***https://doi.org/ 10.33472/AFJBS.6.5.2024. 196-214***

# African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

**Research Paper** **Open Access**

# Myxozoans (Cnidaria: Myxobolidae) parasites of *Labeobarbus batesii* (Boulenger, 1903) (Teleostei: Cyprinidae) in Makombè River, Littoral Region of Cameroon: diversity, ecological status, determinants of monoparasitism and polyparasitism

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**Article History**
Volume 6, Issue 5, Apr 2024
Received: 03 Feb 2024
Accepted: 10 Apr 2024
doi: ***10.33472/AFJBS.6.5.2024. 196-214***

## ABSTRACT

**Purpose:** The degree of association between myxozoan species can reveal how these parasites are contracted and what occur after their recruitment.

**Materials and Methods:** Fish caught were examined for myxozoan infections. The prevalence and mean cysts load were estimated. Analysis of the ecological status of each myxozoan species was done. Dice's and tetrachoric coefficients were used to measure the degree of association between two parasite species. Forbes' index was used to measure the amount of parasites association deviation from expectation.

**Results:** Five *Myxobolus* species were identified. *Myxobolus* sp. and *M. paludinosus* appeared frequent while *M. makombensis, M. njoyai* and *M. dibombensis* appeared intermediate. The mean cyst load was high for *Myxobolus* sp., low for *M. paludinosus* and *M. dibombensis*, very low for *M. njoyai* and *M. makombensis*. In the fish population examined, 5.94% were free of parasite, 19.18% harbor a single parasite species while 74.89% exhibit polyparasitism. Except a single case of negative interaction recorded, increase of prevalence of each species of myxosporidia recorded leads to an increase in the prevalence of others parasites species.

**Conclusion:** Meanwhile strong support for interaction between *Myxobolus* species was provided, the details of this interaction need to be investigated in future studies.

**Keywords:** *Myxobolus*, *Labeobarbus batesii*, ecological status, polyparasitism, parasite association, river Makombè

# 1. Introduction

Aquaculture is a rapidly developing activity in Cameroon. Among the species being considered for fish farming, much interest has been focused on *Labeobarbus batesii* (Boulenger, 1903) (Cyprinidae) due to its high reproductive capacity, rapid growth and high commercial value (Tiogué *et al*., 2013). However, being devoid of defense structures such as toxins, armor or spines; Cyprinid fish such as *L. batesii* are quite vulnerable to predation and parasitism unlike fish of other families (Winfield and Nelson, 2012).

More than half of all living organisms are parasites including myxozoans (Fiala *et al*., 2015). Myxozoans are worldwide distributed, diverse group of microscopic parasites, and belong to the phylum Cnidaria (Fiala *et al*., 2015). They are primarily parasites of freshwater and marine fishes, and some other vertebrates (Sitjà-Bobadilla *et al*., 2016) and invertebrates (Lom and Dyková, 2006). Myxozoans found in freshwater have complex life cycles with alternating forms found mainly in fish (intermediate host) and aquatic oligochaete worms (definitive host). In spite of the concerted effort aimed at their control or eradication, Myxozoans remain a major source of fish problem both in terms of their impact on fish health and their economic significance in aquaculture (Feudjio-Dongmo *et al*., 2022).

It is usual in veterinary medicine to deal with each myxosporidia infection as a separate entity caused by a single pathogen (Lekeufack-Folefack *et al*., 2019 and Feudjio-Dongmo *et al*., 2022**)**. In Africa, multiple infections (polyparasitism) by Myxozoans species are the rule rather than the exception in freshwater fish (Fomena, 1995; Abakar-Ousman *et al*., 2006; Lekeufack Folefack, 2010; Nchoutpouen, 2015; Lekeufack Folefack and Fomena, 2013). However, worldwide investigations on the impacts of this type of infection on individual hosts or host populations are scarce. Moreover, little information is available on the factors structuring the community of parasites in fish individual or fish populations. The few rare cases of available data on polyparasitism by myxosporidia, revealed that each parasite species involved can have a detrimental effect on the host and contribute to the overall disease picture (Holzer *et al*., 2006 and Lekeufack-Folefack *et al*., 2019).

