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Article

Effects of dietary polyunsaturated fatty acid on reproduction and developmental biology of Gangetic Mystus (*Mystus cavasius*) (Hamilton-Buchanan, 1822)

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Abstract: The present study was undertaken to determine the effects of polyunsaturated fatty acids (PUFAs) on enhanced maturation, spawning, embryonic and larval development of endangered Gangetic Mystus (*Mystus cavasius*). Fishes were collected from the Brahmaputra River, Mymensingh, Bangladesh and stocked in the cisterns. Treated group was provided supplemental diet enriched with 1% squid extracted lipids as a source of PUFAs four months whereas controlled group fed the same diet except PUFAs. In comparison with the control group, treated group exhibited higher reproductive performances in spawning trial. The fertilization rate (79.5±1.49), hatching rate (72.29±1.78) and survival rate (64.31±0.48) of offspring were significantly higher in treated group compared to control. Consequently, early embryonic and larval development was observed in the treated fishes. Therefore, the present study indicates the enhanced reproductive and developmental performances of (*Mystus cavasius*) owing to PUFAs supplementation in diet.

Keywords: *Mystus cavasius*; PUFAs; embryonic development; larval development; reproduction

1. Introduction

Mystus cavasius (Hamilton-Buchanan, 1822) is a catfish under family Bagridae of order Siluriformes. It is commonly known as Gangetic Mystus. *M. cavasius* also known as 'Gulsha tengra' or 'Kabashi tengra' is one of the important bagrid catfish and was once available in rivers, oxbow lakes, floodplains, swamps, and canals throughout Bangladesh, India, Burma, Sri Lanka, Nepal, and Pakistan (Rahman *et al*., 2004). It is a favorite food fish to the consumers and therefore has a great demand fetching high prices in the market. It can withstand harsh environmental conditions, such as low oxygen, wide range of temperature fluctuations (Akteruzzaman *et al*., 1991). *M. cavasius* becomes sexually mature at one year age. Females get early maturity than males. Breeding period is March to September. Grayish in color, it becomes yellowish along the abdomen and cheeks with a more or less defined mid-lateral longitudinal stripe. The nutritional condition of broodstock has been found to have effect on the quality of reproduction of cultured fish including the, fertilization, chemical composition of eggs, hatching rates and larval survival rates (Henrotte *et al*., 2008; Henrotte *et al*., 2010). Fecundity and gonadal development are affected by certain essential dietary nutrients, especially in continuous spawners with short vitellogenic periods (Izquierdo *et al*., 2001). Inclusion of PUFAs in the diet proofed with the highest gonadosomatic index (GSI), which gave a sign on fish maturation (Hossen *et al*., 2014a). The fatty acid composition of eggs is directly impacted by the n-3 highly unsaturated fatty acid (HUFA) content of the broodstock diet. A positive correlation was detected between the levels of n-3 HUFA in the diet and eggs with the EPA concentration being more readily affected by dietary n-3 HUFA than DHA. Selective maintenance of DHA has also been seen during embryogenesis (Izquierdo *et al*., 2001) and denoting the essentiality of this fatty acid for the development of embryo and larvae (Reza *et al*., 2013). The percentage of morphologically normal eggs (as a parameter to determine egg viability) has been found to increase with an increase in the n-3 PUFA

content in broodstock diets and an inclusion of these fatty acids as lipid droplets into the eggs (Reza *et al*., 2013; Hossen *et al*., 2014b), thus showing the importance of essential fatty acids for normal development of eggs and embryo. For example, arachidonic acid (AA, 20:4 n-6) is one of the major nutrients to proof reproductive success in many fish species (Tocher, 2010), eicosapentaenoic acids (EPA) and docosahexaenoic acids (DHA) participate in both regulation of prostaglandin synthesis in breeder and the development of brain and retina in larvae (Sargent *et al*., 2002). Fish maturation and steroidogenesis are affected by polyunsaturated fatty acids (PUFAs) with 18 or more carbon atoms directly or through their metabolites (Simon, 2015). The present study describes the effects of dietary PUFAs on reproductive biology and developmental process of *M. cavasius*.

