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SURVIVAL AND GROWTH OF *CLARIAS GARIEPINUS* LARVAE FED WITH FRESHWATER ZOOPLANKTON FROM PIG DUNG

SURVIE ET CROISSANCE DES LARVES DE *CLARIAS GARIEPINUS* NOURRIES AVEC DU ZOOPLANCTON D'EAU DOUCE PRODUIT A PARTIR DES DEJECTIONS DE PORC

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ABSTRACT

Survival and growth performance of *Clarias gariepinus* larvae fed with freshwater zooplankton was compared to those fed with *Artemia salina*. *Clarias gariepinus* larvae at the end of yolk sac resorption with 2.8 ± 0.1 mg initial weight were fed *ad libitum* live zooplankton for 08 days in concrete basins (without water renewal). These latter were regrouped in two treatments (T_1 and T_2) of three replicates each. The treatment T_2 basins were previously fertilized with pig dung for mass production of freshwater local zooplankton. The treatment T_1 larvae were fed with *Artemia* nauplii whereas those of T_2 were fed with freshwater local zooplankton. The average final weight and the specific growth rate of larvae fed with *Artemia* (respectively 9.74 ± 0.20 mg and 15.49 ± 0.26%.day^{-1}) were significantly different ($p < 0.05$) from those raised with freshwater zooplankton (respectively 8.93 ± 0.31 mg and 14.40 ± 0.43%.day^{-1}). Freshwater local zooplankton produced with pig dung was proved efficient for feeding early stage of African catfish *clarias gariepinus* larvae rearing, in fertilized concrete basin without water renewal. It could be used in substitution to the *Artemia* which was not accessible to rural pisciculturists.

Key words: *Clarias gariepinus*, freshwater zooplankton, growth, pig dung, survival.

RESUME

Les performances de survie et de croissance des larves de *Clarias gariepinus* nourries au zooplancton d'eau douce ont été comparées avec celles des larves nourries à *Artemia salina*. Des larves de *Clarias gariepinus* en fin de résorption du sac vitellin de poids initial de 2,8 ± 0,1 mg sont nourries *ad libitum* au zooplancton vivant pendant 08 jours dans des bassins en béton (sans renouvellement d'eau). Ces derniers sont regroupés en deux traitements (T_1 et T_2) de trois réplicas chacun. Les bassins du traitement T_2 sont préalablement fertilisés avec des déjections de porc pour une production massive du zooplancton local d'eau douce. Les larves du traitement T_1 sont nourries aux nauplii d'*Artemia* tandis que celles du traitement T_2 sont nourries au zooplancton local d'eau douce. Le poids final moyen et le taux de croissance spécifique des larves nourries à *Artemia* (respectivement de 9,74 ± 0,20 mg et 15,49 ± 0,26%.j^{-1}) sont significativement différents ($p < 0,05$) de ceux des larves élevées avec du zooplancton d'eau douce (respectivement de 8,93 ± 0,31 mg et 14,40 ± 0,43%.j^{-1}). Le zooplancton local d'eau douce produit avec les déjections de porc s'est révélé efficace pour l'alimentation des premiers stades de l'élevage larvaire du poisson-chat africain *Clarias gariepinus* dans des bassins fertilisés sans renouvellement d'eau. Il peut donc être utilisé en substitution de l'*Artemia* qui n'est pas accessible aux pisciculteurs ruraux.

Mots clés : *Clarias gariepinus,* croissance, déjections de porc, survie, zooplancton d'eau douce.

INTRODUCTION

Larviculture is a very important step in the development of fish production species in controlled conditions (AWAÏSS *et al.*, 1993). It allowed obtaining continuous fingerlings which were provided to pisciculturists or destined to the repopulating of natural medium. Yet, one of the major obstacles in the rearing and marketing of African catfish is the availability of fry and juveniles in continuous (HOGENDOORN, 1980; UYS & HECHT, 1985; LEGENDRE, 1991; OTEME *et al.*, 1996; TABARO *et al.*, 2005). In fact, the larval period which is a transition between the endogenous and exogenous feeding constituted a critical stage in the life of these fish (OGUNREMI & OBASA, 2009).

