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(RESEARCH ARTICLE)

Epidemiological study of *Enterogyrus melenensis*, stomach parasitic helminth of *Hemichromis fasciatus* in the Mefou hydrographic system (South-Cameroon, Africa); effect of the environment

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Abstract

Hemichromis fasciatus is a voracious predator and a prolific breeder used to control Tilapia reproduction. Parasitic diseases can affect growth, reproduction and many other parameters of the dynamics of host populations. The present study aimed to compare the infection dynamics of *E. melenesis* between three ecosystems. Fish were sampled from December 2017 to September 2019 using a gill net, they were euthanized and the parasitological examination was carried out within 24 hours after capture. The abdominal cavity was opened; the sex were identified, then the digestive tract was removed and; the stomach isolated. Monogeneans were dislodged from the stomach wall and mounted in a drop of ammonium picrate–glycerine mixture. Their identification was based on the morphology of sclerotized parts of the haptor and male copulatory organ. Of 302 host specimens examined, 152 were infected by 2590 *Enterogyrus melenensis*. The overall mean intensity was low and did not vary significantly between the three ecosystems studied, while the prevalence globally differed significantly. The mean intensity and prevalence of *E. melenensis* varied with seasons but were not cyclic. The inner location of *E. melenensis* attenuates the direct influence of the water temperature on this endohelminth resulting in its non-cyclical profile. This study highlights the necessity to consider the environment when comparing host parasitism; it also reinforces the practice recommending quarantining native large fish specimens captured in the nature and used as sires in ponds, in order to avoid epizootic outbreaks.

Keywords: Monogenean; Infection; Sex; Length; Season

1. Introduction

Cichlids fishes commonly named Tilapia are important aquaculture organisms in the world they have been introduced to at least 140 countries and have turned into worldwide invasive fishes [1]. *Hemichromis fasciatus* (Peters, 1858) has been used or studied for use in Africanaquaculture; amongst others it is a voracious predator and a prolific breeder used to control Tilapia reproduction [2, 3]. Parasitic diseases can affect growth, reproduction and many other parameters of the dynamics of natural host populations [4, 5, 6, 7]. These hazards can be devastating for both farming systems and natural populations due to stressful conditions linked to food and frequent deterioration in water quality [8, 9, 10].

In Cameroon, *H. fasciatus* is currently parasitized by the Monogenean species *Enterogyrus melenensis* located in the stomach of host individuals [11]. Monogeneans are mainly ectohelminths but some species are endoparasites s.l. and have unusual locations [12,13,14], for example: the oesophagus for *Diplectanotrema* Johnston and Tiegs, 1922, urinary bladder and ureters for and Allison, 1940 and *Urogyrus cichlidarum* Bilong Bilong, Birgi and Euzet, 1994, nasal cavity for *Dactylogyrus nasalis* Strelkov and KhaKi, 1964, ovipositor for *Dactylogyrus* sp. Yukhimenko and Danilov, 1987 and stomach for *Enterogyrus* (Paperna, 1963).The specific morphology of the haptor of *Enterogyrus* spp. and their mode of

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attachment provoke lesions on the gastric epithelium of their hosts and may cause pathological conditions [15, 16]. Due to the relative abundance of *H. fasciatus* in previous fish catches and the fact that it is also highly consumed in our town personal observations, the present study aimed to compare the infection dynamics of *E. melenensis* between three ecosystems.

2. Material and methods

2.1. Study sites

This study was conducted around Yaounde, on the southern plateau of Cameroon characterized by a bimodal humid tropical rainfall regime with four seasons: a long dry season (LDS) from mid-November to mid-march, a short rainy season (SRS) from mid-march to June, a short dry season (SDS) from July to august, and a long rainy season (LRS) from September to mid-November [17]. The annual rainfall is 1650 mm with peaks in May (196 mm) and October (293 mm), minimum values in January (30 mm) and July (37 mm); the atmospheric mean temperature is 23.5 °C and the hygrometry 80% [18]. Three different ecosystems where considered in the Mefou hydrographic system : the Mefou dam in the Ozum village (11°27'N;3°40'E) in the upstream course, Obili (3°51'N;11°29'E) a private semi- intensive pond in the middle course, and two tributaries in the forest downstream course [(Ezazock (11°32'N;3°40'E) and Ekali (11°32'N;3°38'E)].These localities were visited for fishing at least twice a month; no sampling was done in the SDS 2018 due to infrastructural and logistic shortages.