2. Materials and Methods

2.1. Research site and fish stocking

The spawning conducted in the Mini Hatchery Cum Breeding Complex of the Department of Fisheries Biology and Genetics and observation of development was conducted in Laboratory of Fish Genetics and Biotechnology of the Faculty of Fisheries, Bangladesh Agricultural University, Mymensingh-2202**.** Prior to stocking the broodfish, two cisterns (1.22m×2.44m×0.46m) were washed, cleaned thoroughly and filled up with water. Water level was maintained at 30cm. Outlet of each cistern was equipped with net with a metal structured small meshed net. Required number of *M. cavasius* was collected from local fisherman caught from the wild stock of the Brhamaputra river. Upon arrival at the faculty, fish were sexed and conditioned for about 15 days. Then healthy, strong and nearly equal sized fish having initial average length (15.23 ± 1.72) cm, weight of the individual fish (41.75 ± 2.56) g, and gonad (2.34 ± 0.25) g were stocked for the experiment.

2.2. Physicochemical condition of water

Temperature, dissolved oxygen (DO) and P^H of water in each cistern were recorded daily. Temperature, DO and pH were recorded by using a Celsius thermometer, a digital DO meter (multi 340 iset, DO-5509; China) and a portable digital pH meter (MICRO-TEMP, pH 500, Romania), respectively.

2.3. PUFAs enriched lipid extraction and feed preparation

Whole squid was collected from fishery ghat, Cox's Bazar, Bangladesh. Whole squid was first allowed to thaw and washed with clean tap water. The squid was chopped after removing cuttle bone and ink gland. Total lipids enriched with PUFAs from squid were extracted following the method of Bligh and Dyer (1959) with slight modification. The feed containing PUFAs was prepared including the fish meal (40%), rice bran (20%), wheat bran (15%), maize meal (13.5%), vit-B complex (0.5%), total lipids (1%) and wheat flour (10%). While in control, 1% soybean oil was used instead of extracted squid oil. Ingredients were ground finely by a grinding machine and sieved with fine mesh net. After sieving, required amount of each ingredient was weighed as per formulae with a sensitive electric balance (ANDGULF, Model-EK600, UAE) and required amount of lipid were dissolved in ethanol and mixed systematically. After mixing all the ingredients, adequate amount of water was added. Then feeds were formulated as pellet form by machine and dried under sunlight. The feeds were allowed to store in the plastic bag in air tight condition. The brood fish were fed twice daily, Once in the early morning time and then at evening time. Feed were supplied near the shelter made for the fish. The unused food stuff, debris and feces were removed by siphoning method on a daily basis.

2.4. Sampling and GSI estimation

Sampling was done monthly for examining the GSI. During sampling selected fish from each cistern were caught with the help of scoop net after lowering the water level. Then the weight of each fish was taken with a 3 digit sensitive electric balance (AND-GULF, Model-EK600, UAE). For the estimation of gonadosomatic index, 8 fish from each cistern were taken. The weight of each fish was recorded. The fish were dissected and ovaries were than weighed and gonadosomatic index was calculated by using the formula:

$$GSI = (\text{gonad weight}/\text{body weight}) \times 100$$

2.5. Induced breeding, fertilization and hatching

Selected broodfish were kept in the tank for about 6 hours for conditioning prior to injection with PG (Pituitary Gland) extract. Handling and carrying of fish was done very carefully to avoid possible injury and secondary infection. Male and female fish were kept in separate tanks and constant water flow was maintained to ensure proper aeration. In induced breeding of *M. cavasius*, pituitary gland (PG) solution was used as inducing agent. The females were given an injection of 6 mg PG/kg body weight and the male were given an injection of 2 mg PG/kg body weight as 1^{st} dose. After 6h, second dose of PG were given in 6 mg PG/kg body weight of females

and 2 mg PG/kg body weight of males. Eggs were collected from the injected female through gentle pressure on the abdomen and milt was collected by sacrificing the injected male. Eggs and milt were gently mixed with the help of feather for fertilization. Fertilized eggs were collected and transferred and spread as homogenously as possible on trays (101.6 × 40.6 × 12.7 cm^3). Fertilized eggs in trays received gentle shower. The eggs are placed on sieve for assessment of hatching rate. To protect the hatchlings from fungal infection, dead eggs and eggs shells were removed from the incubator by siphoning procedure within one hour of hatching. The hatchlings were left in the incubator till few days post-hatching for observation. The water was replaced every day to maintain good water quality.

