RESEARCH IN
AGRICULTURE, LIVESTOCK AND FISHERIES
ISSN : P-2409-0603, E-2409-9325

Open Access
Research Article

Res. Agric., Livest. Fish.
Vol. 1, No. 1, December 2014: 127-136

INDUCED BREEDING OF ENDANGERED STRIPED DWARF CATFISH (*Mystus vittatus*) AND ITS EMBRYONIC AND LARVAL DEVELOPMENT

Rabeya Yesmin[1], Salina Akhter Sume[1], Md. Nazmul Haque[1], Nargis Sultana[2] and Golam Quader Khan[1*]

[1]Department of Fisheries Biology and Genetics, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh, [2]Department of Fisheries Biology and Aquatic Environment, Bangabandhu Sheikh Mujibur Rahman Agricultural University, Salna, Gazipur-1706, Bangladesh

***Corresponding author:** Golam Quader Khan, e-mail: khanmgq@yahoo.com

ARTICLE INFO

Received
29.09.2014

Accepted
24.11.2014

Online
27.12.2014

Key words:

Mystus vitttus
Induced breeding
Larvae fertilization

ABSTRACT

The present study reports successful induced breeding of endangered striped dwarf catfish *Mystus vittatus* and its different embryonic and larval developmental stages. Three different doses of PG were tested, viz. 17, 15 and 13 mg PG/kg body weight for female and 14, 12 and 10 mg PG/kg body weight for male with maintaining (1:1) male and female ratio. The hormone doses 13 mg/kg for female and 10 mg/kg for male provided the best result i.e. 91.33±2.08% fertilization and 85.00±2% hatching rates. Mean survival percentage of the spawns up to 21 days was 8.00±1%. The fertilized eggs were found to be transparent, demersal, spherical, adhesive and brownish in colour and first cleavage took place within 35-40 min post-fertilization at 29.56± 0.25oC. Hatching took place at 24 h. after fertilization. Newly hatched larvae were 3-4 mm in length and slender, transparent and the yolk sac oval in shape. Anus was situated at almost mid ventrally. Larvae started to feed at 48-72 h post-hatching.

To cite this article: R Yesmin, SA Sume, MN Haque, N Sultana and GQ Khan, 2014. Induced breeding of endangered striped dwarf catfish (*Mystus vittatus*) and its embryonic and larval development. Res. Agric., Livest. Fish. 1(1): 127-136.

INTRODUCTION

Mystus vittatus (Bloch, 1794) is an indigenous catfish species of Bangladesh that belongs to the family Bagridae of the order Siluriformes. It has been listed as an endangered fish species along with the 54 fish species of the inland waters of the country due to overexploitation, aquatic pollution, spread of disease, uncontrolled introduction of exotic fishes, and habitat modification due to industrialization, river-valley projects, excessive water abstraction and siltation due to clearing, the development of breeding technique and so on (IUCN, 2000). The species is locally known as "Tengra" and regarded as a freshwater Small Indigenous Species (SIS) that commonly occurs in inland water areas throughout Bangladesh (Perennou and Santharam, 1990). This SIS contain many minor and trace elements including sodium, potassium, calcium, iron, iodine, zinc, magnesium and phosphorus (Roos et al., 2003). Roos et al. (2003) reported that floodplain fisheries were the main source of fish eaten by rural people of Bangladesh, with SIS contributing the most. They also reported that SIS also dominated the total fish intake in terms of amount and as well as frequency indicating great economic important. Despite its great potential, *M. vittatus* did not receive sufficient attention in aquaculture. Considering its increasing demand and great potentiality, there is a need to start its seed production.

To ensure the availability of fry this species for aquaculture as well as to prevent a fish species from extinction, it is vital to establish a dependable induced breeding and larvae rearing technique. Like other indigenous fish species Tengra is in current threat of extinction for this why to prevent them from extinction and promotion the culture of this species on commercial basis the technique of induced breeding and larvae rearing is badly needed for our commercial aquaculture on an urgent basis and considering this value the present experiment has been carried out.

