Parasitism of *Clarias camerunensis* Lönnberg, 1895 (Siluriformes, Clariidae) by monogeneans in a dense tropical humid forest (Southern Cameroon-Africa) gives more arguments for fish quarantine in breeding using native species

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ABSTRACT: *Clarias camerunensis* is a potential catfish for farming in Cameroon. In order to assess the parasitism of its monogeneans as a function of season and standard lengh, a study was conducted from April 2017 to April 2018 in Lép Mööga stream, of the Nyong river watershed (Southern Cameroon). 179 specimens of *C. camerunsis* were sampled by angling through 5 consecutive seasons. The Prevalence, density, abundance of adults and larvae of the main Monogenean species as well as the condition coefficient K and gonadosomatic index of the female *C. camerunensis* were calculated. The prevalence of the adult *Quadriacanthus* sp. remained equal to or greater than 85% during this study. Its abundance was low and did not show any clear profile pattern; however, its variation peaked during the rainy season. The gonadosomatic index (GSI) and condition factor (K) of female *C. camerunensis* evolved in parallel and showed that this fish lays three times a year, during the short rainy, the short dry and the long rainy seasons. The profile of *Quadriacanthus* sp. larvae showed that this monogenean breeds all year with two peaks during the short rainy season (when the female hosts lay and are weak fry also are in the environment), and in January when hosts are more concentrated in low water. The physiological condition of the fish gradually improved as they grew, when the parasitic density decreased. To limit monogenean outbreaks in farming, this work recommands that fish caught in the wild should be placed in quarantine and dewormed before being exploited.

KEYWORDS: parasitic worms, condition factor, Clarias, gonadosomatic index.

1 INTRODUCTION

The contribution of aquaculture and fisheries to the world economy in 2016 was 362 billion USD, of which 232 billion were obtained from aquaculture [23]. It is worth to note that from 2011 to 2016, the production of fishery resources fell from 92.2 million tons to 80 million tons [23]. As concerns African fish-farming in particular, a clear increase from 1.286 million tons in 2010 to 1.932 million tons in 2016 was observed [23]. In Cameroon, the consumer demand of fish is estimated at 400,000 tons [28]. In 2016 the total imports reached 151,858.5 tons of fish [28], while the national fish production was estimated at 180,000 tons, of which only 5,000 tons were obtained by fish farming [40]. This production by aquaculture clearly increased compared to that of 2012 estimated at 1000 tons [38]. Fish farming is therefore the main solution to increase fish production on the one hand; it also ensures employment for many africans on the other hand as it employed 304, 000 people in 2016 [23]. The breeding of better adapted native fish species is often recommended [2]; [61]. In Cameroon, several catfish species, among which *Clarias camerunensis* Lönnberg, 1895 whose length can exceed 46 cm are potential candidates for domestication and fish farming this is because of their good resistance to environmental conditions such as low oxygen concentration responsible

of death in fish ponds, temperature variations [54], and their rapid growth [53]; [22]. As a prelude to the domestication and breeding of catfish several studies have been undertaken in Africa [19]; [56]; [2]; [60]. In the wild and even more in farming situations, catfish shelter many groups of parasites which can disturb their growth, their reproduction and consequently their yield [7], [30]. Many studies on parasites of siluriformes have been published in Africa [46]; [45]; [41]; [43]; [14]; [32]; [8]; [9]; [5]; [6]; [36]; [37]; [44]. In Cameroon, a few parasotological surveys carried out on catfish as well as on other freshwater fish have taken simultaneously into account the biology of the host and that of its parasites.

The aims of this work are therefore to: (1) follow the variations in the reproductive cycle of *Clarias camerunensis* and that of its xenocommunity of monogenean gill parasites, and (2) establish the relationships between these helminth loads, the condition factor and size of the fish.

2 MATERIAL AND METHODS

The fish were caught at night by angling from the Lép Môôga stream in the dense tropical humid forest of Ndjock Lipan (3 ° 41 '54"N;11 ° 5' 14 " E) in the South centre plateau. Lép Môôga is a tributary of the Kéllé river, the longest tributary of the Nyong, the second longest river in Cameroon [47]. The climate in this ecosystem is a bimodal rainfall with 4 seasons: an irregular short dry season (SDS) from July to August, a long rainy season (LRS) from September to November, a long dry season (LDS) from December to mid-March and a short rainy season (SRS) from mid-March to June [52].

