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Selectivity of zooplanktonic preys in *Heterobranchus longifilis* larvae and fry

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Abstract
The present study was carried out to study the preferred prey in the larval and post-larval stages of *Heterobranchus lonsifilis*. Two hundred and forty larvae and fry of *H. longifilis* aged 2 to 23 days were stored in aquariums of 8 liters, fasted for 24 hours at the end of which they were fed to satiety with freshly collected zooplankton from fishponds**.** The day after feeding, the fish were euthanized and stored for stomach contents examination. The results showed that the zooplanktonic population density of the station ponds was 1356.73 individuals / L. This stand is composed of Cladocerans (455.85 individuals /l), nauplii (289.11 individuals / l), Rotifers (255.47 individuals / l), Copepodites (182.21 individuals / l) and Copepods adults (174.07 individuals /l). Furthermore, examination of stomach contents showed that adult Copepods were the most frequent and abundant prey category in stomach contents in terms of frequency of occurrence (66.7%) and numerical percentage (48.25%). In addition, these preys (adult copepods) constituted the preferred zooplankton prey in larvae and post-larvae of *H. longifilis* with an electivity index (E) equal to +0.5. Apart from the first size classes in which no prey has been observed in the stomach contents, the food preferences of *H. longifilis* larvae and fry do not vary with the size of the individuals. This study showed that adult copepods were the preferred zooplankton prey of *H. longifilis* larvae and fry.

Keywords: Catfish, larvae, zooplankton, preferred prey

1. Introduction

Fish is the main source of animal protein for Ivorian consumers with is 16 kg per inhabitant per year [1]. In Côte d'Ivoire, the need for fishery products is estimated to 600,000 tonnes per year while national production is around 100,000 tonnes. The contribution of aquaculture to fish production, estimated at 4,500 tonnes per year, remains insignificant while it must feed the Ivorian population in a context of population growth and accelerated urbanization.

The tilapia *Oreochromis niloticus*, the catfish *Hetebranchus longifilis* and *Clarias gariepinus*, *Heterotis niloticus* and the jawbone *Chrysichthys nigrodigitatus* are the fish species generally found in Ivorian fish farming [2]. In view of its higher growth potential than those of Chrysichthys and tilapia, the catfish *Heterobranchus longifilis* is a better candidate for fish farming. However, although artificial reproduction techniques no longer present a sticking point, larval rearing remains the most restrictive phase of the *H. longifilis* catfish production. Indeed, Artemia constituting the best food for *H. longifilis* larvae, is not always accessible to fish farmers due to its high cost [3].

Several species of zooplankton are used as live prey in aquaculture [4]. In fact, live zooplanktonic prey contains nutrients such as essential amino acids, fatty acids, carbohydrates, vitamins and minerals [5, 6]. In view of the importance of zooplankton in the diet of fish, many studies have been directed towards the use of various species of zooplankton in the diet of the larvae of *H. longifilis* [7, 8]. In addition, research has shown that it is possible to cultivate zooplankton to feed fish larvae [8, 9].

In addition, we observe a high diversity of zooplankton species reported in Ivorian aquatic environments [10, 11], and a plasticity of the diet according to size in *H. longifilis* [12]. Thus, it appears important to identify the preferred zooplankton prey in this species in order to better guide the peasants in the production of these live prey intended for the larval and post-larval stages. This study was therefore initiated to determine the preferred zooplanktonic prey in the larvae and fry *Heterobranchus longifilis.*

2. Materials and Methods

2.1. *H. longifilis* larvae and fry origin

The larvae and fry used in this study were obtained by artificial reproduction carried out at the research station on inland fisheries and aquaculture in Bouaké (Côte d'Ivoire).

