

Distribution of Gill Parasites of the Freshwater Fish *Barbus martorelli* Roman, 1971 (Teleostei: Cyprinidae) and Tendency to Inverse Intensity Evolution Between Myxosporidia and Monogenea as a function of the Host Age

J. Tombi¹ C.F. Bilong Bilong^{1*}

Key words

Barbus martorelli – Myxosporidia – Monogenea – Gills – Morbidity – Cameroon.

Summary

The study of gill parasite fauna from 558 *Barbus martorelli* in a tropical freshwater course near Yaounde (Cameroon) was undertaken to collect data on the distribution of pathogens in a wild population, which is essential to find means of avoiding catastrophic losses often observed in intensive aquaculture. The analysis revealed the presence of two myxosporidian species (*Myxobolus barbi* and *M. njinei*) and eight monogenean species (*Dactylogyrus bopeleti*, *D. insolitus*, *D. simplex*, *D. maillardi*, *Dogielius martorellii*, *Dogielius* sp., one species of Gyrodactylidae and one larva of Polystomatidae). Four individual fish were found without parasites, whereas 24 and 530 presented monospecific and multispecific infections, respectively. Each infested host harbored an average of four parasite species. As the prevalence and mean intensity reduced with the host size for protozoans, the reverse phenomenon was observed for helminths. A sexual effect was observed on the infestation of *B. martorelli* by the myxosporidian *M. njinei* and by most monogenean species; females were thus more infested than males due to the biology of gravid specimens. Finally, the high parasite species richness appeared to reduce the host weight.

■ INTRODUCTION

Several million people in Africa depend on fish for their livelihood: from fishing, processing, transporting, to retailing. Fish represents a very important food source for low-income populations for whom it is often the only source of protein, particularly in towns where large scale animal husbandry is rare. The African mean consumption is approximately 5.5 to 8.5 kg of fish/person/year (7). But the high incidence of fish diseases

remains a major constraint on successful economic development of this activity. Ectoparasites such as gill myxosporidians and monogeneans figure among parameters which are involved in fish diseases. From a pathological point of view, Gyrodactylidae and Dactylogyridae are the main monogeneans that cause severe gill lesions; Myxosporidia cause destruction and necrosis of much of the tissue, leading to hemorrhage and anemia (21). According to Sindermann (33), Myxozoa constitute one of the two phyla that can have the most severe effects on fish hosts. Apart from their respiratory function, the gills are responsible for regulating the exchange of salt and water, and play a major role in the excretion of nitrogenous waste products. Even a slight structural damage could render a fish very vulnerable to osmoregulatory as well as respiratory difficulties (9). The present study dealt with some biological aspects of the gill parasite fauna of *Barbus martorelli*

1. Laboratory of General Biology, Faculty of Science, University of Yaounde I, PO Box 812, Yaounde, Cameroon

* Corresponding Author
Tel: +237 998 65 45
E-mail: bilong_bilong@yahoo.com

Roman, 1971 (Teleostei: Cyprinidae) with the aim to define in the host population concerned the distribution of pathogens (myxosporidians and monogeneans) as a function of the fish sex and size (age). In fact, the gills of *Barbus martorelli* constitute a biotope for several parasite species. Birgi and Lambert (5) and Fomena et al. (11) described for this host five monogenean species (*Dactylogyrus bopeleti*, *D. insolitus*, *D. simplex*, *D. maillardi* and *Dogielius martorellii*) and two myxosporidian species (*Myxobolus barbi* and *M. njinei*), respectively. These authors laid emphasis on the taxonomic aspect with little or no biology (the distribution and possible interactions) of these pathogens. Yet, Gregory and Keymer (14) stressed that “no animal species exists in isolation”. Similarly, Barbault (2) indicated that considering only specific richness is an insufficient precise measure of the quantitative composition of a community. He then proposed a community pluralist approach, which implies – apart from the determination of specific richness – taking into account the biology of the species concerned. This research was carried out in view of minimizing the different problems that these pathogens could cause in a fish breeding pond.