2.1 CONDUCT OF THE STUDY

Nine sampling campaigns were organized from April 2017 to April 2018: two per season, during the first four consecutive seasons, and one at the start of the second seasonal cycle. As soon as they were captured, fish were immediately immersed in a 10% formalin solution to fix and avoid the release of monogeneans due to an abundant post-mortem mucus secretion [11]. After the fish death, a small (1cm) abdominal incision was made to allow good conservation of its gonads [11]. Fish specimens were then transported to the laboratory.

2.2 MEASURED PARAMETERS

Before dissection, the standard length (SL: distance from the anterior end of the snout to the posterior end of the last vertebra of the fish [51]) was measured using a ruler graduated to the nearest millimeter. The mass of the carcass (Mc) of the fish i.e. stripped of its digestive tract and of the gonads (Mg) only for female individuals were also measured using an electronic Sartorius balance to the nearest hundredth gram.

2.3 RESEARCH, ASSEMBLY AND IDENTIFICATION OF MONOGENEAN GILL PARASITES

Gill arches were removed and placed in Petri dishes containing tap water. Monogeneans attached to the gill filaments were dislodged under a binocular WILD HEERBRUGG stereomicroscope, using a needle, then mounted between a slide and coverslip in a drop of eosin hematoxylin [11]. Parasite species identification was made based on sclerotized parts of the haptor and the copulatory organs after [15], [14] using the Leica DM 2500 microscope. The same preparations were used to count the different monogenean adults species and larvae.

2.4 CALCULATED PARAMETERS

The Prevalence (Pr) and abundance (A) are herein defined after [18], infracommunity and xenocommunity after [20]. The status of each parasite species was determined according to [59]: the species was stated frequent or common or even main or core (Pr> 50%), infrequent or secondary or intermediate ($10\% \le Pr \le 50\%$), and rare or satellite (Pr <10%). The subsequent analysis concerned only the core species since in a community, satellite or rare species are little or no structuring [26]; [20]. The parasitism was categorized based on average helminthic load and according [12]. Therefore, the mean load (abundance) was described as very low (A<10); low ($10 \le A \le 50$); average ($50 < A \le 100$) and high (A > 100). The condition factor (K) was computed according to [58]; its formula is: K = 10Mc / SL; normally K increases regularly as the fish grows. The gonadosomatic index (GSI) indicates the relationship between the female gonads mass (Mg) and the body weight of a fish [58]; its formula is: GSI = Mg / Mc - Mg). In a fish population, the decrease of both K and GSI indicates the laying time [33]; [4]; [27]; [24]. The parasite density (D) is the ratio between the total number of monogeneans (Np) of the infracommunity and the host body weight : D= Np / Mc [18].

2.5 STATISTICAL ANALYSIS

Comparison of prevalences was done using the Chi-square test (χ^2). The one-way analysis of variance (ANOVA) permitted to compare larval mean abundances, K and GSI during the sampling campaigns. For the follow up of the parasite density and the condition factor as a function of the fish standard length, a linear regression model was used. The software SPSS was use during all the analysis. Differences were considered as significant at P < 0.05.

3 RESULTS AND DISCUSSION

3.1 RESULTS

A total of 179 fish were sampled from April 2017 to April 2018, from Ndjock Lipan dense tropical forest: 33 fish in SRS, 42 in SDS, 24 in LRS 58 in LDS and 22 in SRS of the second cycle.

3.1.1 COMPOSITION OF THE MONOGENEAN XENOCOMMUNITY OF CLARIAS CAMERUNENSIS

A total of 4482 monogenean specimens were recorded from *Clarias cameunensis*. These worms belonged to four species: *Quadriacanthussp.* (n_1 = 3938: 87.86%), *Quadriacanthus teugelsi* Birgi, 1988 (n_2 = 36: 0.80%), *Birgiellus kellensis* Bilong Bilong, Nack&Euzet, 2007 (n_3 = 402: 8.97%) and *Gyrodactylus camerunensis* Nack, Bilong Bilong & Euzet, 2005 (n_4 = 106: 2.37%). *Quadriacanthus* sp. was thus numerically predominant.

