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Abstract — An experiment was carried out to evaluate the effects of brine shrimp nauplii (*Artemia* sp.), Cladocera (*Moina* sp.), *Tubifex* worms and a trout starter diet on growth performances and survival rate of Mekong catfish larvae (*Pangasius bocourti* Sauvage, 1880). Larvae accepted all four types of experimental diets, owing to large mouth opening at the first feeding (0.6–1.0 mm at 45° and 90° mouth opening, respectively). *Artemia* nauplii and *Tubifex* worms resulted in the same growth performances and survival rates for a 9-d nursing time with specific growth rates of 36–37 %·d−1 and survival rates of 92–93 %. *Moina* led to a lower growth performance (31 %·d−1) but the survival rate (94 %) was not significantly different to that of larvae fed *Artemia* nauplii or *Tubifex* worms. Trout starter feed, dry pellet, proved relatively less suitable for larval rearing of *P. bocourti* owing to a low survival rate (68 %) and growth performance (21 %·d−1). The study confirmed the feasibility of completely replacing *Artemia* nauplii by *Tubifex* worms. © Ifremer/Cnrs/Inra/Ird/Cemagref/Elsevier, Paris

Larval rearing / Mekong catfish (Pangasiidae) / *Pangasius bocourti* / dry feed / Cladocera / *Tubifex* / *Artemia* nauplii

1. INTRODUCTION

The Mekong catfish, *Pangasius bocourti* Sauvage, 1880 [19], is a major indigenous fish cultured in cages in the Mekong delta, Vietnam. The annual production reached 13,400 tons in 1994 [4] and 22,000 tons in 1998 [18]. Until now, the seed supply depended on catching fry in the wild, upstream of the Cambodian part of Mekong river. Therefore, artificial reproduction and larval rearing represent a bottleneck for the Mekong catfish production and cage culture development in Vietnam. Induced spawning was successfully carried out for the first time in the world in 1995 [5]. Up till then, larval rearing of the species was unknown and also had to be performed for the first time ever.

Brine shrimp (*Artemia* sp.) nauplii are an excellent starter-feed for freshwater and marine fish species [15]. However, use of *Artemia* may not be appropriate in developing countries since the price of *Artemia* cyst is high and its production requires some specialized facilities. Successful rearing of fish larvae using other natural live food has been reported for several species [6, 23]. Among various species of zooplankton, the genus *Moina* of Cladocera is known to be suitable as initial feed for *Chanos chanos* [22] and *Clarias macrocephalus* [7]. *Tubifex* worms have also been successfully used for larval rearing of European catfish (*Silurus glanis*) [20]. It was also reported that some catfish, such as *Clarias gariepinus* and *Heterobranchus longifilis*, can be exclusively reared on artificial feed [2, 15]. However, dry diets often resulted in lower growth and higher mortality than live *Artemia* nauplii.

The present study aims at comparing growth performances and survival rates of *Pangasius bocourti* larvae fed on live *Artemia* nauplii to those of larvae fed *Moina* sp., *Tubifex* worms or a commercially prepared dry diet for larval rearing.

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2. MATERIALS AND METHODS

*Pangasius bocourti* broodfish was cultured in ponds at Can Tho University, Vietnam. Spawning was induced with a human chorionic gonadotropin hormone (HCG) injection. When the hatched larvae were 24 h old, 500 of them were placed into each of twelve 50-L aquariums that were aerated and had a continuous flow of deep well-water at a rate of 0.4–0.5 L min⁻¹. Feeding started from 48 h post-hatch when the yolk sac was not completely absorbed. Weight and total length at that time ranged from 3.7 to 4.0 mg and from 8.7 to 9.0 mm, respectively.

Dissolved oxygen and pH were measured twice a week with DO meter (YSI model 518) and pH meter (Hana HI 8424), ranging from 6.8 to 7.8 mg L⁻¹ and from 7.0 to 7.5, respectively. Ammonia and nitrite, measured by colorimetry method (Aquaquant 14423, 14424), ranged from 0.2 to 0.3 mg N L⁻¹, and from 0.03 to 0.04 mg N L⁻¹, respectively. Temperature, monitored twice a day 08:00 and 15:00 hours, ranged from 28 to 30 °C. Newly hatched *Artemia* were incubated in 10 % saline water for 24 h at a temperature of 30 °C. Newly hatched *Artemia* were kept in aerated saline water. To ensure the nutritive value of the nauplii stage, *Artemia* were used within a period of 12 h. *Artemia* nauplii have a size of 146–250 mm in width and 411–450 mm in total length.