The main difficulty resides in the choice and availability of appropriate food having a good nutritious quality. But the best survival and growth performances of fish larvae were obtained with the utilization of natural food, living (zooplankton). Because, the use of artificial food (integral part of the modern fish farming) for catfish fry production showed its limits (ADEYEMO *et al.*, 1994 ; VERRETH, 1994 ; EVANGELISTA *et al.*, 2005 ; MANDAL *et al.*, 2009 ; CONÇEICAO *et al.*, 2010 ; MOHSENI *et al.*, 2012). *Artemia* is an excellent live food (good nutritious quality) for the freshwater fish larviculture including the catfish (POLLING *et al.*, 1988; AWAÏSS *et al.*, 1993; KERDCHUEN & LEGENDRE, 1994; AJAH, 1997; LEGENDRE & LEVEQUE, 1997; GARCIA-ORTEGA *et al*, 2000; OLURIN & OLOUWO, 2010). Because of its high cost and non-availability in developing countries, particularly for the fish farmer of rural areas (KESTEMONT & AWAÏSS, 1989; IMOROU TOKO *et al.*, 2008), it was then necessary to search local live food of substitution which will be easily accessible and at lower cost for the pisciculturists. For this, some studies of *Clarias gariepinus* larviculture were realized in aquarium with freshwater zooplankton (AWAÏSS *et al.*, 1993; AGADJIHOUEDE *et al.*, 2012). Those different studies will be difficultly applicable by the rural pisciculturists because of the zooplankton monospecific culture provided as the live food and the circulation in continuous of water during the larval rearing. That justifies the realization of the present study in order to develop a simple technique of *Clarias gariepinus* larviculture in basins (without water renewal) of local zooplankton plurispecific production from pig dung. The main objective of the present paper was to compare the survival and growth performances of *Clarias gariepinus* larvae fed with local freshwater zooplankton with those of larvae fed with *Artemia*.

MATERIALS AND METHODS

Source of *Clarias gariepinus larvae*

Larvae of African catfish *Clarias gariepinus* used were obtained by artificial reproduction of captive broodstock at wetlands research station, University of Abomey-Calavi, Benin. Two females (700 g per female) were hormonally induced to spawn using Ovaprim SYNDEL International Inc. Canada (0.5 $ml.kg^{-1}$ body weight). Nine hours after the induction of females, maintained at 28°C, the eggs, reached maturity, were harvested by stripping and fertilized with laitance of two males (500 g per male). These fertilized eggs were incubated in plastic buckets covered with mosquito net and placed in hatcheries according to the procedure described by VIVEEN *et al.* (1985). Hatching was noticed 26 h after incubation at 27°C.

Mass production of freshwater zooplankton

Freshwater zooplankton was mass produced in 06 concrete basins (0.8 x 0.8 x 0.6 m). Two days before the start of experiment, the basins were washed with bleaching water and dried. Each tank was filled with 300 liters of water (drilling water and pond water) and fertilized with 600 $g.m^{-3}$ of pig dung (AKODOGBO *et al.*, 2014b). In fact, 200 liters of drilling water were poured in each basin and fertilized. Three days after fertilization, those basins were seeded in phytoplankton with 100 liters green water of pond filtered under of 50 µm plankton net. Three days after (D_0), zooplankton were harvested in pond with the plankton net of 50 µm and then concentrated in three pill bottles of 100 ml. Each basin was seeded in

zooplankton with 15 ml of each pill concentrate (45 ml); an under-sample of 10 ml of concentrate of each pill was fixed with 5% formaldehyde for a counting under a light microscope (PIERRON, S/N S 294452/ X 4). Thus, each basin had been seeded with an initial density of 79 ind.l^{-1} (28 ind.l^{-1} of rotifers, 44 ind.l^{-1} of copepods and 7 ind.l^{-1} of cladocerans).

Production of *Artemia salina*

Artemia salina nauplii were obtained from the incubation of dry cysts, provided by GSL-INT *Artemia* LLC (USA), under optimal conditions according to manufacturer's protocol. The hatching happened 24 h after incubation. The airing was stopped and the light was moved down the incubation tanks (conical) for 15 mn. Then, the wastes (non-hatched cysts and the cockle of hatched cysts) deposited at the bottom of tanks were evacuated by a quickly game of opening and closing the out gate. N*auplii*, grouped at the bottom of tanks, were finally harvested with a sieve of 100 µm.

