ORIGINAL ARTICLE

TOWARDS AN OPTIMUM PROTOCOL FOR FEEDING OF AFRICAN CATFISH, *Clarias gariepinus* (BURCHELL, 1822), LARVAE WITH DIFFERENT TYPES OF ZOOPLANKTONS PER LIFE STAGE

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ABSTRACT

Background: The main factor constraining Catfish farming consists of a chronic lack of fingerlings which is mostly due to very low larval survival rates. **Objective**: In order to alleviate mass mortalities of larvae, zooplanktons were used as preys for *Clarias gariepinus* larvae. **Methods:** A completely randomized design was set up to test the effects of separate feeding with copepods, cladocerans, rotifers and a 1:1:1 mixture of the three types for 20 days. Ingestibility, survival and growth were evaluated for pre-defined spanning life stages labelled 1, 2, 3, 4 and 5 of four days each. **Results:** Rotifers were more ingested by stage 1 and 2 larvae than cladocerans but stage 3 and 4 larvae ingested more of cladocerans. Larval survivals (100%) were not affected by zooplankton types during stage 1 but were significantly sustained by cladocerans during stage 2, 3, 4 and 5. Only in stage 3 did another zooplankton type, rotifers, stand out as second in sustention of survivals. Larval growth was significantly affected right from stage 1, rotifers, cladocerans and mixtures successively taking turns in excelling each other. Although copepods never excelled, they became increasingly important towards the end of the experiment. **Conclusions:** These results were discussed to enable a conception of recommendations in view of optimizing protocols for applying zooplanktons as preys for *C. gariepinus* larvae.
Keywords: *Clarias gariepinus larvae, Zooplankton, Ingestibility, Survival, Growth.*

1. INTRODUCTION

Clarias gariepinus is the world's biologically best aquaculture species [1]. It can accept and thrive on cheap feeds, it has a high growth rate, it can tolerate high densities under culture conditions, it can resist most diseases, it is highly adapted to tropical climates and it fetches high market prices in most sub Saharan African countries [2-4]. Its culture can therefore constitute a basis for enhancement of food security and alleviation of malnutrition and poverty.

However, despite of the breakthrough reported for its artificial propagation [5,6], the demand for fish seed still outstrips the supply [7]. The main factor constraining its culture in Cameroon consists of a chronic lack of fingerlings. This is mostly due to a shortage of larval food which should not only contain the required nutrients but also enzymes for digesting it [8] given that at this early stage, they lack awell-developed digestive system [9]. The brine shrimp, *Artemia* spp. contains both and is thus widely used for rearing commercially important freshwater and marine fish larvae [10,11,12]. However, its high cost (45 USD/kg in Cameroon) and inaccessibility especially in rural zones [13,14] coupled with the fact that being a marine species, it dies in freshwater within two hours due to osmoregulation [15,16] has resulted in the search of financially accessible and suitable freshwater alternatives. Zooplanktons have been viewed as potential alternatives for Artemia as live starter feed for *C. gariepinus* larvae [17,18,19] because of their excellent morphological, behavioural and nutritional characteristics [20]. Theuse of mixed zooplankton [14-21] and specific species of the rotifer group like *Brachionus calyciflorus* [18-20] and *Moina sp* of the Cladoceran group [21,22]have been used as live starter feed for *C. gariepinus* larvae. However, there is paucity of information of the collective effect of species of each of the main zooplankton groups on the survival and growth of *C. gariepinus* larvae.

The objective of this work was to enable alleviation of the mortalities and thus boost availability of fingerlings through testing the effect of separate feeding with each of the zooplankton groups.

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2. MATERIALS AND METHODS

2.1 Study area

The study was carried out at the aquaculture complex (Pure and Applied Zoology Laboratory) of the Department of Animal Biology and Physiology of the University of Yaoundé I, Cameroon.

2.2 Production of African catfish larvae

Gravid brooders used for the experiment were obtained from a local fish farm in Nkoabang, Yaoundé, Cameroon. Larvae of *Clarias gariepinus* were obtained through a method of artificial reproduction described by de Graaf and Janssen (1996) [23].