2.2. Collection, identification and enumeration of zooplankton

Zooplankton were collected from the 400 m2 ponds of the inland fisheries and aquaculture research station using a plankton net 40 cm in opening diameter, 150 cm in length and 35 µm mesh gap. To collect zooplankton, the plankton net was dragged horizontally in the water column over a length of 18 m. The identification of zooplankton was done according to Dussart [13], Pourriot [14], Rey and Saint-Jean [15], and Kotov [16]. The density of zooplankton was determined according to [17]. Thus the following formula was applied:

Zooplankton density (D) = (N / V1) X (V2 / V3) where n = number of individuals counted; V1= volume of the filtrate collected (3 mL), V2 = volume of the concentrated filtrate (sample volume), V3 = volume of filtered water = π x R^2x d where d = haul distance from the plankton net, and R: the radius of the opening of the plankton net.

2.3. Experimental strategy and analysis of the stomach contents of *H. longifilis* larvae and fry

Two hundred and forty larvae and fry of *H. longifilis* aged 2 to 23 days were used in this study. These fish were stored in eight liters water tanks. After loading, the fish were fasted for 24 hours at the end of which they were fed to their satiety with freshly collected zooplankton from the fish ponds of the Inland Fisheries and Aquaculture Research Station. The day after feeding, the fish were euthanized and stored in a 10% formaldehyde solution for the study of stomach contents. Before dissection for the study of stomach contents, the size was noted. The zooplanktonic compositions of the stomach contents were observed under a WILD M5A stereoscopic microscope, and identified using the identification keys of [13-16].

Stomach contents were analyzed using the following parameters and indices:

Percentage of vacuity (% V) according to [18]:$\%V = \frac{Nse}{N} \times 100$ where N_{se} is the number of empty stomachs and N is the total number of stomachs examined;

- Percentage of occurrence (% F) according to [19]:$\% F = \frac{N_s}{N_{Se}} \times 100$ where N_s is the number of stomachs containing prey i and N_{Se} is the number of non-empty stomachs examined;

- Numerical percentage (% N) according to [20]:$\%N = \frac{N_i}{N_t} \times 100$, where N_i and N_t are respectively the number of individuals of a prey category i and the total number of prey individuals inventoried;

- Electivity index (E) according to Ivlev [21]: $E = \frac{r_i - p_i}{r_i + p_i}$, where r_i is numerical percentage of a prey i in the stomach contents and Pi : numerical percentage of the same prey i in the environment. Electivity index (E) varies between -1 and +1. When E = -1, it is said to be total negative, if E = +1, the selection is said to be total positive, and if E = 0 means that there is no selection.

Size classes were determined according to Sturge's rule [22].

2.4. Statistical analysis

The STATISTICA 7.1 software was used to compare the parameters of the different size classes. Thus, the vacuity and numerical percentages, and the electivity index were subjected to analysis of variance (one-way ANOVA). If there was a significant difference (p< 0.05), the results were subjected to Tukey's multiple comparison tests to establish the differences between the means of the treatments.

3. Results

3.1. Inventory of zooplanktonic prey groups collected in ponds

The faunistic population of plankton in fish ponds is made up of three groups of zooplankton, namely Copepods, Cladocerans and Rotifers. Copepods have been subdivided into three categories based on their size. These are the Nauplii, Copepodites and adult copepods. The zooplanktonic population density of the station ponds is 1356.73 individuals /l. The most abundant preys in this environment were the Cladocerans with a proportion of 33.60% of the total density or 455.85 individuals / l. This category of prey is followed by that of the Nauplii, the proportion of which was 21.31% for 289.11 individuals / l. The proportions of the other prey categories were 18.83% (255.47 individuals / l) for Rotifers, 13.43% (182.21 individuals / l) for Copepodites and 12.83% (174, 07) for adult Copepods.

3.2. General composition of stomach contents of larvae and post-larvae of *Heterobranchus longifilis.*

Of the 240 stomachs analyzed, 210 contained prey and 30 stomachs were empty, for an overall emptiness percentage of 33.3%. Analysis of stomach contents in table 1 showed that adult copepods (% F = 66.7%) were the most frequent prey in stomach contents of larvae and post-larvae of *H. longifilis*. The other prey categories had occurrence percentages of less than 10%. In terms of the numerical percentage, adult Copepods (% N = 48.25) although less abundant in the breeding environment, were the most numerous in stomach contents. They are followed by Rotifers (14.96%), then by Copepodites (% N = 13.25%) and Cladocerans (% N = 11.84%) (Table I). The values of the electivity indices showed that the adult Copepods (E = +0.58), and the Copepodites (E = +0.02) were selected positively. The other prey categories showed negative electivity indices.

