

BIOLOGICAL DEVELOPMENT AND GROWTH OF AMUR CATFISH, *Silurus asotus* REARED UNDER CONTROLLED TROPICAL CONDITION

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ABSTRACT

This study was conducted to investigate the biological development and growth of the Amur catfish, *Silurus asotus* at the early larval stage. The eggs and larvae were obtained via the artificial spawning technique. The findings showed that the eggs of the Amur catfish hatched 27 hr after fertilization (hAF) under temperature conditions of $26.9 \pm 1.3^\circ\text{C}$. The newly hatched larvae (4.71 ± 0.87 mm) had a large green yolk sac (0.99 ± 0.02 mm³), unpigmented eyes, undeveloped mouth, and closed anus with one pair of developed maxillary barbels. At the 30 hAH (7.01 ± 0.41 mm), the yolk sac continued to decrease (0.33 ± 0.08 mm³), the barbels elongated, the mouth opened, the lower jaw was movable, with the peristaltic movement of the small intestine detectable. At this point, the larvae started to feed on rotifer and formulated feed. At 72 hAH, larvae (9.02 ± 0.86 mm) had developed pectoral and dorsal fin and at 12 dAH (34.10 ± 0.89 mm) and entered the juvenile stage. This study concluded that the larval development and growth of the Amur catfish is relatively comparable to other tropical catfishes hence similar rearing techniques can be adopted to enhance the overall growth performance at the later stages.

Key words: Amur catfish, larval stages, growth performance, morphological development, nutritional transition period

INTRODUCTION

The Amur catfish (*Silurus asotus*, Linnaeus, 1758), a freshwater fish species originated from Japan and widely spread across East Asian countries, such as Vietnam, Korea, China, and Taiwan (Kim *et al.*, 2005) that value as a food fish, and as a sport fish. This species is also known as the Far Eastern catfish (Gill *et al.*, 2017), Japanese catfish, and Chinese catfish (Huckstorf, 2012). The Amur catfish is a carnivorous species belonging to the family of Siluridae (Yang *et al.*, 2015).

There are many beneficial features associated with the Amur catfish, which makes it a viable aquaculture species. This species is a popular source of protein in Indo-China, Vietnam, Mongolia, and Japan (Kim *et al.*, 2001), as many have claimed that

they are delicious, and highly nutritious (Park *et al.*, 2004). According to Huckstorf (2012), the production of wild Amur catfish in Vietnam is relatively high, and they are harvested throughout the year from their natural habitat (Gill *et al.*, 2017). This species is also highly resistant to diseases, as it can survive from wounds, and has a maximum 12-year life span (Dulmaa, 1999). This species is commonly found in reservoirs, lakes, and medium-sized streams, as well as rivers with slow running water, and muddy bottoms.

The Amur catfish was introduced in Malaysia three years ago through research collaboration between Universiti Malaysia Sabah (UMS) and Kindai University, Japan. The import permits solely for research of this species were granted by the Sabah state of Fisheries Department in 2016. Understanding the fundamentals associated with larval development and growth is a major concern in developing a suitable protocol for breeding the

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Amur catfish. Breeding of this species was successfully achieved through natural and artificial spawning methods, but a stable mass seed production is yet to be established due to unknown mortality rates at the early larval stage (Baras, 1999). With the establishment of an elaborate breeding technique for this species, attempts to develop such techniques by understanding the fundamental biological aspects of the early larval development stages of the Amur catfish is critically necessary.

It is important to have a clear understanding of the embryonic and larval development of fish species, particularly in the areas of morphological and organ development (Borcato *et al.*, 2004). Embryology and larval development in cultured fishes are important and evident in aquaculture practices, and fish breeding programs (Meijide & Guerrero, 2000). The key to increasing larval growth and maximizing survival is important to help drive the needs of each larval stage. The larval stage can be divided into two phases, which are the pre-larval and post-larval stages (Cloutier *et al.*, 2011). The pre-larval stage starts from the egg to the end of the absorption of the yolk sac. The post-larval stage starts after the absorption is completed to the end of the metamorphosis. The length of the time changes from species to species. It varies according to species, by shape, size, body ratio, fin size, pigmentation across different sizes and orders, shape, and time of the organ formation in the post-larval stage (Aral *et al.*, 2011). Thus, information on the early larval stage of the fishes is needed for increasing the larval growth and maximizing survival.

The morphological studies are needed to help the observation of physical changes in the body of fishes that comprises of the total length, yolk sac absorption, and fin formation. Changes in the morphometry can be used as indices for tissue mobilization and yolk utilization rates, providing inexpensive, real-time information about the relative larval conditions (Rogers & Westin, 1981). Besides that, changes in the total length through endogenous feeding have been used historically as a relative measure of the yolk conversion efficiency and allowed researchers to understand the larval conditions (Watanabe *et al.*, 1998). The Amur catfish had been studied in temperate Korea with temperatures 23 to 25°C in 2017 (Mun *et al.*, 2017). The temperature usually fluctuation during the incubation phase affected the variation in the rates of the fish development in the embryonic, larval, and juvenile stages. Hence, it is important to understand the fundamentals associated with larval morphological development studies to develop proper breeding protocols for the Amur catfish, especially in a tropical area. To develop the proper

breeding protocols for the Amur catfish, the growth and morphological development of the Amur catfish larval needs to be determined initially. This study aims to determine the growth and development morphological characteristics of the early larval stages of the Amur catfish in tropical conditions.

MATERIALS AND METHODS

This study was conducted in the fish hatchery at UMS, Sabah in the green water environment. The brood fish of the Amur catfish used in this study were bred for three years in 20 tonnes of cylindrical fiber reinforced plastic (FRP; 4 m in diameter and 1 m in height), equipped with aeration. The brood fish were fed with enriched prey fish (*Sardinella* sp.) daily until satiation. The water condition was maintained at a temperature of $26.7 \pm 1.3^\circ\text{C}$, with dissolved oxygen (6.5 ± 0.3 mg/L) and a controlled pH (6.8 ± 0.4). In this experiment, the guidelines of care and handlings of laboratory animals were followed according to the Malaysian Animal Handling Code of Conduct and code of practice for the care and use of animals for scientific purposes.

