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Richard Adande
Laboratory of Research on Wetlands (URZH), Faculty of Sciences and Technology, Department of zoology, University of Abomey-calavi (UAC), B.P.526 Cotonou, Benin

Mouhamadou Nourou Dine Liady
Laboratory of Research on Wetlands (URZH), Faculty of Sciences and Technology, Department of zoology, University of Abomey-calavi (UAC), B.P.526 Cotonou, Benin

Comlan Ephrem Tossavi
Laboratory of Research on Wetlands (URZH), Faculty of Sciences and Technology, Department of zoology, University of Abomey-calavi (UAC), B.P.526 Cotonou, Benin

Emile Didier Fiogbe
Laboratory of Research on Wetlands (URZH), Faculty of Sciences and Technology, Department of zoology, University of Abomey-calavi (UAC), B.P.526 Cotonou, Benin

Correspondence
Emile Didier Fiogbe
Laboratory of Research on Wetlands (URZH), Faculty of Sciences and Technology, Department of zoology, University of Abomey-calavi (UAC), B.P.526 Cotonou, Benin

The effect of zooplankton produced using rabbit manure and artemia on the survival and growth of larvae of *Clarias gariepinus, Heterobranchus longifilis* and heteroclarias

Richard Adande, Mouhamadou Nourou Dine Liady, Comlan Ephrem Tossavi and Emile Didier Fiogbe

Abstract
Plurispecific fresh water zooplankton produced with rabbit manure, and dry artemia in powder (artemia Shell free) were used to evaluate survival and growth performances of larvae of *Clarias gariepinus, Heterobranchus longifilis* and heteroclarias witch initial weights are respectively 2.5 ± 0.0, 2 ± 0.14, 2.7 ± 0.1 mg for 12 days. Six experimental diets were tested in out of ponds by triplicate as follow: Czoo (*Clarias gariepinus* fed with fresh water zooplankton), C_{art} (*Clarias gariepinus* fed with artemia), H_{zoo} (*Heterobranchus longifilis* fed with fresh water zooplankton), H_{art} (*Heterobranchus longifilis* fed with artemia), HC_{zoo} (heteroclarias fed with fresh water zooplankton) and HC_{Art} (heteroclarias fed with artemia). At the end of rearing Czoo, Cart, Hzoo, Hart, HCzoo and HC_{art} gave respectively survival rates of 74 and 73.5%, 63.5 and 60% and 70 and 62%. Final mean weights recorded were 15.54 ± 0.37, 13.3 ± 0.97, 34.13 ± 2.31, 36.46 ± 1.16, 18.14 ± 0.71 and 16.25 ± 0.52 mg for Czoo, Cart, Hzoo, Hart, HCzoo and HC_{art} with specific growth rates respectively of 15.22 ± 0.20, 13.91 ± 0.60, 24 ± 1.18, 24.56 ± 0.40 and 15.87 ± 0.11, 14.95 ± 0.45 (%.day^{-1}). Results from the effect of local plurispecific fresh water zooplankton and artemia on the survival and growth of larvae of *Clarias gariepinus* and *Heterobranchus longifilis* and their hybrids (heteroclarias) indicate local fresh water zooplankton can successfully replace artemia in the first life stage of larvae of these two African catfish species and their hybrids.

Keywords: *Clarias gariepinus*, *Heterobranchus longifilis*, heteroclarias, Artemia, plurispecific zooplankton, survival and growth.