2.2. Host sampling and parasitological examination

Fish were sampled from December 2017 to September 2019 using a gill net and transported to the laboratory in isothermal tanks. In the laboratory, they were euthanized; a spike was introduced into the brain of the fish which was then disrupted by the rotary movement of the spike [19]. The parasitological examination was carried out within 24 hours after fish capture. The Standard length [(SL), horizontal distance from front tip of snout to base (articulation) of caudal fin [20] was measured to the nearest millimeter (mm) using a Carbon fiber Calliper. The abdominal cavity was opened using a medio-sagittal section. The fish sexes were determined (as male, female or undetermined), then the digestive tract was removed; the stomach was isolated and placed in a Petri dish containing a wet filter paper. The remaining parts of the digestive tract were fixed in a 10% formalin solution for subsequent studies. Monogeneans were searched under a stereomicroscope then dislodged from the stomach wall using a dissecting needle. Parasites were then mounted on a microscope slide in a drop of ammonium picrate–glycerine mixture according to [21]; the preparation was covered with a cover slip and sealed with Glyceel according to [22]. Worms were identified based on the morphology of sclerotized parts of the haptor and male copulatory organ according to [11], using a Leica DM2500 microscope equipped with a Leica DFC425 camera.

2.3. Data analysis

Data measurements were log transformed to satisfy homoscedasticity or linearity necessary to perform parametric tests [23]. Abundance, intensity, mean intensity, prevalence, xenopopulation and infrapopulation were defined according to [24]. Multiple comparisons of mean intensities were tested using variance analysis (ANOVA). Student's t-test was used to compare mean intensities between male and female. The Tukey's post-hoctest was used to compare helminth loads between two seasons andecosystems. The Chi-square ($\chi 2$) test made it possible to compare prevalence. The Sperman's coefficient "r_s" was used to investigate the correlation between abundance of parasites and hosts' standard length. These analyses were performed using the software PAST.16 and Quantitative Parasitology 3.0 All values of P<5% were considered significant.

3. Results

3.1. Dynamics of *Enterogyrus melenensis* in three ecosystems of the Mefou Hydrographic system

A total of 302 *Hemichromis fasciatus* specimens were examined; their standard length (SL) ranged from 20 to 81 mm. 2590 *Enterogyrus melenensis* individuals were collected from the stomach; this species presented an aggregate distribution (s^2/Im> 1). Among the total host, 150 *H. fasciatus* revealed uninfected. The mean intensity was low (17.04) and did not vary significantly between the three ecosystems studied (F=0.88; P=0.5). On the contrary, the prevalence globally differed significantly (χ^2=110.3, P= 1.15^{E-32}) and varied between the pond (lower) and Mefou dam (P=2.6^{E-32}), the pond and forest downstream course (P=1.4^{E-19}), Mefou dam (higher) and forest downstream course (P=0.006) see table 1.

Table 1 Prevalence, mean intensity, and aggregation of *Enterogyrus melenensis*, stomach parasite of *H. fasciatus* in three ecosystems of the Mefou hydrographic system.

Ecosystems	Fish examined	Fish parasitized	Prevalence (%)	MI ±SE	Aggregation (s2/MI)
PO	101	2	2	22±21	42.01
MD	123	100	81.3	15.61±1.3	15.11
FD	78	50	64.1	19.7±2.42	21.9

SE= Standard Error; PO= pond; MD= Mefou dam; FD= forest downstream course; MI = mean intensity

3.2. Relationship between intensity of *E. melenensis* and the host standard length

In the overall host sample, the intensity of *E. melenensis* slightly decreased significantly (P=0.045) as the length of *H. fasciatus* increased (r= - 0.11). This decrease of the intensity was more apparent in the MD ecosystem (r= -0.34; P= 0.0001; see Fig.1) while in the FD ecosystem, the intensity was positively linked to the LS (r= 0.44; P= 4.3^{E-05}, see Fig.2). No relationship was found between the intensity and LS in the PO (Pond).