Factors known to influence the structure of parasite infracommunity can be broadly classified as being either external to the infracommunity (Adam Ray *et al*., 2015) or due to interactions between the species going to make up the infracommunity (Lekeufack-folefack and Fomena, 2017 and Lekeufack-folefack *et al*., 2019). Interactions between species of parasites infecting a single host may lead to a variety of possible effects on pathogenicity, thus, the greater the number of parasites, the greater the number of potential outcomes. Available data indicate that both positive (Petney and Andrews 1998) and negative (Schmidt-Posthaus *et al*., 2013 and Alarcón *et al*., 2016) interactions can occur between pairs of parasite species.

Moreover, the pathological damage caused by one parasite species can increase the susceptibility of a host to another parasite species (Petney and Andrews 1998). Polyparasitism can therefore play a significant role in the pathogenic processes occurring within the host and are relevant to the evolution of both hosts and parasites species. However, it should be noted that studies of the effect of polyparasitism on pathogenic processes are difficult for animal hosts in natural populations in which the pathogenic changes must be measured against a background of multiple infections which is often already present.

It is essential to analyze the degree of association between myxosporidia species because this can lead to many hypotheses on how these parasites are contracted or on the processes occurring after the recruitment of myxosporidia species (cross-immunization or immunosuppression, inter-specific competition). According to Combes (1983), Dice's coefficient (Dice, 1945), tetrachoric coefficient (Dagnelie, 1960), Forbes' index (Forbes, 1907) and chi-square test provide a satisfactory analysis of the degree of association in a parasitofauna. Although the above association indices have been used as applications of choice in parasitology, in the current state of our knowledge, they have not yet been used in any case of myxosporidia infection.

In this study, we will analyze the impact of polyparasitism and the different types of associations expressed by myxosporidia parasites of *Labeobarbus batesii* sampled from the Makombè River at Nkondjock in Cameroon.

# 2. Materials and methods

## 2.1. Sampling site

Specimens of *Labeobarbus batesii* (Boulenger, 1903) were harvested during a full year from May 2017 to April 2018 in the Makombè River at Nkondjock in Cameroon. Fish were caught in an accessible portion of approximately 8 kilometres long of the Makombè River between 4° 42’ 49’’ - 4° 54’ 36’’ N and 10° 10’ 20’’ - 10° 15’ 41’’ E. The climate in Nkondjock is equatorial of Cameroonian type with two seasons, including one rainy and one dry season. The average annual rainfall varies between 2857 and 3624 millimeters while the average annual temperature varies between 24.8° C and 28.3° C.

## 2.2. Fish sampling and examination

Fish were caught monthly using gill net. Captured fish were euthanized with clove oil solution at a concentration of 0.4 ml $l^{-1}$ of water (Fernandes *et al*., 2017). They were then transported to the Laboratory of Parasitology and Ecology of the University of Yaoundé I in Cameroon for parasitological examination. Identification of sampled fishes based on geographic location, morphology, and metric features was carried out according to De Weirdt *et al.,* (2007). External organs of the fish, including skin, fins, eyes, and operculum, were macroscopically examined and then observed under a binocular stereo microscope (Olympus SZ-ST) for the presence of myxozoan plasmodia. After opening the gills and abdominal cavity of each fish, the kidney, liver, gall bladder, digestive tract, spleen, heart, gills, gonads, urinary duct, and urethra were also examined under a binocular stereo microscope for the presence of myxozoan plasmodia. The contents of the gall bladder, swim bladder, urinary bladder, and urethra were mounted between glass slides and coverslips and examined for the presence of myxospores at 100× magnification of an optic microscope (Ivymen System, Comecta). The length of the kidney, liver, spleen, heart, and gonads of each *L. batesii* specimen was measured to the nearest millimetre, and each of these organs was divided into 3 equal portions. Smears of pieces (0.05 g) from each portion of these organs were mounted between glass slides and coverslips and examined at 100× magnification with the above mentioned optic microscope. Myxospores were identified at 1000× magnification with an optic microscope taking into account the variables proposed by Lom and Arthur (1989).