3.1.2 TEMPORAL VARIATION OF THE PREVALENCE AND ABUNDANCE OF QUADRIACANTHUS SP. (ADULTE STAGE)

The prevalence of the different monogenean species were 99.2%, 84.6% 64% and 42% for *Quadriacanthus* sp., *Birgiellus kellensis,Gyrodactylus camerunensis* Quadriacanthusteugelsirespectively. In this enumeration order, their mean abundances were 22.8, 3.04, 4.29 and 0.20. Therefore, in the Ndjock Lipan ecosystem *Quadriacanthus* sp. was the main (core) species of the *C. camerunensis* monogenean xenocommunity; its prevalence remained very high > 80% (Fig. 1) and was relatively lower in April2017, although these variations were not significant ($\chi^2 = 13.25$; df = 8; P > 0.05). The abundance of this ectohelminth was low over the study period (Fig. 2); it did not show any clear variation pattern despite a slight tendency to increase during the rainy seasons (May 2017 in SRS; October 2017 in LRS and April 2018 in SRS). However these fluctuations were not statistically significant (F = 1.74; df = 8; P>0.05).



Fig. 1. Monthly prevalence of Quadriacanthus sp. during the sampling period



Fig. 2. Adult monthly mean abundance of Quadriacanthus sp. during the sampling period

3.1.3 TEMPORAL VARIATION OF THE ABUNDANCE OF QUADRIACANTHUS SP. (LARVAL STAGE)

Quadriacanthus sp. bred all year round in the dense tropical forest of Ndjock Lipan (Fig. 3). The larval abundance profile was a bimodal curve. The first peak appeared in May 2017 (SRS) and the second, more important, in January 2018 (LDS). The variations in larval mean abundance between the different sampling campaigns were significant (F = 1.84; df = 8; P = 0.007).



Fig. 3. Monthly mean larval abundance of Quadriacanthus sp. during the sampling period

3.1.4 TEMPORAL VARIATION OF THE FEMALE CLARIAS CAMERUNENSIS GONADOSOMATIC INDEX (GSI) AND CONDITION FACTOR (K)

The gonadosomatic index varied between the different sampling campaigns (F = 1.92; df = 8; P = 0.003). It showed two increase periods (June to August 2017 in SDS, November 2017 to March 2018 in LDS) and three decrease periods (April to June 2017 in SRS, August to November 2017: late SDS to early LRS; March to April 2018: early SRS) see figure 4.The condition factor (K) of female *C. camerunensis* varied significantly between the different sampling campaigns (F = 2.00; df = 8; P = 0.001, Fig. 4). It decreased from April to June 2017(SRS).It then increased somewhat in August to October (SDS to the first half of the LRS). In the second half of the LRS, it dropped. Another increasing phase was observed in the LDS followed by a fall from the second seasonal cycle (LDS to SRD 2018).



Fig. 4. Monthly gonadosomatic index GSI and condition factor K during the sampling period

3.1.5 RELATIONSHIP BETWEEN THE PARASITE DENSITY, THE CONDITION FACTOR AND THE HOST STANDARD LENGTH (REGARDLESS OF SEX)

The monogenean density decreased while the host standard length increased according to a linear model (Fig. 5:D = -51×10^4 LS+1.38; r=-0.51; P = 0.0001). As concerns the condition factor, values higher than 4.3 corresponded to those of males. K increased positively with the *C. camerunensis* standard length according to a linear model (see Fig. 6: K = 35×10^3 LS-2.71; r=0.94; P = 0.0001).



Fig. 5. Gonadosomatic index and condition factor as a function of standard lenght of C. camerunensis (regardless of sex)

In the Ndjock Lipan forest ecosystem, the monogenean density decreased when the condition factor of *Clarias* camerunensis increased according to a linear model (D = -0.116K + 0.881). The physiological condition of the host appeared negatively correlated (r = -0.47; P = 0.004) to the helminth load (density).

