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Effects of the parasite *Argulus ambloplites* (Wilson, 1920) on welfare and mouthbreeding eggs capacity of *Oreochromis niloticus* (Linnaeus, 1758) females

Mamadou KONE[1*], Idrissa SYLLA[1], Claver Kémomadjèhi DJIRIEOULOU[1],
Marian BURDUCEA[2] and Dan-Liviu MIRON[3]

[1] *University, Laboratory of Biodiversity and Tropical Ecology, Po. Box 150 Daloa, Côte d'Ivoire,*
[2] *University of Iasi, Research and Development Station for Aquaculture and Aquatic Ecology, Bd. Carol I, No.11, 700506, Iaşi, Romania.*
[3] *University of Agricultural Sciences and Veterinary Medicine of Iasi, Parasitic Diseases Laboratory, Aleea Mihail Sadoveanu nr.3, Iaşi 700490, Romania.*
[*] *Corresponding author; E-mail: bdou2010@gmail.com, Tel.: +225 0747271497*

ABSTRACT

Declines in the reproduction performance of *Oreochromis niloicus* fish have been observed for two decades in the fish farms located on the edge of the Aby lagoon. Research on the causes of these reproductive drops has led to a series of experiments with pathogens harvested from these spawners in a preliminary study in 2012 that found the parasite *Argulus ambloplites* in abundance. Thus, the aim of this study was to know the effects of the two forms of the parasites *A. ambloplites* on the welfare and mouthbrooding eggs capacity of the fish *O. niloticus*. As a method, fish spawners were exposed to an artificial infestation with naupli parasites as treatment one and adult parasites as treatment two in a total randomised design experiment containing three groups triplicated. Fulton's condition factor K, mouth infestation intensity, number of mouthbrooding eggs, and the relationship between mouth infestation intensity and number of mouthbrooding eggs were biological parameters followed and determined. K values were lower in infested fish. Females of control group presented inside their mouth 601 eggs for incubating per female, against 0 egg inside the mouth of females parasitised fish. A strong nonlinear and negative correlation has been established between the absence of eggs in incubation in females and the intensity of mouth parasite infestation. The welfare and eggs mouthbrooding capacity of *O. niloticus* females were negatively affected by the parasite *A. ambloplites* infestation.
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Keywords: Fish, mouth incubation, eggs, capacity, parasite.

INTRODUCTION

Several authors have shown harmful effects of *Argulus* ectoparasites, commonly known as lice of fish, on farmed fish species. Sea lice are considered one of the most destructive and uncontrollable pathogenic species in fish farming (Walker et al., 2004; Hakalahti-Sirén et al., 2008). Parasites of the genus *Argulus* cause skin irritation, tissue atrophy, external bleeding, loss of appetite, loss

of balance and body mass in infested fish with high mortality rates (Lacroix, 2004 ; Walker et al., 2004). Thus, their pathogenicity on several fish species has been demonstrated by some authors (Gault et al., 2002 ; Hakalahti-Sirén et al., 2004a ; Hakalahti-Sirén et al., 2004b ; Pasternak et al., 2000, Hakalahti-Sirén et al., 2008 ; Kumar et al., 2012a ; Kumar et al., 2012b). Reduced zootechnical performances in fish infested with these parasites have also been demonstrated. Authors Koné et al. (2013) have shown specially damages caused by parasites of the genus *Argulus* on the growth, the average daily gain, the food consumption index, the condition coefficient and the mortality rate of *Oreochromis niloticus* males reared in the fish farming installed along lagoons of Côte d'Ivoire. In experiments conducted by researchers (Koné et al., 2013), it was noted that the artificially infested males had in their mouths parasites *Argulus* sp. attached in large numbers to the mouth mucosa. From this observation, these authors asked themselves what consequences could this have on breeding females of this same species of fish. This question is all the more relevant when it is known that females of *O. niloticus* are known for incubating fertilised eggs and larvae in their mouths during their reproduction period. Larvae are kept into their mouths until the total resorption of the yolk bags (Peña-Mendoza et al., 2005). Thus, no studies have been conducted to date on the possible effects of these parasites on the oral incubation capacity of eggs of *O. niloticus* females. The purpose of this work was to show the consequences of mouth infestation of females breeding *O. niloticus* by the parasite *A. ambloplites*.