Table 1: Composition of food prey in the stomach contents of larvae and post-larvae of *H. longifilis*

Prey categories	%F	%N	E
Adult copepods	66.7±44.44	48.25±2,2	+0.58±0.02
Copepodites	9.43±6.28	13.25±1,1	+0.02±0.01
Nauplii	9.43±6.28	11.70±0,8	-0.30±0.04
Cladocerans	6.48±4.32	11.84±1,5	-0.48±0.05
Rotifers	7.61±5.07	14.96±1,9	-0.13±0.03

% F= Percentage of occurrence;% N = Numerical percentage; E= Electivity index

3.3. Composition of stomach contents according to the size of the larvae and post-larvae of *Heterobranchus longifilis*

Two hundred and forty larvae and fry of *H. longifilis*, varying in size from 0.2 to 1.03 cm, were divided into six size classes, according to the sturge rule. Thus, the class intervals were 0.2 - 0.29 cm, 0.3 - 0.39 cm, 0.4 - 0.49 cm, 0.5 - 0.59 cm, 0. 6 - 0.69 cm and 1 - 1.09 cm respectively for classes 1, 2, 3, 4, 5 and 6. Analysis of stomach contents showed that in individuals of size classes 1 and 2, the stomachs contained no prey. Unlike these first two size classes, the stomachs of the other size classes contained prey. The percentage of vacuity for these size classes was therefore 0%.

In the non-empty stomach size classes, the percent occurrence was higher for adult copepods (% F = 100). The other preys had a percentage of occurrence of less than 20% (Table 2). There was no significant difference ($P< 0.05$) between numerical percentage in *H. longifilis* larvae and fry from different size. Adult Copepods were the most abundant with proportions of 52.1; 45.6; 49.1 and 46.2% observed respectively for size classes 3, 4, 5 and 6. For other prey, it was less than 20% for these same size classes (Table 3).

There was no significant difference ($P< 0.05$) between electivity index in *H. longifilis* larvae and fry from different size. Electivity index analysis (E) showed that adult Copepod and copepodites were the positively selected prey by larvae and post-larvae of *H. longifilis* in all size classes of non-empty stomachs. Contrary to Copepods and Copepodites, Cladocerans, Nauplii and Rotifers were selected negatively in all size classes of non-empty stomachs (Table 4).

4. Discussion

The zooplanktonic prey collected in the ponds are Cladocerans, Rotifers and Copepods and their nauplii. The zooplankton stand abundance in the station's fish ponds is 1356.73 individuals / l.

Table 2: Occurrence Percentage (%F) according to *H. longifilis* larvae and fry size

Prey Categories	Size classes of larvae (cm)					
	Class 1 [0.2; 0.29]	Class 2 [0.3; 0.39]	Class 3 [0.4; 0.49]	Class 4 [0.5; 0.59]	Class 5 [0.6; 0.6]	Class 6 [1; 1.09]
Adult copepods	0	0	100	100	100	100
Copepodites	0	0	12.9	13.8	14.2	15.7
Nauplii	0	0	13.2	13.7	14.1	15.6
Cladocerans	0	0	9.8	9.5	9,6	10.01
Rotiferes	0	0	10.7	11.2	11.4	12.4

Table 3: Numerical percentage (%N) according to *H. longifilis* larvae and fry size