2.3 Collection, identification and isolation of zooplanktons

Experimental zooplanktons were captured by towing a plankton net (mesh size of 64µm) through a eutrophic lake. A representative sample of the zooplanktons was analysed right down to the species level as described by Braoini et al., (1983), Chiambeng (2004), Dumont and Negrea(2002), Dussart (1980), Dussart and Defaye (1995), Fernando (2000), Smirnov and Timms (1983), Zebaze (2000) [24,25,26,27,28,29,30,31]. Identified copepods comprised Cyclopoids (*Afrocyclops gibsoni*, *Mesocyclops salinus**, Microcyclops sp., *Tropocyclops confinis*) and copepodite nauplii. Cladocerans comprised Chydorids, Moinids, Daphnids, Macrothricids (Table 1) and rotifers comprised Philodinids, Asplanchnids, Brachionids, Colurellids, Lecanes, Mytilinids, Notommatids, Trichocercids,Bdelloids (Table 2).

Table 1: The table presents the species composition of Cladocerans.

Chydoridae	Daphnidae	Macrothricidae	Moinidae
*Chydorus eurynotus**	*Ceriodaphnia cornuta*	*Guernella raphaelis*	*Moina micrura**
*C. globules**		*Macrothrix spinosa*	
Kurzial ongirostris		*M. laticornis*	
K. latissima			
Pleuroxus denticulatus			
Alona guttata			

*: Most ingested species

Table 2:The table presents the species composition of rotifers.

Philodinidae	Asplanchnidae	Brachionidae	Colurellidae	Notommatidae	Trichocercidae
*Rotaria citrine**	*Asplanchna brightwelli**	*Anureopsis fissa*	*Mytilina mitica*	*Cephalodella gibba*	*Trichocerca bicristata*
Rotaria sp.	*A. priodonta*	*Brachionus angularis**	*M. mucronata*	*C. bottgeri*	*T. chattoni*
		*B. calyciflorus**		*Notommata codonella*	*T. stillata*
		*B. falcatus**		*N.pseudocerberus*	*T. tchadiensis*
		*B. quadridentatus**		*Macrochaetus sp.*	*Trichotria tetractis*
		B. leydigi			
		Keratella quadrata			
		Platyias leloupi			
		P. quadriconnis			

*: Most ingested species.

The collected water samples were filtered using 64µm mesh size plankton net and rotifers were retained. The samples were later filtered using a 100µm mesh size net to collect copepods and cladocerans. In order to reduce contamination by unwanted organisms, the various zooplankton group samples were thoroughly rinsed with distilled water.

2.4 Experimental setup

The experimental set-up consisted of a completely randomized design for testing effects of copepods (Treatment 1), cladocerans (Treatment 2), rotifers (Treatment 3) and a 1:1:1 mixture of the three zooplankton groups (Treatment 4), on average ingestibility, survival and growth spanning life stages (5) of four days each of *Clarias gariepinus* larvae. It should be noted that the growth spanning ages defined (1, 2 3, 4 and 5 of every four days each) in the present study was to be able to ease the presentation of results and explanations.

Initial average weight (3mg) of experimental larvae was determined by measuring the parameters of a random sample of 30 three-day old larvae which had just completely absorbed their yolk sacs and were ready for exogenous feeding. Weighing was done by using a sensitive electronic balance (Sartorius, ±1mg).

Prior to experimentation, nursing water (3liters) contained in each of twelve plastic basins was exposed to air for 24hours to favour vaporization of chlorine usually used to sterilize the water. Each basin was then stocked with fifty larvae corresponding to a density of almost17larvae/litre. Thereafter, three of the basins, chosen at random, were assigned to each of the four treatments. Throughout the experiment (20 days), water temperature, pH, dissolved oxygen, and total dissolved solids (TDS) as monitored every four days, remained acceptable for larval rearing [20-32, 33].Water temperature varied from 24.8 to 25°C, pH revolved around neutrality (7CU), dissolved oxygen ranged from 5.18 to 7.06mg/l while total dissolved solids ranged from 51.33 to 63.1mg/l.