Experimental plan

Nine concrete basins (0.8 x 0.8 x 0.6 m); containing each 300 liters of non-renewed water, 06 of which previously fertilized for the freshwater local zooplankton mass production were used. Larvae aged two days (D_2 after hatching), of *Clarias gariepinus* were transferred in 06 basins (03 fertilized and 03 non-fertilized) twelve days (D_{12}) after the seeding in zooplankton (AKODOGBO *et al.*, 2015b). The fertilized basins contents were filtered with a silk of 500 µm, 24 h before larviculture starting in order to eliminate big size organisms, larvae potential predators and stimulate rotifers production (BONOU *et al.*, 2015).

Two treatments (T_1 and T_2) were applied to these six laviculture basins which received each 150 *Clarias gariepinus* larvae (individual mean weight 2.8 ± 0.1 mg) fed *ad libitum* with living zooplankton during 08 days (VERRETH & VAN TONGEREN, 1989). The larvae of treatment T_1 were fed with *Artemia* nauplii four times a day (08 h, 12 h, 16 h and 20 h); those nauplii were rinsed with water before being served to larvae. The ones of treatment T_2 were fed with local zooplankton content in the culture medium. Every two days after the transfer of larvae, each basin of treatment T_2 received the 50% of the zooplankton population harvested in a basin of zooplankton mass production. These basins were refertilized every three days with the third of the initial dose (200 g.m^{-3}) of pig dung (AKODOGBO *et al.*, 2015b). The average zooplankton density in treatment T_2 basins the day of larvae loading was 1974 ± 167 ind.l^{-1} zooplankton per basin and was composed of 74% of rotifers, 21% of copepods and 5% of cladocerans.

Follow-up of water physico-chemical parameters

The physico-chemical parameters such as pH, temperature and dissolved oxygen in the water of larvaculture basins were measured, *in situ*, three times a day (8 h, 12 h and 17 h) and every two days. They were respectively appreciated with a pHmeter ATC (pH-009-III - Temperature Display) and an Oxymeter ANNA (HI 9143 Microprocessor Auto Cal Dissolved Oxygen Meter). Likewise, 500 ml of water from each basin were taken into plastic bottles for diverse chemical analyses (ammonium, nitrates and nitrites test, respectively by methods of Nessler-380, to Cadmium-335 reduction and Diazotation-371) with the spectrophotometer HACH.

Larvae growth and survival Rates

Larvae growth control was carried out every 02 days by an aleatory sampling of 20 larvae per basin, 60 larvae per treatment, which were weighed. At end of raising (D_{10} after hatching), all the larvae were weighed after passing them quickly through rag pulp to eliminate the body water weight. An electronic scale (Proscale – HC-600AX), sensitive at 0.01 g was used to take the weight of larvae. The dead larvae were taken out every day and counted. At the end of the experimentation, a systematic counting of all the larvae was made in order to evaluate the effect of treatments over their survival. The survival rate (S), daily weight gain (DWG) and Specific Growth Rate (SGR) were calculated as follow:

S (%) = 100 x Nf/Ni with Ni = initial Number

of individuals and Nf = final Number of individuals.
GDW ($mg.day^{-1}$) = (Wf - Wi)/t with Wi = initial Weight, Wf = final Weight, and t = time in days.
SGR ($\%.day^{-1}$) = [100 (ln (Wf) –ln (Wi)]/t with ln = neperian logarithm.

Statistical analyses

The statistical analysis of obtained results was performed with statistic logical SAS version 9.2 by analysis of variance method with one classification criteria (ANOVA I) (SCHERRER, 1984; DAGNELIE, 1984). The LSD (Least Significant Difference) of Fisher (SAVILLE, 1990) was used to compare the different average of growth and survival performances data. The hypothesis null is at every time rejected to 5%.

RESULTS

Table 1 summarizes the physico-chemical parameters of rearing medium of *Clarias gariepinus* larvae fed with *Artemia salina* nauplii and freshwater local zooplankton.