MATERIEL AND METHODS
Experimental design used for infestation of fish with parasites

This work was carried out from June 2018 to December 2019 in three (3) different periods (as triplication experiment in the time) in a commercial fish farming (05 23' 830" N and 03 51' 287" W) located in Bingerville, Côte d'Ivoire.

The experimental device put in place was carried out with nine (9) concrete basins of dimensions $3 \times 2.5 \times 1$ m arranged in parallel system and spaced from one another by one (1) meter (Figure 1). Basins were arranged in three (3) triplicate groups. Thus, a control groups containing three (3) basins (Basin C.1, Basin C.2 and Basin C.3 in triplicate tests), a second group of three (3) basins (Basin 1.1, Basin 1.2 and Basin 1.3 in triplicate tests), a last group containing also three (3) basins (Basin 2.1, Basin 2.2 and Basin 2.3 in triplicate tests) were put in place in a total randomization design by generating random numbers (Graphpad, 2018). The basins of all groups were water-fed with the same wellbore. Basins used for this experiment were placed into a 200 m^2 building used as a nursery. Basins of each group were designed as showing by the Figure 1.

Choice of experimental spawners

During these experiments, the fish were treated according to the instructions indicated by ARRIVE directives of animals use for experiments (ARRIVE, 2018).

Females and males of *Oreochromis niloticus* adult specimens with mean body weights of 60 ± 2 g and 100 ± 3 g respectively were taken. Fish with desired body mass and with absence of targeted parasites on the body were selected from fisheries in the rearing ponds of future spawners on the SAPPE fish farming. One thousand (1000) spawners were stored in five (5) large basins according to the sex. For each experiment, a total of seventy-five (75) males and a total of two hundred and twenty-five (225) females were stoked. The stocking density was four (4) fish per square meter (Lacroix, 2004). All fish were dewormed into 500 ppm potassium permanganate baths for one hour. During fifteen (15) days storage period, fish were fed with a feed containing 35% crude protein at 3% of their biomass with a daily distribution frequency of two (2) (morning at 9 am and evening at 3 pm) (Bamba et al., 2008).

Choice of parasites *Argulus ambloplites*

During this experiment, two (2) physiological stages of *Argulus ambloplites* parasites were used. These were adults and larval individuals (immature individuals called naupli). They have been identified through the identification key for gill parasites (Rushton-Mellor, 1994).

Adult parasites were collected from naturally infested fish in the floating cages of commercial fish farming established on the Adjin Lagoon. Parasites harvested on fish using a spatula. They were kept alive on live fish placed in aquaria arranged in the laboratory (Hakalahti-Sirén et al., 2004a). The average length of the adult parasites was 4.5 ± 1 mm.

For immature parasites, naupli were obtained from hatching of eggs laid by the adult parasites. Two hundred (200) mature parasites, took on fish placed in the laboratory conditions, were previously placed in petri dishes (60 mm in diameter) containing distilled water as indicated by some authors (Steckler et al., 2012). The average length of naupli (immature parasites) used in this experiment was 1 ± 0.6 mm.

Random assignation of subjects to experimental groups

Three (3) days before the start of each of three (3) experiments, fish stored in the adaptation tanks were caught by blinding and observed under a hand magnifying glass to research possible targeted parasites. After that, fishes were put into each group until obtaining a number of thirty fish per group using the technic of random assignation of subjects to groups (Graphpad, 2018). Within each experimental group, seven (7) reproductive nucleuses were placed at a sex-ratio of one (1) male for three (3) females. A stocking density of four (4) fish / m^2 / basin was applied, i.e. 30 breeding fish corresponding to seven (7) males for twenty-three (23) females in each basin (Lacroix, 2004). Two hundred and seventy (270) spawners were used at each experiment period. A total of eight hundred and ten (810)

fish spawners were necessary for whole three periods of experiment.

Realization of artificial infestation of fish by the parasite *Argulus ambloplites*

Three (3) days before the start of each experiment, fish were observed under a hand magnifying glass to look for possible targeted parasites.

As treatments, immature parasites of the species *Argulus ambloplites* called naupli were taken for treatment one (1), and adult parasites of the same species were taken for treatment two (2). Each dose of treatment was composed of one hundred and fifty (150) individuals. Thus, treatments one (1) and two (2) were applied separately to fish of treatment groups using the technic of random assignation of subjects to groups (Graphpad, 2018) (Figure 2). The cohabitation method of infestation was used to infest fish of treatment groups (Saurabh et al., 2011).

