THE BIOLOGICAL DIVERSITY AND AQUACULTURE OF CLARIID AND PANGASIID CATFISHES IN SOUTH-EAST ASIA

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LARVAL REARING OF THE ASIAN CATFISH, *PANGASIUS BOCOURTI* (SILURIFORMES, PANGASIIDAE): *ARTEMIA* ALTERNATIVE FEEDING AND WEANING TIME.

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Abstract

Three experiments were carried out to evaluate larval rearing in *Pangasius bocourti*. The survival rates of larvae fed *Artemia* nauplii, *Moina* sp. (Cladocera) or tubifex (red blood worms) were not significantly different, being comprised between 91 and 93%. *Artemia* nauplii and tubifex led to similar growth rates (35-36%.d^{-1}), while *Moina* sp. gave an inferior growth rate in comparison to the two former feeds. Commercial trout starter feed gave the lowest survival rate (67%) and growth rate (20%.d^{-1}). However, the use of a dried feed based on yeast improved the survival in comparison to the trout starter diet. Larvae fed dried diets or decapsulated *Artemia* cysts had the same survival rate but a lower growth rate than those fed *Artemia* nauplii. The stomach content analysis showed that the reduced growth in larvae fed decapsulated cysts in comparison to live *Artemia* may reflect more a difference in feed ingestion and preference than a difference in nutritional quality of the feed.

Pangasius bocourti larvae requires 3 days of *Artemia* feeding before being shifted to trout starter feed without negative effects on growth performance. The stomach attains its functional and physiological achievement 3 days after the first feeding. It is obvious that digestive tract development of *P. bocourti* larvae is still going on during the initial feeding.

INTRODUCTION

The Asian catfish, *Pangasius bocourti* Sauvage, 1880, is a major indigenous fish cultured in cages in the Mekong Delta, Vietnam. The annual production reaches about 13 400 tons (Cacot, 1994). The availability of seed has been dependent on fry catching in natural water bodies. Therefore, artificial reproduction and larval rearing represent actually a bottleneck for the production and cage culture development of the species in Vietnam. Induced spawning of the species was successfully carried out for the first time in the Mekong Delta in 1995 (Cacot, 1999). So far, information on the larval rearing of this species is lacking.

Brine shrimp nauplii (*Artemia* sp.) often proved to be an excellent start feed for freshwater and marine fish species (Léger *et al.*, 1986). However, *Artemia* use may not be appropriate in developing countries since *Artemia* cyst price is quite high and it requires some specialised facilities to produce. Successful rearing of fish larvae using live zooplankton was reported for several species (Watanabe *et al.* 1983, Dabrowski, 1984). Among various species of zooplankton, the genus *Moina* (Cladocera) is known to be suitable as initial feed for *Chanos chanos* (Villegas, 1990) and *Clarias macroceplalus* (Fermin & Bolivar, 1991). Tubifex worms have been also used successfully for European catfish (*Silurus glanis)* larval rearing (Ronyai & Ruttkay, 1990). The use of decapsulated *Artemia* cysts, rather than live *Artemia* nauplii, presents several advantages: eliminating the inconvenience of producing live food (Pector *et al.*, 1994), decapsulated cysts are disinfected and have a higher dry weight and energy content (Vanhaecke *et al.*, 1983). They do

not leach nutrient in water like formulated feed and its particle size is appropriate for most fish species (Verreth *et al.*, 1987). As a result, they have been considered as a reference diet for nutritional study of *Clarias gariepinus* (Verreth *et al.*, 1987). It was also reported that some freshwater fish species can be exclusively reared on artificial diets from exogenous feeding such as *Clarias gariepinus*, *Coregonus sp.*, *Cyprinus carpio* and *Heterobranchus longifilis* (Appelbaum *et al.*, 1988; Bergot *et al.*, 1986; Charlon *et al.*, 1986; Legendre *et al.*, 1995).

In hatchery operation and research activity, shifting from live to artificial feed was done as soon as possible when it does not affect anymore growth performance and survival of larvae. It seems that after onset of exogenous feeding, fish larvae require a certain time to develop their ability to adapt to dry feed. Freshwater fish are fairly large at hatching, and thus can accept dry diet at earlier time than marine species. The time depends on the quality of dry feed as well as the physiological and functional development of the digestive tract at the larval stage.

So, the aim of the present study is to evaluate different types of live feed, artificial diet or decapsulated *Artemia* cysts on the acceptability, growth and survival rate of *Pangasius bocourti* larvae. The work also aims at studying the digestive tube development in order to find a relationship between the weaning time and the development of digestive organs.