■ MATERIALS AND METHODS

B. martorelli is one of the common fish species in Southern Cameroon (36). Highly consumed by rural human populations in this part of the nation, it thus constitutes one of the animal protein sources. It also happened to represent the dominant ichthyological species in the samples of the study. Specimens were caught from February 1998 to August 1999 using a 1 cm by 1 cm mesh gillnet in the Foulou watercourse in Nkolfoulou locality, i.e. in the outskirts of Yaounde, capital of the Republic of Cameroon. In this site, the bottom of the river is sandy and rocky; during the sampling period the depth varied from 0.5 to 1.5 m with an average width of 5 m. The vegetation is made up of mosaic savannah-forest, mesophyllitic forests and preforest savannahs of tall herbs (34). Except in February and March 1998, two samples were collected monthly and an effort was made to obtain a minimum of 30 fish per month. After the catch, the host and their parasites were immediately fixed in 10% formalin. At the laboratory, the size of each specimen was measured and its sex determined. The gills from both sides were dissected and examined with a stereo-microscope. Myxosporidian cysts, present on the filaments or on the bony arch, were counted. The monogeneans were collected and mounted on slides in a drop of hematoxylin (eosin). The different species were identified using a microscope: magnification x 40 and x 100 for Monogenea and Myxosporidia, respectively. All individual hosts were grouped in seven different length classes at 10 mm amplitude. The standard length (SL) hereby corresponds to the length from the extremity of the muzzle to the last vertebra. The prevalence, intensity (I) and mean intensity (Im) of parasites were defined according to Margolis et al. (19). On the prevalence basis and according to Valtonen et al. (35), the parasite species were termed common (prevalence > 50%), intermediate (10 ≤ prevalence ≤ 50%), and rare (prevalence < 10%). These categories may correspond to what other authors such as Hanski in Koskivaara and Valtonen (16) designated as core, secondary, or satellite species, respectively. Considering the intensity, the classification adopted was that of Bilong Bilong and Njiné (4): I < 10 = very low; 10 < I ≤ 50 = low; 50 < I ≤ 100 = medium; I > 100 = high. The χ^2 test was used to compare prevalences, while the Mann Whitney (U) and Kruskal Wallis (K) tests permitted to compare two and more than two mean intensities, respectively. Except for any subsequent indications, the degree of security retained here was 95%, i.e. P < 0.05.

■ RESULTS

A total of 558 *B. martorelli* individuals were caught at a female-to-male sex ratio of 0.9. From the overall sample, two species of Myxosporidia (*Myxobolus barbi* and *M. njinei*), and eight of Monogenea (*Dactylogyrus bopeleti*, *D. insolitus*, *D. simplex*, *D. maillardi*, *Dogielius martorellii*, *Dogielius* sp., polystomatid larva and *Gyrodactylus* sp.) were collected (Table I). *D. bopeleti*, *D. insolitus*, *D. simplex* and *M. barbi* were common, *D. maillardi*, *M. njinei* intermediate, and *Dogielius* sp., *Do. martorellii*, *Gyrodactylus* sp. and the polystomatid larva rather rare (Table I). It was noted that no mature polystomatid parasite was found on *B. martorelli*. Of the 46,056 myxosporidian cysts observed on the different individual hosts, 97.28% were those of *M. barbi* alone. The percentage distribution of 8840 collected worms was as follows: 32.56% *D. bopeleti*, 32.67% *D. insolitus*, 27.68% *D. simplex*, 5.49% *D. maillardi*, 0.7% *Dogielius* sp., 0.02% *Do. martorellii*, 0.49% Gyrodactylidae, 0.4% Polystomatidae. Globally, the mean intensities of the different parasite species were very low except for *M. barbi*, one of the common (or core) species (Table I). In Nkolfoulou host population, all core and intermediate species presented an aggregate distribution ($s^2 > Im$), while the rare ones were dispersed ($s^2 < Im$). In the total host sample, only four specimens were uninfected and 24 others presented a monospecific infection: 23 by core species (*D. bopeleti*, *D. insolitus*, *D. simplex* and *M. barbi*) and 1 by an intermediate species (*D. maillardi*). The remaining 530 fish presented each a multispecific infection (from 2 to 8 parasite species). The overall average number of parasite species per host was about 4 (3.98 ± 1.32) (Figure 1).

It should be noted that the rare species were hereafter excluded from the analysis for their rare occurrence and very low intensities. Concerning the prevalence, only *D. bopeleti* and *D. insolitus* were evenly distributed throughout the length classes (Figure 2: a and b) ($\chi^2 = 3.21$ and 8.68, respectively; df = 6). Moreover, the protozoan prevalences decreased progressively with the host size (Figure 2: e and f), while the reverse phenomenon was observed for helminths (Figure 2: a through d).