## 2.3. Epidemiological indices, ecological status of parasites species and statistical analyses

According to Bush *et al.* (1997), the prevalence was estimated as the number of individuals of *L. batesii* infected with one or more individuals of a particular Myxozoan species divided by the total number of *L. batesii* examined. Referring to the mean intensity defined by Bush *et al*. (1997), the mean cysts load was calculated as the average number of cysts of a particular species of Myxozoan among the infected members of *L. batesii* found in the sample divided by the number of *L. batesii* infected with that Myxozoan species.

Analysis of the ecological status of each Myxozoan species was made according to Valtonen *et al*. (1997). Thus, the species are qualified as frequent (or common or principal) if prevalence > 50%, Less frequent (or secondary or intermediate) if 10% ≤ prevalence ≤ 50%, and scarce (or satellite) if prevalence < 10%. Following Bilong Bilong and Njiné (1998), the mean cysts load ($\bar{x}$) was judged very weak if $\bar{x} < 10$, weak if $10 \leq \bar{x} < 50$, average if $50 \leq \bar{x} \leq 100$ and very high if $\bar{x} > 100$.

Dice's coefficient (D) (Dice, 1945) was used to measure the degree of association between two parasites species. The value of D varied from 0 to 1. When D < 0.1 the association is very weak, 0.1 ≤ D < 0.25 indicated weak association, 0.25 ≤ D < 0.5: revealed moderate association, and D ≥ 0.5 means very strong association (Combes, 1983).

The tetrachoric coefficient (φ), also called phi coefficient (Dagnelie, 1960) was used to evaluate the degree of association between two given species (Combes, 1983). The tetrachoric coefficient varies from -1 to +1. φ = -1 correspond to perfect exclusion (one parasite excludes another); φ = +1 refer to perfect association (one parasite favors the presence of another); φ = 0 when the presence of one parasite does not influence that of the other (Combes, 1983).

The Forbes' index (F) (Forbes, 1907), measure the amount of association deviation from expectation between two parasites species. When F < 1, the association is less frequent than expected by chance; if F = 1, the association is in accordance with the laws of chance; when F > 1, the association is more frequent than expected (Combes, 1983).

Using the statistical package SPSS version 16.0 for Windows, the chi-square test ($\chi^2$) was used for the comparison of the prevalence of myxozoan species. All statistical tests were considered significant at P<0.05.

# 3. Results

## 3.1- Parasites species richness, prevalence, mean cyst load and ecological status of myxosporidia species recorded on *Labeobarbus batesii*

Examination of 438 specimens of *Labeobarbus batesii* revealed the presence of five species of myxosporidia, all belonging to the genus *Myxobolus* Bütschli, 1882. They are: *Myxobolus* sp., *M. makombensis* Feudjio-Dongmo, Lekeufack-Folefack, Tene-Fossog, Fomena, Wondji, Yurakhno, Alomar, Mansour, 2022; *Myxobolus dibombensis* Lekeufack-Folefack, Abdel-Baki, Onana, Fomena and Mansour, 2019; *Myxobolus njoyai* Nchoutpouen and Fomena, 2011 and *Myxobolus paludinosus* Reed, Basson and Van AS, 2002. Their prevalence's range is 11.87% to 87.44% (Table 1) in the fish population examined. *Myxobolus* sp. and *M. paludinosus* appeared frequent or primary (prevalence > 50%) while *M. makombensis*, *M. njoyai* and *M. dibombensis* appeared intermediate or secondary (10% ≤ prevalence ≤ 50%).