MATERIAL AND METHODS

P. bocourti broodfish were cultured in ponds at the University of Can Tho, Vietnam. Spawning was induced by treatment with human chorionic gonadotropin hormone (hCG). When hatched larvae were 24 hours old, 500 of them were placed into each of twelve 50 L aquarium that were aerated and had a continuous flow of well water at a rate of 0.4-0.5 $L.mn^{-1}$. Feeding started from 48 hours post hatching when the yolk sac was not completely absorbed. Fish weight and total length at that time ranged from 3.7 to 4.0 mg and from 8.7 to 9.0 mm, respectively.

Water current through aquarium was maintained constant at 0.4-0.5 $L.mn^{-1}$ and an aeration of water was done during the experiment. Dissolved oxygen and pH were measured twice a week with DO meter (YSI model 518) and pH meter (Hana HI 8424). Ammonia and nitrite, were measured by colorimetry method (Aquaquant 14423, 14424). Temperature, monitored twice a day at 8h.00 and 15h.00, ranged from 28 to 30°C. Dissolved oxygen concentration was always higher than 5 $mg.l^{-1}$. pH values varied in a range of 7.0-7.5. Ammonia and nitrite were from 0.1 to 0.3 $mg.l^{-1}$ and from 0.01 to 0.04 $mg.l^{-1}$, respectively.

Three experiments were carried out in the present study to evaluate different feed on growth performances, survival rates in *P. bocourti* larval rearing and the weaning time with a trout feed starter.

1. The first experiment tried four diets, including *Artemia* nauplii, tubifex worms, cladocerean and trout feed starter.
2. The second experiment tested three diets, including *Artemia* nauplii, decapsulated *Artemia* cysts and dry diet based on beef liver and yeast.
3. The third experiment determined the suitable weaning time in *P. bocourti*. In the experiment, fish were fed either *Artemia* nauplii or a trout starter diet. There were eight treatments which were assigned to 0, 1, 2, 3, 4, 5, 6 and Art. For instance, treatment 2 means that larvae were fed during two days with *Artemia* nauplii before being shifted directly to dry diet. Treatment "O" or "Art" means that larvae were fed exclusively with dry diet or *Artemia* nauplii respectively.

   Each treatment had three replications.

Artemia nauplii obtained from cysts (San Francisco Bay strain), were inoculated and cultured in Vietnam since 1982. Cysts were incubated in $10g.L^{-1}$ saline water for 24 hours at a temperature of 30°C. Newly hatched *Artemia* were kept in aerated saline water. To ensure the provision of the nauplii stage, *Artemia* were used within a period of 12 hours. These *Artemia* nauplii have a size of 146-250 µm in width and 411-450 µm in total length.

Cladocereans were cultured in earthen ponds fertilised with pig manure. They were daily collected and treated with formalin for 1-2 minutes to eliminate disease germs. They were still alive and moved actively after treatment. A small part of other zooplankton organisms were recorded in the *Moina* collection, including *Eucyclops* and some

ephippial eggs. Cladocereans were mostly *Moina sp.* with a size of 288-300 µm in width and 850-900 µm in total length.

Tubifex worms (*Tubifex tubifex*) were collected on river bank. They were treated with formalin for 1-2 minutes and then chopped into small pieces of 800-900 µm in width and 900-1000 µm in length.

Dry diet used in the first experiment, was a trout starter diet (Aqualim, France) composed of fish meal, cereal, terrestrial animal product, fat, oil, vitamin and mineral premix. The proximate composition of the dried feed was 55% protein, 18% lipid, 8% mineral. The size of feed particles was 0.2-0.4 mm.

Another dry feed (dry diet 2), based on beef liver and yeast with the following formula (INRA) and the proximate composition, was used in the second experiment. The formula was:

- «Protibel» yeast powder	50%
- Beef liver	35%
- Soybean oil	5%
- Vitamin premix	5%
- Mineral premix	5%

Proximate composition (dry basis):

- Moisture	7.83%
- Crude protein	35.69%
- Crude lipid	10.97%
- Ash	11.73%

Artemia cysts were decapsulated in 25% hypochloride solution for 5-10 minutes until the orange colour appearance and were then washed in freshwater until disappearance of the chloride odour. Decapsulated cysts were then kept in refrigerator (10°C) for daily feeding. Fish were fed six times a day at 8h:00, 12h:00, 16h:00, 20h:00, 24h:00 and 4h:00. Live *Artemia* nauplii, *Moina* and tubifex were fed at 160% of fish biomass (wet feed basis), based on the last fish sampling and increased arbitrarily by 50% at each following day. The adjustment was made on the basis of fish weights registered every three days. The dry diet was distributed at 20% of fish biomass and increased by 50% at each following day. Every three days, 30 larvae were randomly sampled. They were placed on paper towels, in order to absorb water and weighed in batch of 30 fishes at an accuracy of 0.1 mg, according to the procedure of Kerdchuen and Legendre (1994). Weighed larvae were not further used in the feeding experiment. At the end of the experiment, 50 fishes were sampled per aquarium. On each sampling day, five supplementary larvae were also caught 30 minutes after feeding and fixed in formalin 10% for further gut content analysis and mouth size measurement.

