sites

As indicated in Figure 7A, out of 10 infected organs, only 3 organs (kidneys, liver and spleen) were both mono and poly infected. Meanwhile the 7 other organs only experienced mono infection, and the prevalence for these organs was very low (less than 5%). The highest (p =0.001) infection rate was observed in the kidneys as compared with the liver and spleen.

As for the categories of polyinfection, Figure 7B shows that the number of cohabiting myxosporean species in the kidneys varied from 2 to 5 and the prevalence of different categories dropped sensitively (p= 0.001) as the number of coexisting species increased. On the contrary, the liver and spleen were coinfected with only 2 and 3 species respectively. The different combinations of tetra and penta specific associations found in the kidneys are summarized in Table 2. Four and 3 parasite combinations were noticed for tetra and penta specific polyinfection. Moreover, *Myxobolus tilapiae* was the most cosmopolitan species since it occurred in all the combinations of both tetra and penta specific infections. On the contrary, *Myxobolus agolus* appeared to be the less associated parasite, since it was recorded in a single tetra specific infection.

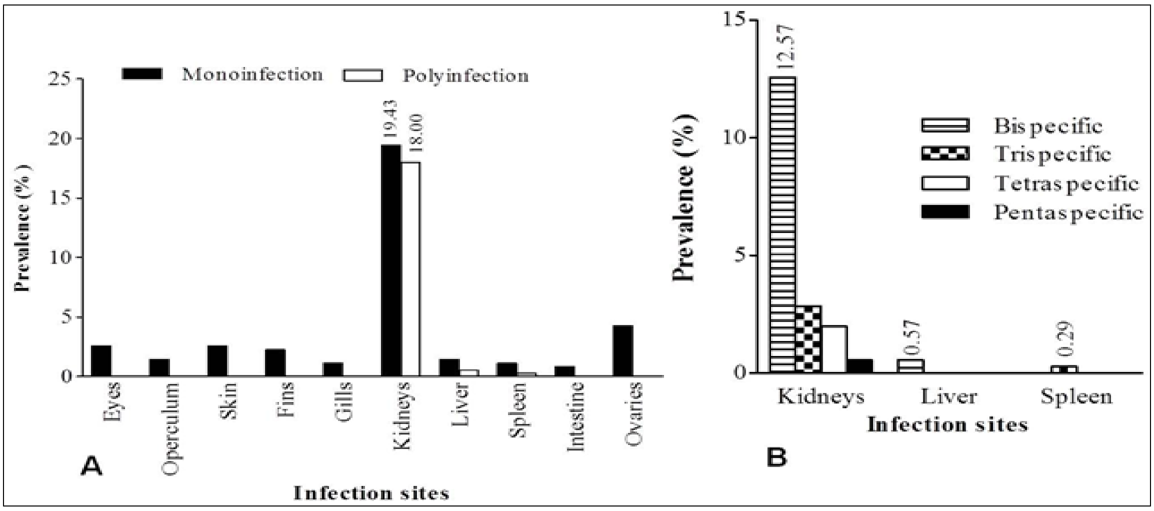


Fig 7: Prevalence in relation to *Oreochromis niloticus* infection sites; **A:** mono and polyinfections; **B:** polyinfections categories

Table 2: Tetra and penta specific combinations of parasite species in the kidneys

Categories of polyinfections	Combinations of parasite species	Prevalence (%)
Tetra specific	*M. heterosporous + M. agolus + M. tilapaie + M. pseudodispar*	25.00
	M. camerunensis + M. tilapiae + M. brachiosaurus + M. pseudodispar	50.00
	M. israelensis + M. tilapiae + M. brachiosaurus + M. mapei	12.50
	M. israelensis + M. tilapiae + M. brachiosaurus + M. ellipsoid	12.50
Pent specific	*M. israelensis + M. tilapiae + M. brachiosaurus + M. mapei + M. pseudodispar*	25.00
	M. camerunensis + M. israelenis + M. tilapiae + M. brachiosaurus + M. ellipsoid	50.00
	M. israelensis + M. heterosporous + M. tilapiae + M. brachiosaurus + M. ellipsoid	25.00

3.5 Seasonal prevalence of mono and polyinfections

The seasonal prevalence of mono and polyinfections (Figure 8) show that fish were mono infected and poly infected during both the dry and the rainy seasons. Polyinfections (28.76%) were insignificantly more frequent ($X^2 = 0.82$; $p = 0.364$) than mono infections (24.18%) during the dry season. As far as the rainy season was concerned, the prevalence of mono infections and polyinfections were comparable ($X^2 = 0.26$; $p = 0.613$). Furthermore, fish were significantly ($X^2 = 4.82$; $p < 0.05$) more poly infected during the dry season than the rainy season.