Stripping and fertilization

For artificial stripping purposes, the brood fish (1 female and 3 male) weighing 314.20 ± 48.06 g were treated with the pituitary gland (PG) hormone. The dosage was 5 mg/kg of the total body weight of the fish. Hormones were injected via intramuscular and after 6 hr, the eggs and sperms were collected at the peak of the ovulation. The total weight of the egg was (60.0 ± 1.9 g). The fertilized eggs were greenish in color and oval in shape. The fertilized eggs were then spread into 60 L plastic tanks until hatching. The tank was equipped with a sufficient aeration supply ($8\text{ cm}^3/\text{s}$). The hatching was expected to be around 27 hr after the fertilization occurred. 60 L of rectangular plastic tanks equipped with recirculating aquaculture systems (RAS) throughout the incubation period with a water temperature of $26.9 \pm 1.3^\circ\text{C}$ and dissolved oxygen (6.7 ± 0.3 mg/L) with a controlled pH of 7.0 ± 0.4 was used. The egg development was observed under a microscope (Compound light microscope, Omax, China).

Larval rearing

A total of 10,886 Amur catfish larvae were collected for 20 days after the eggs hatched in five 60 L plastic tanks equipped with RAS. The water quality was at a temperature of $27.0 \pm 1.3^\circ\text{C}$, with a dissolved oxygen level of 6.6 ± 0.3 mg/L and pH of 7.2 ± 0.4 . Bottom cleaning was carried out and 20% of water was exchanged every day. Newly hatched larvae were fed with a combination of live

feed such as Rotifer (*Brachionus plicatilis*) and *Moina* sp., and formulated pellets (Otohime, Nisshin Maru, Japan), following the schedule as shown in Figure 1. The larvae were fed up to six times per day until satiation. The number of mortality events was measured by counting the dead larval. The embryonic (fertilization rate (%), development rate (%), and hatching rate (%)) were simply done by sampled 200 ± 5 eggs in a petri dish and calculated according to Fukuhara (1986). The larval data also was measured as follows according to Fukuhara (1986):

$$\text{Fertilization rate (\%)} = \frac{\text{Total number of fertilized eggs}}{\text{Total number of eggs}} \times 100$$

$$\text{Development rate (\%)} = \frac{\text{Total number of survived eggs}}{\text{Total number of fertilized eggs}} \times 100$$

$$\text{Hatching rate (\%)} = \frac{\text{Total number of larvae hatched}}{\text{Total number of fertilized eggs}} \times 100$$

$$\text{Survival (\%)} = \frac{\text{Final number of fish}}{\text{Initial number of fish}} \times 100$$

$$\text{Mortality (\%)} = \frac{\text{Final number of fish died}}{\text{Initial number of fish}} \times 100$$

Larval morphological observation

Once the embryonic development stage was completed, sampling ($n=10$) was done for larval morphological observation. The hatching time was recorded and indicated as 0 hAH. The observation for larval development was sampled every 6 hr interval from 0 until 90 hAH. After that, larvae continued sampled from 5 until 20 dAH with 1 and 2 days intervals. The larvae were observed under a microscope (Compound light microscope, Omax, China) with a magnification of 40x to examine the

morphological development. The morphological changes that were observed included fins developed, mouth and anus opening, the appearance of the barbels, and pigmented eyes. All photos were taken using a digital camera and sketched. The larvae were then measured for their total length, and the yolk sac absorption using a microscope (Digital microscope, Dino-lite, Taiwan). The formula for yolk sac volume (YSV) was measured as follows according to Fukuhara (1986):

$$\text{YSV} = \frac{\pi}{6} \times L \times H^2$$

L: Yolk sac length
H: Yolk sac height

RESULTS

Embryonic observation

A total of 14,400 eggs (1.6 ± 0.1 mm) were collected and were found to be greenish, with negative buoyancy, sinking to the bottom of the tank. Fertilization and development rates were recorded at $90.0 \pm 4.9\%$ and $80.0 \pm 4.9\%$, respectively. The larval hatching rate was $75.6 \pm 4.7\%$. The water temperature, dissolved oxygen, and pH during egg incubation ranged between $27.9 \pm 1.4^\circ\text{C}$, 6.5 ± 0.5 mg/L, and 7.1 ± 0.4 , respectively. Hatching took place at approximately 27 hAF.

Yolk sac absorption and larval growth

The initial volume of the yolk sac for the newly hatched (0 hAH) was observed at 0.99 ± 0.02 mm³ and decreased to 0.90 ± 0.13 , 0.77 ± 0.05 , and 0.65 ± 0.15 mm³ at 6, 12, and 18 hAH, respectively. After that, the yolk sac volume decreased gradually to 0.47 ± 0.03 and 0.33 ± 0.08 mm³ at 24 and 30 hAH. The first feeding time occurred at 30 hAH while, the yolk sac volume was still visible and decreased at

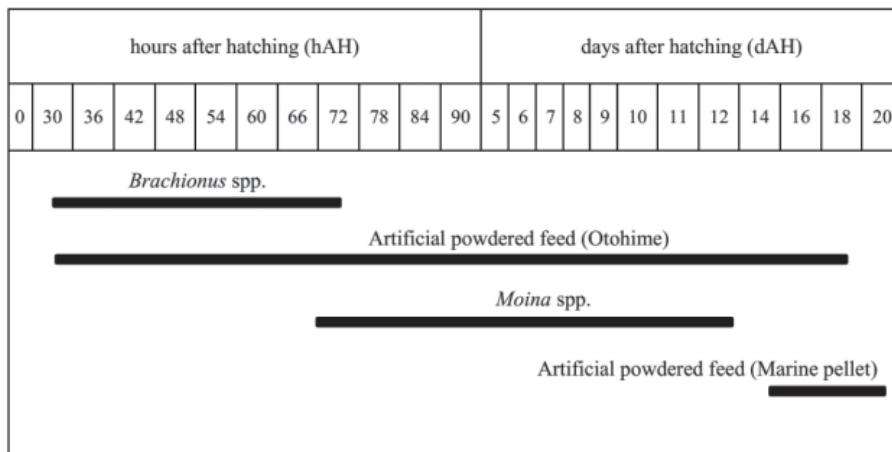


Fig. 1. Feeding regime of Amur catfish larvae for 20 days.