Introduction
Current development of aquaculture in West Africa focus essentially on certain species such as Nile Tilapia (*Oreochromis niloticus*) and African catfishes (*Clarias gariepinus* and *Heterobranchus longifilis*) [1, 2]. In most of African countries, fish farming encounters many difficulties despite the presence of traditional production systems of catfish (claridae) though the demand is high [3]. Indeed, the non-availability of fries and juveniles of claridae constitutes a choc to fish farming development [4-8]. In addition, larval rearing (determinative stage) always doesn't lead to expected results due to insufficient feeding and non-adaptation of feed to first stage of development [9, 3]. Moreover, the passing of larvae from endogenous feeding (vitellin sack) to an exogenous feeding with the appearance of digestive organs constitutes an important stage to be considered in fish life [10, 11]. Currently, the mastery of larval rearing is necessary for the production of fries in order to satisfy demand about these two catfish species. Nowadays, the major difficulty of larvae rearing is the choice of best quality of feeding for fresh water fish larvae [12]. It will be better to use suitable feed for larvae not only to optimize the survival and growth but to enable reduction of production cost [13]. Certain authors [14, 15] present artemia like the ideal feed for fresh water fish larval rearing. Although its availability, accessibility and cost constitute serious problems for fish farming in developing countries. To remedy this, [3, 16-19] have shown in their studies the use of the live feed produced from organic fertilizers of animal origin (poultry droppings, cow dung, pig dejection) use of artemia for larviculture. By the same way, rabbit manure is rich in mineral elements able to provoke the production of fresh water zooplankton and phytoplankton for larvae feeding [20]. Unfortunately to this day, the effect of zooplankton produced with rabbit manure on the survival and growth

of larvae of claridae has never been studied. We were glad to try larval rearing based on local plurispecific zooplankton produced with rabbit manure in order to promote catfish farming in rural areas. Therefore, in this study, we propose to compare the effect of zooplankton produced with rabbit manures and artemia on the survival and growth of larvae of *Clarias gariepinus, Heterobranchus longifilis* and heteroclarias.

Materials and methods

Origin of larvae and installation

Larvae of *C. gariepinus*, *H. longifilis* and heteroclarias were obtained from artificial reproduction on the station of research on the diversification of fish farming of the Laboratory of Research on Wetlands (LRZH) of the University of Abomey-Calavi (UAC). The experiments were carried out from 22 June to 4 July 2017. Two specimens of *C. gariepinus* (1 kg weight per individual) and *H. longifilis* (a female of 3 kg and a male of 1.5 kg) were used in artificial reproduction. Larvae of heteroclarias were obtained from *C. gariepinus* ♀ X *H. longifilis* ♂ weighting respectively 0.5 and 1.2 kg. To provoke spawning, an extract of ovaprim (0.5 and 0.25 mL/kg of body weight respectively for the female and the male) was used for oocytes and spermatozoids maturing [21, 22]. Ten hours (10 hours) after injection, eggs were stripped and fertilized with milt obtained from male. Fertilized eggs were hatched with baskets in hatchery tanks and in ponds at free air. Eggs hatching happened 27 hours after hatchery at 26.02 ± 0.91°C. Two days after hatching, larvae were distributed in circular ponds (50 cm radius x 8 cm height) in triplicate and fed for 12 days [9]. An initial density of 200, 45, 100 larvae respectively for *C. gariepinus* (initial weight 2.5 ± 0.00 mg), *H. longifilis* (initial weight 2 ± 0.04 mg) and heteroclarias (initial weight 2.5 ± 0.06 mg) were constituted in six triplicate as follow:

C_{zoo}: *Clarias gariepinus* fed with fresh water zooplankton,
C_{art}: *Clarias gariepinus* fed with artemia,
H_{zoo}: *Heterobranchus longifilis* fed with fresh water zooplankton,
H_{art}: *Heterobranchus longifilis* fed with artemia,
HC_{zoo}: heteroclarias fed with fresh water zooplankton,
HC_{art}: heteroclarias fed with artemia.
Larvae were fed three times daily such 7 a.m., noon and 7 p.m. [2].

Production of fresh water zooplankton

Fresh water zooplankton was produced in plastic buckets of 80 liters capacity containing 40 liters of drilling water fertilized with 600 g/m^3 of rabbit manure (optimal dose). Three days after fertilizing, 10 liters of earthen pond of polyculture (Clarias and Tilapia) were filtered carefully using plankton net of 25 µm in order to eliminate zooplankton and then completed to the 40 liters of fertilized water (phytoplankton inoculation). Seventy-two hours after phytoplankton seeding, zooplankton were seeded with an initial density of 20 ind/L. Zooplankton seeded were made of 4 ind/L of nauplii of copepods, 5 ind/L of adult's copepods, 3 ind/L of cladocerans and 8 ind/L of rotifers.