Table 1: Physico-chemical parameters of rearing medium of *Clarias gariepinus* larvae fed with *Artemia salina* nauplii and freshwater local zooplankton.

	pH	Temperature (°C)	Dissolved Oxygen ($mg.l^{-1}$)	$N0_3^-$ ($mg.l^{-1}$)	$N0_2^-$ ($mg.l^{-1}$)	NH_4^+ ($mg.l^{-1}$)
Artemia	6.87 ± 0.40^{a}	28.53 ± 1.46^{a}	6.33 ± 1.2^{a}	5.28 ± 0.25^{a}	0.035 ± 0.003^{a}	0.107 ± 0.098^{a}
Local Zooplancton	7.92 ± 0.62^{b}	28.27 ± 1.5^{a}	5.87 ± 1.3^{b}	9.34 ± 1.58^{b}	0.077 ± 0.008^{b}	0.163 ± 0.086^{b}

The values affected with the same letter in exponent on the same line were not significant different ($p > 0.05$).

Much fluctuations were not observed in the evolution of temperature, according to the table 1, during the experimentation; the average of temperatures of all the basins was 28.5 ± 1.5°C. The water pH mean value was more important in the fertilized basins (7.2 ± 0.62). The average of concentrations of dissolved oxygen was higher in non-fertilized basins (6.03 ± 1.2 $mg.l^{-1}$) whereas those of dissolved salts were higher in fertilized basins.

Table 2 summarizes the survival and growth performances of *Clarias gariepinus* larvae fed with *Artemia salina* nauplii and freshwater local zooplankton.

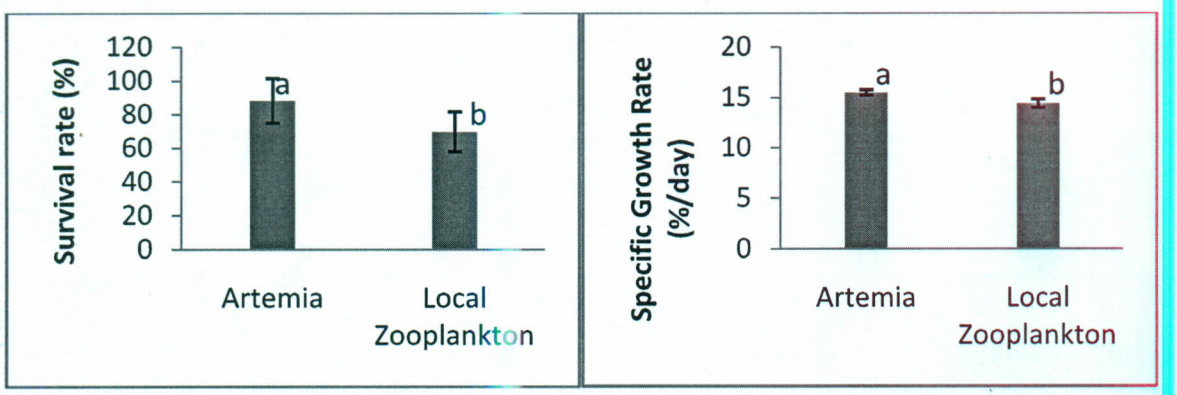
Table 2: Survival and growth of *Claris gariepinus* larvae fed With *Artemia salina* and freshwater local zooplankton

Parameters	*Artémia Salina*	Local zooplankton
Initial larvae number	150 ± 00^{a}	150 ± 00^{a}
Final larvae mean number	132.33 ± 19.86^{a}	104.67 ± 17.78^{b}
Survival (%)	88.22 ± 13.24^{a}	69.78 ± 11.86^{b}
Initial larvae mean weight (mg)	2.82 ± 0.10^{a}	2.82 ± 0.10^{a}
Final larvae mean weight (mg)	9.74 ± 0.20^{a}	8.93 ± 0.31^{b}
Mean weight gain per day ($mg.day^{-1}$)	0.86 ± 0.02^{a}	0.76 ± 0.04^{b}
Specific Growth Rate ($\%.day^{-1}$)	15.49 ± 0.26^{a}	14.40 ± 0.43^{b}

Survival and growth of *Clarias gariepinus* larvae fed with freshwater zooplankton from pig dung.

The values affected with the same letter in exponent on the same line were not significant different ($p > 0.05$).

Figure 1 presents the growth and survival of *Clarias gariepinus* larvae fed with *Artemia* and freshwater local zooplankton



a- Survival b- Specific Growth Rate

Figure 1: Effect of *Artemia* and freshwater local zooplankton on the survival and growth of *Clarias gariepinus* larvae reared during 08 days.