Fish in the control group had not received a treatment. Tests were triplicated in control and treatment groups. After the application of treatments, no handling was carried out on fish of different groups for thirty (30) days. This allows fish to be acclimatised to experiment devices.

Basins containing experimental fish were water-fed with the drilling water free of parasites. During 6 months, fish from different experimental groups were fed with the same feed. The feed, with 35% crude protein, was distributed to fish at a rate of 3% of their biomass and twice daily distribution frequency (8 am and 4 pm).

Eggs laid by females from control groups were artificially incubated and yielded approximately 190 000 fish weighing 300-500 g after rearing in fish-farming structures.

Parameters evaluated on experimental fish

Using the millimetre and an Aquamerck kit, the temperature, hydrogen potential, dissolved oxygen and nitrate levels of the water-fed in the basin groups were followed and monitored every day.

Parameters evaluated on experimental fish were the female spawners mouth infestation intensity (Iib) by parasites, the number of eggs harvested in the mouth per female spawners (Noe), the Fulton's condition factor K of female spawners, and the correlation between female mouth infestation intensity by parasites and the number of eggs harvested in the mouth per female (Iib / Noe).

Intensity of female spawners' mouth infestation by parasites

The intensity of female spawners' mouth infestation by parasites was determined every fifteen (15) days after thirty (30) days of post-infestation acclimatization.

For that, after fishing in each experimental group, each female was manipulated and the mouth was opened to count parasites inside without harvesting them. The intensity of female spawner mouth infestation (I_{ib}) was calculated by the formula (Bush et al., 1997):

$$Iib = \frac{Tot.\,Nbr.\,Para.}{Nbr.\,Inf.\,Fem.}$$

with:
- Tot. Nbr. Para. = Total number of parasites counted in the mouth of a female spawner ;
- Nbr. Inf. Fem. = Number of mouth infested female spawners.

Number of eggs harvested in the mouth per female spawner

The number of eggs harvested in the mouth per female spawner was determined every fifteen (15) days after thirty (30) days of post-infestation acclimatization. For that, after fishing in each experimental group, each female was manipulated and the mouth was opened to harvest eggs into labelled buckets. It was obtained by standard oocyte counting method as indicated by the following formula:

$$Noe = \frac{Nbr.\,eg.\,sam.\ \times\ Tot.\,Mass.\,eg.\,har.}{Mass.\,eg.\,sam.}$$

with:
- Nbr. eg. sam. = Number of eggs counted in a sample based on the total number of eggs harvested in the mouth of a female ;

- Tot. Mass. eg. har. = Total mass of eggs harvested in the mouth of a female ;
- Mass. eg. sam. = Mass of eggs in the sample from the total of eggs collected in the mouth of a female.

Fulton's condition factor K of female spawners

For the Fulton's condition factor K, it was determined at the end of experiments. Concerning the Fulton's condition factor K of female spawners, each female was weighed and measured in standard length during control fisheries. It was calculated with the following formula:

$$K = \frac{BMass.Fem.}{L^3}$$

with:
- BMass.Fem. = Biomass of a female ;
- L = Total length of a female.

Correlation between female mouth infestation intensity by parasites and the number of eggs harvested in the mouth per female

The indicative correlation model given by the number of eggs harvested in the mouth per female spawner based on the intensity of mouth infestation of females or condition factor of breeding females was established with data from three (3) experiments carried out at three (3) different periods. The model was determined through the simple regression plot.

Statistical analyses of experimental data

The Kruskal-Wallis test, after verification of the non-applicability of the one-factor ANOVA, was used to determine whether there were significant differences in ranks between the three experimental groups for each of the variables (parameters) studied. Once this difference was established ($p < 0.05$), the multiple comparison test was subsequently performed to show groups between which they had differences.

A correlation model was sought to determine the type and degree of the relationship between the different variables. This was done after the establishment of a correlation matrix in which the different

correlation values existing between the variables studied were determined. The model was designed with variables with a correlation value of 0.8 or higher. The regression equation used was the one given by the scatter plot that best reflected the look of the given graph.