Mouth height was the distance from lower jaw to upper jaw, for larvae with the mouth open at 45 or 90°. Mouth width was the width of the lower jaw. Measurement were performed under a binocular lens with an accuracy of 0.01 mm. Survival rates were calculated by taking into account the remaining and discarded larvae.

Histological study was carried out on larvae fed exclusively on *Artemia* nauplii in a separated aquarium with temperature range of 28-30°C. Sampling of 10 larvae occurred at 0, 24, 36, 48 hours post hatching and later on 3, 4, 5, 7 and 10 days old. Larvae were fixed in either buffered formalin (10%) or Hollande Bouin and then dehydrated and embedded in paraffin and cut at sections of 5-10 µm. The section was coloured with Hematoxycline-Eosin or PAS. The pH gut measurement was carried out by injecting a methyl red or Congo red fluid into the gut of larvae.

Dead larvae were recorded and siphoned out two times a day at 8h:00 and 20h:00. The observed mortality was defined as the observed dead larvae ratio to the larvae amount. The cumulative mortality rate was denoted as the cumulate of daily observed mortality. The cannibalism rate was also defined in the present study as the missing larvae during the experiment, cannibalism rate % = 100 – (survival rate % + observed mortality %).

Mean weight, specific growth rate and survival rates were subjected to one way ANOVA, followed by Duncan's Multiple Range test to determine the significant difference among treatments with the help of the software Statgraphics version 5.0.

RESULTS

Comparison between Artemia *nauplii, tubifex worms, Cladocerean and trout starter feed*

The larvae fed voraciously all experimental diets during the rearing period. They swam actively at the moment of feeding, searching feed on the bottom. Gut content analysis showed that *P. bocourti* larvae started to feed 48 hours after hatching at the temperature of 28-30°C, while yolk

sac has not yet completely absorbed. At first feeding, the mouth height opening at 45° and 90° was 0.55 mm and 0.95 mm respectively and the width of lower jaw was 1.00 mm (Table 1). It is clear that *Pangasius bocourti* have a mouth size large enough to ingest different types of natural diets including *Moina* sp and tubifex worms. Cladocereans were always alive and available in the water column while dry feed and tubifex worms settled down quickly. *Artemia* nauplii remained alive for 5-6 hours in freshwater but they all concentrated at the bottom of aquarium.

Mean weight, specific growth rate and survival rate at the end of the experiment showed that *Artemia* nauplii and tubifex worms were excellent diets for *P. bocourti* larval rearing (Table 2). During the three initial days of feeding, *Artemia* nauplii led to the highest growth performances compared to tubifex worms. Nevertheless, larvae fed tubifex worms grew fast in further days and caught up the growth of larvae fed *Artemia* nauplii (Fig. 1). Starting eight days after hatching (6 days from exogenous feeding) onwards, larvae fed tubifex did not show any significant difference in mean weight when compared to larvae fed *Artemia*. Cladocereans led to a lower growth rate than those fed *Artemia* nauplii or tubifex worms. Artificial diet led to lower growth performances than all live feeds. This low growth was already apparent after the first 3 days of feeding (D5).

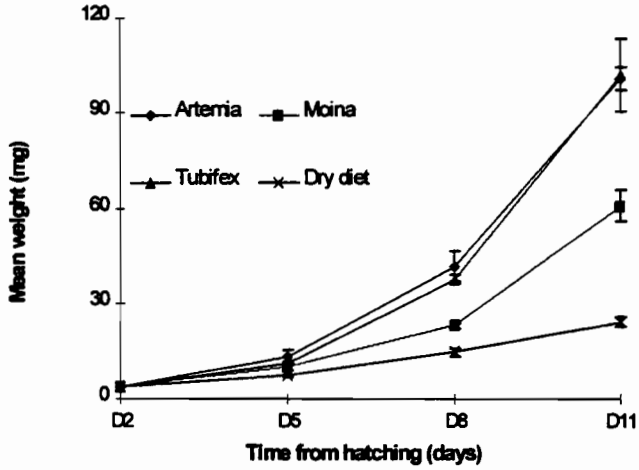


Figure 1: Growth curves of *P. bocourti* larvae fed live brine nauplii (*Artemia sp.*), *Moina sp.*, tubifex worms and a trout starter diet in the first experiment.