2.6. Determination of fertilization, hatching rate and survival rate

For calculation of fertilization and hatching rates of fertilized eggs produced by the broods, a portion of fertilized eggs were taken and incubated on sieve. Soon after fertilization, the embryonic development started and the fertilized eggs looked blackish or watery and slightly transparent while unfertilized eggs look opaque and whitish in color. After completion of hatching, the number of newly hatched larvae of each sieve was counted.

Percent fertilization and hatching rates were recorded as indices of the effectiveness of diet using following formulae:

$$\% \text{ fertilization} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs (fertilized + unfertilized)}} \times 100$$

$$\% \text{ hatching} = \frac{\text{No. of eggs hatched}}{\text{Total no. of eggs}} \times 100$$

$$\% \text{ survivability} = \frac{\text{No. of survived offspring}}{\text{Total no. of offspring}} \times 100$$

2.7. Observation of early embryonic and larval development, and image capturing

The fertilized egg samples were collected randomly from the hatching trays with the help of a dropper and were taken in a petridish containing water for studying the embryonic development stages of *M. cavasius* at every 15 minutes of interval till completion of the development. Microscopic observation was continued till first feeding of fry. Embryonic and larval developmental stages were photographed (40x) under a binocular microscope OLYMPUS CX41RF, Tokyo, Japan with a digital camera (Pixel 5.0) OLYMPUS DP22 Tokyo, Japan.

3. Results and Discussion

3.1. Water quality parameters

For physiological activities like breeding and developmental stages of fish temperature is a major factor. During entire experimental period temperature range was 26.5 ± 2^oC and did not vary significantly. The dissolve oxygen (DO) and pH were also at optimum level 6.7 ± 0.5 ppm and 7.4 ± 0.2, respectively. The result is shown in Table 1. Similar water quality parameters were found for the suitable health condition and survival rate of different species of fish and other aquatic organisms (Jahan *et al.,* 2020; Pervin *et al.,* 2020a; Pervin *et al.,* 2020b; Mollah and Hossain, 1995; Mollah and Hossain, 1998; Islam *et al.,* 2018; Hossain *et al.,* 2018).

3.2. Gonadosomatic Index (GSI) calculation

Results of GSI gave a clear indication about the gonadal development as well as breeding season of fish. The highest average GSI value was found in PUFAs treated groups was 5.59 and 3.81 at the month of June and the lowest were found 2.34 and 2.09 at the month of February in PUFAs treated and control group, respectively (Figure1)**.**

Rational amount of PUFAs were supplied to the food chain in the experiment to ensure the n-3 and n-6 fatty acid requirement to the fish. The dietary supplement of squid extracted PUFAs exerted a significant increase in GSI of treatment groups compared to control fed with no PUFAs in diet. The highest average GSI (%) was observed in the month of May in PUFAs fed fish. However, it was found that the GSI of both sexes increased monthly and attained its maximum level in May. A similar result has been also found (Hossen *et al.,* 2014a) GSI of female was relatively greater than those of male. A similar result has been also found (Malla and Banik, 2015) except the highest average GSI (%) 15.582 and 2.185 in female and male of *O. bimaculatus*, respectively. GSI functioned as an important indicator of gonadal development or maturity of fish. As same ingredients of feed other than 1% lipids were fed in both the treated and control fish, improvement in GSI of treated group might be due to the presence of sensible amount of n-3 and n-6 fatty acids of the treated diet.