All of these statistical tests and graphs were performed using the R studio statistic software version 3.6.0 which was available for free access.
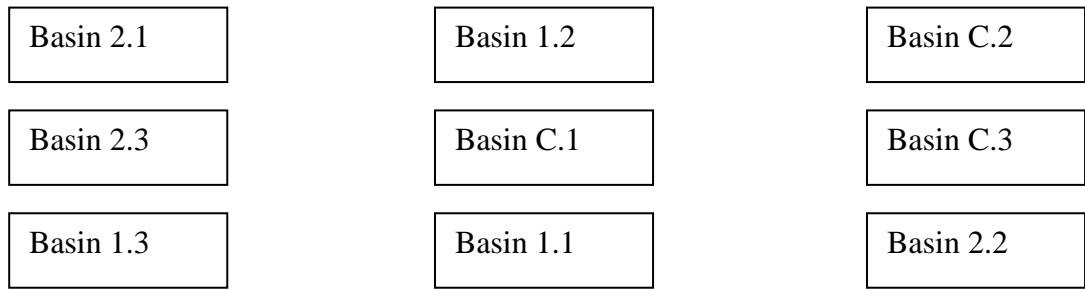
Basin 2.1		Basin 1.2		Basin C.2
Basin 2.3		Basin C.1		Basin C.3
Basin 1.3		Basin 1.1		Basin 2.2

Figure 1: Schematic random experimental design adopted with basins.

Treatment 2 → Basin 2.1: (30 fish)
Treatment 1 → Basin 1.2: (30 fish)
No treatment → Basin C.2: (30 fish)

Treatment 2 → Basin 2.3: (30 fish)
No treatment → Basin C.1: (30 fish)
No treatment → Basin C.3: (30 fish)

Treatment 1 → Basin 1.3: (30 fish)
Treatment 1 → Basin 1.1: (30 fish)
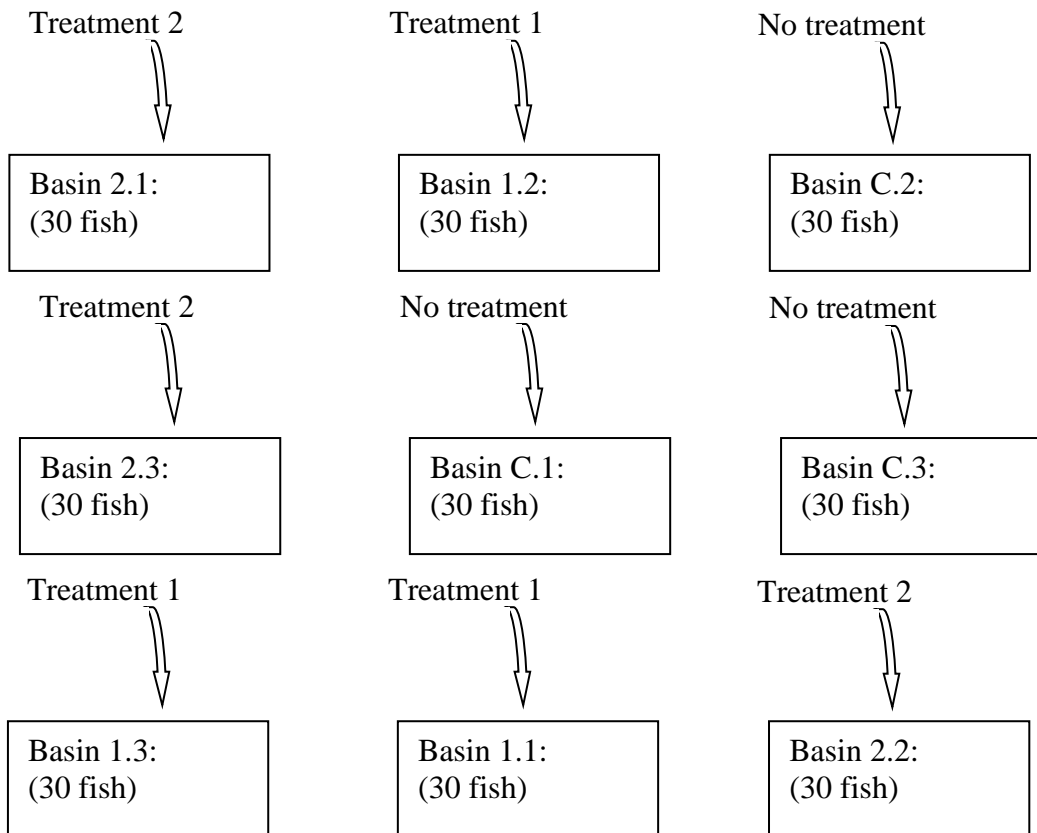Treatment 2 → Basin 2.2: (30 fish)

Figure 2: Schematic random of artificial experimental infestation of fish spawners.