For feeding of larvae, the zooplanktons were captured every other day and separated into copepods, cladocerans and rotifers, using a micropipette and a pair of binoculars (WILD M5). Thereafter, 10ml of separated concentrate, respectively averaging 362 copepods, 3134 cladocerans and 2816 rotifers; were collected with a syringe and thoroughly washed with tap water prior to feeding [14] larvae of Treatments 1, 2 and 3 respectively. For feeding Treatment 4 with mixed zooplanktons, 3.3ml from each of the separate concentrates, containing a total of about 2018 zooplanktons, was used. All larvae were fed thrice a day (07H00, 15H00, and 18H 00). Morning rations were applied after cleaning of holding basins and partial (about 66%) replacement of holding water with fresh one. Cleaning and reduction of water were done by siphoning with a rubber tube (2mm).

2.5 Data collection and analysis

For determination of ingestibility, the number of zooplanktons remaining in the holding basins per day was estimated by counting the number contained in homogenized samples (10ml) of holding water. Ingestibility was defined, within the context of this work, as the ratio of the difference between the zooplankton number that was injected the previous day (ni) and the number that was found the following day (nf) to the number that was injected (ni):

$$\text{Ingestibility} = ((ni-nf)/ni)x\ 100 \qquad (1)$$

It should be noted that copepods were difficult to capture due to their dodgy character, hence their leftovers were only estimated on the last day of the experiment. Therefore, it was not possible to determine ingestibilities for treatments 1 and 4- it was only done for treatments 2 and 3. Dead larvae removed on a daily basis were counted to enable calculation of daily survivals per treatment. Overall, survivals were calculated after counting all the larvae (nf) remaining on day 20 according to the following formula:

$$\text{Percentage survival} = (Nf/50)\ x100 \qquad (2)$$

As for growth performance, 10 larvae were randomly sampled every four days, from each basin and their weights at the beginnings and ends of each stage (Wt_1 and Wt_2) were measured using an electronic balance (Sartorius, ±1mg). Specific growth rates (SGR) were then calculated according to the following formula:

$$SGR = ((Ln\ Wt_2 - LnWt_1)\ x\ 100)/20 \qquad (3)$$

Ingestion, survival and growth parameters of the various treatments were subjected to ANOVA using SPSS (version 17.0). Differences were considered significant at $p<0.05$.

3. RESULTS

The ingestibilities of rotifers and cladocerans by *Clarias gariepinus* larvae per stage are shown in Table 3. Rotifer ingestibilities were negatively correlated (correlation factor = -0.49) with the age of larvae while those of cladocerans were positively so (correlation factor=+0.12). The lowness of the correlation factors can be explained by the facts that the decreasing trend of rotifer ingestibility was reversed at a larval age of 8-12 days (growth spanning ages 1,2 and 3) while the increasing trend of cladocerans ingestibility was reversed at a larval age of 13-23 days (growth spanning ages 4 and 5).

Table 3: The table presents the ingestibilities of cladocerans and rotifers by *C. gariepinus* larvae per four-day period.

Larval Age	Rotifers	Cladocerans
4 - 8 days	83.5	72.77
9 - 13 days	76.75	75.21
14 - 17 days	86.06	95.84
18 - 21 days	78.03	81.42
22 - 25 days	75.94	72.12

The effect of feeding with copepods, cladocerans, rotifers and a 1:1:1 mixture of the three zooplankton groups on the survival of *C. gariepinus* larvae is shown in Figure 1.