MATERIALS AND METHODS

Sample collection and rearing

Fish sample were collected from different locations (Figure 1 and Table 1) in Bangladesh during March to August, 2012. Broodstock domestication was done into the Fisheries Faculty Field Laboratory Complex, Bangladesh Agricultural University, Mymensingh-2202, Bangladesh. Live sample was stocked and reared in the previously prepared separate three rectangular ponds (size 18×14 m^2 and average depth of 1.3 m) of Field Laboratory Complex, Faculty of Fisheries with acclimatization. A special feed (vitamin premix) was applied to fish at the rate of 7-8% of their body weight twice a day (Table 2). In every week growth was measured in terms of length and weight (g). Brood fish are rearing up to their sexual maturation.

Breeding trail

After brood fish rearing up to their sexual maturation and breeding was conducted in fiber plastic tank (1 x 2 x 1 ft^3) using ready to breed fish and physico-chemical conditions of water as follows temperature, DO and pH of water in different tanks ranged between 27.17^{0}C -29.04^{0}C, 2.03-4.03 ppm and 7.19-7.93 respectively with little variation) under three different treatments (T_1, T_2, T_3) each of them have three replication (R_1, R_2, and R_3) and each tank contain 150 individuals (females : males) in each replication. The average total weight of males and female in each treatment was 47gm and 35gm respectively. The females under treatment T_1, T_2 and T_3 were treated with carp PG extract (prepared by homogenization of PG with a small volume of distilled water and that was carefully transferred to a centrifuge tube by using distilled water and centrifuged for 5 min at 3000 rpm to ensure complete transfer using following formula amount of PG required was calculated, Weight of carp PG (mg) (wt) = $W_b \times P_t/100$; Where W_b represents total of the body weight of all the fishes injected and P_t represents the rate in mg of carp PG injected/kg body weight under a particular

treatment) at the doses of 17, 15 and 13 mg/kg body weight respectively whereas males were treated at the doses of 14, 12 and 10 kg/body weight (Table 3) and through a 1ml hypodermic syringe the freshly prepared solution was injected intramuscularly to the fish on the dorsal side above the lateral line. The dose was divided into two volumes (40 % & 60 %) and injected to the broods with 6 hours interval. During injection needle was inserted at about 45^{o} angles.

Table 1. Collection sites of fish samples (*M. vittatus)* from different stocks in Bangladesh

Sl. No.	Date	Sources	Stocks	No. of Individuals
1.	12-02-2011	Brahmaputra river, Sutiakhali, Mymensingh	Brahmaputra river	750
2.	18-12-2011	Chamtaghat Kishorganj	Kishorganj Haor	550
3.	29-12-2011	Brahmaputra river, Somvhogongh, Mymensingh	Brahmaputra river	700
4.	27-02-2012	Chamtaghat Kishorganj	Kishorganj Haor	450
5.	28-02-2012	Brahmaputra river, Somvhogongh, Boraikandi	Brahmaputra river	115

Table 2. Composition of experimental feed ingredients

Ingredients	Inclusion level (%)	Preparation of 200 g	Preparation of 500 g
Wheat flour	10	20	50
Wheat bran	15	30	75
Rice bran	20	40	100
Maize meal	13.5	27	67.5
Fish meal	40	80	200
Vitamin-B	0.5	1	2.5
Vitamin-E	2.5 IU/g	500 IU	1250 IU

Table 3. Lay out of the experiment for PG doses in three treatments

Treatments	Replication	Stocking density(no./Tanks)	Gender	Dose(mg/ kg/body weight)		
				1st	Interval	2nd
	R_1	150	Female	3	6h	14
T_1	R_2	150				
	R_3	150	Male	6		8
	R_1	150	Female	2	6h	13
T_2	R_2	150				
	R_3	150	Male	5		7
	R_1	150	Female	1	6h	12
T_3	R_2	150				
	R_3	150	Male	4		6

Table 4. Average fertilization, hatching and survival rates of *Mystus vittatus* at different treatment

Treatment	Fertilization rate (Ave± Sd)	Hatching rate (Ave± Sd)	Survival rate (Ave± Sd)
T1	67±17.77	55±4.58	4±3.60
T2	74±7.93	67.66±7.23	4.66±2.51
T3	91.33±2.08	85±2	8±1.00