The survival and cannibalism rate of larvae fed live feed including *Artemia* nauplii, tubifex worms and cladocereans were not significantly different, while trout starter led to the lowest survival rate (67.5%) and the highest cannibalism (10.4%). Cumulative mortality (Fig. 2) indicated that larvae fed live feed had high mortality during the three initial days of feeding (D2 to D5), compared to further days (D5 to D11). By contrast, artificial feed caused an increased mortality until the end of the experiment. However, the mortality of larvae fed artificial diet tended to diminish after the 5th day, in comparison to the period from D2 to D5.

Age from hatching	Total length (mm)	Mouth size (mm)		
		opening at 45°	opening at 90°	width of lower jaw
48 h (D2)	8.7	0.5	0.9	1.0
72 h (D3)	9.6	0.7	1.2	1.3
96 h (D4)	10.6	0.8	1.3	1.3

Table 1: Mouth size of *Pangasius bocourti* larvae at first feeding. (48 hours after hatching). Distance from lower to upper jaw was measured for larvae with mouth opened at 45° or 90°.

Feeding treatments	*Artemia* nauplii	*Moina* sp	Tubifex worms	Trout starter
Initial weight at D2	3.7	3.7	3.7	3.7
Weight at D5	13.3 ± 1.7 ^{a}	9.9± 1.2 ^{b}	11.0 ± 0.4 ^{b}	7.2 ± 0.4 ^{c}
Weight at D8	41.5 ± 4.6 ^{a}	22.9 ± 0.7 ^{b}	37.6 ± 1.3 ^{a}	14.6 ± 1.3 ^{c}
Final weight at D11	100.7 ± 0.7 ^{a}	60.8 ± 5.1 ^{b}	101.5 ± 11.7 ^{a}	24.3 ± 1.7 ^{c}
SGR (%. d^{-1})	36.0 ± 0.4 ^{a}	31.0 ± 0.9 ^{b}	36.7 ±1.3 ^{a}	20.8 ± 0.8 ^{c}
Survival rate (%)	91.7 ± 5.2	93.7 ± 2.4	92.7 ± 2.1	67.5 ± 13.6
Cannibalism rate (%)	3.9 ^{a}	1.6 ^{a}	2.6 ^{a}	10.4 ^{b}

Figures in the same line having same superscripts are not significantly different ($p<0.05$). Mean ± SD.
SGR = 100x(Ln(W2)-Ln(W1))/(T2-T1)

Table 2: Mean weight (mg), specific growth rate (SGR, %.day^{-1}) and survival rate (%) of *Pangasius bocourti* larvae fed artificial diet or live feed (*Artemia* nauplii, *Moina* sp., tubifex worms) after nine days of experiment from first feeding.

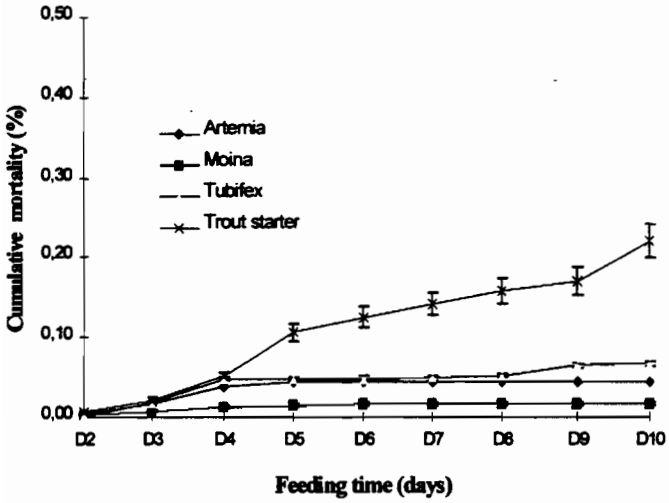


Figure 2: Cumulative mortality of *P. bocourti* larvae in response to live feed or trout feed starter. Feeding started 48 hours after hatching (D2).

Comparison between* Artemia *nauplii, decapsulated* Artemia *cysts and dry diet based on yeast and beef liver.