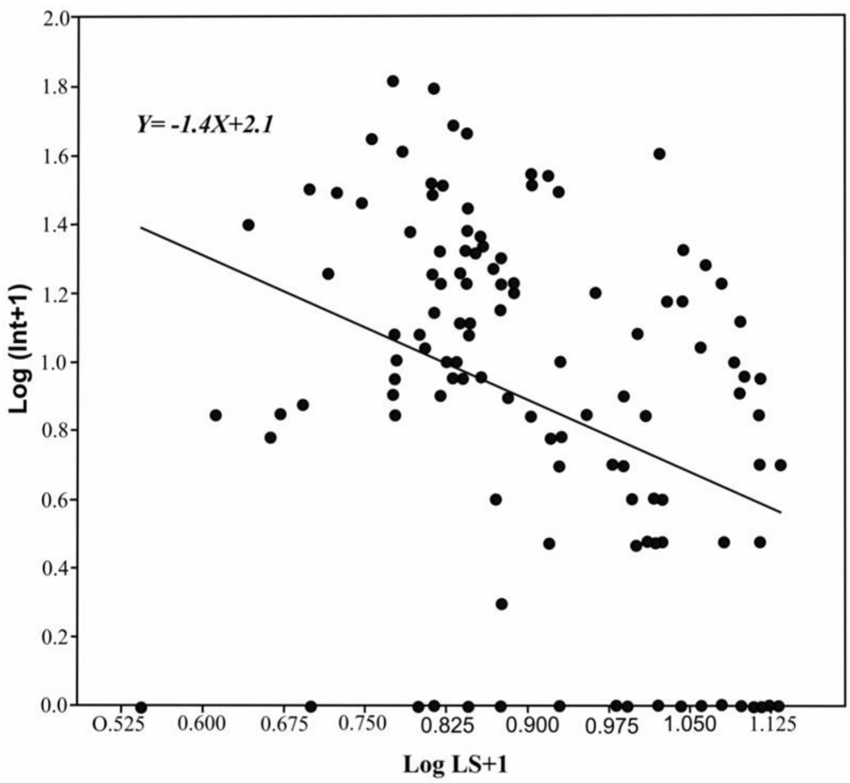

Figure 1 Regression curve between *E. melenensis* intensity and *H. fasciatus* Standard Length in the Mefou dam