Table 5. The physico-chemical conditions of water in experimental bowls under different c with different PG doses

Treatment	Parameters	Initial sampling	1st sampling	2nd sampling	3rd sampling
T_1	Average temp. ºC	28.00±0.5	27.82 ± 0.21	27.17± 0.05	28.87± 0.25
	Average DO ppm	3.54± 0.08	3.89 ± 0.27	4.03 ± 0.14	3.93 ± 0.14
	Average pH	7.47± 0.19	7.94 ± 0.23	7.38 ± 0.07	7.19 ± 0.09
T_2	Average temp. ºC	28.30±0.13	27.54 ± 0.07	27.18± 0.08	27.6 ± 0.17
	Average DO ppm	3.47 ± 0.12	3.81 ± 0.24	3.73 ± 0.15	3.25 ± 0.16
	Average pH	7.59 ± 0.19	7.70 ± 0.10	7.45 ± 0.23	7.66 ± 0.09
T_3	Average temp. ºC	27.86 ±024	28.05 ± 0.08	27.45± 0.22	28.4 ± 0.15
	Average DO ppm	3.59 ± 0.04	3.75 ± 0.61	3.42 ± 0.73	2.55 ± 0.46
	Average pH	7.32 ± 0.28	7.42 ± 0.07	7.44 ± 0.18	7.53 ± 0.11

Determination of Ovulation, fertilization and hatching rate

For determination of fertilization and hatching rates of fertilized eggs produced by each treatment, a portion of eggs from each female was taken separately and incubated in bowls of 15 liters. Soon after fertilization, the embryonic development started and the fertilized eggs looked watery and slightly transparent. Within 1 h of incubation, the numbers of fertilized and unfertilized eggs from each bowl were counted based on the color of the eggs. The unfertilized eggs turned opaque and whitish in color. No. of all fertilized eggs was counted contained in the bowls. After completion of hatching, the number of larvae from each bowl was counted by siphoning them out. Percent ovulation fertilization and hatching rates were calculated using following formulae:

$$\frac{No.\ of\ fish\ ovulated}{Total\ no.\ of\ fish\ injected}$$

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

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

$$\text{\% hatching = } \times 100$$

Observation of the embryonic and larval development

After the egg samples were collected randomly from the bowls with the help of a dropper and were taken in a petridish containing water for studying the embryonic developmental stages of *M. cavasius* at every 15 min, 30 min and 1 h interval till completion of morula, gastrula and hatching stage respectively. Then larvae samples were collected from the incubator. Initially samples were collected at daily intervals. At least 10 eggs and larvae undergoing embryonic and larval developmental process were observed by microscope (Optica C×41) and digital camera together with software (Magnus MIPS- Microsoft Image Processing System) for embryonic and larval developmental to obtain precise information about developmental stages.

First feeding

Although the hatchlings of *M. cavasius* get nutrition from the yolk sac upto 3 days after hatching, the larvae were provided first feeding from 3rd days (approximately 70h) after hatching at ambient temperature of 27-29ºC. Hard boiled chicken egg yolk was provided as first feed for the hatchlings upto satiation level. Three days after fertilization live zooplankton (Tubified worms) were supplied as food. The larvae reared up to 21 days and then transferred to nursery pond for further rearing.

Statistical analysis

For statistical analysis of data, a one-way analysis of variance (ANOVA) was followed. Significant results were further tested by using Tukey's Multiple Comparison test to identify significant difference among the means. The statistical data analysis was carried out with the aid of the computer software SPSS version 17 (SPSS, 1999).

RESULTS

Ovulation rate

Females treated with three different doses of PG extract showed no difference in the effectiveness of the doses on including ovulation in females. All of treated females were ovulated.