RESULTS

Effects of the parasite *Argulus ambloplites* on *Oreochromis niloticus* female spawners

Fulton's condition factor K

On Figure 3, it appears that values of Fulton's condition factors of *Oreochromis niloticus* female spawners are significantly different ($p < 0.05$) between experimental groups of female spawners according to the Kruskal-Wallis test. Median values are 2.67; 2.38 and 2.53 for the breeding females of the control group (GC), the parasitised group with naupli (Giwip) and the parasitised group with mature parasites (Giwmp). Naupli-infested breeding females have the lowest condition factor values.

Number of mouth eggs havested per female spawners

For the values for the number of mouth eggs harvested per breeding female, it is clearly visible, in Figure 4, that fishe spawners parasitised with naupli (Giwip) and with adult parasites (Giwmp) have not eggs in their mouths (NEFM = 0). However, the breeding females of the control group (CG) show eggs inside their mouth with a median value of 601 eggs per female spawner, in comparison with 0 eggs in the parasitised group with naupli (Giwip) and with the parasitised group with mature parasites (Giwmp). The Kruskal-Wallis test shows a significant difference ($p < 0.05$) between the rank values of the number of buccal eggs found in the breeding females of different experimental groups.

Intensity of the mouth parasites infestation

The Figure 5 presents values of intensities of the female spawners mouth infestation by parasites. This parameter is zero (0) in the female spawners from control group.

They are eight (8) parasites and six (6) parasites as median values, respectively in the group of female spawners parasitised with naupli (Giwip) and in the group of those parasitised with mature parasites (Giwmp). Median rank values of buccal parasitic infestation intensities were not significantly different ($p > 0.05$) in both treated groups according to the Kruskal-Wallis test.

Correlation model between different variables evaluated

The table below shows the values of the correlation coefficients existing between our dependent variables studied.

The analysis of this table shows that there is a correlation ($r \neq 0$) between all variables studied which are the condition factor of females, the number of buccal eggs harvested per female, and the intensity of buccal infestation of breeding females by parasites. The highest correlation coefficient value ($r \geq 0, 8$) observed is negative ($r < 0$). It is established between the number of mouth eggs harvested per female (NEFM) and the intensity of buccal infestation of breeding female by parasites (NPFM). Correlation values are mentioned on following Table 1.

The model shown in Figure 6 shows a correlation between the number of harvested mouth eggs per female spawner and the intensity of mouth infestation of breeding females by parasites. The layout of the model $Y = 0,11 X^4 - 4,03 X^3 + 54,23 X^2 - 306,59X + 613,48$ illustrates this correlation very well ($R^2 = 0.98$).

Thus, we see that as the number of mouth parasites increases, the number of eggs and larvae in the mouth cavity of female incubators decreases.
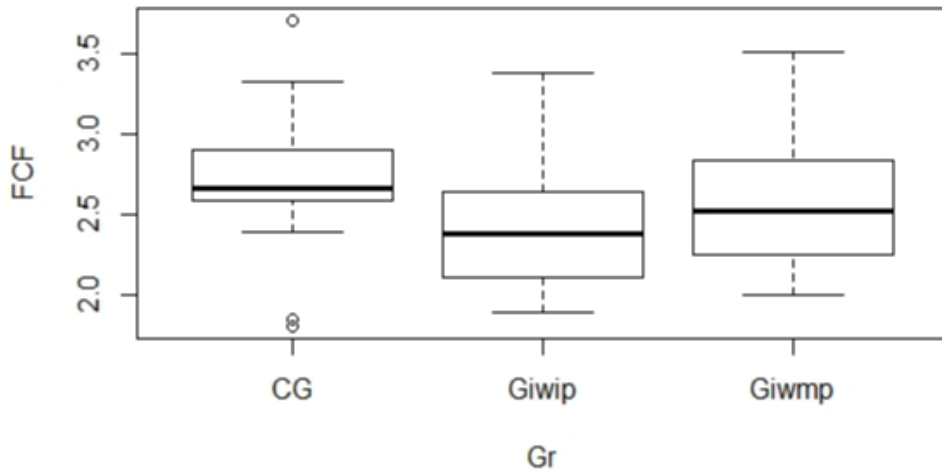
Figure 3: Comparison of Fulton's condition factor of female spawners according to experimental groups.