The artemia used

Dry artemia powder (artemia Shell free) is a marine crustacean canned by Aqua-Lush (China, NAFADAC, REG, N°A9-0275) and sold in Beninese markets. According to manufacturer indications, it is made of 58% of protein, 5.8% of ash, 6.0% moisture, 2.2% of fiber and 16% of fat.

Physico-chemical quality of water and rearing ponds maintenance

In order to maintain good rearing conditions, dead fishes were removed, weighted and counted every morning in each pond. Wastes were siphoned before fishes feeding. Temperature, pH, conductivity and dissolved oxygen were controlled daily and *in situ* with a multi-parameter device CALYPSO (Version Soft/2015, SN-ODEOA 2138) at ± 0.1 °C close to mg/L.

Larvae survival and growth

The monitoring of growth was carried out every three days on 200, 45 and 100 larvae respectively of *C. gariepinus*, *H. longifilis* and heteroclarias. At each monitoring process, half part of individuals was caught randomly with a net. The biomass of individuals was taken waterless with a scale (sensitivity 0.01 mg). Dead specimens were counted daily in each pond to evaluate larvae survival.
Recorded data were used to determine the following zootechnical parameters: Mean weight gain (MWG = (final biomass - initial biomass) / rearing duration); Specific Growth Rate (SGR = [ln (final mean weight)-ln (initial mean weight) / rearing duration)] x 100; Survival Rate (SR = final fish number / initial fish number) x 100 and Production Index (mg/j) (PI = Survival Rate x [final weight – initial weight / rearing duration)] [23, 24].

Statistical analysis

Data recorded were analyzed with STATISTICA software (Statsoft inc., Tulsa, OK). Different means were compared by using a one way analysis of variance (ANOVA). All significant levels were fixed at $p < 0.05$. In case of need, significance of differences among means was tested using the test LSD of Fisher.

Results

Despite the acid pH level during experiment period, temperature, conductivity, dissolved oxygen, salinity and TDS in different rearing ponds fluctuated weakly and are mentioned in Table 1.

Table 1: physico-chemical parameters of the water.

Parameters	Variations	Mean and Range
T (°C)	27.30 - 26.02	26.02±0.91
DO (mg/L)	6.70 - 7.40	7.40±0.49
Conductivity (µs/cm)	183.60 - 184.80	184.80±0.85
pH	6.00 - 6.90	6.90±0.64
Salinity (mg/L)	0.10 - 0.11	0.11±0.01
TDS	95.34 - 94.41	94.41±0.66

Zootechnical performances of fish larvae

Zootechnical parameters of different species and hybrids are mentioned in Table 2 with a significant difference ($F_{(25,31,22)}$ = 14.33, p<0.05). The highest survival rates of both two species and hybrids were recorded in C_{zoo} (74%) followed by HC_{zoo} (70%) with a significant difference ($F_{(5,12)}$ = 191.47, p<0.05). In return, there is no significant difference between C_{zoo} (74%) and C_{art} (73.5%) on the one hand and H_{zoo} (60.3%) and H_{art} (60%) on the other hand (p>0.05). Concerning hybrids, the survival rate of HC_{zoo} (70%) is higher than HC_{art} (62%) with a significant difference (P<0.05). So, the survival rates (63.5-74%) of larvae of these two species and their hybrids fed with plurispecific fresh water zooplankton produced by using rabbit manure are comparable to those fed with artemia (60-73.5%). The mean weight gain (MWG) of C_{zoo} (1.08 ±

0.03 mg/day) is higher than C_{art} (0.90 ± 0.08 mg/day) with a significant difference (p<0.05). Besides, the mean weight gain (MWG) of H_{art} (2.87 ± 0.86 mg/day) is higher than H_{zoo} (2.28 ± 0.20 mg/day) with a significant difference (p<0.05). Also, the highest PI was recorded in H_{art} with a significant difference ($F_{(5,12)} = 45.80$, p<0.05). In return, there is no difference between the PI of H_{zoo} and H_{art} (P>0.05). The same trend was observed in hybrids. But, the PI of C_{art} was less than C_{zoo} with a significant difference (p<0.05).