0.29 ± 0.04 , 0.22 ± 0.06 , 0.19 ± 0.10 , 0.14 ± 0.09 , 0.10 ± 0.01 and 0.0 mm^3 at 36, 42, 48, 54, 60 and 66 hAH, respectively. The yolk sac was completely exhausted at 66 hAH. The nutritional transition period (NTP) of the Amur catfish was 36 hr (Figure 2).

The total length (mm) of the newly hatched larvae (0 hAH) was 4.71 ± 0.87 mm, reaching 5.51 ± 0.25 , 5.57 ± 0.25 and 5.86 ± 0.70 mm at 6, 12 and 18 hAH (Figure 3). After that, the total length of the Amur catfish increased slightly to 6.44 ± 0.69 and 7.01 ± 0.24 mm at 24 and 30 hAH. The total length increased steadily at 7.26 ± 0.41 , 7.48 ± 0.05 and finally 7.82 ± 0.37 mm at 36, 42 and 48 hAH, respectively. Then, the total length of the Amur catfish increased slightly to 7.85 ± 0.99 , 8.32 ± 0.51 ,

8.90 ± 0.46 , 9.02 ± 0.86 , 9.27 ± 0.32 , 9.90 ± 0.26 and 11.78 ± 0.41 mm at 54, 60, 66, 72, 78, 84 and 90 hAH, respectively. The total length increased steadily at 12.12 ± 0.68 , 13.48 ± 0.16 , 15.02 ± 0.45 , 15.47 ± 0.13 , 16.09 ± 0.64 , 16.65 ± 0.11 , 18.35 ± 0.04 and finally 20.22 ± 0.09 mm at 5, 6, 7, 8, 9, 10, 11 and 12 dAH, respectively. After that, total length increased at 14, 16, 18 and 20 dAH with 23.28 ± 0.13 , 26.44 ± 0.17 , 30.52 ± 0.50 and 34.10 ± 0.89 mm, respectively.

Larval survival and mortality

All Amur catfish larvae attained $100.0 \pm 0.0\%$ survival at 0 and 6 hAH (Figure 4). After that, the survival of larvae decreased gradually to 99.9 ± 0.2 , 99.9 ± 0.1 , 99.9 ± 0.17 , 99.8 ± 0.6 and $99.4 \pm 0.2\%$

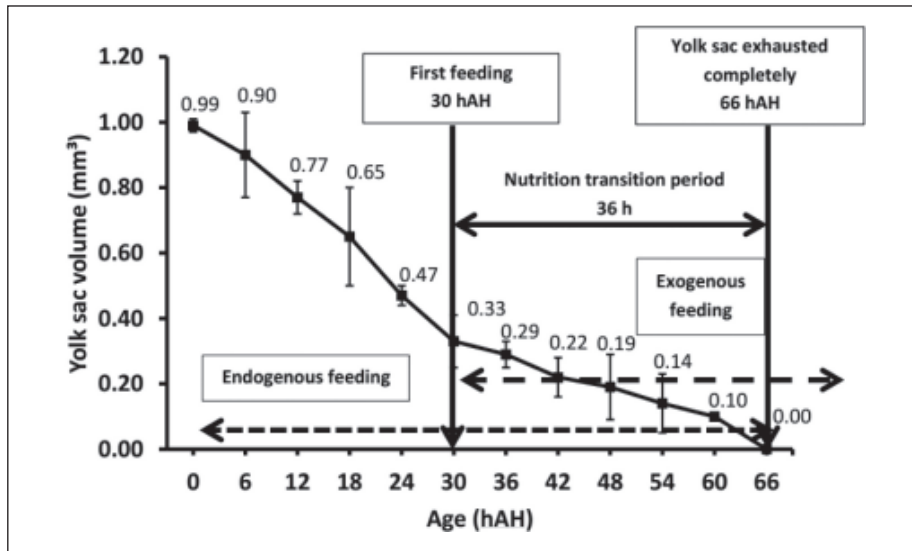


Fig. 2. Yolk sac volume (mm^3) of Amur catfish from 0 until 66 hAH. First feeding started from 30 hAH and yolk sac completely exhausted at 66 hAH with 36 hr NTP.