All myxozoans species recorded from *L. batesii* developed cysts. The mean cyst load is high ($\bar{x}$ > 100 cysts) for *Myxobolus* sp., low (10 ≤ $\bar{x}$ ≥ 50 cysts) for *M. paludinosus* and *M. dibombensis*, very low ($\bar{x}$ < 10 cysts) for *M. njoyai* and *M. makombensis*. In the *L. batesii* population examined, excluding *Myxobolus* sp., each myxosporidia species developed a minimum of 1 cyst per host individual while the maximum ranged from 12 to 179 cysts. Meanwhile a specimen of *L. batesii* has hosted at least 64 cysts of *Myxobolus* sp. and up to 230 cysts (Table1). *Myxobolus paludinosus*, *M. njoyai* and *M. dibombensis* had standard deviation values higher than the mean cyst load (table 1).

**Table 1. Prevalence, mean cyst load and ecological status of myxosporidia species parasites of *Labeobarbus batesii***

| Parasite species | Prevalence (%) | $\bar{x}$ (σ)$x_{min} - x_{max}$ | Ecological status based on: | |
|---|---|---|---|---|
| | | | prevalence | mean cyst load |
| *M. dibombensis* | 44.98 | 11.8(19.14)1-75 | less frequent | weak |
| *M. makombensis* | 11.87 | 3.38(3.31)1-12 | less frequent | very weak |
| *M. njoyai* | 21.46 | 7.79(14.62)1-101 | less frequent | very weak |
| *M. paludinosus* | 55.48 | 25.8(28.94)1-179 | frequent | weak |
| *Myxobolus* sp. | 87.44 | 107.45(32.82)64-230 | frequent | very high |

*M.* : *Myxobolus* ; $\bar{x}$: mean cyst load; σ: standard deviation; $x_{min}$: minimum value; $x_{max}$: maximum value

**Table 2: Types of parasitism by myxosporidia in *Labeobarbus batesii***

| Type of parasitism | | | n | Prevalence |
|---|---|---|---|---|
| **Parasitism by one species** | | *M. njoyai* | 1 | 0.23 |
| | | *M. paludinosus* | 10 | 2.28 |
| | | *Myxobolus* sp. | 73 | 16.67 |
| | | **Subtotal** | **84** | **19.18** |
| **Parasitism by several species** | **Parasitism by two species** | *M. njoyai-M. makombensis* | 1 | 0.23 |
| | | *M. dibombensis-M. makombensis* | 2 | 0.46 |
| | | *M. paludinosus-M. dibombensis* | 3 | 0.68 |
| | | *M. paludinosus-M. njoyai* | 4 | 0.91 |
| | | *M. makombensis- Myxobolus* sp. | 9 | 2.05 |
| | | *M. paludinosus-M. makombensis* | 9 | 2.05 |
| | | *M. njoyai- Myxobolus* sp. | 16 | 3.65 |
| | | *M. dibombensis- Myxobolus* sp. | 48 | 10.96 |
| | | *M. paludinosus- Myxobolus* sp. | 67 | 15.3 |
| | | **Subtotal** | **159** | **36.3** |
| | **Parasitism by three species** | *M. njoyai-M. dibombensis-M. makombensis* | 1 | 0.23 |
| | | *M. dibombensis-M. makombensis- Myxobolus* sp. | 2 | 0.46 |
| | | *M. njoyai-M. makombensis- Myxobolus* sp. | 4 | 0.91 |
| | | *M. paludinosus-M. makombensis- Myxobolus* sp. | 4 | 0.91 |
| | | *M. njoyai-M. dibombensis- Myxobolus* sp. | 11 | 2.51 |
| | | *M. paludinosus-M. njoyai - Myxobolus* sp. | 20 | 4.57 |
| | | *M. paludinosus-M. dibombensis- Myxobolus* sp. | 76 | 17.35 |
| | | **Subtotal** | **118** | **26.94** |
| | **Parasitism by four species** | *M. njoyai-M. dibombensis-M. makombensis-Myxobolus* sp. | 1 | 0.23 |
| | | *M. paludinosus-M. njoyai-M. dibombensis-M. makombensis* | 2 | 0.46 |
| | | *M. paludinosus-M. dibombensis-M. makombensis-Myxobolus* sp. | 10 | 2.28 |
| | | *M. paludinosus-M. njoyai-M. dibombensis-Myxobolus* sp. | 31 | 7.08 |
| | | **Subtotal** | **44** | **10.05** |
| | **Parasitism by five species** | *M. paludinosus-M. njoyai- M. dibombensis -M. makombensis-Myxobolus* sp. | 7 | 1.6 |
| | | **Subtotal** | **7** | **1.6** |
| | | **Total** | **412** | **94.06** |