Whatever the season, 4 categories of polyinfections were noticed, with penta and hexa specific categories being present only during the dry and rainy seasons respectively. Whatever the season, the prevalence significantly (p=0.001) dropped from the lowest parasite association (bispecific) to the highest parasite association (hexa specific). Since penta and hexa specific categories were season specific, the detailed combinations is provided in Table 3. It appears that *Myxobolus brachiosaurus* was the most cosmopolitan since it was found in all the three combinations.

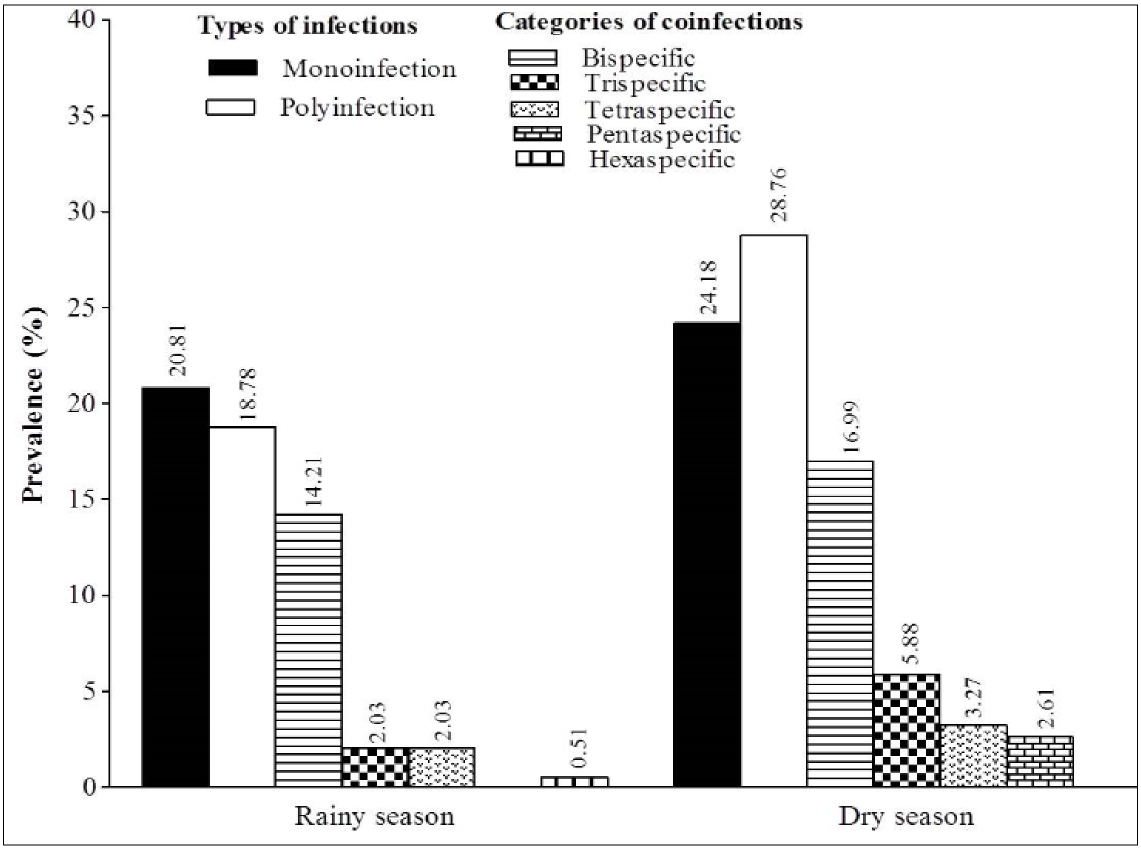


Fig 8: Seasonal prevalence of mono and polyinfections

Table 3: Penta and hexa specific combinations of parasite species in relation to season