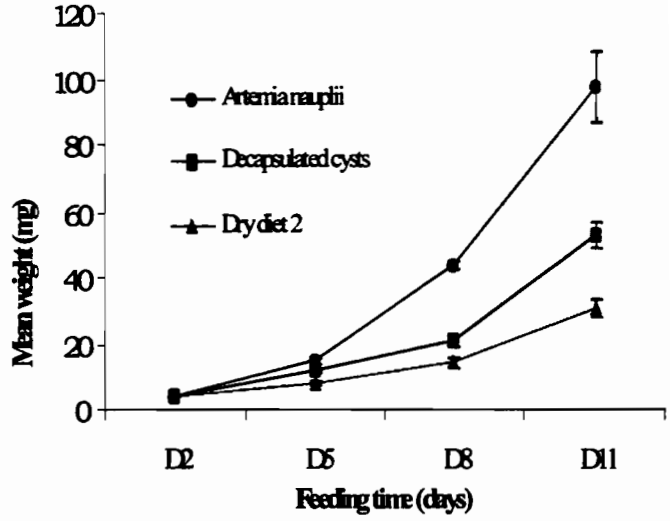
In the second experiment, The SGR of larvae fed on *Artemia* nauplii was 35.4%.d^{-1}, higher than those obtained with decapsulated cysts (28.7%.d^{-1}) or dry diet based on yeast (22.7%.d^{-1}). Thus, the growth performance of fish fed decapsulated cysts or dry diet based on yeast, was inferior to that of fish fed Artemia nauplii (Table 3; Fig. 3). However, survival rates in fish fed decapsulated cysts or dry diet based on yeast, were 90.4% and 86.6% respectively, not significantly different to that of fish fed Artemia nauplii (90.5%). Cannibalism rates were not significantly different among the three treatments.

When growth performance and survival rates in fish fed trout starter or dry diet based on yeast and beef liver were compared, it was clear that the latter ameliorates the survival rate while the growth was similar with the two dry diets.

Weaning time and digestive tract development

Weaning time

After 9 days of feeding, the highest growth performance was found in larvae fed exclusively on *Artemia* nauplii (101.9 mg and 36.7%.day^{-1}). However, the final mean weight of larvae weaned to artificial feed after 4 days and 6 days of *Artemia* feeding were 101.3 mg and 99.7 mg respectively, not significantly different from those fed *Artemia* nauplii. Larvae weaned after 3 days had a mean weight of 94.8 mg, relatively lower than those fed exclusively on *Artemia* but not significantly different from those weaned after 5 days.



Vertical bars refer to standard error.

Figure 3: Growth of *P. bocourti* larvae fed *Artemia* nauplii, decapsulated *Artemia* cysts or dry diet based on yeast and beef liver (dry diet 2) in the second experiment.

It was not surprising to notice that larvae fed exclusively on artificial diet or weaned as early as 1 day of *Artemia* feeding resulted in the lowest growth performances with final mean weights of 49.3 mg and 48.0 mg and specific growth rates of 28.6%.day^{-1} and 28.3%.day^{-1}, respectively. Larvae weaned after 2 days presented an intermediate growth rate between the group fed on artificial diet and the group fed on *Artemia*. In general, larval mean weights at the end of the experiment illustrated a trend curve that reached approximately an horizontal line starting with larvae weaned after 3 days of *Artemia* feeding (Fig. 4).

Larvae fed exclusively on a commercial trout feed, had the lowest survival rate (67.2%). Nevertheless, weaning after 1 day or 2 days resulted in improving the survival (78.0% and 76.0% respectively). With the exception of larvae that fed exclusively on artificial diet or weaned after 1 or 2 days of *Artemia* feeding, survival rates were consistently high and varied between 88.1% and 96.2%. Figure 4 also pictured the trend of survival rates of different treatments that reached approximately an horizontal line starting with larvae weaned after 3 days of *Artemia* feeding.

Feeding treatments	*Artemia* nauplii	Decapsulated *Artemia* cysts	Yeast and beef liver
Initial weight (mg)	3,7	3,7	3,7
Final weight (mg)	$97,6 \pm 10,7^{a}$	$53,0 \pm 3,8^{b}$	$30,9 \pm 2,6^{c}$
SGR (%. d^{-1})	35.3 ± 2.2^{a}	28.7 ± 0.8^{b}	22.7 ± 0.2^{c}
Survival rate (%)	$90,5 \pm 4,8^{a}$	90.4 ± 7.7^{a}	$86,6 \pm 1.5^{a}$
Cannibalism rate (%)	3.2 ± 4.2^{a}	5.5 ± 4.8^{a}	$7.0 + 1.4^{a}$

Figures in the same line having same superscripts are not significantly different. ($p<0.05$). Mean ± SD.
SGR = 100x(Ln(W2)-Ln(W1))/(T2-T1)

Table 3: Mean weight (mg), specific growth rate (%.d^{-1}) and survival rate (%) of *P. bocourti* larvae fed *Artemia* nauplii, decapsulated *Artemia* cysts or dry diet based on yeast and beef liver.