*M.* : *Myxobolus* ; n : number of parasitized hosts;

### 3.2. Monoparasitism and polyparasitism by myxosporidia species in *Labeobarbus batesii*

In the population of *L. batesii* examined, 5.94% were free of parasite, 19.18% harbor a single parasite species while 328 fish (74.89%) exhibit polyparasitism. In cases of polyparasitism, the number of myxosporidia species hosted by fish ranged from 2 to 5 (Table 2).

- **Parasitism by a single myxosporidia species**

The parasites species implicated in this type of parasitism are *M. paludinosus*, *M. njoyai* and *Myxobolus* sp. (Table 2). With 16.67% of cases, infection with *Myxobolus* sp. alone dominated while infection with *M. njoyai* being reported alone in 0.23% of case was least common.

- **Parasitism by two myxosporidia species**

With 36.3% of case registered, parasitism by two species of myxosporidia was the most frequently type of polyparasitism encountered in the present study. Of the nine bispecific combinations registered, the coalition formed by *M. paludinosus-Myxobolus* sp. was the most frequent (15.30% of cases) (Table 2). *Myxobolus paludinosus*, *M. makombensis* and *Myxobolus* sp. were involved in 4 of the 9 combinations types while the other two species (*M. njoyai* and *M. dibombensis*) appeared only in 3 cases of association with two parasites species (Table 2).

- **Parasitism by three myxosporidia species**

Simultaneous infestation by three myxosporidia species was found on 26.94% of examined fish (Table 2). The association made by *M. paludinosus-M. dibombensis-Myxobolus* sp. found in 17.35% of fish examined is the most common (Table 2). *Myxobolus* sp. is involved in 6 of the 7 types of three-species associations; *M. njoyai*, *M. dibombensis* and *M. makombensis* participate in 4 of these associations while *M. paludinosus* is involved in only three cases of association with three parasites species (Table 2).

- **Parasitism by four Myxosporidia species**

Cases of parasitism by 4 myxosporidia species were registered on 10.05% of the population of *L. batesii* examined. The combination made by *M. paludinosus-M. njoyai-M. dibombensis-Myxobolus* sp. recorded on 7.08% of fish individuals is the most common. *Myxobolus dibombensis* appears in all associations made by four species of myxosporidia while other parasites species involved in this type of parasitism occur in only three associations (Table 2).

- **Parasitism by five myxosporidia species**

Simultaneous infection by five species of myxosporidia was found in only 1.6% of examined fish. This combination which involves all parasites species recorded in the examined fish population is the least common type in *L. batesii*.

We summarize the number of times each myxosporidia species was found in each type of parasites association in *L. batesii* (Table 3). This number peaks for: (1) *M. paludinosus*, *M. makombensis* and *Myxobolus* sp. in associations involving two parasites species; (2) *Myxobolus* sp. in parasitism which involved the presence of three parasites species; (3) *M. dibombensis* in parasitism where four species of myxosporidia are simultaneously implicated.