The mean intensity of infection by monogeneans and protozoans remained very low for all the length classes, except for *M. barbi*

Table I

Data on *Barbus martorelli* infection during the study period

Parasite species	Prevalence (%)	Im (σ) minV – maxV
Protozoa		
<i>Myxobolus barbi</i> ***	79.03	101.63 (211.73) 1 – 1613
<i>M. njinei</i> **	27.70	8.49 (10.76) 1 – 65
Helminths		
<i>Dactylogyrus bopeleti</i> ***	80.64	6.42 (5.38) 1 – 32
<i>D. insolitus</i> ***	84.41	6.15 (5.01) 1 – 32
<i>D. simplex</i> ***	70.79	6.28 (7.71) 1 – 62
<i>D. maillardi</i> **	33.15	2.60 (2.30) 1 – 20
<i>Dogielius</i> sp.*	9.32	1 (0) 1
<i>Do. martorellii</i> *	0.36	1.18 (0.32) 1 – 2
Polystomatidae (larva)*	5.91	1.06 (0.24) 1 – 2
Gyrodactylidae*	5.55	1.37 (0.75) 1 – 4

Im: mean intensity; σ : standard deviation; minV: minimum value; maxV: maximum value

***, **, *: common, intermediate, and rare species, respectively

for which the mean intensity appeared to be high for relatively shorter individual hosts, i.e. when the host size (SL) was lesser than 8 cm, and it was low when $8 \leq SL < 10$ cm, and very low when $SL > 10$ cm (Figure 3: a through f). In addition, it was noted that *M. barbi* and *M. njinei* each formed cysts on the gill filaments and on the host arch bones, respectively. Concerning gill filament pathogens at the parasite phylum level, the average total number of helminths increased with the host size (Kruskal Wallis statistics $K = 14.26$; $df = 6$) and was associated with a progressive decrease in the number of protozoan cysts ($K = 29.41$; $df = 6$) (Figure 4).

A striking feature of this work was the different behavior of both protozoans and helminths in the colonization of the gill biotope and as a function of the host size. The parasitism of *B. martorelli* by myxosporidians appeared sex dependent only for *M. njinei*, significantly more prevalent in females than in males ($\chi^2 = 56.00$). Concerning monogeneans, the infection rates of *B. martorelli* revealed differences between sexes only for *D. bopeleti* and *D. insolitus* ($\chi^2 = 11.62$ and 15.13 , respectively). Although the mean infection intensity was generally very low, it appeared [except for *D. insolitus* (148.5 ; $P = 0.180$)] significantly greater in females.

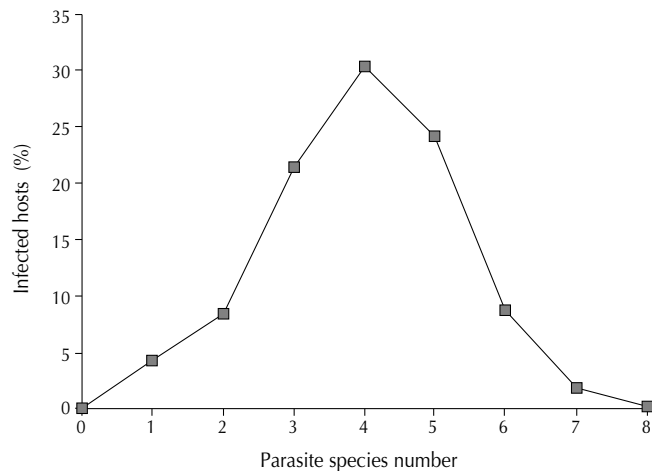


Figure 1: Relative proportion (%) of infected hosts as a function of parasite species number in Foulou.

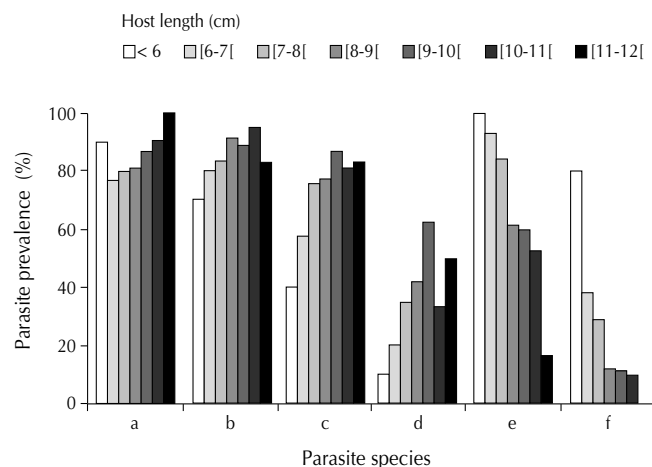


Figure 2: Prevalence (%) of parasite infections of *Barbus martorelli* gills from Foulou as a function of host length classes; a: *Dactylogyrus bopeleti*; b: *D. insolitus*; c: *D. simplex*; d: *D. maillardi*; e: *Myxobolus barbi*; f: *M. njinei*.