The mean weight of C_{zoo} was higher than C_{art} at the beginning of the third day (fig 2). Those of larvae H_{art} was slightly higher than larvae H_{zoo} (fig 3). Among hybrids, the weight of HC_{art} was higher till the ninth day from which HC_{zoo} became slightly higher (fig. 4).

Table 2: Zootechnical parameters of larvae of *C. gariepinus*, *H. longifilis* and heteroclarias.

Parameters	C_{zoo}	C_{art}	H_{zoo}	H_{art}	HC_{zoo}	HC_{art}
Ni	600	600	135	135	300	300
Nf	451	441	90	82	210	187
SR (%)	74a	73,5a	63,5be	60b	70b	62ac
IMW (mg)	2.5±0.00a	2.5±0.00a	2±0.14a	2±0.14a	2.7±0.1a	2.7±0.1a
FMW (mg)	15.54±0.37b	13.3±0.97a	34.13±2.31e	36.46±1.16f	18.14±0.71cd	16.25±0.52bc
MWG/day (mg/day)	1.08±0.03a	0.90±0.08b	2.28±0.20a	2.87±0.86b	1.28±0.05a	1.12±0.04b
SGR (%/day)	15.22±0.20a	13.91±0.60b	24±1.18c	24.56±0.40c	15.87±0.11b	14.95±0.45ab
PI (mg/day)	80.41±4.42b	66.15±3.75a	170.02±28.10c	172.25±10.30c	90.06±6.01b	70.00±2.19ab

[ab] values bearing same letters on the same line between difference species and hybrids are not significantly different at p<0.05.

Ni = initial number of larvae, Nf = final number of larvae, SR = survival rate; IMW= initial mean weight, FMW = final mean weight, Mean weight gain (MWG), SGR = specific growth rate, PI = production index.

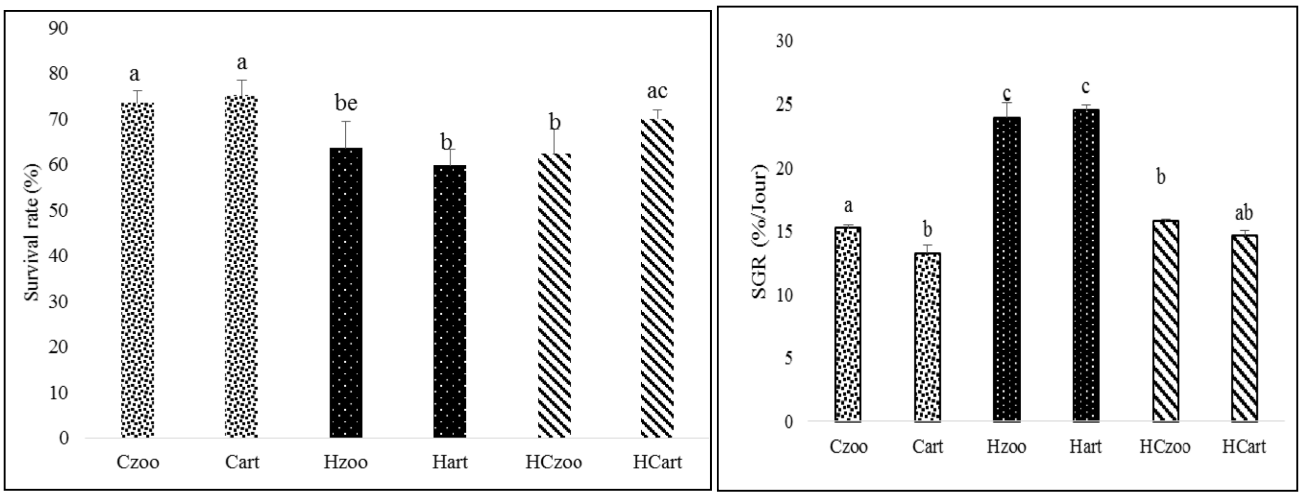

Fig 1: Effect of zooplankton and artemia on the survival and growth of larvae of *C. gariepinus*, *H. longifilis* and heteroclarias for 12 days.