3.3. Fertilization rate, hatching rate and survival rate of *M. cavasius*

For good reproductive performance, the fertilization rate, hatching rate and survival rate are important indicator. Fertilization rate (79.5 ± 1.49) and hatching rate (72.29 ± 1.78) of PUFAs treated *M. cavasius* is highly significant (P<0.01) than that of control (Figure. 2). The survival rate of larvae was found significantly higher (P<0.1) in treated group than control group fed with no PUFAs (Figure 2). In the present study, the fertilization rate and hatching rate and survival rate in treated PUFAs group were found significant in number. This higher reproductive performance is clearly attributable to the effect of additional PUFAs supplementation in the diet of treated group. Similar results also found in *M. cavasius* (Hossen *et al.,* 2014b). This result is in conformity with the findings of Reza *et al*. (2013) and Izquierdo *et al*. (2001), who reported better reproductive performances of *Nandus nandus* as well as increased fecundity, fertilization rate, egg quality and hatching rate of gilthead seabream and European sea bass respectively as a result of PUFAs supplementation in the broodstock diets. It is well known that, following digestion of dietary PUFAs in the small intestine, it is subjected to absorb, transport in the blood and then subsequent assimilation within body tissues (brain, retina, heart, testis, sperm and other tissues) for enhancing their physiological response which was in fact supportive for showing better reproductive performances.

3.4. Observation of the early embryonic development of *M. cavasius*

The embryonic period starts when the egg is fertilized by the sperm and involves a constant synthesis or the building up of those elements that are vital to the normal process of the development of individual. Early embryonic development of *M. cavasius* has shown in Table. 2 with short description. Relatively few studies have inspected on the effect of dietary PUFAs on the growth, reproductive perfomance, embryonic and larval development of *M. cavasius* broodstock. In this study, *M. cavasius* was fed with n-3 and n-6 fatty acid (derived from squid extracted lipids) enriched diet at 1% level of inclusion level with other feed ingredients. In the present observation, unfertilized eggs were blackish in colour, opaque and not adhesive in nature (Figure 3). Fertilized eggs were very transparent, demarsal, spherical and brownish in colour and had a reddish brown spot on one side which is easily recognizable with naked eye (Figure 3). Whether Kohinoor *et al*. (1997) found fertilized eggs colour in light pinkish. Fertilized eggs were adhesive in nature. Adhesive nature also found in *M. cavasius* (Rahaman *et al*., 2004). Adhesive nature is similar to those of other catfish species such as *O. malabaricus* (Vijayakumar, 2010), *Heteropneustes fossilis* (Puvaneswari *et al*., 2009). In the current study, cleavage stage started from 30 min and completed within 1.5 h, early morula reached within 3.0 h at 26-27^{o}C of post fertilization. Rakhi *et al.* (2015) have similarly found the first cleavage of *Nandus nandus* eggs at 0.3±0.01 h post fertilization at 26°C water temperature. Chakrabarty *et al*. (2008) and Hossen *et al*. (2014c) observed first cleavage in about 30 min after fertilization followed by 16 celled stages in 70 min. Tumpa *et al.* (2020) have found gastrula stage in *O. pabda* within 4.50 to 5.00 h. According to Dey *et al*. (2015) gastrula period were observed to be from 3.05 to 6.33 h in *Botia lohachata* fish. In a prior study, Puvaneswari *et al*. (2009) also reported that *H. fossilis* took 7 h to reach in gastrula stage. Vigorous thrashing movements of the embryo were noticed about 1-2 h before hatching and finally the embryo hatched out in about 21 h. Similar result has also been reported by Rahaman *et al.* (2004) that *M. cavasius* took 17 to 21 h for hatching.