Categories of polyinfection	Combinations of parasite species	Season	Prevalence (%)
Penta specific	*M. camerunensis + M. israelensis + M. tilapiae + M. brachiosaurus+ M. ellipsoid*	Dry	60
	M. camerunensis + M. brachiosaurus + M. kainjiae + M. tchadanayei+ M. pharyngeus		25
Hexa specific	*M. israelensis + M. tilapiae + M. brachiosaurus + M. kainjiae + M. mapei + M. pseudodispar*	Rainy	15

4. Discussion

The polyinfection of fish is in accordance with Combes (1995) [3] who observed that pathogenic effects are hardly caused by a single parasite species. The trophic behaviour of *O. niloticus* can also explain its polyinfection. Indeed, algae, planktons, mud and sludges which serve as food for *O. niloticus* (Lauzanne, 1988) [13] are carriers of various actinospores.

The drop in the prevalence for polyinfection categories with the increasing number of associated parasites species probably is provoked by the interspecific competition. The higher the number of associated species, the higher the intensity of interspecific competition leading to the shortage of resources and subsequently, lower the prevalence of the association. The lowest prevalence recorded for the hexa specific association may be due to the synergistic action between parasite species which finally kills the host. The association between *Myxobolus* species probably increases their virulence.

Myxobolus species occur alone without competition (mono infection) or in association (polyinfection). In case of polyinfection, association with fewer species (bispecific infection for instance) is more advantageous because the competition is reduced. The significance of an option (mono infection or polyinfection) probably depends on the intrinsic factors (fish sex, class size and infection sites) and extrinsic factors (season). For instance, fish were significantly more poly infected during the dry season compared to the rainy season while fish 50 to 100 mm long were significantly mono infected compared to other body class sizes.

Ibrahim and Soliman (2010) [12] revealed that there is neither inter nor intra competition between myxosporean species. This is possible only if one of the ecological niches do not overlap and the resource supply is greater than the demand. The unfulfillment of the previous conditions may result in the competitive exclusion according to Gause's principle (Holmes, 1973) [11].

The specificity of some *Myxobolus* species associations to a particular organ may be explained by the fact that, this organ provides a suitable microbiotope for optimal development of each species. The heterogeneity of biotopes (organs) generates different infections sites which are habitat options for parasites (Ibrahim and Soliman, 2010) [12]. The segregation of the infection sites may originate from the migration or translocation of some parasites following the competition. By so doing, *Myxobolus* species adapt themselves to a new microbiotope by creating new ecological niches leading to speciation (Holmes, 1973) [11]. The cohabitation between different parasite species in a given organ could result in the hybridation in the case of trematodes.

Kidneys harbored the highest number of polyinfection categories (4 categories) because they filter blood and secrete many solutes (Ellis *et al*., 1978) [6] that encourage parasites to converge there for the metabolites they need. In the kidneys, *Myxobolus tilapiae* was the most competitive i.e. the most adapted species since it was found in all combinations of the tetra and penta specific associations. On the contrary, *Myxobolus agolus* was the less adapted since it appeared only in one combination of the tetra specific category. One may therefore deduce that this latter parasite is less competitive to survive when associated with four others species.

The polyinfection of fish during the dry season may be due to the high temperature of water and mud. In fact, myxospores easily sink in high water temperature where they get aging and become mature in mud or sludge so as to be able to infect the new host (Obiekezie and Okaeme, 1990; Uspenkaya, 1995) [20, 23]. No matter the season, *Myxobolus brachiosaurus* was the most associated parasite since it was found in all the three combinations of penta and hexa specific associations. Season therefore did not decrease the competitiveness of that parasite. Thus, one may conclude that this parasite is the most competitive of all the *Myxobolus* species found in *O. niloticus* in the study area.

5. Conclusion

Five categories of polyinfection i.e. bi, tri, tetra, penta and hexa specific were observed in *O. niloticus*. Their prevalence dropped with the number of combined species no matter the fish sex, class size, infection site and season. The polyinfection of *O. niloticus* probably affects its health and also renders the control strategies more difficult. The recorded data is helpful for developing the control methods since effective broad spectrum drugs against myxosporidiosis are unavailable.

6. Financial Support

This research received no specific grant from any funding agency, commercial or not-for-profit sectors

7. Conflicts of interests

None

8. Ethics approval

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

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