Fertilization rate

Fertilization rates of ovulated eggs in three different treatments (T_1, T_2, and T_3) showed marked difference in the effectiveness among three doses of PG extracts (Table 3). Fertilization rates of eggs were obtained from females treated with treatment T_1, T_2 and T_3 showed 67.00±17.78, 74.00±7.94 and 91.33±2.08% fertilization, respectively. The highest fertilization rate (91.33%) was recorded in T_3 (13 mg PG/kg/bw in female, 10 mg PG/kg/bw in male) whereas the lowest fertilization rate (67%) was found in T_1 (17 mg PG/kg/bw in female, 14 mg PG/kg/bw in male). Duncan's New Multiple Test indicates that T_1 was significantly (P<0.05) lower than T_3 and T_2 but there was no significant difference between T_3 and T_2. (Table 4)

Hatching rate

There was marked difference in the hatching rates of fertilized eggs in three different treatments (T_1, T_2 and T_3) (Table 4). Hatching rates of fertilized eggs obtained from females treated with treatment T_1, T_2 and T_3 were 55.00±4.58%, 67.67±7.23% and 85±2%, respectively. Duncan's New Multiple Test for hatching rate showed that T_3 was significantly (P<0.05) higher than T_3 (13 mg PG/kg/bw in female, 10 mg PG/kg/bw in male) was significantly (P<0.05) higher than T_1 (17 mg PG/kg/bw in female, 14 mg PG/kg/bw in male) and T_2 (15 mg PG/kg/bw in female, 12 mg PG/kg/bw in male) but there was no significant difference between T_1 and T_2.

Survival rate

The survival rate was found to be 4.00±3.6%, 4.66±2.52% and 8.00±1% in treatment T_1, T_2 and T_3, respectively after 21 days of experimental period and (Figure 5). Duncan's New Multiple Test revealed a significantly (P<0.05) higher survival rate in T_3 (15 mg PG/kg/bw in female, 12 mg PG/kg/bw in male) than T_2 and T_1 but there was no significant difference between T_2 and T_1.

Physico-chemical condition of water

The physico-chemical conditions of water in experimental bowls under different treatments with different PG doses are shown in Table 5. Temperature, DO and pH of water in different bowls ranged between 27.17^{0}C -29.04^{0}C, 2.03-4.03 ppm and 7.19-7.93, respectively with little variation.

Observation of the embryonic and larval development of *M. vittatus*

Changes in the pattern of the entire structure of an organ or of a specific organ in relation to the environment are decisive for evaluating the developmental patterns of a species (Balo, 1999). Changes in structure emphasize the thresholds between embryonic, larval, and post-larval development from the onset of cleavage or epiboly, or at the time of organogenesis, respectively (Kovac, 2000; Carlos et al., 2002).

DISCUSSION

Optimization of the dose of PG for induced breeding of *M. vittatus*

Dose optimization is an important aspect for successful breeding programme. To standardize the dose of PG for successful ovulation, many scientists attempted to conduct experiments [9] as the catfishes do not spawn in the laboratory condition but readily respond to injection of fish and frog pituitary gland extract and to mammalian gonadotropins (Haniffa and Sridhar, 2002) but there are remains ambiguity among the doses reported by various workers. At doses combination 6-12 mg PG/kg body weight, females respond to ovulation for at 1:2 male and female ratios where male was treated 3-6 mg PG/kg body weight revealed 80% fertilization and 56% hatching rates. Mean survival percentage of the spawns up to 10 days was 60% [11] which is slight a bit lower than the present experiment in terms of doses, fertilization (91.33±2.08%), hatching (85.00±2%) rates but opposite is true for survival rate (8.00±1%) as the days pass by this may due to temperature, that is optimum (24-30°C), and causes the hatching rate increased and ranging from 48.0±0.118, 74.33±0.232 in *Cyprinus carpio* (El-Gamal, 2009) neutral pH as reported (Nchedo and Chijioke, 2012) and other factors such as (a) age and physical state of fish, (b) the seasonal variation, (c) environmental parameters such as water temperature, dissolved oxygen etc. (d) source of fish (wild or farmed) and most importantly (e) the source, age and maturity of the donor of PG used in the experiment. Whereas (Mijkherjee et al., 2002) [14] with different doses (1-2.5 ml/kg body weight) of PG at 1:2 females and males ratios of catfishes (Pabda, *Ompok pabda* and Tengra *Mystus guillo*) and stated that 2.5 ml/kg body weight of female showed maximum ovulation, hatching (80%) rate but all the females did not show 100% ovulation may be due to species difference.