3.5. Observation of the larval development of *M. cavasius*

After hatching, larval development was observed until first feeding of *M. cavasius.* Larval development has been shortly described in Table 3. The hatchlings of *M. cavasius* were yellowish black in colour and measured 2 mm in length (Figure 4). Similar result found Rahaman *et al*. (2004) reported 2.59 to 2.62 mm in *M. cavasius*. The lengths of newly hatched hatchlings of *M. montanus* were 3.0 ± 0.1 mm (Arockiaraj *et al*., 2003). Observation made on the newly hatched larvae of *H. fossilis* recorded the length of 2.5 ± 0.2 mm, which was transparent and faintly brown in colour (Puvaneswari *et al*., 2009). Mukherjee *et al*. (2002) reported the length of 5-6 mm in *Ompok pabo*, while. Marimuthu and Haniffa (2007) revealed that length variation in different species can be related to the size of eggs. Improved egg quality has been associated with higher total n-3 fatty acids content in European sea bass fed a pelleted diet enriched with high quality fish oil (Navas *et al*., 1996), whereas the comparison between brackish water and seawater cod eggs showed that AA and DHA, EPA contents in the PL fraction of eggs are positively correlated with egg symmetry and viability (Pickova *et al*., 1997). In some species, such as halibut (*Hippoglossus hippoglossus*), the n-3 PUFAs are also regarded as major energy sources during early embryonic development (FalkPetersen *et al*., 1989). As whole squid meal and 1% lipids were supplied to the treatment group in their diet in the current study, there might be the effects of PUFAs diluted in squid lipids to enhance the reproductive performance as well as the early and larval development.

Table 1. Physicochemical parameters of water.

Parameters	Range (mean±SD)
Temperature	26.5 ± 2°C
pH	7.4 ± 0.2
DO	6.7 ± 0.5

Table 2. Early embryonic development of *M. cavasius.*

Developmental stage	Time	Observed Characteristics	Figure
Unfertilized eggs	**0 min**	Unfertilized eggs were blackish in color, opaque, demarsal and spherical. A large, blackish in yolk sphere is located in the center of each unfertilized egg.	**3A**
Fertilized eggs	**0 min**	Fertilized eggs were transparent, demarsal, spherical and brownish in color and had a reddish brown spot on one side which is easily recognizable with naked eye.	**3B,3C**
Cleavage	**30-1.5h**	The blastodisc was divided into 2 cells blastomeres. Then continues to divide into 4, 8, 16, 32 cells blastomeres.	**3D**
Morula	**3-4 h**	Blastomeres after repeated cleavage results into 64 cells called early morula stage.	**3E,3F**
Gastrula stage	**6-10h**	Blastoderm flattened down into yolk sphere and resulted a dome shaped structure. Gastrulation ring was formed after 5h of fertilization.	**3G,3H**
Neurula stage	**11-13h**	Yolk sphere was nearly covered by thin blastoderm. The head and tail turned into oval shape, optic bud was appeared on each side of the cephalic end.	**3I. 3J**
Somite stage	**14-19h**	Anterior-posterior end distinguishable. Heart beating actively. Notochord appeared.	**3K**
Just before hatching	**19h**	Caudal region was active. Egg membrane lost its shape. Active movement of embryo within the egg membrane was observed.	**3L**

Table 3. Larval development of *M. cavasius.*

Developmental stage	Observed Characteristics	Figure
Newly hatched larvae	The embryo hatched out in about 19h to 21h. Newly hatched larvae were slender, transparent and devoid of pigmentation in mouth and pectoral fins. The anal fin fold extended up to the yolk sac.	**4A**
6 h old larvae	The length of larvae measured averagely 3.10mm. The brain was slightly visible. Prominent notochord and barbell partially appeared.	**4B**
12 h old larvae	Two pairs of mandibular barbells had appeared. Eyes visible. The pectoral fin fuds and mouth cleft had formed. Brain becomes distinct.	**4C**
24 h old larvae	Three pairs of barbell appeared. The yolk sac was reduced. Mouth was clearly visible.	**4D**
2 days old larvae	Mouth cleft was distinguished clearly. Yolk sac completely absorbed. A pouch like stomach formed. Eyes fully pigmented. Pelvic and pectoral fin clearly visible.	**4E**
4 days old larvae	The head became round in shape. The dorsal fin fold had broadened to the caudal fin. Anal fin was formed.	**4F**
6 days old larvae	Anal fin was separated from anus. Head more pigmented than ventral part of the body. Adipose fin was not free from caudal fin. Spine of pectoral fin was well developed.	**4G**