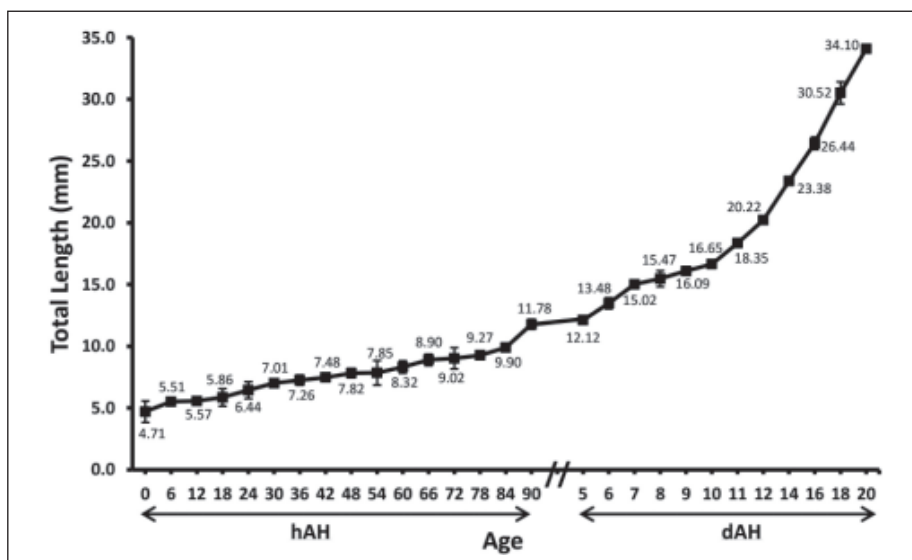


Fig. 3. Growth changes in total length (mm) of Amur catfish from 0 hAH to 20 dAH.

at 12, 18, 24, 30 and 36 hAH, respectively. Survival of the Amur catfish continued to decrease at 99.0 ± 0.3 , 99.0 ± 0.1 , 98.9 ± 0.2 , 98.5 ± 0.4 , 98.3 ± 0.3 , 98.0 ± 0.3 and $97.7 \pm 0.2\%$ at 42, 48, 54, 60, 66, 72 and 78 hAH, respectively. At 84 hAH, the larvae attained $97.3 \pm 0.3\%$ survival, and finally decreased to $97.0 \pm 0.1\%$ of survival at 90 hAH. Then, the survival of Amur catfish continue decreased to 96.5 ± 0.3 , 96.3 ± 0.3 , 96.0 ± 0.1 , 95.8 ± 0.1 , 95.5 ± 0.2 , 95.3 ± 0.2 and $95.2 \pm 0.3\%$ at 5, 6, 7, 8, 9, 10 and 11 dAH, respectively. After that, the survival of larvae decreased gradually to 95.2 ± 0.1 , 95.0 ± 0.2 , 94.7 ± 0.2 , 94.3 ± 0.1 and $94.0 \pm 0.2\%$ at 12, 14, 16, 18 and 20 dAH, respectively.

The mortality of the Amur catfish larvae are shown in Figure 5. Mortality was first observed at 12 hAH with $0.1 \pm 0.0\%$. At 18, 24, 30, 36, 42 and 48 hAH, mortality rates increased with 0.1 ± 0.0 , 0.1 ± 0.0 , 0.2 ± 0.01 , 0.6 ± 0.01 , 1.0 ± 0.05 and $1.0 \pm 0.03\%$, respectively. Mortality attained 1.1 ± 0.04 , 1.5 ± 0.02 , 1.7 ± 0.04 , 2.0 ± 0.01 , 2.3 ± 0.03 , 2.7 ± 0.04 and $3.0 \pm 0.05\%$ at 54, 60, 66, 72, 78, 84 and 90 hAH, respectively. At 5, 6, 7, 8, 9, 10 and 11 dAH, the mortality incidence increased slightly at 3.5 ± 0.02 , 3.7 ± 0.05 , 4.0 ± 0.01 , 4.2 ± 0.04 , 4.5 ± 0.07 , 4.7 ± 0.05 and $4.8 \pm 0.02\%$. After that, the mortality of the larvae increased slightly at 4.8 ± 0.06 , 5.0 ± 0.01 , 5.3 ± 0.01 , 5.7 ± 0.01 and $6.0 \pm 0.01\%$, at 12, 14, 16, 18 and 20 dAH, respectively.

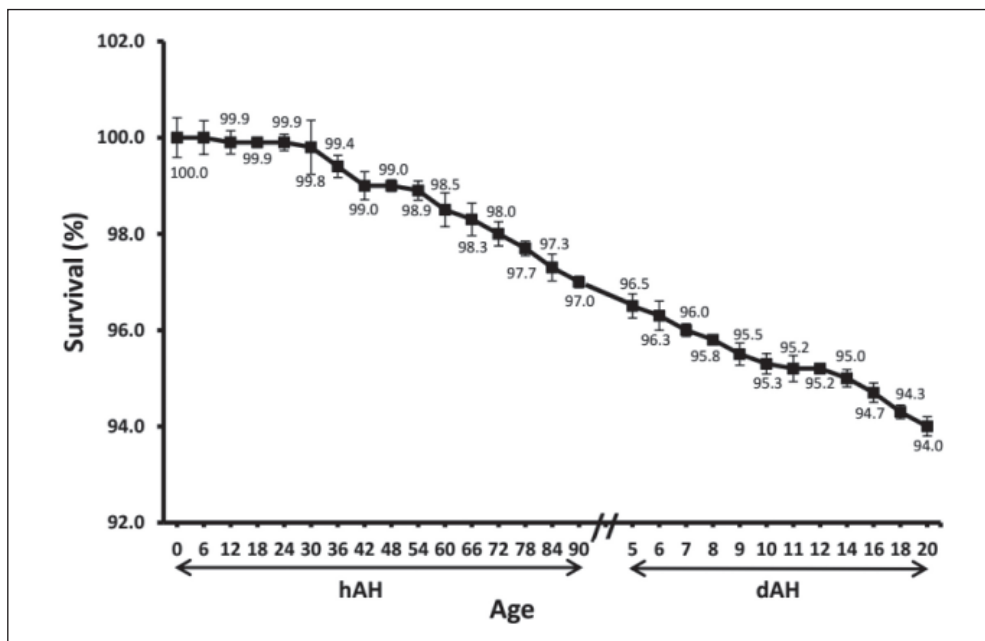


Fig. 4. Survival (%) of Amur catfish from 0 hAH to 20 dAH.