Cladocera were produced in an earthen pond fertilized with pig manure. They were collected daily and treated with formalin for 1–2 min to eliminate disease germs. They were still alive and moved actively after that time. Mouth height was the distance from lower jaw to upper jaw, when larvae have a mouth opening at 45 or 90°; mouth width was the width of the lower jaw. Measurements 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.

The artificial diet consisted of a trout starter diet (Aqualim, France) composed of fish meal, cereal, animal fat and vegetal oil, vitamin and mineral premix. The proximate composition of the dried feed was 55 % protein, 18 % lipid and 8 % mineral. The size of feed particles was 0.2–0.4 mm.

Tubifex worms (*Tubifex tubifex*) were collected from various river banks close to Can Tho City. They were treated with formalin for 1–2 min and then chopped into small pieces of 800–900 mm in width and 900–1000 mm in length.

The artificial diet consisted of a trout starter diet (Aqualim, France) composed of fish meal, cereal, animal fat and vegetal oil, vitamin and mineral premix. The proximate composition of the dried feed was 55 % protein, 18 % lipid and 8 % mineral. The size of feed particles was 0.2–0.4 mm.

Fish were fed six times a day at 08:00, 12:00, 16:00, 20:00, 24:00, and 04:00 hours. Live *Artemia* nauplii and Tubifex were fed at 160 % fish biomass (wet feed basis), based on the last fish sampling and increased arbitrarily by 50 % on each subsequent day. The adjustment was made on the weights registered every 3 d. The dry diet was distributed at 20 % of fish biomass and increased by 50 % on each subsequent day.

Every 3 d, 30 larvae were randomly sampled. They were placed on paper towels in order to absorb water and weighed in batches of 30 fishes to an accuracy of 0.1 mg, according to the procedure used in *Heterobranchus longifilis* larval rearing [14]. 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, another sampling of five larvae was also caught 30 min after feeding and fixed in 10 % formalin for further gut content analysis and mouth size measurements. Mouth height was the distance from lower jaw to upper jaw, when larvae have a mouth opening at 45 or 90°; mouth width was the width of the lower jaw. Measurements 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.

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.

3. RESULTS AND DISCUSSION

The survival rates of larvae fed with live feed including *Artemia* nauplii, *Moina* or *Tubifex* worms were not significantly different (90–95 %) while the artificial diet gave the lowest survival rate (67.5 %), *P* < 0.05.

Mean weight, specific growth rate and survival rate at the end of the experiment showed that *Artemia* nauplii and *Tubifex* worms provide an excellent diet for *P. bocourti* larval rearing (table 1, figure 1). During the three initial feeding days, *Artemia* nauplii gave the highest growth performance when compared to *Tubifex* worms. However, larvae fed with *Tubifex* worms grew faster over the subsequent days so as to catch up with larvae fed with *Artemia* nauplii. As a result, larvae fed on *Tubifex* did not have any significant difference in weight when compared to larvae fed with *Artemia* from 8 d onwards. *Moina* gave a lower growth than *Artemia* nauplii and *Tubifex* worms. The artificial diet is inferior to all other live feed in growth performance.

In the study, *Artemia* nauplii and *Tubifex* worms proved to be an excellent feed for larval rearing of *P. bocourti*, in both growth and survival. The other live food, *Moina*, resulted in the same survival as *Artemia* nauplii but lower growth. This implies that the use of live feed other than *Artemia* is possible for *P. bocourti*. The use of *Artemia* for successful larval rearing was reported for several species. *Clarias gariepinus* fed with live *Artemia*, or a combination of *Artemia* with dry diet, showed a superior result [9]. *Heterobranchus longifilis* larvae fed live or frozen *Artemia* nauplii showed the best growth performance when compared
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Table I. Mean weight (mg), specific growth rate, SGR, (%·d⁻¹), survival rates (%) of *Pangasius bocourti* larvae fed with an artificial diet or live feed (*Artemia* nauplii, *Moina* sp., *Tubifex* worms) in a 9-d experiment.

<table>
<thead>
<tr>
<th>Feeding treatments</th>
<th><em>Artemia</em> nauplii</th>
<th><em>Moina</em> sp.</th>
<th><em>Tubifex</em> worms</th>
<th>Trout starter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial weight at day 2</td>
<td>3.7</td>
<td>3.7</td>
<td>3.7</td>
<td>3.7</td>
</tr>
<tr>
<td>Weight at day 5</td>
<td>13.3 ± 1.7a</td>
<td>9.9 ± 1.2b</td>
<td>11.0 ± 0.4b</td>
<td>7.2 ± 0.4c</td>
</tr>
<tr>
<td>Weight at day 8</td>
<td>41.5 ± 4.6a</td>
<td>22.9 ± 0.7b</td>
<td>37.6 ± 1.3a</td>
<td>14.6 ± 1.3b</td>
</tr>
<tr>
<td>Final weight at day 11</td>
<td>100.7 ± 0.7a</td>
<td>60.8 ± 5.1b</td>
<td>101.5 ± 11.7a</td>
<td>24.3 ± 1.7b</td>
</tr>
<tr>
<td>SGR (%·d⁻¹)</td>
<td>36.0 ± 0.4a</td>
<td>31.0 ± 0.9b</td>
<td>36.7 ± 1.3a</td>
<td>20.8 ± 0.8c</td>
</tr>
<tr>
<td>Survival rate (%)</td>
<td>91.7 ± 5.2a</td>
<td>93.7 ± 2.4a</td>
<td>92.7 ± 2.1a</td>
<td>67.5 ± 13.6b</td>
</tr>
</tbody>
</table>