Observation of the embryonic development of *M. vittatus*

Stage	Phase	Time after fertilization	Developmental landmarks	Fig. No.
I	Unfertilized egg	00 min	Opaque, demarsal, spherical and whitish in colour	1(a)
II	Fertilized egg	00 min	Transparent, demarsal, spherical and brownish in colour	1(b)
III	Cell division	35-40 min	Cleavage	1(c)
IV	Morula	2h	Cleavage resulted into 64 cells and were arranged in 3-4 layers	1(d)
V	Blastula	2h 35min	Spherical shape, flat border between blastodisc and yolk	1(e)
VI	Gastrula	2h 55min	Cleavage resulted into 64 cells and were arranged in 3-4 layers	1(f)
VII	Head and tail bud formation	9h 10	Head and tail rudiment visible. Notochord became visible, auditory and optic bud developed	1(g)
VIII	Just before hatching	23h 30min	Embryo encircled the whole yolk. The olfactory pits and auditory vesicles were prominently visible. Melanin pigmentation was developed. Continuously beat the egg shell by the caudal region especially around the middle part of the body	1(h)
IX	Newly hatched larvae	24 h	Slender, transparent and the yolk sac oval in shape. Anus situated at almost mid ventrally. The length of newly hatched larvae about 3-4 mm	1(i)

Observation of the larval development of *M. vittatus*

Stage	Age of larvae	Characteristics	Plate No.
I	1h 55 min	Mouth was not yet developed. Heart became more distinct. Larvae tried to move by propelling the tail.	2 (a)
II	5h	Melanophores appeared on the head, around the yolk sac. The anterior part began to thicken and stronger.	2 (b)
III	9 h	A tubular pulsating heart appeared. Eye and anus slightly visible.	2 (c)
IV	18h	Eye spot with a dark pigmented area and barbells were found in the forms of tiny knobs. Pectoral fin buds were seen. Prominent chromatophore was present on the head region. Larvae swam haphazardly.	2 (c)
V	26h	Distinct heart functioned actively; reddish blood was seen around the heart region. Mouth was formed as a small opening and the anal pore also opened.	2 (c)
VI	30h	Pectoral fin rudiment faintly visible.	2 (d)
VII	48h	Burbles appeared. Brain lobe clearly distinguished. The heart functioned actively.	2 (e)
VIII	72h	Yolk sac completely disappeared and larvae started feeding	2 (f)