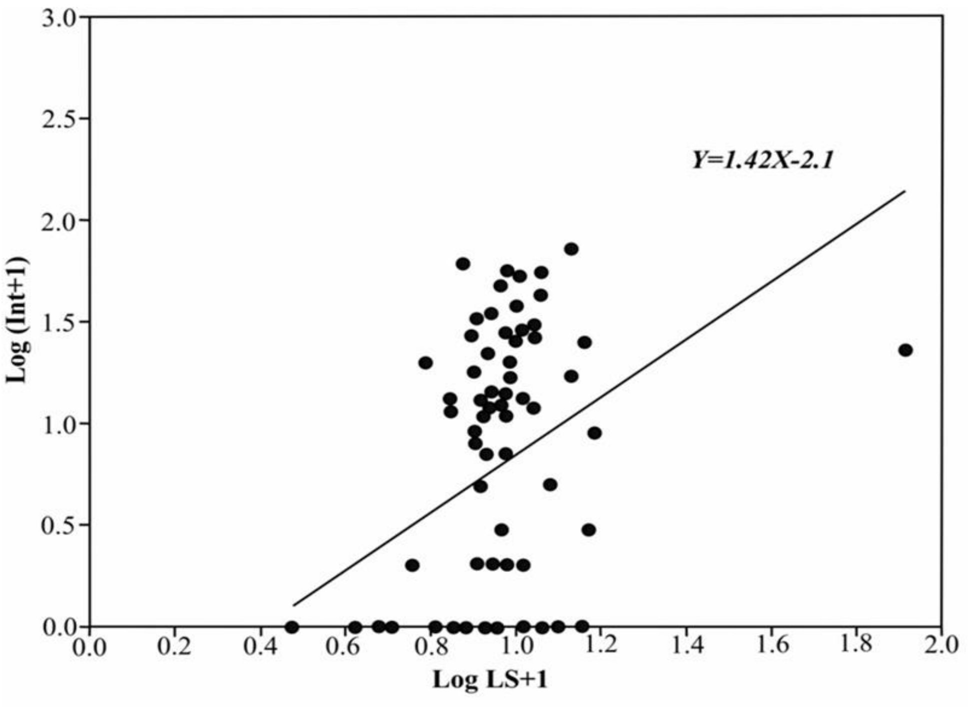

Figure 2 Regression curve between *E. melenensis* intensity and *H. fasciatusStandard* Length in the Forest downstream course

3.3. RelationshipbetweenE. melenensisbetween the intensity and host sex

Among the total host sample (302 *H. fasciatus*), the sex of 277 individuals was determined. The intensity and the prevalence of *E. melenensis* were not sex dependent (table 2).

Table 2 Prevalence and mean intensity of *E. melenensis* as a function of the host sex

Sex	Number examined	Number infected	Prevalence (%)	MI ± SE
Males	123	67	54.4	16.79±1.79
Females	154	80	51.9	16.9±1.76
Statistics -			$\chi2=0.13$; $P=0.67$	$t= 0.05$; $P=0.95$

MI = mean intensity

3.4. Seasonal variation of the parasitism by*E. melenensis*

Enterogyrus melenensis parasitized its host population during all the sampling periods (table 3).

Table 3 Prevalence and mean intensity of *E. melenensis* as a function of the seasons

Seasons	Fish examined	Prevalence (%)	MI ±SE
LDS 2017	44	47	4.9±0.37
SRS 2018	11	45	21±10.5
LRS 2018	20	90	13.4±3.09
LDS 2018	63	57	20.3±2.6
SRS 2019	75	48	16.8±2.47
SDS2019	45	33	15.4±3.19

MI = mean intensity

Both epidemiological indexes (mean intensity and prevalence) varied with seasons, but without an obvious profile, and were not cyclic.

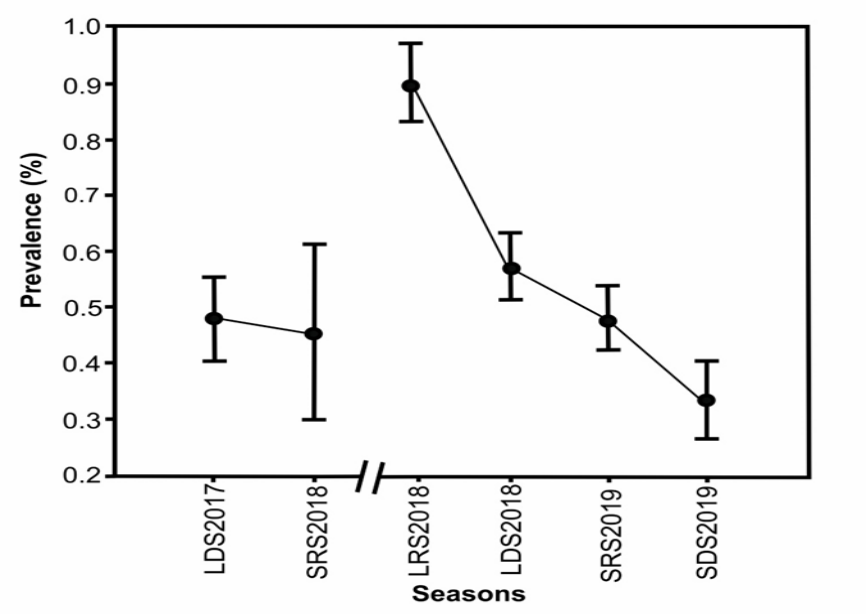

Figure 3 Seasonal variation of prevalence of *E. melenensis*

For a given season, the values of these indexes significantly differed, from one year to another (table 3; Fig.3 and Fig.4)