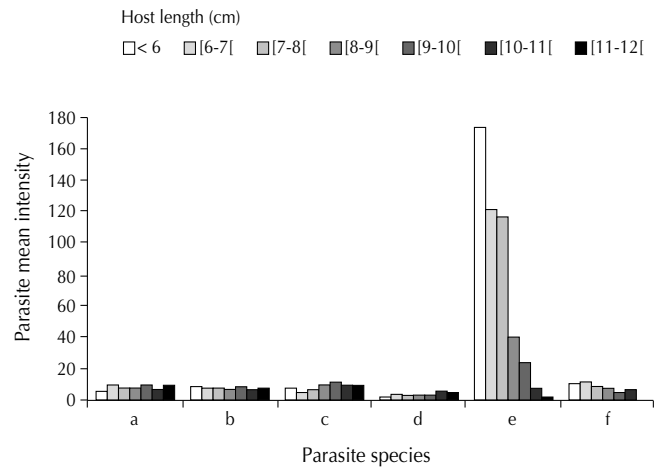


Figure 3: Mean intensity as a function of the host length classes; a: *Dactylogyrus bopeleti*; b: *D. insolitus*; c: *D. simplex*; d: *D. maillardi*; e: *Myxobolus barbi*; f: *M. njinei*.

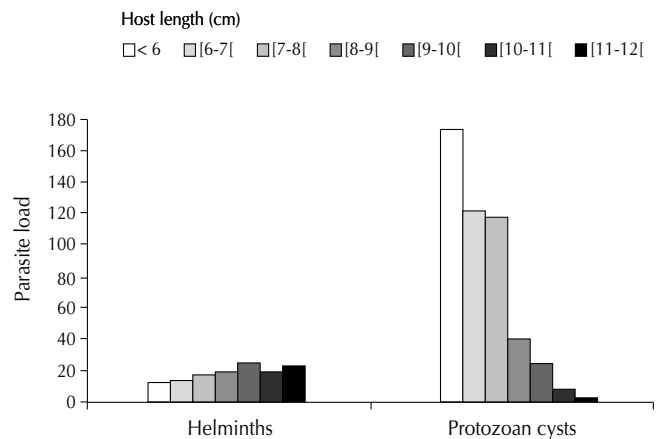


Figure 4: Average total number of helminth and protozoan cysts of the host gill filaments as function of host length classes.

DISCUSSION

B. martorelli gill parasite fauna was diversified and composed, on the one hand, of two myxosporidian species of the genus *Myxobolus* Bütschli, 1882, and, on the other hand, of eight monogenean species belonging to three families: Dactylogyridae with two genera (*Dactylogyrus* Diesing, 1850 and *Dogielius* Bychowski, 1936), Gyrodactylidae, and Polystomatidae (larva stage). The simultaneous infections by several congeneric species already observed for Dactylogyridae by Birgi and Lambert (5), and for Myxosporidia by Fomena et al. (11), were hereby confirmed. In this component community, there was often an association between any parasite species of either helminth or protozoan with any other protozoan or helminth. It is worth noting that a detailed analysis of the parasite community structure is in the making; nevertheless, some authors like Koskivaara and Valtonen (17) have already reported on the coexistence of three congeneric monogenean species, with one glochidia, one copepod, and one protozoan on the roach *Rutilus rutilus*. Adams (1) also found out that a correlation existed between two monogenean species and