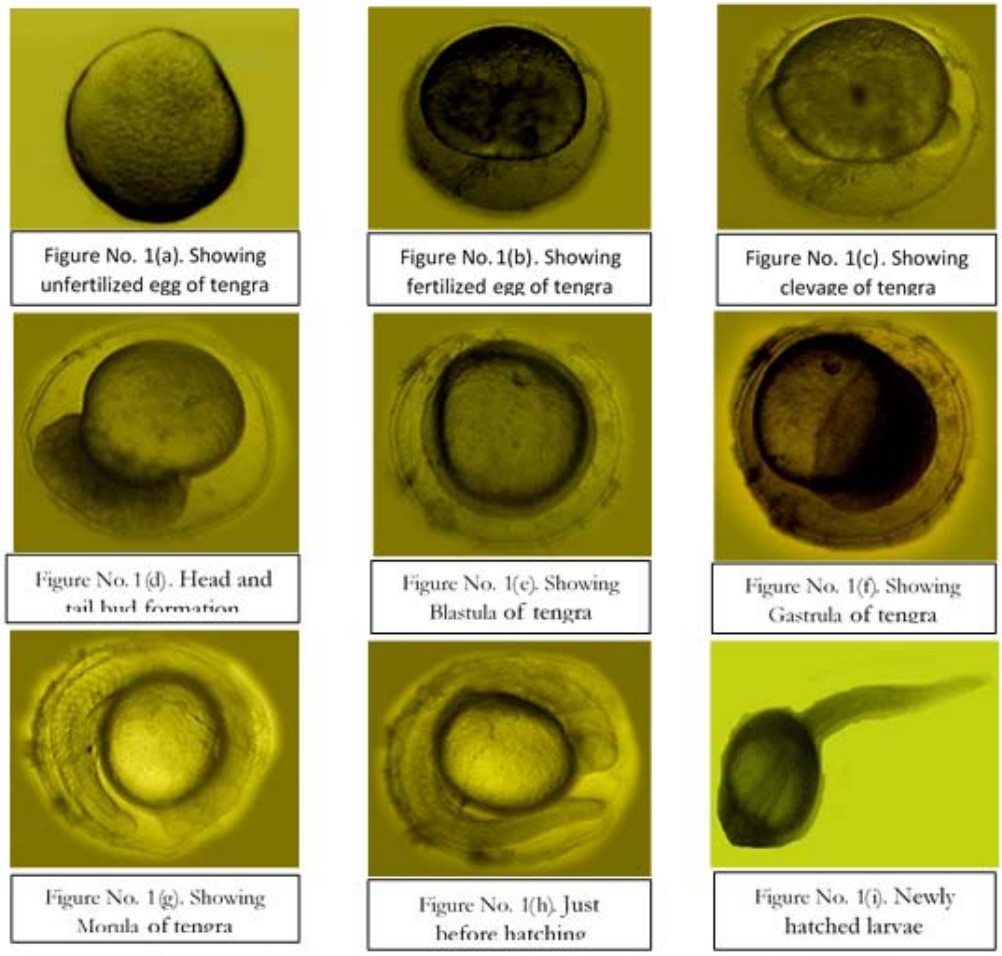

Figure 1. Stages of the embryonic development of *M. vittatus*

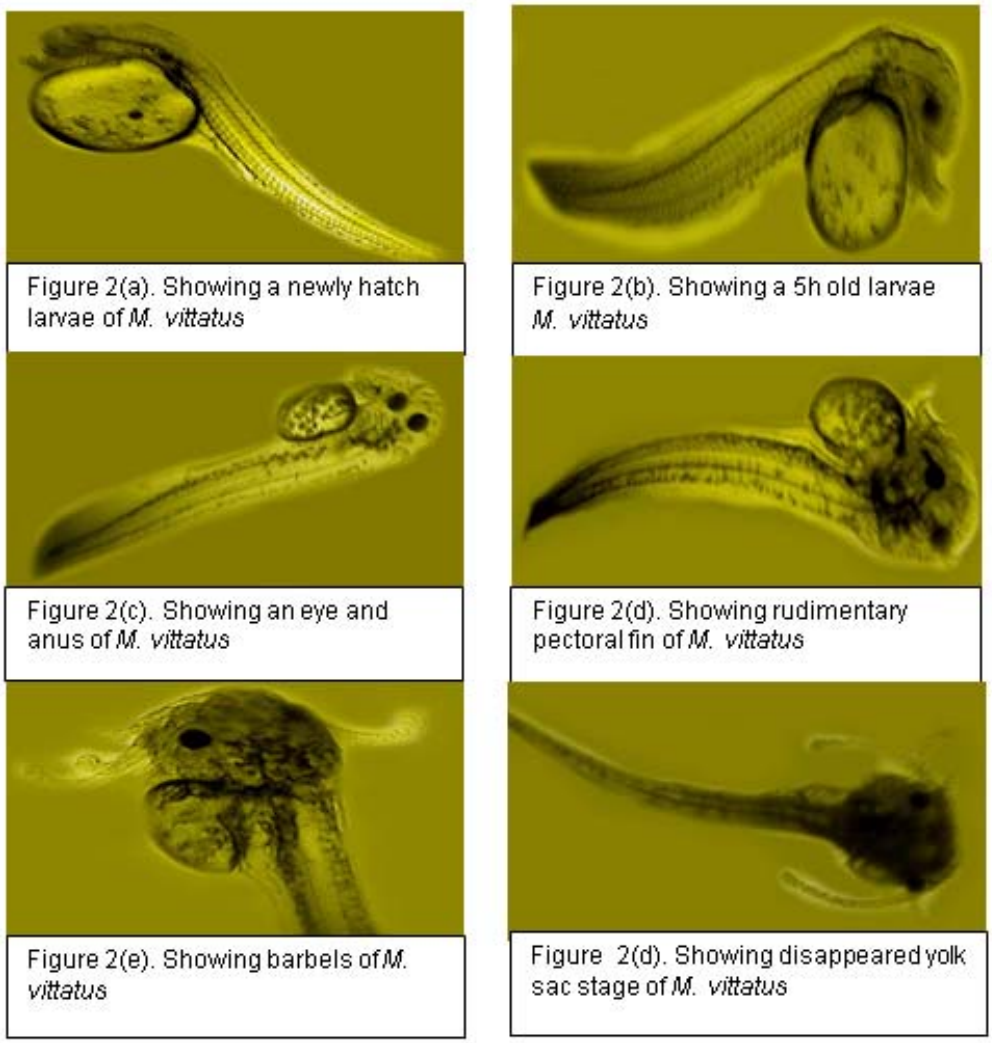

Figure 2. Stages of the embryonic development of *M. vittatus*

Observation of the embryonic and larval development

To expand catfish culture, knowledge of early larval development and feeding is imperative. But the embryonic and larval development of this fish is poorly understood. Therefore the present study was conducted to investigate and also to provide detailed information about the embryonic and larval development of this important fish species.

The unfertilized eggs of *M. vittatus* were adhesive, whitish in colour and slightly smaller while the fertilized ones were transparent, demersal, spherical, adhesive and brownish in colour which agreed with the findings of (Puvaneswari et al., 2009) except in the reported colour which they found as brownish green. This difference in colouration may be due to the species difference or may be due to colour of the background container. The fertilized eggs of *M. vittatus* were adhesive. The adhesiveness of eggs is the special character of other catfish species such as *Clarias gariepinus* (Osman et al., 2008), *Mystus montanus* (Arockiaraj et al., 2003) and *Pangasius sutchi* (Islam, 2005).

In the present study, the diameter of the fertilized eggs ranged between 1.0 and 1.3 mm. Variation in egg size was also recorded for the eggs of *Clarias gariepinus* (Osman et al, 2008). This variation might be attributed to the species variation and brood size. In this study, first cleavage took place within 25-30 min post-fertilization at the water temperature of 27-28^{o}C but in *Clarias gariepinus*