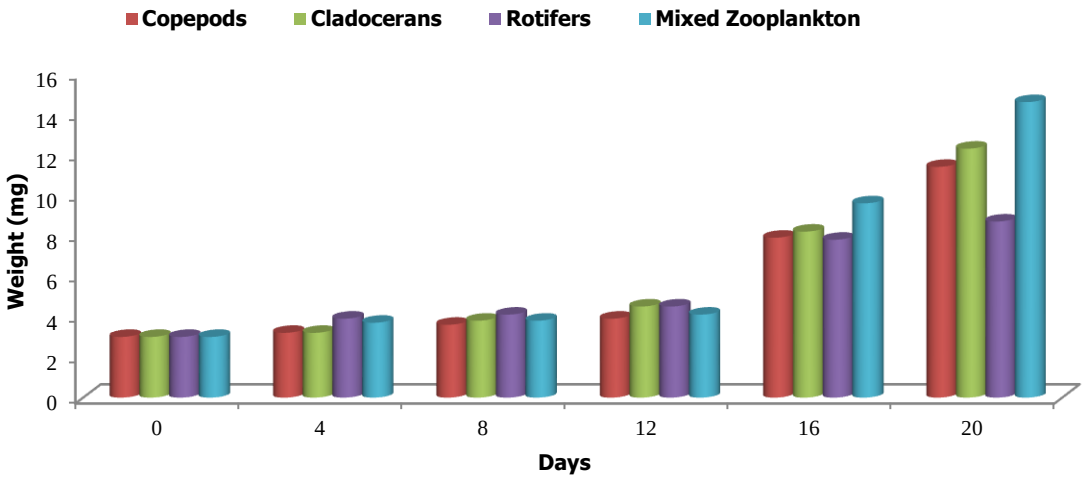

Figure 1: The figure presents the effect of feeding with copepods, cladocerans, rotifers and mixed zooplanktons on survival (%) of *C. gariepinus* larvae per period of four days.

Irrespective of treatments, survival remained 100% for larvae of upto9 days old (growth spanning ages 1 and 2) and dropped gradually for those of 10 and 11 days old (growth spanning age 3), with the sharpest drops being registered at an age of 12 days old. Not until13 days did treatments begin to affect larval survival? Cladocerans-fed larvae suffered the least mortality while copepod-fed ones suffered the greatest. This trend of significantly higher survival ($p<0.05$) of cladoceran-fed larvae was maintained right up to an age of 23 days (growth spanning age 5). Ingestion of cladocerans thus significantly sustained the survival of larvae. With respect to survival sustention, rotifers seconded cladocerans during stage 3. Irrespective of the stage, there were no significant differences ($p>0.05$) in the survival-sustention effects of copepods and mixtures.

The effect of the treatments on the growth of *C. gariepinus* larvae per stage is presented in figures 2 and 3. Rotifers conferred a significantly higher ($p<0.05$) SGR to larvae of 3 to 7 days old than all the other treatments. Cladoceran-conferred SGRs were significantly higher ($p<0.05$) for larvae of 8 to 16 (growth spanning ages 2, 3 and 4) days old, a rank which was taken over by mixture conferred SGRs for larvae of 16 to 23 days old(growth spanning ages 4 and 5). In general, rotifers, cladocerans and mixtures successively took turns in excelling each other. They were respectively seconded by mixtures, copepods and cladocerans. Over the 20-day experimental period, attained final weight and specific growth rate decreased as diet changed from mixed zooplankton through cladocerans and copepods to rotifers.

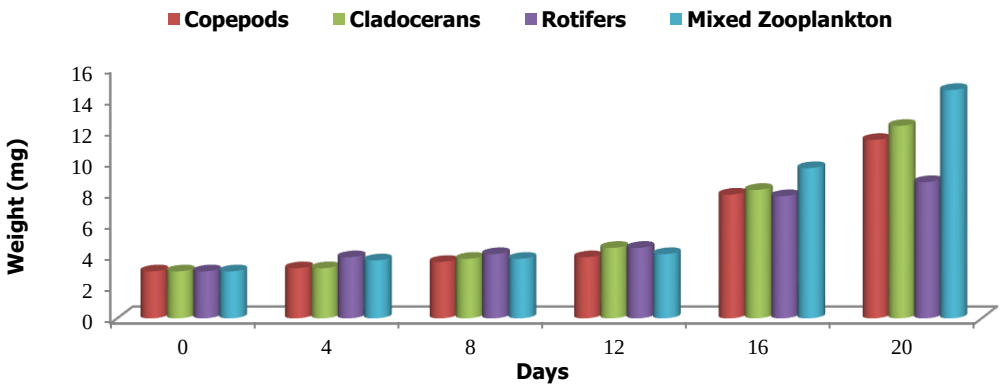

Figure 2: The figure presents the effect of feeding with copepods, cladocerans, rotifers and mixed zooplanktons on weight (mg) gain by *C. gariepinus* larvae per four-day period.