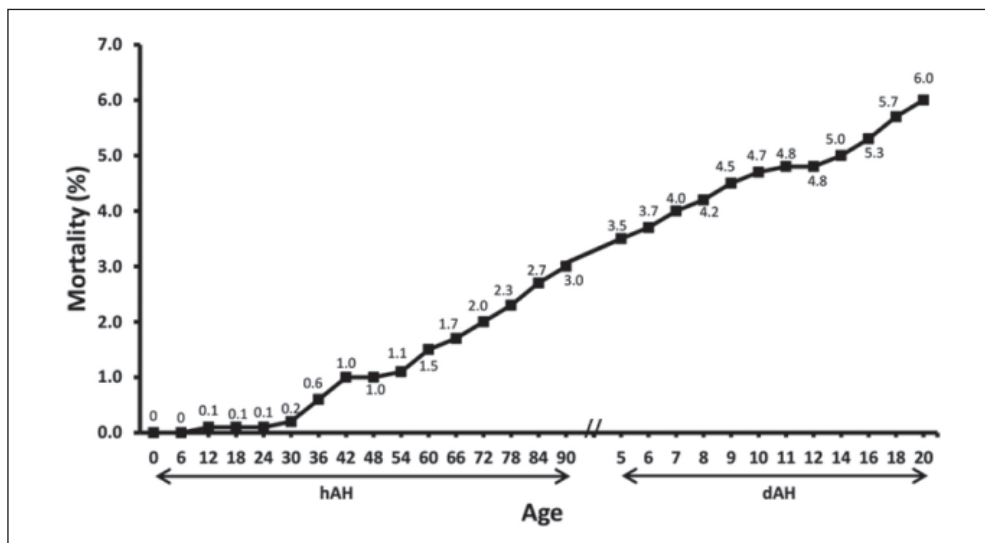


Fig. 5. Mortality (%) of Amur catfish from 0 hAH to 20 dAH.

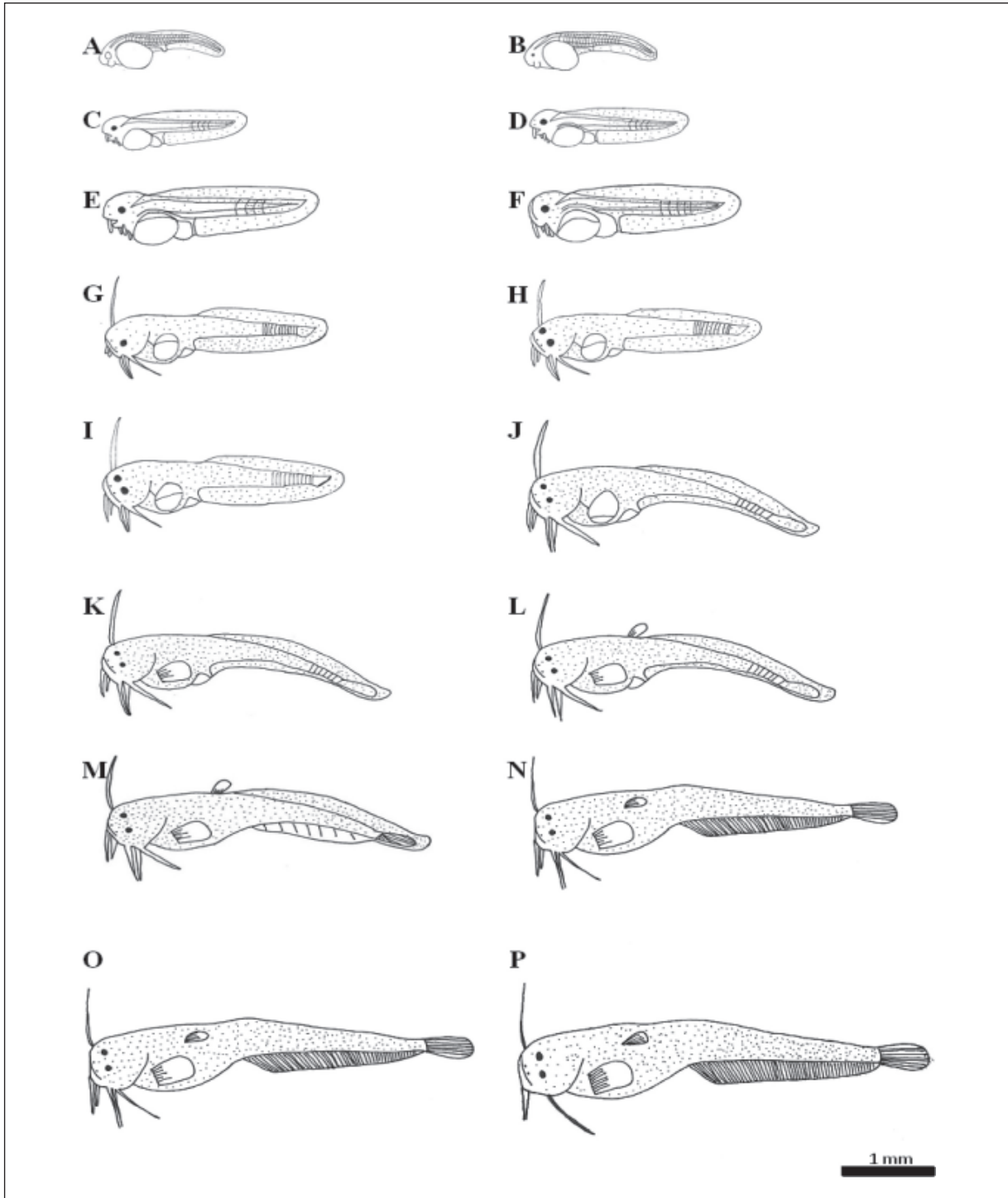


Fig. 6. Morphological ontogeny in the Amur catfish larvae. A) 0 hAH; B) 6 hAH; C) 12 hAH; D) 18 hAH; E) 24 hAH; F) 30 hAH; G) 36 hAH; H) 42 hAH; I) 48 hAH; J) 60 hAH; K) 66 hAH; L) 72 hAH; M) 5 dAH; N) 9 dAH; O) 12 dAH; P) 20 dAH (Bar: 1 mm).