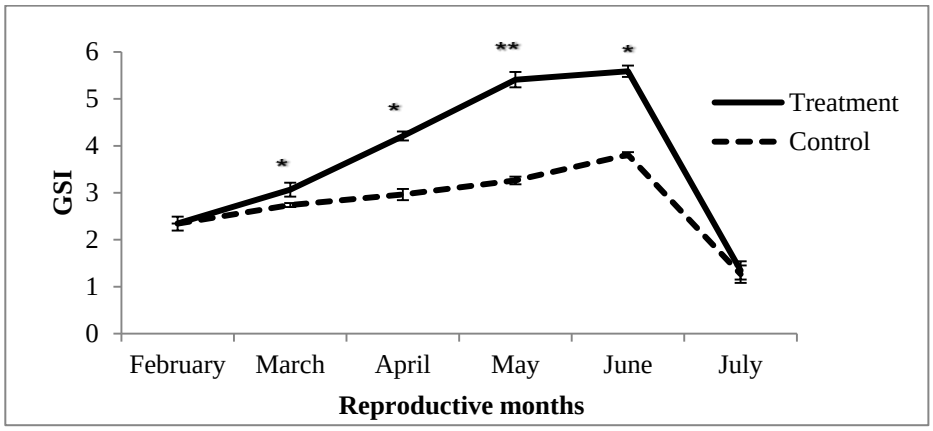

Figure 1. Monthly variation of average gonadosomatic Index (%) of *M. cavasius* in treatment and control groups. Values with *(P<0.05) and ** (P<0.01) are significantly different.

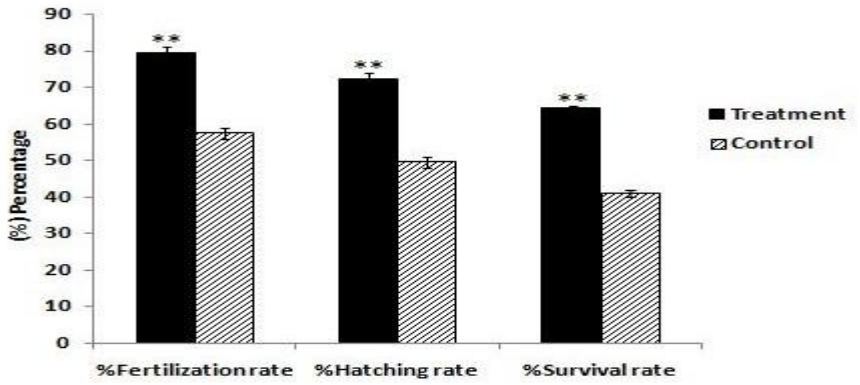

Figure 2. Percentage (%) fertilization rate, hatching rate and survival rate of *M. cavasius* in treatment and control groups. Values with ** (P<0.01) are significantly different.