Prey categories	Size classes of larvae (cm)					
	Class 1 [0.2; 0.29]	Class 2 [0.3; 0.39]	Class 3 [0.4; 0.49]	Class4 [0.5; 0.59]	Class 5 [0.6; 0.6]	Class 6 [1; 1.09]
Adult copepods	$0±0^{a}$	$0±0^{a}$	$52.1± 3.5^{b}$	$45.6±2,9^{b}$	$49.1±1,9^{b}$	$46.2±3.6^{b}$
Copepodites	$0±0^{a}$	$0±0^{a}$	$12.3±0.9^{b}$	$13.5±0.9^{b}$	$12.9±1.2^{b}$	$14.3±0.9^{b}$
Nauplii	$0±0^{a}$	$0±0^{a}$	$11.4±1.9^{b}$	$13.1±1.1^{b}$	$10.2±2.1^{b}$	$12.1±0.9^{b}$
Cladocerans	$0±0^{a}$	$0±0^{a}$	$10.1±2.7^{b}$	$14.1±1.8^{b}$	$11.7±1.8^{b}$	$11.5±2.5^{b}$
Rotifers	$0±0^{a}$	$0±0^{a}$	$14.2 ± 1.9^{b}$	$13.7±1.4^{b}$	$16.1±1.6^{b}$	$15.9±1.4^{b}$

Values represent means and standard deviations. The values that are not assigned the same letter are significantly different (Anova, $p< 0.05$) for each row of the table.

Table 4: Electivity index (E) according to *H. longifilis* larvae and fry size

Prey categories	Size classes of larvae (cm)					
	Class 1 [0.2; 0.29]	Class 2 [0.3; 0.39]	Class 3 [0.4; 0.49]	Class4 [0.5; 0.59]	Class 5 [0.6; 0.6]	Class 6 [1; 1.09]
Adult copepods	$0±0^{a}$	$0±0^{a}$	$0.6±0.03^{b}$	$0.56± 0.1^{b}$	$0,58± 0.1^{b}$	$0.56± 0.09^{b}$
Copepodites	$0±0^{a}$	$0±0^{a}$	$0.01±0.01^{b}$	$0.02±0^{b}$	$0.03±0.02^{b}$	$0.02±0,02^{b}$
Nauplii	$0±0^{a}$	$0±0^{a}$	$-0.3±0.2^{b}$	$-0.23±0.18^{b}$	$-0.35±0.27^{b}$	$-0.27±0.19^{b}$
Cladocerans	$0±0^{a}$	$0±0^{a}$	$-0.53±0.31^{b}$	$-0.4±0.35^{b}$	$-0.48±0.29^{b}$	$-0.49±0.4^{b}$
Rotifers	$0±0^{a}$	$0±0^{a}$	$-0.15±0.07^{b}$	-0.15±0.09	$-0.14±0.09^{b}$	$-0.08±0.08^{b}$

Values represent means and standard deviations. The values that are not assigned the same letter are significantly different (Anova, $p< 0.05$) for each row of the table.

This abundance is much higher than those of the Ivorian continental shelf and coastal rivers (Ehiania, Bodoua and Boulo) estimated respectively at 10.85 individuals /l [23] and 12.17 individuals /l [10]. Compared to the latter two environments, the zooplankton abundance (1217 individuals / l) recorded by Soro *et al.* [24] in the upper Bandama basin, is close to that observed in the present study. This high numerical abundance observed could be explained by the fact that the ponds are rich in minerals (N. P. C) resulting from the decomposition of food leftover and faeces from fish. Indeed, a high concentration of minerals stimulates a high production of phytoplankton, which promotes the development of zooplankton. Because phytoplankton constitute the bulk of the zooplankton diet.