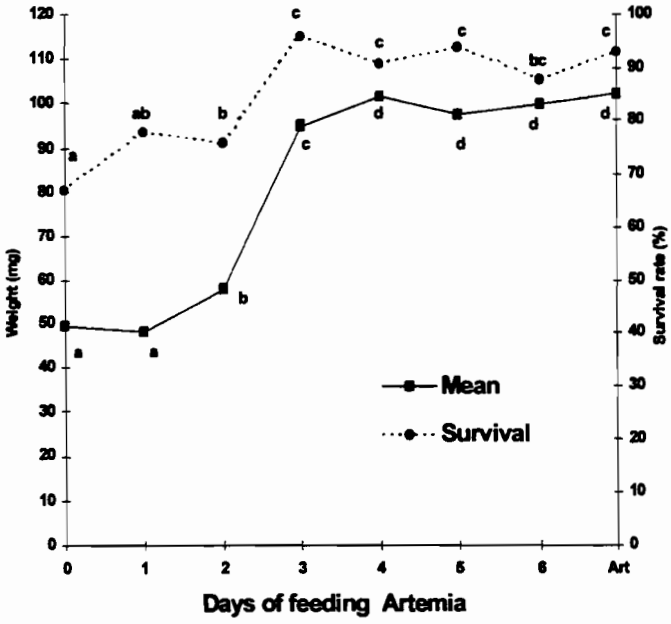


Figure 4: Mean weight (mg) and survival rate in *P. bocourti* larvae weaned at different days after hatching. Fish were fed either *Artemia* nauplii and then shifted directly to a commercial trout starter at different age.

Digestive tract development

At hatching, the digestive tube is only a rudimentary undifferentiated segment lying on yolk sac. Twenty-four hours later (D1), pancreas and liver are present as a clump of cells in posterior part of the digestive tract. At the exogenous feeding (D2), the stomach is an enlarged part with isometric epithelium cells, which are different from elongated cells in intestinal part. The stomach is also well distinguished with intestine due to the presence of a thick circular muscular layer in stomach and high folds of epithelium in intestine. Also at that time, zymogens were detected in pancreas in form of purple coloured granules in Hematoxycline-Eosin section. Gastric glands were not yet found in the stomach at that moment. It is only 24 hours later that these glands started to develop and were well distributed beneath the gastric epithelium on the day 4 post hatching (D4). Like other carnivorous species, surface epithelial cells in the stomach of *P. bocourti* contain muco-polysaccharides. Therefore, these cells may be responsible for the PAS positive coloration, which is visible on the stomach section on the day 5 (D5). At that time, the gastric secretion pH falls to 3.3 (changed colour of Congo red indicator) and the pyloric sphincter was found.

These observations indicated that the stomach then attained its morphological and probably functional completeness. In respect to absorption, droplets of lipids were detected twenty-four hours after feeding in the posterior part of intestine and liver cells had basal nucleus, showing that liver started to accumulate stored materials.

DISCUSSION

Growth performances and survival rates in response to different type of feed

In the first and second experiment, *Artemia* nauplii proved to be an excellent feed in terms of growth and survival rates for larval rearing of *P. bocourti*. Nevertheless, the survival rate of larvae fed *Artemia* was not significantly different from those fed other live food such as Cladocerean or tubifex. That implies that the use of other live feed than *Artemia* is possible. Using *Artemia* for successfully larval rearing was reported for several species. Hogendoorn (1980) reported superior results for larval rearing of *Clarias gariepinus* when using live *Artemia*, or a combination of *Artemia* and dry diet, as first feeds. Kerdchuen and Legendre (1994) showed the best growth

performance in *Heterobranchus longifilis* larvae fed live or frozen *Artemia* nauplii when compared to other diets. For these species, the specific growth rate (SGR) after a 14-day period was 40%.d^{-1}, which is close to the one obtained in *P. bocourti,* 36%.d^{-1} for an 11-day duration. Fermin and Bolivar (1991) who carried out experiment on *Clarias macrocephalus* also demonstrated that *Artemia* plus dry diet led to the best growth performance, even if the SGR was only 12.4%.d^{-1}. Knud-Hensen *et al.* (1990) concluded that *Clarias batrachus* larvae fed *Artemia* during 7 days displayed the best growth performance and survival in comparison to other live and dry feed. Thus, like most other tropical catfish, *P. bocourti* larvae had a maximal growth and an optimal survival rate when fed live *Artemia* nauplii.