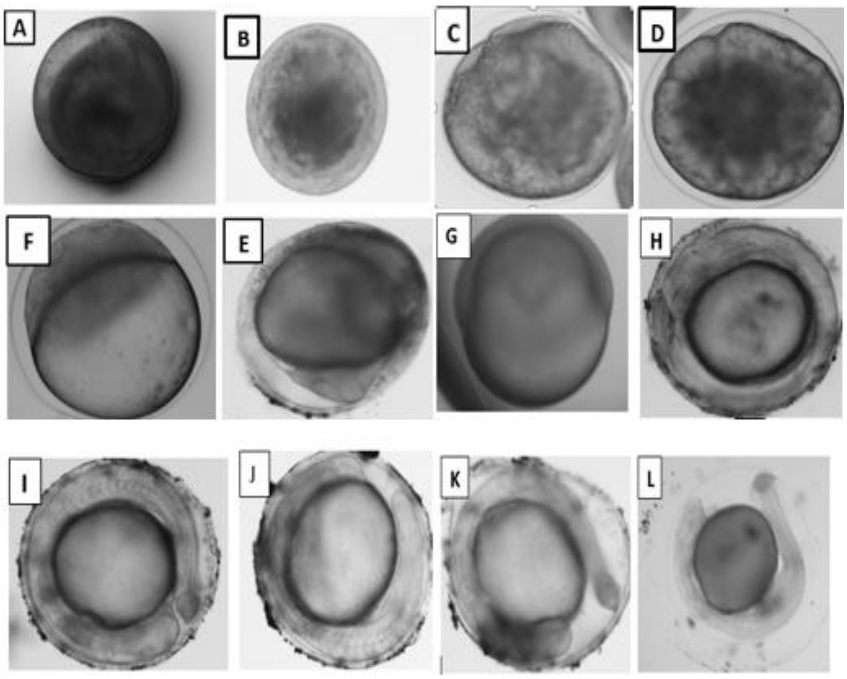

Figure 3. Early embryonic development of *M. cavasius*, A. Unfertilized egg (0 min), B. Fertilized egg, C. Blastodisc just formed (15 min), D. Cleavage stage (30 min to 1.50 h), E. Early morula stage (1.5 h to 2.0 h), F. Morula stage (3.0 h to 4.0 h)G. Gastrula stage (6h to 10h), H. Yolk plug stage (10 h), I. Nerula stage (11h), J. Somite stage (11-19 h), K. Twisted movement (19 h) and L. Just before hatching (21 h).

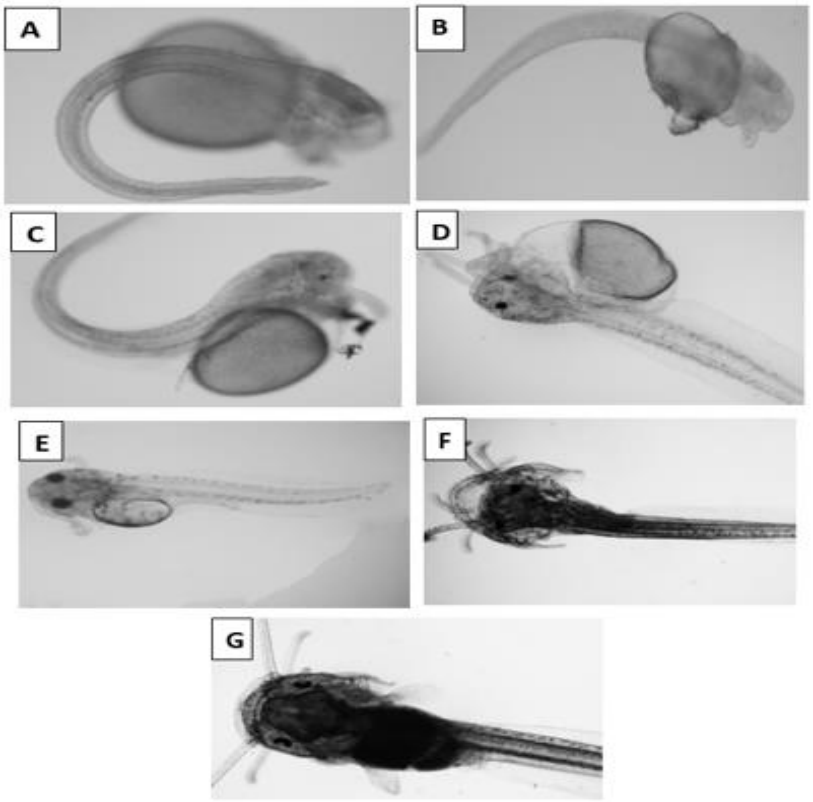

Figure 4. Larval development of *M. cavasius*, A. Newly hatched larva, B. 6 h old larva, C. 12 h old larva, D. 24 h old larva, E. 48 h (2 d) old larva, F. 96h (4d) old larva, G. (6 d) old larva.

4. Conclusions

Adverse climatic effects like pollution, degradation and decline of wetland resources and application of pesticides are the reason behind production of poor quality fish seed and extinction. For enhancing production and restricting resources declining, development policy must be undertaken prioritizing improvement of biological management and application of synthetic hormone. Great influence of bio-functional compounds like PUFAs on improvement of fish's reproduction is well documented. As the main source of n-3 fatty acids are marine environment, it is difficult to make them available for freshwater teleosts. Squid is considered as by catch and are of no use in our country, are used n-3 fatty acids source in this study.

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Conflict of interest

None to declare.

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