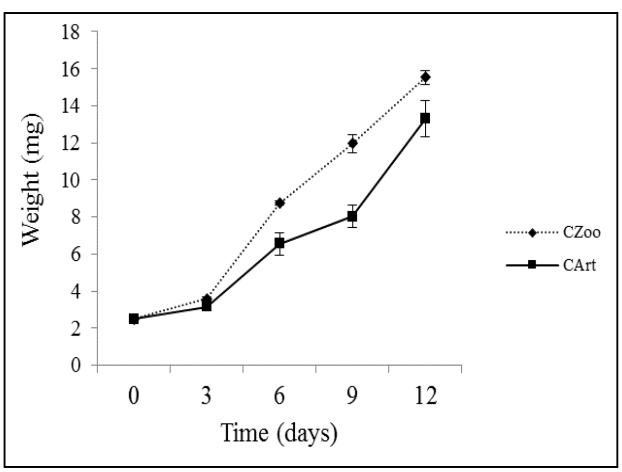

Fig 2: weight progression of *C. gariepinus* larvae fed for 12 days with zooplankton and artemia.

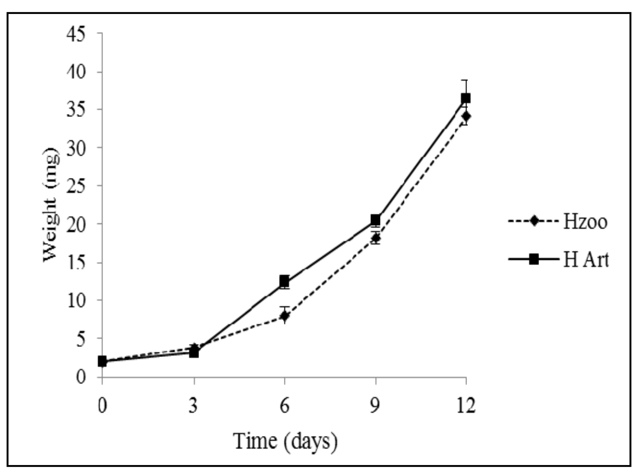

Fig 3: Weight progression of *H. longifilis* larvae fed for 12 days with zooplankton and artemia.

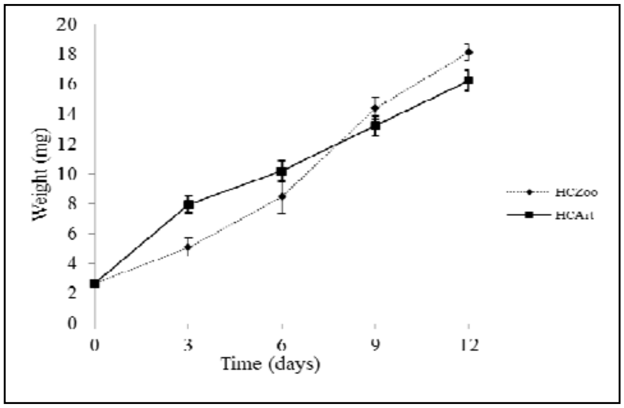

Fig 4: Weight progression of heteroclarias larvae fed for 12 days with zooplankton and artemia.