Larvae fed *Moina* had a lower growth performance than those fed *Artemia* nauplii and tubifex worms. The SGR was only 22.9%.d^{-1}. The same conclusion was reported in *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994). When fed exclusively on *Moina macrocopa,* Fermin and Bolivar (1991) showed that *Clarias macrocephalus* also had a lower growth than fish fed a mixture of *Artemia* and dry feed. However, Adeyemo *et al.* (1994) had an opposite conclusion in two other catfishes, *Heterobranchus bidorsalis* and *Clarias gariepinus*. They reported that fish fed *Moina dubia* had a higher growth and survival than those fed *Artemia* nauplii. However, in their experiments, the SGRs of fish fed *Artemia* were surprisingly low, 5.1%.d^{-1} and 6.1%.d^{-1} for a 7-day duration in *Heterobranchus bidorsalis* and *Clarias gariepinus* respectively. Kerdchuen and Legendre (1994) observed the presence of numerous undigested ephippial eggs in the digestive tract of *Heterobranchus longifilis* fed *Moina,* which could lead to the low growth. *Moina* used in the present experiment, were collected in earthen ponds. We observed some *Eucyclops* and ephippial eggs in larval stomach content analysis. Then, their presence may explain, at least for a part, the lower growth observed in *P. bocourti* larvae as ephippial eggs are resistant to digestion in many fish species (Mellors, 1975).

Tubifex worms have been used as a live feed for nursing European catfish (*Silurus glanis*) in Hungary. Horvath *et al.* (1981) proposed the application of tubifex worms in large scale rearing which proved to be the most economical diet. However, many fish larvae cannot ingest such a large prey. Based on the measurement of mouth opening at 45° and 90°, food size suitable for first feeding of *P. bocourti* larvae was 0.6-1.0 mm. Therefore, mouth opening in *P. bocourti* permits to ingest tubifex worms if they are chopped. Starting from 8 days of age, larvae fed tubifex did not present any growth differences with the ones fed *Artemia.* Nevertheless, their mean weight at 5 days of age was still lower than those of fish fed *Artemia* (Table 1). Hence, the study confirmed the feasibility of completely replacing *Artemia* by tubifex worms for larval rearing of *P. bocourti.*

The poor growth and survival of *P. bocourti* larvae fed a commercial trout starter diet are in agreement with findings in other catfish such as *Clarias gariepinus* (Hogendoorn, 1980; Verreth & Van Tongeren, 1989) and *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994). This may be related to the feed quality and the digestibility of the dry diet or to the primary development of digestive systems at the first feeding. When fed trout starter feed, larvae of *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994) and *Clarias gariepinus* (Msiska, 1981) showed low survival rates of 32% and 12% respectively. In the present study, *P. bocourti* apparently showed a better survival rate of 67.5%. This indicates that this species has a high potential for using artificial diet.

In the present study, the yeast and beef liver based dry diet was well accepted by *P. bocourti* larvae and led to survival rates as high as that obtained with *Artemia* nauplii or other live feed. Dry diet based on yeast proved comparative to *Artemia* nauplii in term of survival rate in several fish species such as *Heterobranchus longifilis* (Kerdchuen & Legendre, 1994), *Clarias gariepinus* (Applebaum & Van Dame, 1988). The improved survival of yeast based dry feed could be attributed only to better digestibility of protein in the diet. Secondly, the inactive yeast are roasted during the manufacturing process, thereby, denaturing the protein to a significant degree, which in turn facilitates their digestion (Hecht, 1996). This would explain the unsatisfactory result obtained when using other protein sources as dry feed ingredients for the *P. bocourti* larval rearing.

The use of decapsulated cysts as a direct food source for an initial stage of larvae has been

proposed as a reference diet in larval nutritional studies (Verreth *et al.*, 1987). However, in the present study, live *Artemia* nauplii resulted in higher growth than decapsulated *Artemia* cysts. Highly nutritional value of decapsulated cysts was confirmed by several workers. They contain 30-50% energy higher than freshly hatched nauplii (Vanhaecke *et al.*, 1983).

It is clear that the nutritive value of feeds did not account for the difference observed in body gain of larvae fed live *Artemia* nauplii and decapsulated cysts. In the present study, *P. bocourti* larvae showed a higher feed ingestion of live nauplii than decapsulated cysts (Fig. 5). This may be linked to movements of live *Artemia* nauplii and quick sedimentation of decapsulated cysts that become less available for the larvae. Therefore, the stomach analysis can elucidate the reduced growth in larvae fed decapsulated cysts in comparison to live *Artemia*. The same phenomenon was also observed in *Heterobranchus longifilis* (unpublished data). It reflects a difference in feed ingestion and preference more than in nutritional quality of the feed.

Trout starter feed in the first experiment resulted in a higher cannibalism rate (10.4%) when compared to live food (1.6-4.7%). Fermin and Bolivar (1991) also observed a high cannibalism rate in a dry diet treatment (21.7%) in comparison to a combination of *Artemia* with dry diet (9.7%).