**Table 3: Number of occurrences of parasites species in each type of parasites association in *Labeobarbus batesii***

| Parasite species | Type of parasite association | | | |
|---|---|---|---|---|
| | two species involved (9) | three species involved (7) | Four species involved (4) | Five species involved (1) |
| ***M. paludinosus*** | 4 | 3 | 3 | 1 |
| ***M. njoyai*** | 3 | 4 | 3 | 1 |
| ***M. dibombensis*** | 3 | 4 | 4 | 1 |
| ***M. makombensis*** | 4 | 4 | 3 | 1 |
| ***Myxobolus* sp.** | 4 | 6 | 3 | 1 |

( ) : number of combinations

### 3.3. Binary association between myxosporidia species

Measurement of the degree of interspecific association between myxosporidia revealed three levels of association: weak association, moderate association, and strong association. Weak association was established between *M. paludinosus-M. dibombensis* (D=0.21), *M. njoyai-M. makombensis* (D=0.23), *M. dibombensis-M. makombensis* (D=0.20) and *M. makombensis-Myxobolus* sp. (D=0.17). Moderate association was noted between *M. paludinosus-M. njoyai* (D=0.35); *M. njoyai-M. dibombensis* (D=0.36) ; *M. njoyai-Myxobolus* sp. (D=0.36). A strong association exists between *M. paludinosus-M. dibombensis* (D=0.59), *M. paludinosus-Myxobolus* sp. (D=0.69), *M. dibombensis-Myxobolus* sp. (D=0.66).

The tetrachoric coefficient (φ) revealed a negative (-0.18) and significant correlation between *M. makombensis-Myxobolus* sp.. Positive and almost null correlation ($0 \leq \varphi < 0.1$) was noted between several parasite species (*M. paludinosus-M. njoyai, M. paludinosus-M. dibombensis, M. paludinosus-Myxobolus* sp., *M. njoyai-Myxobolus* sp. and *M. dibombensis-M. makombensis*), while positive correlation was recorded between some species (*M. paludinosus-M. dibombensis, M. njoyai-M. dibombensis, M. njoyai-M. makombensis, M. dibombensis-Myxobolus* sp.).

Measuring the amount of association deviation from expectation between two parasite species, the Forbes' index (F) revealed that: associations between *M. paludinosus-M. dibombensis*, *M. paludinosus-Myxobolus* sp., *M. njoyai-Myxobolus* sp. and *M. dibombensis-M. makombensis* respond to the laws of chance (F≈1); while associations between *M. paludinosus-M. njoyai, M. paludinosus-M. dibombensis*, *M. njoyai-M. dibombensis, M. njoyai-M. makombensis* and *M. dibombensis-Myxobolus* sp. appeared more often than chance would predict (F>1). The association between *M. makombensis-Myxobolus* sp. occurred less frequently than predicted by chance (F = 0.81).

**Table 4 : Ecological indexes of binary association between myxosporidia species**

| Type of myxosporidia binary association | Ecological indexes | | | |
|---|---|---|---|---|
| | D | φ | F | χ² |
| *M. paludinosus-M. njoyai* | 0.35*** | 0.08 | 1.13[a] | 0.01+++ |
| *M. paludinosus-M. dibombensis* | 0.59**** | 0.19 | 1.19 [a] | 9.66++ |
| *M. paludinosus-M. dibombensis* | 0.21** | 0.03 | 1.07 [a] | 0.02+++ |
| *M. paludinosus-Myxobolus* sp. | 0.69**** | 0.06 | 1.02 [a] | 0.01+++ |
| *M. njoyai-M. dibombensis* | 0.36*** | 0.11 | 1.23 [a] | 54.59+++ |
| *M. njoyai-M. makombensis* | 0.23** | 0.10 | 1.52 [a] | 14.5+++ |
| *M. njoyai-Myxobolus* sp. | 0.36*** | 0.05 | 1.03 [a] | 0.04+++ |
| *M. dibombensis-M. makombensis* | 0.20** | 0.02 | 1.07 [a] | 0.01+++ |
| *M. dibombensis-Myxobolus* sp. | 0.66**** | 0.26 | 1.11 [a] | 0.02+++ |
| *M. makombensis-Myxobolus* sp. | 0.17** | -0.18 | 0.81 [b] | 0.05+++ |