Legend:
- Gr = Experimental groups ;
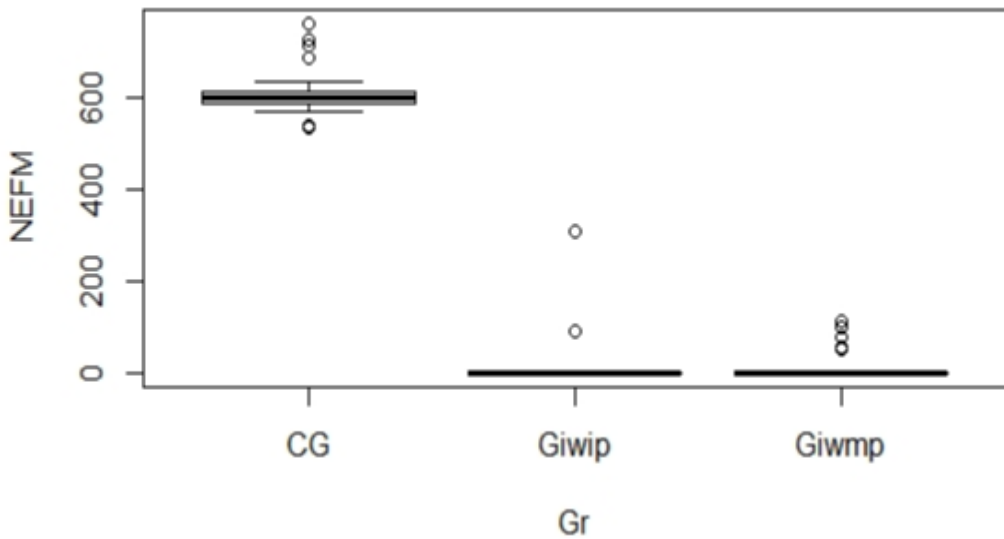- CFC = Condition factor (K) of breeding females

Figure 4: Comparison of the number of mouth eggs harvested per female spawners according to experimental groups.

Legend:
- Gr = Experimental groups ;
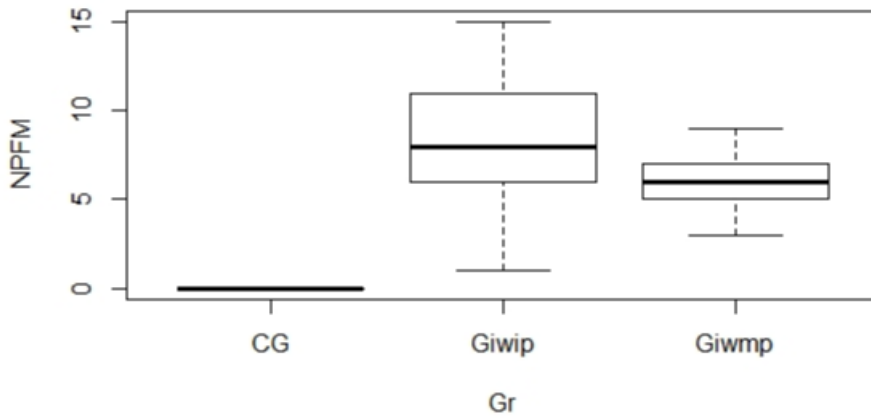- NEFM = Number of mouth eggs harvested per female spawner

Figure 5: Comparison of intensities of the mouth parasites infestation according to experimental groups.