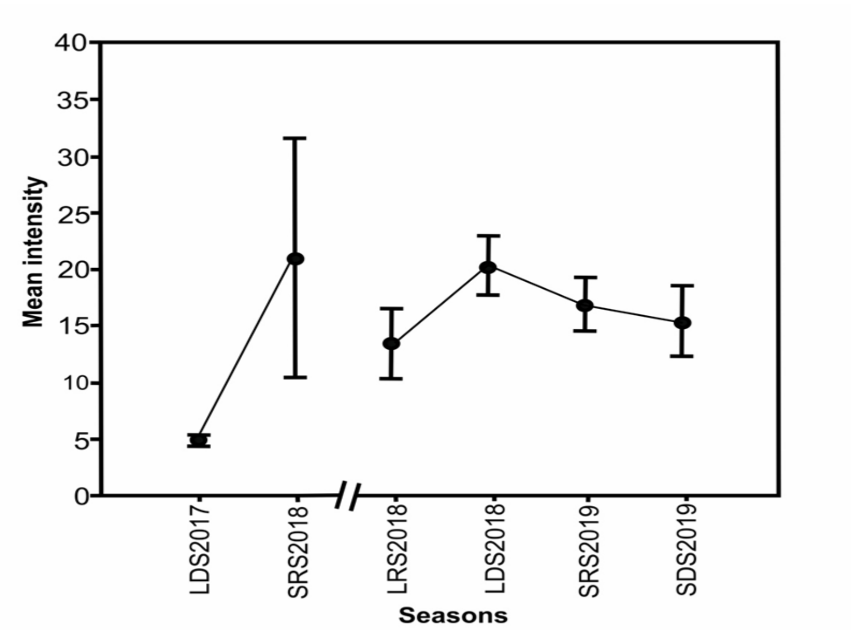

Figure 4 Seasonal variation of the intensity of *E. melenensis*

Differences in intensities between seasons were significant: SRS2018 and LDS2017 ($K=4.38$; $P=0.02$), LDS2018 and LDS2017 ($K=4.21$; $P=0.03$) (Table 4).

For the prevalences, significant differences were noted between: LDS2017 and LRS2018 ($\chi^2=7.25$; $P=0.001$); SRS2018 and LRS2018 ($\chi^2=4.1$; $P=0.008$); LRS2018 and LDS2018 ($\chi^2=4.8$; $P=0.001$); LRS2018 and SRS2019 ($\chi^2=8.26$; $P=0.001$); LRS2018 and SDS2019 ($\chi^2=13.14$; $P=0.001$) (Table 4).

Table 4 Multiple comparisons of seasonal intensities (A) and prevalences (B) of *E. melenensis*

A

LDS2017		0.01;0.90	7.25;0.001*	0.68;0.34	0.00;0.97	1.36;0.17
SRS2018	4.38;0.02*		4.1;0.008*	0.37;0.48	0.01;0.88	0.38;0.46
LRS2018	2.32;0.56	2.05;0.69		4.8;0.001*	8.26;0.001*	13.14;0.001*
LDS2018	4.21;0.03*	0.17;1	1.88;0.76		0.85;0.28	4.4;0.015
SRS2019	3.25;0.19	1.12;0.96	0.93;0.98	0.95;0.98		1.8;0.11
SDS2019	2.85;0.32	1.52;0.89	0.53;0.99	1.35;0.99	0.39;0.99	
	LDS2017	**SRS2018**	**LRS2018**	**LDS2018**	**SRS2019**	**SDS2019**

B

Intensity (K; Pvalue), Prevalence (χ2; Pvalue)
*= significant comparison

4. Discussion

The present study deals with the dynamics of *E. melenensis,* a stomach parasite of *H. fasciatus* from three ecosystems in the Mefou hydrographic system.This monogenean was aggregated within the host population. Most of the host individuals carried a small proportion of parasites, while a small number of fish carried a larger proportion of parasites. This distribution pattern agrees with [25] who stated that in most cases, parasites are almost universally aggregated between their hosts. According to [26] the aggregative distribution may indicate heterogeneity in the relationship between the host and the parasite populations. This distribution pattern could be advantageous for the parasite insofar as it increases the opportunities for mating [27].