and *Mystus cavasius* first cleavage took place within 40-50 min post-fertilization reported from (Khan and Mollah, 1998; Rahman et al. 2004) at 28.5 and 26°C respectively. This variation might be due to species difference and other environmental factors. In the present observation, morula stage reached within 2.40 h post-fertilization. Gastrula stage was found in *M.vittatus* at 7.30 to 9.30 h of fertilization at 27-29°C. Previous report from (Puvaneswari et al., 2009) in *Heteropnuestes fossilis* also indicated the duration of 7 h to reach the gastrula stage. Just 1-2 h before hatching, the embryo of *M. vittatus* showed twisting movements inside the egg capsule. The similar hatching behaviour was found in different fishes reported by (Puvaneswari et al. 2009; Osman et al., 2008).

In the present study, hatching commenced from 24 h at 28.87±0.25°C which is similar to other findings reported from (Arockiaraj et al., 2003). In *Clarias gariepinus* hatching started after 18 h at 28.5°C and was completed within 22 h which is lower than the present observation (Khan et al, 1998). The development of embryo and the variability of hatching time in fertilized eggs of most of the fish are generally influenced by the temperature of water (Mollah and Tan, 1982). At lower temperature the hatching started late and the duration of hatching was longer. In *Clarias macrocephalus* hatching started within 22 h at 30°C and 34 h was required to hatch at 25°C. But at 20°C no hatching was observed (De Graaf and Janssen, 1996). Length of the newly hatched larvae of this species was around 1.0 mm which is in the ranges of the findings of (Arockiaraj et al., 2003). Variation in length of the newly hatched larvae was recorded by several scientists. Ogunji and Rahe (1999) recorded the length of newly hatched larvae of *H. longifilis* vary from 4.09 to 4.9 mm. These variations may be related to the size of the eggs. According to (Bagarinao and Chua, 1986), egg diameter is positively correlated with larval length and weight at hatching. In the present study, larvae of *M. vittatus* started to feed at 48 h after hatching which is similar to other previously reported studies stated by (Puvaneswari et al., 2009).