Legend:
- Gr = Experimental groups ;
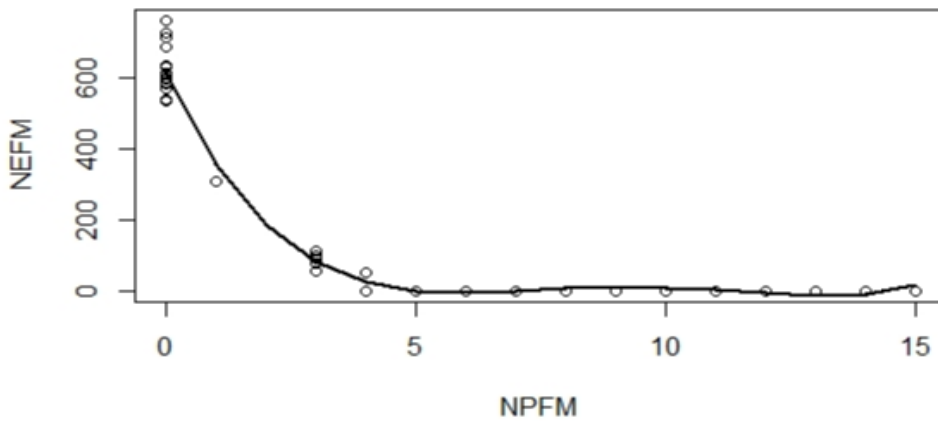- NPFM = Intensity of mouth infestation of a female spawner by parasites

Figure 6: Polynomial regression derived from the number of harvested mouth eggs per breeding female as a function of the intensity of mouth infestation of breeding females by parasites.
Legend:
- NEFM = Number of mouth eggs harvested per female spawner ;
- NPFM = NPFM = Intensity of mouth infestation of a female spawner by parasites

Table 1: Correlation matrix between three variables studied.

	FCF	NEFM	NPFM
FCF	1	0.25	-0.32
NEFM	0.25	1	-0.86
NPFM	-0.32	-0.86	1

- CFC = Fulton condition factor (K) of breeding females
- NEFM = Number of mouth eggs per female spawner
- NPFM = Intensity of mouth infestation of a female spawner by parasites

DISCUSSION

Fulton's condition factor K values recorded through our experiments (2.67; 2.38 and 2.53), respectively in control group, in group infested with naupli and those infested with mature parasites indicated that the parasite *Argulus ambloplites*, regardless of their physiological condition, negatively affected Fulton's condition factor K observed in breeding females of *Oreochrmis niloticus*. Thus, the naupli forms caused a lower condition factor value in *O. niloticus* females than the mature forms. Fulton's condition factor K is an important indicator of fitness, performance, survival and reproductive effectiveness in fish (Kondoh & Okuda, 2002). This factor informs us about the physiological development level of a fish because it focuses on the distribution of metabolic energy towards fish growth and reproduction functions (Walker et al., 2004). When K value is one (1), the metabolic energy used for gonad reproduction and maturation functions is negligible. The value of the condition factor can be negatively influenced by chemical, physical and biological pollution of fish through their environment.

It should be noted that values of physicochemical parameters of water recorded in the different experimental groups of our study were kept constant. As a result, we can say that *Argulus ambloplites* parasites, especially the immature form (naupli), cause a poor distribution of metabolic energy in *Oreochromis niloticus* females, resulting in a low value of Fulton's condition factor K. We can therefore say that the presence of parasites *A. ambloplites* on *O. niloticus* females, at a certain infestation intensity, would cause a deviation of the metabolic energy intended for the reproductive and survival functions of the fish to be diverted to its functions of the immune system in order to combat the parasites. The low Fulton's condition factor K values observed in females infested with different forms of the parasite *A. ambloplites* may be explained by the reduction of the metabolic energy for these two functions. This observation is consistent with that made by

some authors (Bryan & Luca, 2004) on males of the fish *Lepomis macrochirus* infested naturally by parasites of different species. These authors observed a decrease in Fulton's condition factor K in parasite-infested males as the intensity of parasite infestation increased. However, contrary observations (Sumuduni et al., 2014) found a negative correlation between Fulton's condition factor of the golden carp species *Carassius auratus* and the common carp *Cyprinus carpio* and the external parasite density of these fish. Among ectoparasites identified by these authors, parasite densities of the genera *Trichodina* and *Apiosoma* were indicated to result in low Fulton's condition factor values in the two hosts mentioned above. Other studies (Lizama, 2006) have shown that ectoparasites *Rhinonastes pseudocapsaloideum* (Monogenea), *Saccoelioides magnorchis* (Digenea) and *Gamispatulus* sp. (Copepoda) had not effect on the Fulton's condition factor of the fish *Prochilodus lineatus*.