Myxosporidia on the *Fundulus kansae* gills. In Central Finland, Koskivaara et al. (18) reported that Dactylogyridae are common monogenean parasites, which are usually numerous and have a high species density on the gills of cyprinid fishes. In the present study carried out in a tropical zone, the prevalences of *Dactylogyrus* species on *B. martorelli* gills were also high, except for *D. maillardi* (an intermediate species) and for the other rare monogenean species (including some Dactylogyridae). The mean intensities of all helminths remained very low and this could be partly due to the water current velocity. Other parasite community intrinsic or extrinsic factors may change or affect the parasite shelter. Ergens (10) argued that the degree of infection depends on the conditions and density of the hosts; the current is often evoked as a factor limiting the recruitment of infective larva stages, thus reducing the intensity of parasitism (31). In Israel, Paperna (29) also reported that the rate of infection (mainly with ectoparasites) was high in the lake of Galilea, in small water reservoirs, as well as in fish ponds, while fishes in swiftly flowing streams were found to be either free of infection or only slightly infested. According to Gerashev and Staravoitov (13), the water current might have a double effect on monogeneans: it brings their infective larvae on the hosts or it helps these latter get rid of them (infective larvae and mature individuals). Obiekezie (22) also noted that in wild fishes, monogeneans occurred at low intensities and in apparent equilibrium with their hosts, whereas under cultured conditions (in which fishes are concentrated more than they are in nature) these pathogens build up heavy worm burdens which provoke epizootics. Comparatively, in an oligotrophic water course, Bilong Bilong (3) found out that all monogenean species of the *Hemichromis fasciatus* gills were common and occurred in very low mean intensities. While in an eutrophic lake all these parasites remained common except one (*Cichlidogyrus dageti*), which disappeared; their mean intensities also remained very low for all but one (*Onchobdella voltensis*), whose infrapopulations increased slightly (4). These findings tend to illustrate the fact that host confinement may change the parasite status. In the present paper, the *B. martorelli* gill monogeneans from an oligotrophic river revealed to be either common (prevalence > 50%), intermediate (10 ≤ prevalence ≤ 50%) or rare (prevalence < 10%), whereas their mean intensities were very low (< 10).

In simultaneous infections of *B. martorelli* by protozoans, *M. barbi* was the core species parasitizing its host with a high mean intensity, while *M. njinei* appeared to be an intermediate pathogen species with a very low mean intensity. These data are quite similar to those of Fomena et al. (11), but on a different host sample. These authors were only interested in single prevalences and found 54.68 and 25% of *B. martorelli* parasitized by *M. barbi* and *M. njinei*, respectively.

It has been shown that the average number of cysts decreased while the SL of the host increased. Similar results were obtained in Nigeria by Obiekezie and Enyenihi (23), who pointed out a high prevalence and a high mean intensity of *Henneguya chrysichthysi* in the juveniles of *Chrysichthys nigrodigitatus*. As observed by the previously mentioned authors, the present study confirmed the fact that high rates of infection (and probably high intensities) seem to characterize the parasitism of young teleosts by gill Myxosporidia. In fact, the rapid growth of gill epithelium in this age group favors the development of myxosporidian plasmodia (20). Conversely, low prevalences and low mean intensities of these myxosporidian infections observed in large size hosts could partly be justified by the rupture of old age cysts. In Melen fish pond station (Cameroon), Fomena (12) also frequently observed that old cysts on the *Oreochromis niloticus* gill filaments were split open but without spores. Dykova and Lom in Obiekezie and

Enyenihi (23) argued that elevated ambient temperatures enhancing the immunological response of fish could lead to the destruction of *Henneguya* cysts; whether this applies to *M. barbi* and *M. njinei* in Foulou, where temperatures on the average are below 23°C, is questionable. This work had already shown that the average total helminth load increased with the host size. As a matter of fact, it is likely that the rupture of myxosporidian cysts should also be consecutive to perforations due to monogenean hooks.

The difference in infections of male and female hosts by *M. njinei*, whose cysts seemed not affected by monogenean hooks, could be explained by the host biology. An explanation could be that this myxosporidian species is present on gill arches, whereas worms are present on gill filaments, as observed during the study. In this field study, female *B. martorelli*, in the spawning period (females in gravid stage), formed shoals (of about 10 individuals) and sheltered in small rocky cavities becoming readily accessible to parasites in the actinosporean stage. Kent et al. (15) called to mind that an important discovery in the 1980s, i.e. the life cycle of *Myxobolus cerebralis* requires development of an actinosporean stage in the oligochaete *Tubifex tubifex*, led to the elucidation of the life cycles of several other myxozoans. Furthermore, according to these authors it is admitted that in the course of this cycle, the actinospore attaches to the surface of the fish and injects its sporoplasm, while the fully developed myxospores released from the fish host are ingested by annelids. But the direct fish-to-fish transmission without the requirement of alternate actinosporean development may occur in some species. In comparison, Fomena (12) observed no sex influence on the infestation of *O. niloticus* gill filaments by the different myxosporidian species.