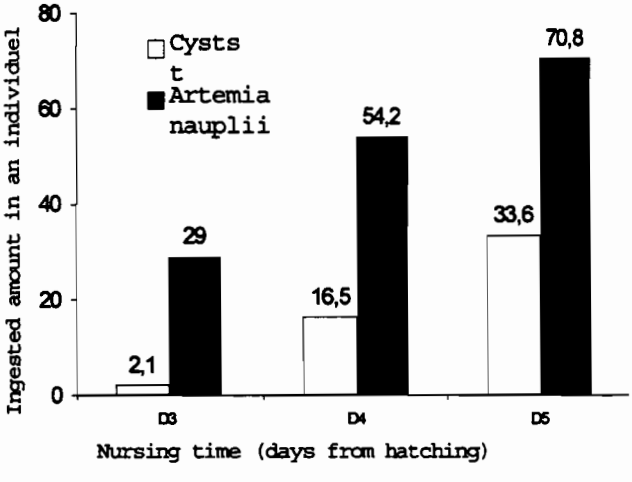


Figure 5: Amount of *Artemia* nauplii or decapsulated cysts found in stomach of 3 to 5 days old *P. bocourti* larvae 30 minutes after feeding.

Cannibalism was reported in most larval rearing. Hecht and Appelbaum (1987) demonstrated that cannibalism in *Clarias gariepinus* contributed greater to larval mortality than natural mortality.

Weaning time and digestive tract development

From results of the third experiment, it seems that *P. bocourti* larvae require 3 days of *Artemia* feeding before being shifted directly to trout starter feed without effects on growth performance. In respect to survival rate, it requires only one day. When compared to other freshwater species, *P. bocourti* larvae tend to have an earlier weaning time since at the temperature of 30°C, Verreth and Tongeren (1989) indicated that *Clarias gariepinus* could be weaned at 4.1 days and 1.8 days in respect to growth performance and survival respectively. Fermin *et al.* (1995) reported the weaning time of the Asian catfish (*Clarias macrocephalus*) to be 4 days after live zooplankton feeding. Bryant and Matty (1981) demonstrated that common carp larvae may be reared on a commercial trout starter from initial weight of approximately 15 mg, which is what they called "adaptation weight". The weaning size of different species were reported as 5-6 mg in Chinese carps, 18 mg in African catfish and 11-14 mg in Asian catfish (Dabrowski, 1984; Verreth & Tongeren, 1989; Fermin *et al.* 1995). In *P. bocourti* larvae, the earliest weaning size was 10-13 mg. Cyprinids larvae have a lowest weight at weaning (5-6 mg); however, when taking weaning time into account, the adaptation weight correspond to 4 days in common carps and 8 days in grass carp (*C. idella*) and silver carp (*H. molitrix*).

A question arises concerning the use of dry feed versus live feed for larval rearing; why fish larvae require live feed during the initial feeding? There has been a debate on the matter since the publication of Appelbaum's papers for larval rearing of carps (Hecht, 1996). According to Dabrowski and Culver (1991), larvae of some catfish have no functional stomach or gastric gland at initial feeding but whole digestive system differentiates during a complex metamorphosis. Therefore, the fish would depend on exogenous enzymes in live food to compensate for the impaired activity of proteolysis enzymes during the initial feeding (Dabrowskii & Glogowski, 1977; Lauff & Hofer, 1984). Moreover, Dabrowski (1992) indicated that the weaning period often corresponds to the moment at which the stomach becomes functional, with a switch from a digestion

exclusively intestinal to a mainly digestive active stomach. However, *Cyprinus carpio* does not have a functional stomach during its larval stage nor throughout its life. Theoretically, therefore, the larvae of carps should not be able to digest dry diet. However, the work undertaken by Appelbaum (1976a, b), Appelbaum and Dor (1978) and Dabrowskii *et al.* (1978) conclusively showed that carp larvae can indeed be reared as successfully on dry feed as on live feed.

In the present study, at the exogenous feeding fish larvae had a functional pancreas. Yet, larval gastric glands in stomach wall do not develop until 2 days after exogenous feeding. In addition, the stomach attains the functional and physiological perfection together with the appearance of a pyloric sphincter and a fall in pH value to 3.3 occurring 3 days after first feeding. When compared to the development of digestive tract in *Clarias gariepinus* larvae, *P. bocourti* larvae have a functional stomach earlier, 3 days after feeding instead of 5 days in *C. gariepinus*. It is obvious that digestive tract development of *P. bocourti* larvae is still going on during the initial feeding.

However, more fundamental studies are needed to elucidate whether the reduced growth of *P. bocourti* larvae fed on dry diet is due to a deficiency in gastric proteolysis enzyme during its initial feeding or to other reasons. That needs the mentioned debate. Yet, few work have paid attention to the fact that larval preference of live feed rather than dry diet may be due to the movement of live prey. As a result, feed intake of dry diet feeding is constantly lower than that obtained in live feeding, especially during the initial feeding when larval fins are not yet well developed. This is possibly an important reason for the unsuitable dry diet for larval rearing in many fish species.

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