The difference between our results and those of authors cited could be explained by the species of host and the state or nature of the aquatic environment. In fact, the physiology of most fish species is complex and poorly known, the future (orientation and amount) of all metabolisable energy, depending on the major functions of the organism, it cannot be determined precisely from one fish species to another. For example, a species of fish that has the capacity to store excess energy for reproductive purposes will not suffer the same adverse effects of a parasite that attacks reproductive organs as a species with less storage capacity. As regards the species of parasite, it is known that all species of parasites have not the same virulence or pathogenicity on their hosts. And even considering a species of parasite, this virulence is depending of the physiological state of the host beings and the environment in which they live.

This pathogenicity, in the specific case of our study, is evidenced by a high intensity of buccal infestation and a low number of buccal eggs in *Oreochromis niloticus* females artificially infested with both forms of the

parasite *Argulus ambloplites*. Our results also indicate that from the two forms of parasite, the naupli form infesting the mouth of fish, results in a low number of eggs collected from breeding females. Our experience shows that the naupli of the parasite *A. ambloplites*, located in the mouth cavity of breeding females of *O. niloticus*, prevents them to incubate their eggs into the mouth. This fact explains the low number of eggs collected from these females. Also, it should be noted that the nauplius form of the parasite that infests the mouth cavity of females causes more eggs loss in females than adult forms. Previous studies have shown the very high virulence of naupli on several species of fish relative to the adult form. This is due to the fact that the naupli, during their larval life, feed exclusively on epithelial cells of the host. This is not the case for adults who are hematophagous (Tam & Avenant-Oldewage, 2006). These two preferred types of nutrient cells would cause the two forms of the parasite to feed differently. The nauplius form, which feeds on epithelial cells, would cause more spatial lesions on the skin or mucosa of the host, while the adult form, which is more hematophagous, would seek to perforate the skin or mucosa of the host at a point in order to access the blood vessels and finally to take the hematices. This would result in less extensive point injuries. Thus, the nauplius form would cause skin lesions greater than the adult form; hence the absence or low number of mouth eggs collected from naupli-infested females. Thus, the absence or low number of buccal eggs observed in incubating females infested with the parasite *A. ambloplites* was noted, unlike those of incubating females not infested with the parasite (control group). In fact, this could be explained by mouth lesions caused by the parasite A. ambloplites to host females. These lesions would lead to painful inflammations and mouth irritations that would force affected females to propel eggs or fish larvae from their mouths. Authors (Heins & Baker, 2003), in their study of nine lakes in the United States of America (USA), showed that eggs of nine (9) populations of female fish *Gasterosteus aculeatus* infested naturally by

the parasite *Schistocephalus solidus* had reduced masses, compared to those of eggs of female fish of the same host species of uninfested fish from the same lakes. According to the same authors, the difference in mass between infested and uninfested fish females was 8 to 32% based on the intensity of parasite infestation. This study showed a significant negative non-linear correlation between the number of mouth eggs and the intensity of parasitic mouth infestation. They (Heins & Baker, 2003) found a significant negative linear correlation between egg mass of infested females and parasite infestation intensity.

Conclusion

At the end of our study, it was found that the reproductive females of *Oreochromis niloticus* artificially infested with the parasite *Argulus ambloplites* in two of its physiological forms (nauplius and adult) showed the lowest Fulton's condition factor K values. In other words, the parasite *A. ambloplites* causes to females of *O. niloticus* a bad physiological condition.

Thus, the mouth infestation of *O. niloticus* females by this parasite results in the loss of eggs that they carry in incubation. This loss of incubating eggs is strongly negatively related to the intensity of mouth parasitic infestation. The more parasites in the mouth cavity induce the fewer eggs to incubate in the mouth of female breeders. The resulting relationship is represented by the following non-linear model equation: $Y = 0{,}11\,X^4 - 4{,}03\,X^3 + 54{,}23\,X^2 - 306{,}59X + 613{,}48$.

AUTHORS' CONTRIBUTIONS

MK designed the experiment. IS and CKD carried out samples. Data analyses were done by MB and D-LM prepared all the figures. All authors contributed to data interpretation. MK wrote the draft of the

manuscript. All authors reviewed the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.

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