Analysis of stomach contents showed that adult copepods, although less abundant in the rearing environment (12.83%), are the main prey for larvae and post-larvae of *H. longifilis*. This result gives the larvae and post-larvae of *H. longifilis* the preference for adult Copepods given the high values of the percentage of occurrence (% F = 66.7), of the numerical percentage (% N = 48.25 ± 2. 2) and the electivity index (E = +0.5). Indeed, the larvae of *Heterobranchus longifilis* preferably consume large prey. The selection of the prey was exerted primarily on species of large sizes, which are easy to detect, which explains the strong selectivity of the adult copepods whose size varies between 0.3 and 8 mm against 0.1 and 1 mm for the Rotifers and 0.2 and 3 mm for Cladocerans. This is in agreement with Lazzaro [25] who asserts that contrary to what was observed in fish larvae where the size of the prey captured is a function of the size of the mouth, in *H. longifilis* the larvae first select the large preys that are easily detected by their barbels. Hem *et al.* [26] state that larvae, with a mouth width is about 1 mm, are already capable to ingest large planktonic prey such as adults of Moina and Cyclopides. Apart from size class 1 and 2, which did not contain prey, stomach contents were not different according to size classes. According to Gilles [27] the absence of prey in the stomach contents of these larvae could be related to their two-day ages. Indeed, the larvae of this age would still have their yolk reserves, which serve as natural food. In addition, at two days

of age, the larvae have much more difficulty moving around and hunting prey. For example, Hunter and Kimbrell [28] stated that the efficiency of larval predation depends on several factors, including their swimming speed and their efficiency in capture. However, stomachs of size classes 3, 4, 5 and 6 contained prey. The presence of prey in the stomachs of individuals belonging to these size classes would be due to their age and especially to their larger size, which is between 0.48 and 1.03 cm. These individuals over two days old and tall would have emptied their yolk reserve, which served them as food. In these size classes, adult Copepods were the most frequent prey and the most numerous in the stomach contents although they were less abundant in the environment. Their electivity indices showed that they were the most selected prey in the environment by the larvae and post-larvae of *H. longifilis* (with E ≥ + 0.5) from all size classes of not empty stomach (size class 3, 4, 5 and 6).
The higher numerical percent values and positive values of the electivity indices of adult Copepods indicate that they were the preferred prey in larvae and post-larvae of *H. longifilis*. The other categories of prey were very little ingested because of their small size. This strong selection exerted on adult Copepods could be explained by the fact that the larvae of *Heterobranchus longifilis*, whatever their age and size, first select the larger preys [25]. Likewise, Braum [29] demonstrated that Copepods constitute the main prey consumed during the 25 days following their start of exogenous feeding. He explains this preference by the fact that the Copepods flight reaction stimulates the larva and makes it easier to capture. In addition, predation appears rather to be selective.
Regarding the other categories of prey (Rotifers (18.83%), Copepodites (13.43%), Cladocerans (33.6%) and Nauplii (21.31%)) very abundant in the environment, they have were very little present in the stomach contents, whereas they should have been if the predation was nonselective. This low capture was probably linked to the small size of these preys. Finally, these observations agree [30]. Indeed, according to this author, preys selection by fish larvae depends on the density and vulnerability of the preys. Thus, vulnerable species were the most diet of larvae when predation is selective. While the most abundant species were the most diet when predation is non-selective. Predation did not appear to have a pronounced impact on the community of these prey categories. In addition, the larvae and fry of *H. longifilis* preferably selected adult Copepods because the fish were fed during the day only. Indeed, Hem *et al.* [26] showed that copepods and ostracods are highly selected during the day, and during the night, selection is mainly on Moina in Catfish larvae.

5. Conclusion

The present study was carried out to study the preferred prey in the larval and post-larval stages of *Heterobranchus lonsifilis*. Thus, at the end of the research work carried out on the different categories of prey. In terms of numerical abundance, Cladocerans dominate with a density of 1190.31 individuals/l while copepods have a low numerical percentage with the density of 454.51 individuals/l. The results show that predation is selective in larvae and post-larvae of *H. longifilis*. Adult Copepods were the most common and abundant prey category in stomach contents in terms of frequency of occurrence (66.7%) and number percentage (48.25%). Moreover, these prey (adult copepods) constitute the preferred zooplanktonic prey in larvae and post-larvae of *H. longifilis* with an electivity index (E) of +0.58. Apart from the first size classes in which no prey was observed in the stomach contents, the food preferences of the larval stages and larval post of *H. longifilis* do not vary with the size of the individuals. As this study is a preliminary work, it would be interesting to study the growth performances of *H. longifilis* larvae and fry fed with adult copepods.

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