**Legend : D** : Dice index ; **F**: Forbes index; ***M.*** : *Myxobolus*; **χ²** : Chi square ; **φ** : Tetrachoric coefficient; ** : weak association ; *** : Moderate association ; **** : Strong association; **a** : Association more frequent than expected by chance ; **b** : Association less frequent than expected by chance ; ++: significant with P value < 0.01, +++ : significant with P value < 0.001.

## 4.Discussion

All Myxosporidia species recorded on *Labeobarbus batesii* are histozoic. The most common phenomenon encountered in histozoic Myxosporidia is the encapsulation of the vegetative stage (plasmodium) in various host organs by layers of connective, fibrous or epithelial tissue, isolating the parasite and preventing its dispersal into the surrounding tissue (Davies and Sienkoswki, 1988). Prevalence and mean cyst load, traditionally used to qualify myxosporidia populations or the severity of infestation, are subject to variation. Poulin (2006) believes that for a given parasite species, the proportion of infested hosts is not fixed across its geographical range. Therefore, the prevalence of myxosporidia in fish fluctuates greatly in the wild and generally ranges from 0 to 100% (Foott *et al*., 2010 and True *et al*., 2013). Based on the classification of ecological status of parasite species proposed by Valtonen *et al*. (1997), *Myxobolus paludinosus* and *Myxobolus* sp. appeared frequent or principal (P > 50%) while *M. njoyai*, *M. dibombensis* and *M. makombensis* appeared intermediate or secondary (10% ≤ P ≤ 50%). Given that the status of parasite species varies according to environmental conditions (El Tantawy, 1989) and host species (Brummer-Korvenkontio *et al*., 1991), the high prevalence recorded for the majority of myxosporidia species collected in the present work would be associated to the reduction of the host species defense capacity.

In general, myxosporidia infestations elicit little or no cellular response from the host (Lom and Dyková, 1992). Host immune responses against these parasites vary according to parasite species, target tissue, species and host individual (Gómez *et al*., 2014). The best-known host immune response is to encapsulate and isolate myxosporidia plasmodium from the surrounding host tissue (Sitjá-Bobadilla *et al*., 2015). This probably reflects the result of a co-evolutionary process involving parasite adaptations to host immune responses and host adaptations to parasite virulence. However, the effectiveness of encapsulation in eliminating parasite is limited (Koehler *et al*., 2004). Evidence of such failure would be the high mean cyst loads recorded for *Myxobolus* sp.. Cases of low or very low mean cyst loads were recorded for the other parasites species. This may be due to the fact that sampling of heavily parasitized fish is rare in the wild because such host individuals are quickly eliminated by predators (Miller *et al*., 2014). Cysts of *M. paludinosus*, *M. njoyai*, and *M. dibombensis* were aggregated in some fish individuals. Assuming equal exposure of fish specimens to actinospores in the Makombè River, parasite aggregation suggests susceptibility differences between host individuals. It is however important to recall that parasite aggregation is a typical phenomenon in parasite-host relationships (Shaw and Dobson, 1995).