Figures in the same line having the same superscript are not significantly different (P < 0.05). Mean ± SD.

SGR = 100 × [(ln(W2) - ln(W1))/(T2 - T1)].

to other diets [12], and its specific growth rate for a 14-d period was 40 %·d⁻¹, close to that obtained in *P. bocourti*, 36 %·d⁻¹ for a 11-d duration. The study on Asian catfishes, *Clarias* *macrocephalus* and *Clarias batrachus*, also demonstrated that *Artemia* gave the best growth performance, even though the SGR was only 12.4 %·d⁻¹ [7, 13]. Thus, as with most other tropical catfish, *P. bocourti* larval rearing has an excellent growth and survival when fed with live *Artemia* nauplii.

As obtained in *P. bocourti* larval rearing, *Moina* led to a lower growth performance in *Heterobranchus longifilis* when compared with *Artemia* nauplii [12]. The fish had a SGR of 23 %·d⁻¹ with *Moina* feeding. *Clarias macrocephalus* fed with *Moina macrocopia* also showed a lower growth than fish fed a mixture of *Artemia* and dry feed [7]. On the other hand, a contradictory conclusion was reported in two other catfishes, *Heterobranchus bidorsalis* and *Clarias gariepinus* [1], such that *Moina dubia* gave a higher growth and survival than *Artemia* nauplii. However, the SGRs of fish fed *Artemia* were surprisingly low, only 5.1 and 6.1 %·d⁻¹ for a 7-d duration, respectively. The lower growth in *Heterobranchus longifilis* fed with *Moina* can account for the presence of numerous undigested ephippial eggs in the digestive tract of the larvae [12]. In the present study, we observed some *Eucyclops* and ephippial eggs in larval stomach content analyses. This could be the cause of growth retardation in *P. bocourti* since ephippial eggs are resistant to digestion in many fish species [16].

*Tubifex* worms have been used as a live feed for nursing European catfish (*Silurus glanis*) on a large scale in Hungary owing to its economic value [10]. Since fish larvae cannot ingest such a large prey as *Tubifex*, its use in larval rearing is quite limited. Based on the measurement of mouth opening at 45 and 90°, the food size suitable for first feeding of *P. bocourti* larvae was 0.6–1.0 mm. Therefore, mouth opening in *P. bocourti* permits ingestion of *Tubifex* worms only if they are chopped. From 8 d old, *P. bocourti* larvae fed with *Tubifex* did not show any growth differences from those fed with *Artemia*. Nevertheless, the mean weight at 5 d old was still lower than that of fish fed *Artemia* (table I). 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 with a commercial trout starter diet are in accordance with other catfish: *Clarias gariepinus* [9, 17, 21], *Heterobranchus longifilis* [12]. This may be related to the quality and the digestibility of the dry diet or to the primary development of digestive systems at the first feeding. When fed with trout starter feed, *Heterobranchus longifilis* and *Clarias gariepinus* showed low survival rates of 32 and 12 %, respectively [12, 21]. *P. bocourti* in the present study apparently showed a better survival rate of 67.5 %. Therefore, it indicated that *P. bocourti* larvae has a high potential for using the artificial diet. Recently, the use of dry diets based on yeast has successfully completely replaced *Artemia* nauplii in some freshwater fishes [11]. This throws light on the possibility of using a dry diet for complete larval rearing of Mekong catfish.

Cannibalism was reported in most larval rearing especially in artificial feeding. For example, the cannibalism in *Clarias gariepinus* larval rearing contributed more than natural mortality [8]. Conversely, the *P. bocourti* larvae displayed a low cannibalism even in the artificial feeding treatment. One reason should be the homogeneity of larvae size observed during the experiment. As a result, the high survival in rearing may be partly linked to the behaviour.

Figure 1. Growth curve of *P. bocourti* larvae fed with live brine nauplii (*Artemia* sp.), *Moina* sp., *Tubifex* worms and a trout diet over a 9-d nursing time.

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