Discussion

Average values of physico-chemical parameters of the water were in range of those in larval rearing of African Catfish *C. gariepinus* and H. *longifilis* despite constant pH value (6.90±0.64) [25, 26, 3, 18, 2]. The highest survival rate (74%) recorded in *C. gariepinus* larvae fed with plurispecific fresh water zooplankton in pond is less than the 97% obtained by Agadjihouèdé *et al.* [19] in larvae of *C. gariepinus* fed with fresh water zooplankton and the 99.7% recorded by Awaïss *et al*. [13] in larvae of *C. gariepinus* fed with *Brachionus calyciflorus* produced in mono-specific culture. In return, survival rates of 60-74% recorded in this study are in the range of 71-76% obtained respectively by Otémé and Gilles [5] and Atsé *et al.* [27] in larvae of these two African catfish and their hybrids fed with zooplankton. Indeed, the main reasons of survival rate decrease during larval rearing of *C. gariepinus* and H. *longifilis* are either tied to cannibalism or territorial aggression [28]. By the same way, these results are similar to those obtained by Smith *et al*., Folkvord *et al*. and Coulibaly *et al*. [29-31] who recorded that mortality would be due to the presence of initial weak larvae. Besides, in the present study, this mortality might be also due to the acidity of drilling water pH (6 - 6.90). Similar observations were made by Tossavi *et al*. [32] during the rearing of *Schilbe intermedius* fries in the same production systems. The high mortality rate recorded in *H. longifilis* larvae fed with artemia could be explained by larvae fragility during the first stages of development contrary to *C. gariepinus* larvae. Indeed, according to Keremath *et al*. [33], that is the reason of the crossing of these two species.

For 12 days, larvae of *C. gariepinus, H. longifilis* and hybrids fed with zooplankton achieved respectively 15.54 ± 0.37; 36.46 ± 1.16 and 18.14 ± 0.71 mg as mean final weight. *H. longifilis* is a species with more rapid growth than *C. gariepinus* [5, 34]. In addition, according to Otémé *et al*. [5] and Atsé *et al*. [2], food availability (zooplankton) is a determinative factor to growth and height homogeneity of *H. longifilis* larvae thus, they would weight more than *C. gariepinus*. Specific growth rates (SGR) recorded were higher than the 8.9% obtained by Okunsebor *et al*. [15] in larvae of *C. gariepinus* fed with *Brachionus calyciflorus, Ceriodaphnia reticulata* and artemia. By the same way, the SGR recorded in these two species and hybrids (Table 2) are comparable to those obtained in larvae of *Clarias macrocephalus* [35], *Labeo parvus* [11], *H. longifilis* [2], C. *gariepinus* [36] and hybrids [37] fed with dry feed, artemia and live food (fresh water zooplankton). Indeed, that shows the performance of live food used (zooplankton). Although, SGR recorded in hybrids was largely higher to the 0.78%/D obtained in hybrids (*H. longifilis* ♂ x ♀ *C. gariepinus*) by Keremath *et al.* [33]. According to Ndimele *et al*. [38], specific growth rate would be certainly tied to the type of crossing. Good growth performances and survival recorded in larvae of *H. longifilis, C. gariepinus* and hybrids fed with zooplankton in the present study were influenced by the quality of live food. Our results are similar to prior studies that showed the performances were due to good digestibility of live food [39, 2]. Besides, according to Lauff *et al*. [40], the live food (zooplankton) contributes in 80% to the digestion due to the quality and the quantity of protease it contains. Therefore, zooplankton remains the best live food for fish larvae contrary to artificial feed [41-44], what justify good survival performances and growth recorded in the present study. Moreover, the production index (PI) indicates the optimal production performance compared to the survival rate, density, availability and food quality. PI of H_{zoo} and H_{art} respectively 170.02 ± 28.10 and 172.25 ± 10.30 corroborate those 136.28 and 166.28 mg/day recorded by Atsé *et al* [2] in larvae of *H. longifilis* fed three times daily with artemia. These could be due to good growth performances of *H. longifilis* larvae.

Conclusion

Results from the present study showed that larvae of *C. gariepinus, H. longifilis,* and hybrids fed with plurispecific fresh water zooplankton produced using rabbit manure gave good survival rate and growth. Larvae fed with zooplankton gave survival rate ranging 63.5 - 74% and growth rate 15.22 ± 0. 20; 24 ± 1.18 and 15.87 ± 0.11%.day^{-1} respectively for larvae of *C. gariepinus, H. longifilis,* and hybrids. Thus, plurispecific fresh water zooplankton can freely replace artemia during larval rearing of these catfish and their hybrids. It would be important to future studies to determine the influence of the biochemical composition of zooplankton on survival and growth parameters of these larvae and to evaluate these performances with fresh water macroinvertebrates produced by using the same organic fertilizer.

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