Larval morphological development

The morphological changes to the Amur catfish larvae are illustrated in Figure 6. Newly hatched larvae had a large visible greenish yolk sac, unpigmented eyes, mouth and anus which was not open, and one pair of maxillary barbels. At this stage, the pigmentation on the body started to

appear (Figure 6A). At 6 hAH, the eyes were pigmented, and the anus started to develop, and the yolk sac decreased in size (Figure 6B). At 12 hAH, the eyes of larvae were deeply pigmented, the mouth and anus were open, and one pair of the mandibular barbels had developed (Figure 6C). The pigmentation on the body increased at this stage.

At 18 and 24 hAH, the yolk sac of the Amur catfish larvae decreased, and the barbels became much longer (Figures 6D and 6E).

At 30 hAH, the barbels become longer and there were four barbels, and the yolk sac decreased. The larvae had a mouth that was opened, and the lower jaw was movable (Figure 6F). The larvae were observed to be morphologically prepared for the first feeding, and rotifer was observed as there was a peristaltic movement of the small intestine. At 36 hAH, the larvae had another pair of mandibular barbels (Figure 6G). At 42 hAH, black pigmentation became more obvious. Six barbels appeared around the mouth, the mouth opening was more pronounced, and the lower jaw was movable (Figure 6H). At 48 hAH, the larvae's body pigmentation increased. The upper and lower jaws were fully developed (Figure 6I).

At 60 hAH, the tip of the tail started to bend at 45°, and pigmentation on the body more intense (Figure 6J) while at 66 hAH, the yolk sac finished, and pectoral fin in round shape appeared at the larvae body (Figure 6K). At 72 hAH, stem began to develop in the dorsal fin, and pectoral and dorsal fin appeared at the body (Figure 6L). At 5 dAH, larvae showed anal and pelvic fins appeared (Figure 6M), and at 9 dAH, caudal fin start developed and fin rays start to be seen at anal and caudal fin (Figure 6N). Amur catfish reached the juvenile stage as external morphology starts similarly with adult fish at 12 dAH (Figure 6O). At 20 dAH, another one pair of mandibular barbels degenerates on Amur catfish fish (Figure 6P).

DISCUSSION

Embryonic development of fertilized eggs

The fertilized eggs of the Amur catfish were greenish, with a uniform spherical shape. Greenish eggs are a common indicator for many tropical catfishes eggs, and this can be a key identification to distinguish catfish from other freshwater fish species, such as the yellowish eggs of the river catfish (*P. hypophthalmus*) (Baidya & Senoo, 2003) and bagrid catfish (*M. nemurus*) (Muchlisin, 2017). Meanwhile, it is evident that this species also has bigger egg diameters (1.6 ± 0.01 mm) compared to other tropical catfish, such as the yellowtail catfish, *Pangasius pangasius* (1.2 until 1.45 mm) (Ferosekhan *et al.*, 2015) and the African catfish (*Clarias gariepinus*) measured 1.26 to 1.44 mm (Baidya & Senoo, 2002).

The egg size could increase the potential for survival of the individual fish, as large larvae tend to have lower mortality rates, and can feed on larger prey, and swim actively and evidently. This should increase the larvae's resistance to starvation and,

thereby, increase its probability of encountering a favorable environment (Duarte & Alcaraz, 1989). According to McGurk (1986), bigger egg sizes could have a much bigger yolk sac of larvae, that provides sufficient feed in the endogenous stage, and longer nutritional transition periods, hence the larvae can have ample time to complete early development of the morphology and sensory organs. Eggs produced by one individual female tend to be more uniform in size, and that is also an indicator of the good quality of the eggs (Bagenal, 1969). The selection of good female broodstock for artificial spawning purposes was a critical factor that contributed to the uniform and good quality of the eggs in this study.

This study obtained good quality eggs through fertilization with a rate of $90.0 \pm 4.9\%$, and a development rate of $80.0 \pm 4.9\%$, as well as a hatching rate of $75.6 \pm 4.7\%$. This contributes to the comprehensive broodstock management, such as strict monitoring of the quality of the water, extensive feeding management, and stress-minimized handling techniques. A complete broodstock diet is necessary to improve spawning quality and consistency (Izquierdo *et al.*, 2001). In the present study, broodstock of the Amur catfish was fed omega enriched prey fish (*Sardinella* sp.) to support maturation, and to later produce good quality eggs in the subsequent stages. The brood stockfish were usually were fed at 1 – 2% of their body weight per day, according to the FAO (2010). However, in this study, the Amur catfish were fed approximately 4 – 6% of their body weight to a satiation level to maximize the maturation quality of the broodstock, which later contributed to the availability and quality of the seeds in the subsequent stages of the fish seed production (Samad *et al.*, 2013).

One of the most significant findings in this study revealed an extremely short larval hatching time at 27 hours ($27.9 \pm 1.4^\circ\text{C}$), compared to those reported by Mun *et al.* (2017) on similar fishes, of which, the Amur catfish took 48 hr to hatch in Korea with lower water temperature (15.8 until 26.7°C). Undoubtedly, the temperature was accountable for the significant difference in the hatching hour, of which, in this study, the larvae hatched in warmer waters, which presumably has a much higher induced metabolic rate to hatch. Temperature is a major abiotic factor for controlling the rate of morphogenesis in fish. This would be beneficial to producing Amur catfishes with temperatures above 27°C , as the time to hatch can be shortened when incubated at this temperature. This finding has shown that the production of Amur catfishes can also be performed continuously all year round, in tropical regions, owing to the stable and high temperatures between 27 to 28°C . A previous study by Haylor and Mollah (1995) showed that African

catfish eggs can be successfully hatched in ambient water temperatures between 20 and 35°C with different hatching times.