3.2 DISCUSSION

During the current study, no significant temporal variation in the abundance or prevalence of adults *Quadriacanthus* sp., the core species of this monogenean xenocommunity, was observed, although the abundance profile seemed to show 3 peaks during the rainy seasons. Moreover, the variation in abundance of *Quadriacanthus* sp. larvae indicated that this species bred throughout the year but mainly in two periods: May (SRS) and January (LDS). This result is close to that of [10] on *Gyrodactylus*

linstowi in Tajikistan (Central Asia) in a stream with constant temperature; these authors also didn't note any clear pattern in the parasitism as a function of season. Although with insignificant differences, our observation is similar to those by [46], [12], [13], [16], [3] and [39] respectively, for monogenean gill parasites of Tilapia sp. in Ghana Chrysichthys nigrodigitatus in Nigeria, Hemichromis fasciatus (now correctly renamed H. elongatus [51] and Barbus martorelli in Cameroon, Sarotherodon melanotheron and Oreochromis niloticus in Côte d'Ivoire, Cyprinus carpio in Algeria; these different monogenean communities showed outbreaks during the rainy seasons and spring. These results differfrom those of [13] who did not find any model in the occurence of monogenean larvae of Barbus martorelli in Cameroon. The constant prevalence and the low parasite abundance of Quadriacanthus sp. in the present study can be justified by the constancy of ambient and / or water temperature [10]; [46]. The decline of the gonadosomatic index (GSI) and condition factor (K) allows to identify the fish laying time [27]; [1]; [24]. Those of female Clarias camerunensis appeared seasonal, and cyclical and indicated that this catfish breeds three times a year during the short and long rainy seasons. Our results somewhat do not agree with those of [31] who pointed out that, in general the species of Clarias have only one laying period limited to a few months. However, they also claimed that, considering the bimodal distribution of their oocytes, these fish can breed all year round when conditions allow. In Nigeria [29] observed that Parachanna obscura bred most of the year (except in February, March and April) with two peaks in May and July. Quadriacanthus sp. larvae were more abundant in May 2017 and January 2018; it is thus suggested that this ectohelminth could have selected these periods because host fish are available. In the short rainy season, these larvae hatch when female hosts lay and their physiological condition and therefore their immune defenses are reduced; these weakened females, as well as the fry, are easy targets for the parasites. This is the reason why [55] argued that the ephemeral nature of the host as a habitat has forced parasites to find ways to colonize new individuals. They also indicated that the hatching of monogenean eggs is closely linked to environmental cycles and direct host stimuli. The relative outbreak of larvae in January in the Ndjock Lipan ecosystem can be explained by the availability of host individuals. In LDS the probable physicochemical modifications of the environment due the shallow water causes an aggregation of the hosts which become easily accessible to infesting stages of parasites [49].

In this study, the condition factor (K) of *C. camerunensis* increased regularly as it grew, while the parasite density (D) decreased. Therefore, as the fish grows it acquires immunity limiting the parasite load. [34] and [35] also found a negative correlation between the total number of two gill monogenean parasites of *Piaractus mesopotamicus* and two others of *Arapaima gigas* respectively and the host condition factor K. Similarly, [50] observed in *Thymallus thymallus*, parasitized by *Gyrodactylus salaris* that the host gradually acquires an immune response limiting infestations. In contrast, [57] found no difference between the condition factors of the parasitized *Corydoras aeneus* individuals and those not parasitized. As concerns [25], they obtained a positive correlation between the abundance of four monogenean species and the condition factor of *Hoplias aff. malaboricus* in Brazil.

The parasite density decreased as a function of the standard length of *Clarias camerunensis*. [35] also observed a decrease in the number of monogeneans as a function of the body weight of *Arapaima gigas* but they didn't explain why. In the current work, the drop in the parasite density as a function of the standard length of *Clarias camerunensis* can be more explained by the fact that *Clarias* species have a rapid growth [17]; [53], since it was shown that the monogenean mean abundance was not host length dependant. Indeed, [48] and [21] respectively showed that in *Cyprinus carpio* and *Salmo gardneri*, the rapid multiplication of parasites is counteracted by the rapid growth of these fish limiting the damage.

4 CONCLUSION

This work, carried out in a dense tropical humid forest less anthropized, suggests that monogenean gill parasites of *Clarias camerunensis,* mainly *Quadriacanthus* sp. the core species of this xenocommunity, adopt strategies for a sustainable colonization of their host. This observation is illustrated by: a wide dispersal within the host population (high prevalence) and low abundance which do not reach a dangerous threshold throughout the year.Monogeneans reproduce when young fishes with weak immunity arrive in the environment, when the physiological condition of adult fish decreases and when all the fish population is more accessible because of increased concentration in Shallow water during in the dry season. To limit parasite outbreaks in farming, we suggest that the fish caught in the wild be placed in quarantine and undergo deworming before being exploited.

CONFLICT OF INTEREST

The authors declare no conflicts of interest.

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