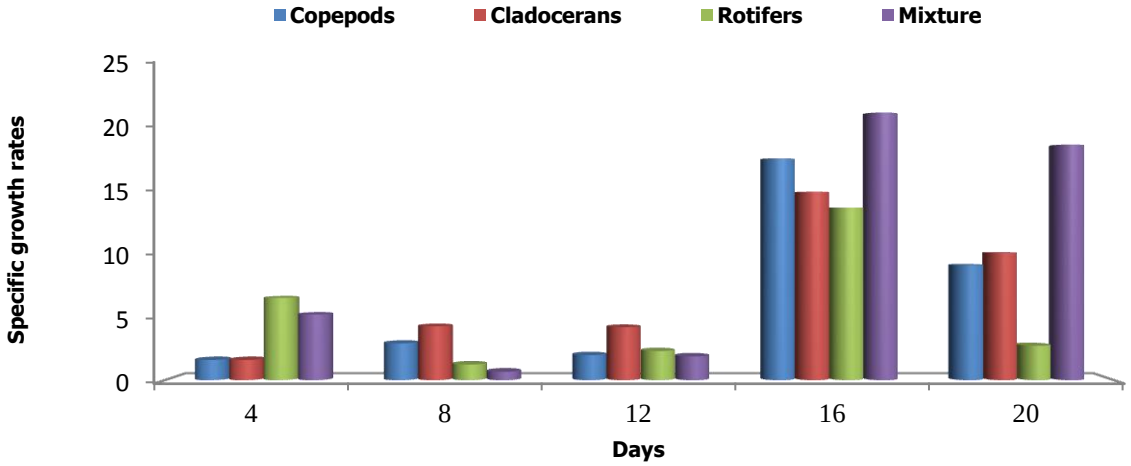

Figure 3: The figure presents the effect of copepods, cladocerans, rotifers and mixed zooplankton on the evolution of specific growth rates (%) of *C. gariepinus* larvae with time.

4. DISCUSSION

Reversals of rotifers and cladocerans ingestibilities with respect to growth spanning ages are believed due to larvae's non-selective filter feeding habit and to the capacity of more developed larvae to also ingest copepods. It would appear that *Clarias gariepinus* larvae kept ingesting more cladocerans than rotifers until they were also able to prey on the dodgy copepods. This preference for larger preys in spite of their high swimming activity could be attributed to the high swimming activity and predation of the fish larvae as well [34,35].

Results demonstrated for the first time that the survival and growth of zooplankton-fed *C. gariepinus* larvae can only be optimized if the kinds of the zooplanktons are tailored to specific life stages of larvae. While survival of up to two-week-old larvae seemed to be more dependent on extra experimental factors, those of more than two-week-old portrayed cladocerans as the best feed. This was corroborated in terms of growth as it excelled the other diets for larvae of 7 to 15 days old and remained competitive for larvae of 16 to23 days old. However, it should be noted that rotifers excelled for larvae of 3 to 6 days while mixtures excelled in those of 16 to 23 days. That the suitability of rotifers for 3 to 6 days old larvae was closely seconded only by the mix diet in which they were also present underscores their importance as preys for this stage of larvae. Such larvae should therefore be fed with pure rotifers or with diets in which they are abundantly represented. This was in conformity with the works of Agadjihouèdéet al., (2012)[14] who used a mixture of the three zooplankton groups to feed *C. gariepinus* larvae with rotifers representing the highest percentage (78%).

That rotifers stood out as the best zooplankton preys at the onset of exogenous feeding confirms the findings of Arimoro, Awaïss et al., (1993) and Wang et al., (2005) who observed that freshwater rotifers can be successfully used as starter feed for *C. gariepinus* larvae [20,36,37]. This can also be attributed to the match between their small sizes and the small mouth gapes of the larvae. It would appear that whenever gapes can permit, larvae will go for bigger than for smaller preys [38], even abstaining from smaller preys at the risk of starving as indicated by the negative correlation between ingestion of rotifers and age of larvae here observed.