According to table 2 and figure 1, the survival rate is very high in larvae fed with *Artemia* (88.22 ± 13.24%) from those fed with freshwater local zooplankton (69.78 ± 11.86%). The larvae fed with *Artemia* had a final weight (9.74 ± 0.20 mg) more important than the ones fed with local zooplankton (8.93 ± 0.31 mg). The specific growth rate (SGR) of larvae feeding *Artemia* (15.49 ± 0.26%.day^{-1}) was slightly higher than those of larvae fed with local zooplankton (14.40 ± 0.43%.day^{-1}). There was a significant difference ($p < 0.05$) between the survival and growth performances of the larvae of two treatments.

The figure 2 shows the body average weight evolution of *Clarias gariepinus* larvae fed with freshwater local zooplankton and *Artemia* in function of time.

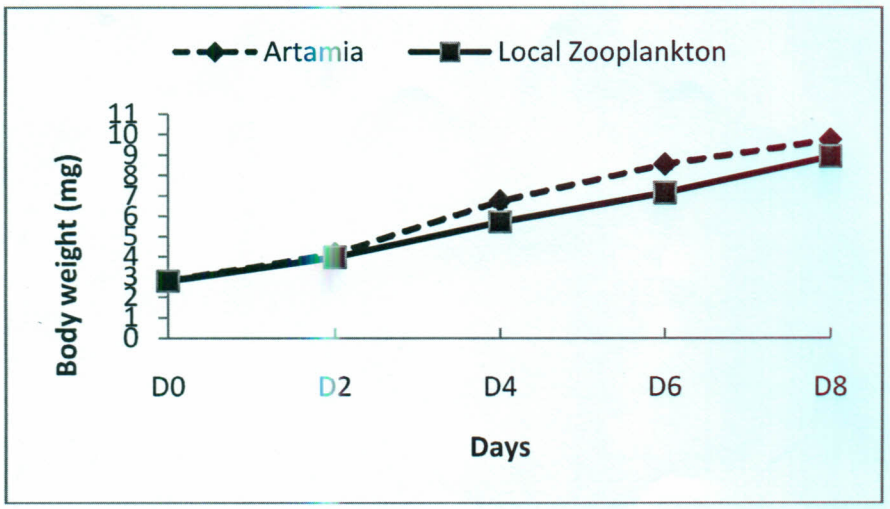


Figure 2 Body average weights evolution of *Clarias gariepinus* larvae feeding with freshwater local zooplankton and *Artemia* in function of time.

The average weights of produced larvae increased linearly during all the period of

experimentation. In fact, during the first two days of rearing, the larvae almost have the same body weight. But after the second day, the body weights of larvae fed with *Artemia* were more important to those raised with local zooplankton, until the end of the experiment.

DISCUSSION

Physico-chemical parameters of larviculture basins water were conform to the values allowing a good survival and growth of *Clarias gariepinus* larvae (TICLER *et al.*, 1979 ; SCHWELDER *et al.*, 1985 ; LEGENDRE & TEUGELS, 1991 ; LUQUET *et al.*, 1993 ; AWAÏSS & KESTEMOND, 1998 ; GILLES *et al.*, 2001 ; AGADJIHOUEDE *et al.*, 2014).

The difference between survival rates of *Clarias gariepinus* larvae fed with freshwater local zooplankton (69.78 ± 11.86%) and those fed with *Artemia* nauplii (88.22 ± 13.24%) was due to the quality of culture medium. In fact, the larvae rearing basins of treatment T_2 were fertilized with pig dung whereas treatment T_1 were not. This led the increase of dissolved salts in fertilized basins due to the fertilizer mineralization (AKODOGBO *et al.*, 2014a). Survival rate of larvae fed with freshwater local zooplankton was better than the one obtained by AGADJIHOUEDE *et al.* (2014) after 07 days of *Heterobranchus longifilis* larvae rearing in basins fertilized with chicken droppings (52 ± 0.49%) at a density of 0.5 larvae/liter. This low survival rate of *H. Longifilis* was explained by the lack of food and the predation by the macroinvertebrates. Likewise it was the double of the one obtained by YILMAZ *et al.* (2006) during the feeding of *Clarias gariepinus* larvae with freshwater zooplankton (31.20%) for 07 days. The presence of big size organisms explained the low survival rate in the feeding of larvae whereas our zooplankton productions were richer in small size organisms, mostly the rotifers. Our results were inferior to those obtained by AGADJIHOUEDE *et al.* (2012) during the feeding of *Clarias gariepinus* larvae with *Artemia* (98%) and freshwater zooplankton (97%) in aquarium. They were also low compared to the ones obtained by AWAÏSS *et al.* (1993) during the *Clarias gariepinus* larviculture with the freshwater rotifer, *Brachionus calyciflorus* (99.7%) in aquarium. These differences were due to the experimental conditions. In fact, the larvae reared in aquarium were disposed in the laboratory with the continuous renewal of water which was oxygenated whereas the ones rearedin the basins were displayed at free air and without water renewal.