Parasitism by several species (polyparasitism) was more frequent than infestations by a single parasite species (monoparasitim). Polyparasitism by myxosporidia is well documented in central African freshwater fish (Fomena, 1995; Abakar-Ousman *et al*., 2006; Lekeufack Folefack, 2010; Nchoutpouen, 2015 and Fonkwa *et al*., 2018), where cases of two-, three-, four-, five-, six- and seven-species parasitism have been recorded. Out of central Africa, few cases of polyparasitism by Myxosporidia have been reported. Holzer *et al*. (2006) found a five-species parasite combination (*Tetracapsuloides bryosalmonae* + *Sphaerospora truttae* + *Chloromyxum schurovi* + *Chloromyxum truttae* + *Myxobolus* sp.) in *Salmo trutta* in Scotland. Studying parasitism of the sea bream *Sarpa salpa* in Tunisia, Laamiri (2014) noted cases of parasitism by two species (*Ceratomyxa herouardi* + *Ceratomyxa pallida*). No case of monoparasitism due to *M. dibombensis* and *M. makombensis* was recorded while *Myxobolus* sp. has the greatest number of appearances in cases of monoparasitism and polyparasitism in *L. batesii*. These observations suggest a possible weakening of the immune response of this Cyprinid fish by *Myxobolus* sp., which is probably the first species to establish itself in this host species (Koskivaara and Valtonen, 1992 and Tombi, 2005). Weakening of the host immune response would favor colonization by other species of myxosporidia therefore pathogenesis condition which is rarely caused by a single parasite species (Combes, 1995). In the case of polyparasitism, natural selection may favor different levels of virulence among parasite species (Bordes and Morand, 2011). Consequently, immunity would be the key element in co-infestation because, by structuring parasites community, it would affect the positive or negative interactions between parasites species (Bordes and Morand, 2011). In the case of polyparasitism, the epidemiological indices of one (or more) parasite species can be reinforced by those of the other (others) parasites species (Fenton *et al*., 2010).

The unique case of negative interaction has been recorded between *Myxobolus* sp. and *M. makombensis*. Peeler *et al*. (2008) report a strong negative interaction between *Tetracapsuloides bryosalmonae* and *Ceratonova schurovi* in *Salmo trutta* in England. In Switzerland, *T. bryosalmonae*, a myxosporidia responsible for proliferative nephropathy in wild trout, is negatively associated with the nematode *Raphidascaris acus* (Schmidt-Posthaus *et al*., 2013). Another case of negative interaction was recorded in Norway between the myxosporidia *Kudoa islandica* and the microsporidia *Nucleospora cyclopteri* in *Cyclopterus lumpus* (Alarcón *et al*., 2016). *M. makombensis* and *Myxobolus* sp. share the same habitat and the coincidences of them being found together in the same host individual are fewer than expected by chance ($F < 1$). Moreover, the negative association between these two parasites species provides information to speculate on the type of relationship that might exist between them. Therefore, we can think of no other explanation for this deviation than interspecific competition. This negative interaction indicates that infestation by one parasites species could reduce the probability of infestation by another. Except the single case of negative interaction mentioned above, increase of prevalence of each species of myxosporidia recorded leads to an increase in the prevalence of others parasites species. According to Petney and Andrews (1998), a large number of factors have been shown to be involved in the determination of either the number of parasite species infesting a host individual or the probability of a particular parasite species infesting a host individual. These factors include environmental, physiological, immunological, population dynamics, behavioral, genetic, and stochastic processes. Thus, pathological damage caused by one parasite species may also increase the host susceptibility to another parasite species. The deleterious effects in this case may be linked to higher misuse of resources by parasite and/or cumulative damage registered on the host due to overall parasite loads (Holmstad *et al*., 2008). The presence of different Myxozoan species in *L. batesii* means that, they have overlapping ecological niches within Makombe River. Moreover, most of these parasites co-occur more frequently than expected. This may happen because the same factors promote their presence in the host fish, not because they are interacting synergistically (Petney and Andrews 1998).

## 5. Conclusion

The mechanism for the relationship between Myxozoans species parasite of *L. batesii* cannot be exactly determined, as the current data do not indicate whether the interaction is direct or host mediated. However, the analyses provide in this study are credible evidence for the presence of interspecific parasite interactions throughout a xenocommunity of *Myxobolus* species, suggesting that *L. batesii*’s immunity may have a role in shaping that xenocommunity.

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