Growth of Amur catfish larvae

The total length of the newly hatched larvae of the Amur catfish was 4.71 ± 0.87 mm and grew to 34.10 ± 0.89 mm at 20 dAH. Hence, this can be categorized as a fast-growth species. Contradicting the study reported by Mun *et al.* (2017), this showed that a similar species was only 3.60 mm upon hatching, and increased to 8.00 mm at 72 hAH, with a temperate of 23 to 25°C in Korea. The temperature fluctuation during the incubation phase affected the variation in the rates of fish development in the embryonic, larval, and juvenile stages. A decrease in the rate of ontogeny caused by a change in temperature that resulted in a much longer larval time and increased exposure to the larval environment was observed (Atkinson, 1996). Temperatures can influence the size of the organism, at which ontogenetic transformation occurs, such as physiological and behavioral capabilities of an organism, which can exhibit effects of a changing environment (Rijnsdorp *et al.*, 2009).

Small temperature changes could have an excessively greater impact on the development of tropical fish larvae, than larvae in temperate systems with naturally large temperature variations (Green & Fisher, 2004). In tropical regions where water temperatures lie between 27 to 28°C, catfish larval sizes are much larger, as obtained in this study. This was observed for the African catfish (4.50 ± 0.20 mm in total length) (Mukai *et al.*, 2008). Swimming speed and behavior are an example of performance capabilities, as it is important to dispersal, prey capture, and predator avoidance. Temperature affects the swimming speed and activity which can influence the growth and morphological changes (Green & Fisher, 2004). In the present study, the Amur catfish swam vertically and horizontally in the middle layer of the water column and swam for most of the period on the bottom during the daytime. They swam throughout the surface and middle layers more actively at night time, which they are categorized as nocturnal species. Therefore, less energy may be required for fishes that allow for an increase in protein and fat deposition in the fish bred in the tank (Mat Nawang *et al.*, 2019). This might be the factor that is responsible for the improved growth of the Amur catfish.

First feeding of Amur catfish larvae

The onset of the first feeding was observed at 30 hAH, as was similarly reported in some catfishes, such as the bagrid catfish (30 hAH) (Rahmah *et al.*, 2011) and the African catfish (36 hAH) (Baidya & Senoo, 2004). Amur catfishes were able to attain

successful initiation of the first feeding when the rotifer was introduced in the breeding tank, creating larvae with completely pigmented eyes, open mouths, movable lower jaws, intestines that depicted peristaltic movement, and an open anus. A similar study reported the use of rotifer to compensate for delays in successful initiation of first feeding for catfish fish larvae, such as the African catfish (Ing & Chew, 2015), freshwater carp, *Cyprinus carpio* (Lubzens *et al.*, 1984), and sutchi catfish, *P. hypophthalmus* (Mukai *et al.*, 2010).

In the present study, the yolk sac volume of the newly hatched Amur catfish larvae was 0.99 ± 0.02 mm³. Larvae hatched from larger eggs tend to prolong the onset of exogenous feeding (Gisbert *et al.*, 2000). According to Ching *et al.* (2012), smaller sized larvae possessed a smaller yolk sac, and could only provide endogenous feeding in a short period. Thus, the larvae experienced a shorter time to change from endogenous to exogenous feeding and potentially underwent shorter yolk sac absorption. First feeding periods of the Amur catfish larvae ranged from 30 to 66 hAH, with the yolk sac remaining at 78.3% of its initial volume, and had 36 hr NTP. Therefore, the short yolk sac absorption was disadvantageous for larval survival and growth strategies at the early stage, as larvae had insufficient time to develop their feeding ability, and physiological mechanisms, before first feeding (Kailasam *et al.*, 2007).

The size, age, and condition of the broodstock increased the egg size, as larger eggs with larger yolk sacs generally yielded offspring with a higher probability of survival (Puvaneswari *et al.*, 2009). Commonly, larval yolk sac absorption rates varied according to the species (Jordaan & Kling, 2003), salinity (Swanson, 2005), temperature (Berlinsky *et al.*, 2004), and first feeding time (Yoseda *et al.*, 2006). The Amur catfish in temperate regions such as Korea was recorded to initiate first feeding at 3 dAH with *Artemia* sp. when the yolk sac was completely absorbed (Mun *et al.*, 2017). The length of time from hatching to first feeding depended upon the nutrients stored in the yolk sac (Hodson & Blunt, 1986), and environmental factors (Houde, 1974). First feeding of the larvae tilapia (*Tilapia sparamanii*) started as the water temperature decreased. Feeding started on day 2 at 30°C, on day 3 at 27°C, and day 6 at 24°C (Ishibashi, 1974). The nutritional period, which started from endogenous nutrient supply from the yolk to the exogenous feeding on its prey is a very important stage in the early larval development.

Survival and mortality of Amur catfish larvae

Survival of the Amur catfish showed a decreased pattern of survival from 6 hAH to 20 dAH, with 100 to 94%, which is commonly seen in many other fish

species. The present study also showed an increased pattern of mortality, from 6 hAH to 20 dAH ranging from 0 to 6% mortality. Many factors can influence the larval survival and mortality rates, such as nutrients stored, and the size of the yolk sac, temperature, size heterogeneity, food density, light intensity, and time of the first feeding (Dou *et al.*, 2002). In this study, cannibalism was responsible for larval survival and mortality rates, since the larvae had cannibalistic behavior.