The mean intensity of *E. melenensis* in the three ecosystems was always low (10<IM≤ 50) according to the categorization of parasitism in our environment [28]. High prevalence was observed both in the Mefou dam and forest zone of the Mefou. Only 2 fish individuals out of 101 from the pond were parasitized. It is suggested that regular pond emptying, which eliminates part of the infecting larvae (oncomiracidia) and eggs of the parasite, and fishing may reduce the xenopopulation of this endoparasite. As far as ectoparasitic monogeneans are concerned, according to [29] the degree of infection depends on the condition and density of the hosts; according to [30] the strong water current is also often a factor limiting the invasion of such infective larva stages thus reducing the intensity of parasitism. Therefore the reduced or low water current in the Mefou dam, and the apparent higher host density favor the higher prevalence observed, compared to the forest downstream course where the water current is more rapid, fish more scattered and therefore their density apparently lower.

Globally the intensity of *E. melenensis* slightly decreased as the length of the host increased, although in the forest ecosystem, we found the inverse effect. This contrary result seems unintelligible concerning the same host/parasite and hydrographic systems. Many investigations on the relationship between the intensity of ectoparasitic monogeneans and the size (or age) of the host exist. Most authors found that the intensity of monogeneans increased with fish standard length [31, 32, 30, 33]; this result is always explained by the fact that larger fish offer large surface areas for parasites to colonize [31, 34], [35, 36]. Infrequently, it is stated that the intensity of monogeneans decreases as the host size (or age) increases [37]. In this case, according to [38] the host immunity to explain such result, according to [39] this result is attributed to the self-cure mechanism triggered by the increase of the parasite population. We suggest that the reduction of an infrapopulation by the process of acquired immunity in large host [37] or the self-cure mechanism [39] is preceded by the parasite accumulation in a host up to a certain threshold which stimulates its defense. This last phenomenon could explain the positive correlation between the intensity and the host size in the forest downstream course. Moreover, according to also [36] the relationship between the number of endoparasites and host size is less clear compared that to with ectoparasites. But in general, acquired immunity tends to induce convex changes in mean worm load with host age where the peak levels of infection occur in younger hosts.

The prevalence and the mean intensity of *E. melenensis* were not host sex dependent. The same observation was obtained for ectoparasitic monogeneans of *Oreochromis niloticus* [40, 41].According to [13] the sedentary lifestyle of *H. fasciatus* individuals of both sexes during the spawning period justifies the equal level of infestation between males and females. This explanation is palatable in the case of *Enterogyrus melenensis* also transmitted directly by a free infective larva (oncomiracidium).

Enterogyrus melenensis parasitized its host population throughout the year with seasonal variations but without a clear profile. In the same climatic zone, clear seasonal profiles have been defined for ectoparasitic monogeneans of *H. fasciatus*[13],*Barbus martorelli*[42]. In both cases and over consecutive years, the prevalence and intensity of the ectohelminths were highest during the rainy seasons, lowest in dry seasons especially in LRS. In this study, the infection level was not cyclical. The water temperature is always assumed to be an important factor in controlling the occurrence of ectoparasitic monogeneans [43]. Although fish are ectotherms, *E. melenensis* being an endoparasite s.l., it is suggested that its inner location attenuates the direct influence/effect of the water temperature on the metabolism of this helminth, resulting in the non-cyclical profile observed.

5. Conclusion

It emerges from this study that a high prevalence of *E. melenensis* was observed in the Mefou dam and in the forest zone of this river hydrographic system, the values being higher in the dam where the water current is reduced and the host density apparently higher. Well managed, pond emptying could be an optimal strategy to diminish monogenean infective larval loads preventing epizootic events. The gathering of parasites, over time, in *H. fasciatus* specimens could stimulate the host defense (immunity) to lessen the worm load (intensity). The inner location of *E. melenensis* attenuates the direct influence of the water temperature on this endohelminth resulting in its non-cyclical profile.This study also highlights the necessity to consider the environment when comparing host parasitism. These findings reinforce the practice recommending quarantining native large fish specimens captured in the nature and used as sires in ponds, in order to avoid epizootic outbreaks.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declares no conflict of interest.

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