Rohde (30) stated that only in a few cases preference for one sex of host by monogeneans has been demonstrated. In this work, the gravid female sedentary lifestyle and the high host population density also optimized the monogenean infective larva recruitment. Similar behavior observed in a temperate zone during the spawning period of the seabass (*Dicentrarchus labrax*) justified the highest infection intensities of diplectanids on female fishes (31, 32). In the tropical area (Cameroon) no behavioral difference has been noticed between males and females of *Hemichromis fasciatus* (8), for which Bilong Bilong (3) found no sexual preference by these host gill monogeneans.

B. martorelli parasitism displayed a reduction or disappearance of gill epithelium as well as faded filaments and was partly or fully covered with cysts. Inevitably, this caused breathing problems and the host became unfit due to a high parasite load. In this work, some fish specimens were infected by up to 1600 *M. barbi* cysts. Fomena et al. (11) showed that *M. barbi* cysts are often ovoid and spherical and located in secondary gill strips, as observed in this study.

Dactylogyridae monogeneans and, more rarely, Gyrodactylidae and polystomatid parasites were also found in the gills of *B. martorelli*. Gyrodactylidae are fairly common on African fishes (27). They are able to reproduce extremely rapidly if conditions are favorable; their occurrence is epizootic, in a cultured fish population, and is generally a sign of poor husbandry conditions (21). Physical and chemical parameters showed that Foulou water is oligotrophic, meaning that pollution is insignificant (Dr Kengne, pers. commun.); also, the host density was often apparently not high, except in the spawning period. These two factors combined could explain the limited occurrence of gyrodactylids in this study. Moreover, Needham and Wotten (21) noticed that dactylogyrids could be dangerous for hosts, especially for young fishes, even under low intensities. The lethal and sublethal effects of pathogens

(myxozoans, monogeneans, etc.) is well documented (15, 22, 24–28, 30). Rohde (30) sums up the importance of fish parasites as follows: they may reduce population numbers by causing mortalities and sometimes mass mortalities, they may affect the reproductive organs and reduce the number of offsprings and thus the population size, and they may reduce the weight of fish. Combes (6) also stressed that the pathogenic effect is rarely caused by one parasite species. It was hereby shown that *B. martorelli* monogenean community was made up of six species of Dactylogyridae; taking into account all parasite species (protozoa and helminths), infracommunity sizes could be considerable and the sum of pathogenic effects so important that it could cause cases of morbidity or mortality even in a natural environment. In this study, the weight of hosts which harbored less than four parasite species increased as a function of the standard length, while this increase was slightly obvious in fish infested by more than three parasite species (Figure 5).

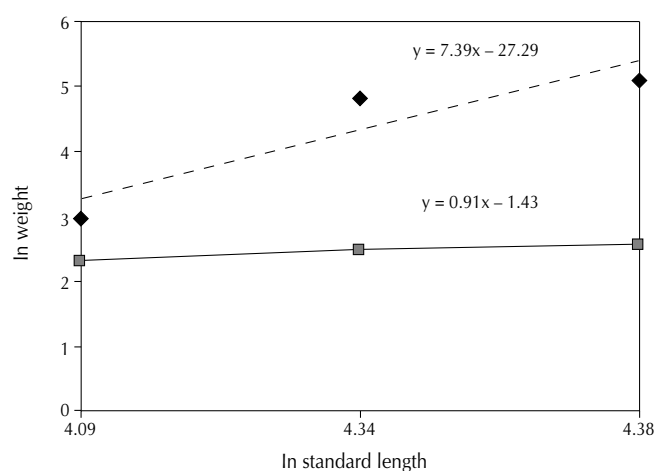


Figure 5: Weight logarithm as a function of standard length logarithm for non infected fish and fish infested by 1 to 3 parasite species ($y = 7.39x - 27.29$), and fish infested by more than 3 parasite species ($y = 0.91x - 1.43$).