The final average weight of *Clarias gariepinus* larvae fed with the local zooplankton (8.93 ± 0.31 mg) during the experimentations was conform to the one obtained by AWAÏSS & KESTEMONT (1998) after 07 days of *Clarias gariepinus* larvae feeding with freshwater rotifers, the *Brachionus calyciflorus*. This confirmed the high density in rotifers of zooplankton population in the rearing basins of treatment T_2. This weight was superior to the one obtained by Uys and Hecht (1985) during the feeding of *Clarias gariepinus* larvae with the live food for 08 days (06.81 mg). That difference was explained by the presence of Chironomidae larvae some of which were fish larvae predators. Likewise, it was better to the one obtained by YILMAZ *et al.* (2006) during the feeding of *Clarias gariepinus* larvae with local zooplankton (4.50 ± 0.42 mg) for 07 days. It was due to the presence of big size zooplankton organisms which were not accessible to fish larvae. However, that weight was inferior to the one obtained by AGADJIHOUEDE *et al.* (2012) during the feeding of *Clarias gariepinus* larvae with local zooplankton (11 ± 0.003 mg) for 10 days. That difference was due to the duration and environmental conditions of the experiments.

The specific growth rate of the larvae fed with freshwater local zooplankton (14.40 ± 0.43%.day^{-1}) was higher than the one of *Clarias gariepinus* larvae reared for 10 days, with freshwater zooplankton (11.28 ± 0.3%.day^{-1}) by AGADJIHOUEDE *et al.* (2012). That difference was due to the experimental duration. In fact, after 08 days of rearing, the larvae fed with freshwater zooplankton weight have not increased significantly. Likewise the rate was higher to

the one obtained during the feeding of *Clarias gariepinus* with live aliment, by UYS & HECHT (1985) during 08 days (10.71%.day^{-1}) and by YILMAZ *et al.* (2006) during 07 days (7.03%.day^{-1}). These differences were due to the final low weight of larvae reared by the authors. Our results were in concordance with those obtained by AWAÏSS & KESTEMONT (1998) after 07 days feeding of *Clarias gariepinus* with freshwater rotifers, *Brachionus calyciflorus* (14.29%.day^{-1}).

The growth performances of larvae fed with *Artemia* were higher than the ones fed with local zooplankton because, probably, the proteins rate and the amino acids composition of *Artemia* were more important than those of local zooplankton (ALLA *et al.*, 2011). The good growth and survival performances of larvae fed with freshwater local zooplankton obtained in the present experiment resulted from the good digestibility and good nutritious quality of those live preys. According to LAUFF & HOFER (1984); KOLKOVSKI (2001); MITRA *et al.*, (2007) ; HAZMAN & GÖKÇEK (2014); the main interest of the live food in larviculture was the good digestibility thanks to proteases they contained and the availability of larvae. The freshwater local zooplankton produced with pig dung proved efficient for the feeding of early stages in catfish *Clarias gariepinus* larvae rearing, at end of yolk sac resorption. That live food can then be used in substitute of *Artemia* for that fish larvae nutrition, in fertilized basins without water renewal.

CONCLUSION

The good survival and growth performances obtained at the end of this experiment showed clearly that freshwater local zooplankton produced with pig dung was proved efficient for the feeding at the early stages of African catfish *Clarias gariepinus* larvae rearing at the end of yolk sac resorption. That live food could be used in substitute of *Artemia* for this fish larvae feeding. These latter could be reared in fertilized medium of freshwater zooplankton mass production. It is therefore possible to carry out that larviculture with simple technique adapted to rural conditions in order to allow pisciculturists of developing countries to have fry permanently.

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