One of the factors that influenced the cannibalism of fishes depends on the stocking density and size heterogeneity. Furthermore, insufficient food that is supplied in the culture system leads to decreased survival of the larval and increased mortality rates. The absence of sufficient food may lead to stress of the larval, which leads to cannibalism in the culture system. On the other hand, a work by Mollah *et al.* (1999) reported that the African catfish cannibalism can be reduced by regular grading in the larval rearing system. The Asian catfish (*P. hypophthalmus*) showed heavier losses of larval caused by the temperature and cultured fishes at suitable temperatures, which can maximize the larval growth and survival rates. Unsuitable temperatures might also induce deformation in the larval stages, including the commonly found absence of pectoral fins, which avoids the fish from moving backward (Baras *et al.*, 2011).

Morphological development of Amur catfish larvae

Newly hatched larvae developed maxillary barbels (one pair barbel) at 0 hAH, and this was similarly reported for newly hatched larvae of the bagrid catfish, which first appeared with adhesive characteristics (Ooi, 2010). Furthermore, the development of the first pair mandibular barbel for the Amur catfish was at 12 hAH, and continued to a second pair mandibular barbel at 36 hAH. The mandibular barbels functioned as gustatory senses for feeding during the early larval stages. The Amur catfish had fully pigmented eyes at 6 hAH, and this early development, in particular, the larval eyes, was in agreement with Mun *et al.* (2017), who stated that the demersal egg's larvae, like the Amur catfish, developed eyes and others organ much quicker, compared to the pelagic larvae. This is undoubtedly beneficial, as it is an improvement to how predators can be alluded to due to the early maturation of the visual system, as well as the improvement in the mobility itself (Brown, 1986). The formation of pigmentation in the eye provides a phototaxis negative reaction towards the light.

Newly hatched larvae are usually transparent, but some larvae have some visible pigment spots. Pigment spots are often used for species identi-

fication, and the developmental patterns of various organs differ from species to species (Iwai, 1980). Pigmentation of the Amur catfish larvae starts to appear upon hatching, in which they are recorded much earlier than other catfish larvae. According to Iwai (1980), the bagrid catfish larvae depicted pigmentation at 12 hAH on the head and yolk sac surfaces. This characteristic can be used to differentiate the Amur catfish and other catfishes, upon hatching. In terms of the taste buds development of the Amur catfish, it is found to be rather similar, as the taste buds increased in number and also in size, as the development is much faster in freshwater, compared to marine species. The taste buds appeared at 0, 12, and 36 hAH in the Amur catfish, which was before the onset of the first feeding, although it is considered as a rather preliminary form of the taste buds. Taste buds play an important role in fishes to detect and distinguish different kinds of chemical compounds, and this further justifies the active prey detection for the Amur catfish in the early stages. This behavior is similarly seen in the larval of the African catfish, which showed a feeding frenzy when they were stimulated with the rotifer, even before the development of the taste buds (Mukai *et al.*, 2008).

The Amur catfish was not able to commence its first feeding until the lower jaw was fully developed, although the mouth was open much earlier. The Amur catfish was seen with an open mouth at 12 hAH, but the lower jaw was only functional at 30 hAH. The anus started to open around this period, and hence, the first feeding started. Ramezani-Fard *et al.* (2011) reported that the larval mouth of the Malaysian mahseer (*Tor tambroides*) was first seen opened with a functional lower jaw as early as 1 dAH. The mouth of the Amur catfish larvae opened with a size of 150 μm at 30 hAH. The mouth size determines the maximal and optimal prey size to be ingested by the fish larvae (Rønnestad *et al.*, 2013). Fish larvae can ingest prey with similar sizes into its mouth, but they prefer smaller prey, as the preferred prey size increases as the mouth size increases (Tucker, 2000). In the present study, the Amur catfish were fed rotifer (100 to 400 μm) for the first feeding, and after 66 hAH, the Amur catfish were fed moina (500 to 1000 μm) and a grinded formulated pellet (Otohime, Nisshin Maru, Japan). If the feed provided is larger than the mouth size, the larvae will certainly be unable to feed, and consequently die of starvation (Qin & Hiller, 2000). Thus, it is suggested that the feed needs to be offered according to the mouth size of the Amur catfish larvae.

The pectoral fin of Amur catfish starts developed in round shape at 66 hAH that function as help to steer and control depth (Puvanewari *et al.*, 2009). After that, at 72 hAH, pectoral and

dorsal fin appeared at the body with the right shape of the fin. Dorsal and anal fin produce balancing torques during steady swimming (Standen & Lauder, 2005). The anal and pelvic fin appeared at the body at 5 dAH. Pelvic fin function as stabilizing and stopping when larvae swimming (Puvaneswari *et al.*, 2009). Caudal fin appeared at 9 dAH, and fin rays start to develop on the anal and caudal fin. The development of the extended caudal fin can aid in the active movement during prey capturing or predator escaping (Puvaneswari *et al.*, 2009).

At 12 dAH, Amur catfish reached the juvenile stage as the morphology of the Amur catfish same as adult fish with a total length, 20.22 ± 0.09 mm. A similar study reported in Mun *et al.* (2017) that Amur catfish reached the juvenile stage at 12 dAH with a total length, 16.7 ± 0.35 mm. One pair of mandibular barbels degenerates at 20 dAH when the total length, 34.10 ± 0.89 mm, and called temporary barbels. Another one pair of mandibular and one pair of maxillary barbels possesses permanent. The regenerative ability of the barbels may diminish gradually according to the increase in the total length of the larvae. According to Sato and Katagiri (1966) showed that the regeneration of both permanent and temporary barbels of larvae in catfish species measuring 20 and 30 mm in total length, respectively.

CONCLUSION

In summary, the present study provided important information on the successful early larval breeding of the Amur catfish. Early larval development of the Amur catfish showed higher fertilization, development and hatching rates, and better growth rates, which was relatively comparable to other tropical catfishes. The Amur catfish also showed lower mortality with a suitable breeding protocol applied. The development of the morphology of the early larval stages provided possible improvement of the larvi-culture management for the Amur catfish. Further studies should be carried out, particularly to enhance the growth and survival rates of this species.

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