CONCLUSION

Owing to the danger these parasites represent to such an important food resource that are fishes in Africa, one can quote these words from Obiekezie et al. (25): “There is no reason to doubt that the same diseases will occur when *B. martorelli* will be kept in culture, probably at still higher prevalences (and intensities) due to dense stocking. An understanding of the causes of temporarily high disease prevalences in the natural stock is essential in order to find ways of avoiding catastrophic losses in planned intensive aquaculture”. In this work, it was found that even in a natural environment, the accumulation of many pathogen species in *B. martorelli* was associated with the specimen weight reduction. This fish juveniles were more infected by myxosporeans than by monogeneans. In a culture situation (this teleostean fish has recently been observed in two rural fish ponds in Mandoumba and Mom II villages), cementing the hatching basins, on the one hand, could diminish oligochaete biomass; these organisms are actually considered as reservoirs of actinospores which infect fish hosts. On the other hand, washing the basins periodically could reduce helminth eggs and infective larvae (*Onchomiracidia*).

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Résumé

Tombi J., Bilong Bilong C.F. Répartition des parasites branchiaux du poisson d'eau douce *Barbus martorelli* Roman, 1971 (Teleostei : Cyprinidae) et tendance à une évolution d'intensité contraire des myxosporidies et des monogènes en fonction de l'âge de l'hôte

L'étude de la parasitofaune branchiale de 558 *Barbus martorelli* a été menée dans un cours d'eau douce aux alentours de Yaoundé (Cameroun) en milieu tropical, afin d'obtenir des informations sur la répartition des agents pathogènes dans une population d'hôtes sauvages ; ces données sont indispensables dans la recherche de moyens pour éviter les pertes énormes souvent observées en pisciculture intensive. Cette analyse a révélé la présence de deux espèces de myxosporidies (*Myxobolus barbi* et *M. njinei*) et de huit espèces de monogènes (*Dactylogyrus bopeleti*, *D. insolitus*, *D. simplex*, *D. maillardi*, *Dogielius martorellii*, *Dogielius* sp., une espèce de Gyrodactylidae et une larve de Polystomatidae). Quatre poissons n'ont pas présenté de parasites, 24 ont présenté une infection monospécifique et 530 une infection plurispécifique. Chaque individu infesté a hébergé en moyenne quatre espèces de parasites. Alors que la prévalence et l'intensité moyenne parasitaire diminuaient avec la taille de l'hôte pour les protozoaires, le phénomène inverse a été observé pour les helminthes. L'effet sexe a été noté dans l'infestation de *B. martorelli* par la myxosporidie *M. njinei* et par la plupart des espèces de monogènes. Les femelles ont ainsi été plus infestées que les mâles en raison de leur biologie en période de frai. Enfin, la richesse des espèces parasitaires a semblé affecter négativement le poids des hôtes.

Mots-clés : *Barbus martorelli* – Myxosporidia – Monogenea – Branchie – Morbidité – Cameroun.

Resumen

Tombi J., Bilong Bilong C.F. Distribución de los parásitos de branquias en el pez de agua dulce *Barbus martorelli* Roman, 1971 (Teleostei: Cyprinidae) y la tendencia a una evolución de intensidad inversa entre Myxosporidia y Monogenea en función de la edad del huésped

Se llevó a cabo un estudio del parásito de branquias a partir de 558 *Barbus martorelli*, en un cauce de agua dulce cerca de Yaoundé (Camerún), con el fin de recolectar datos sobre la distribución de patógenos en una población silvestre, lo cual es esencial para encontrar los medios de evitar las pérdidas catastróficas que a menudo se observan en la acuicultura intensiva. El análisis reveló la presencia de dos especies de mixosporidios (*Myxobolus barbi* y *M. njinei*) y ocho especies monogénicas (*Dactylogyrus bopeleti*, *D. insolitus*, *D. simplex*, *D. maillardi*, *Dogielius martorellii*, *Dogielius* sp., una especie de Gyrodactylidae y una larva de Polystomatidae). Cuatro peces fueron encontrados sin parásitos, mientras que 24 y 530 presentaron infecciones mono específicas y multi específicas, respectivamente. Cada huésped infestado presentó un promedio de cuatro especies parasitarias. Mientras que la prevalencia y la intensidad media se redujeron con el tamaño del huésped para los protozoarios, el fenómeno inverso fue observado para los helmintos. Se observó un efecto sexual en la infestación de *B. martorelli* por el mixosporidio *M. njinei*, así como por la mayoría de las especies monogénicas; las hembras fueron más infestadas que los machos, debido a la biología de las especímenes grávidas. Igualmente, la alta riqueza en especies parasitarias pareció reducir el peso del huésped.

Palabras clave: *Barbus martorelli* – Myxosporidia – Monogenea – Branquia – Morbosidad – Camerún.