That cladocerans were not so consumed at the beginning and that they took over as the best preys for larvae of 7 to 14 days old could have resulted from the match between their sizes and mouth gapes of larvae. Therefore, larvae of 7 to 14 days old should be fed with cladocerans. These findings are similar to the works of Adejoke (2015) who reported that *C. gariepinus* larvae fed with cladocerans exhibited good growth performance and survival [39].

The confirmation of the decisiveness of the match between prey size and mouth gape came from the fact that copepods conferred higher and higher growth rates as the larvae grew older and older (16 to 23 days old). This growth performance of copepod-fed larvae could be due to the better nutritional value (higher essential fatty acids) when compared to the other live feeds [40]. The relative performance of copepods and cladocerans during this period suggests that it is mostly thanks to the two that the mix diet performed best. The African catfish *C. gariepinus* larvae of 16 to 23 days old should therefore prefer larger preys like cladocerans and copepods. This preference for larger size preys has also been observed in *Heterobranchus longifilis* larvae, in juvenile Pollock (*Theragrachalco gramma*) and in larval yellow perch by Ajah (2010), Brodeur (1998) and Graeb et al., (2004) respectively [41,42-38]. The question why predators should prefer larger preys to many small one amounting to the same weight begs for further research. Growth performance obtained for mixed zooplankton- fed larvae was more important than those reported by Awaïss et al., (1993) and Agadjihouèdé et al., (2012) [36-14].

The mass mortalities of week-old *C. gariepinus* larvae reported by Yong-Sulem (2011) recurred in this experiment regardless of the treatment [21]. Had it been due to clogging of gills or physical attack suspected by Yong-Sulem (2011), treatment 3 with only rotifers which neither clog nor attack would not have suffered them [21]. Even treatment 2 with only cladocerans would have been spared. Only the large sized copepods have been reported to constitute a nuisance of any sort to larvae: -Schäperclaus (1992) reported that fish larvae are generally attacked by adult copepods and advanced copepodite stages resulting in serious lesions of fins, head, nares and particularly the gills [43]. Therefore, the hypothesis of Yong-Sulem (2011) does not apply to all groups of preys [21]. The mortalities observed by Yong-Sulem (2011) and in the present experiment are more likely to do with lack of nutrients by certain species of zooplanktons from certain biotopes [21]. This is corroborated by the fact that zooplanktons from a similar source (hyper-eutrophic reservoir) equally sustained low survival of *C. gariepinus* larvae, 31% as early as 10 days old [34]. According to Agadjihouèdé et al., (2012), good survival and growth performance of *C. gariepinus* larvae fed with freshwater zooplankton results from the excellent digestibility and good nutrient quality of the prey [14].

Ajah (2010) proved that the gustatory and chemoreceptor organs of *C. gariepinus* larvae were developed during the second week of life and it is thought that such development requires more nutrients than were present in those of un-enriched zooplanktons [41]. To avoid survival crashes during the period of organogenesis, prey zooplanktons should be enriched. Such enrichment is known to more than double survival of African catfish *C. gariepinus* [20] and thought to be able to optimize both survival and growth if compounded with tailoring to specific life stages.

5. CONCLUSION

These results demonstrated that the survival and growth of zooplankton-fed *Clarias gariepinus* larvae can only be optimized if the kinds of the zooplanktons are tailored to specific life stages of larvae. Rotifers were the best preys of 3 to 6 day old larvae, followed by cladocerans of 7 to 14 day old larvae and as they grow older a mixture of cladocerans and copepods of 16 to 23 day old larvae should be given as proven by their high growth and survival performances. The low survivals observed during this experiment could be attributed to the poor nutrient quality of the preys. In order to avoid such survival crashes, preyed zooplankton should be enriched as such enrichment has shown to double survival of the African catfish *C. gariepinus*. Experiments are required to optimize enrichment regimes for desired intends and purposes.

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