The present work generated some information on induced breeding, embryonic and larval development of *M. vittatus*. The study consisted of two experiments. The first experiment dealt with the optimization of the doses of carpPG extract on breeding performance of *M. vittatus*. The second one was conducted to know embryonic and larval development of the same species.

CONCLUSION

It is however, difficult to pinpoint the reason for such differing results because a number of factors affect the biological experiment particularly involving hormones. Several major factors that may have bearing on the result are: But upon all consideration a PG dose of 13 mg/kg may be recommended for this species.

REFERENCES

1. IUCN, Bangladesh, 2000. List of threatened animals of Bangladesh. Paper presented in the special workshop on Bangladesh Redbook of threatened animals, 22 February 2000, Dhaka. pp. 13.
2. Perennou C and V Santharam, 1990. An anthropological survey of some wetlands in south-east India. Journal of Bombay National Historical Society, 87: 354-63
3. Roos N, MM Islam and SH Thilsted, 2003. Small indigenous fish species in Bangladesh: contribution to vitamin a, calcium and iron intakes. Journal of Nutrition, 133 4021S-4026S.
4. SPSS, 1999. Software Program of Statistical Analysis, Version 17.0 Edition for Windows. SPSS Incorporation Chicago IL US.
5. Balo K, 1999. Alternative ways how to become a definitive phenotype or a juvenile (and on some persisting linguistic offences). Environmental Biological Fisheries, 56: 17-38.
6. Kovac V, 2000. Early development of Zingel streber. Journal Fisheries Biology, 57: 1381-1403.

7. Carlos AM, MC Sanchez, GS Papp, AD Parra and LG Ross, 2002. Observations on spawning, early development and growth of the puffer fish *Sphoeroides annulatus*. Journal of Aquaculture Tropical, 17: 59–66.
8. Thakur NK and P Das, 1974. Length weight relationship of Shinghi, *Heteropneustes fossilis*. Journal of Inland Fish Society Indian, 6: 95-96.
9. Haniffa MAK and S Sridhar, 2002. Induced spawning of spotted murrel (*Channa punctatus*) and catfish (*Heteropneustes fossilis*) using human chorionic gonadtropin and synthetic hormone (ovaprim). Veterinary Archive, 72: 51-56.
10. Rahi Md, SK Lifat, I Shahinur and MS Shah, 2011. Study of fecundity and induced breeding of *Mystus vittatus*. Bangladesh Journal of Zoology, 39: 205-212.
11. Abd El-Hakim and E El-Gamal, 2009. Effect of Temperature on Hatching and Larval Development and Mucin Secretion in Common Carp, *Cyprinus carpio* (Linnaeus, 1758). Global Veterinaria, 3: 80-90.
12. Nchedo AC and OG Chijioke, 2012. Effect of pH on Hatching Success and Larval Survival of African Catfish (Clarias gariepinus). Nature and Science, 10: 21-25.
13. Mijkherjee M, A Praharaj and S Das, 2002. Conservation of fish stocks through artificial propagation and larval rearing technique in West Bengal, India. Aquaculture Asia, 7: 8-11.
14. Puvaneswari S, K Marimuthu, R Karuppasamy, MA Haniffa, 2009. Early embryonic and larval development of Indian catfish, *Heteropneustes fossilis*. EuroAsia Journal of Biological Science, 3: 84-96.
15. Osman AGM, S Wiertz, IA Mekkawy, J Verreth and F Kirschbaum, 2008. Early development of the African catfish *Clarias gariepinus* focusing on the ontogeny of selected organs. Journal of Applied Ichthyology, 24: 187-195.
16. Arockiaraj J, MA Haniffa, S Seetharaman and SP Singh, 2003. Early development of a threatened freshwater catfish *Mystus montanus* (Jerdon). Acta Zoologica Taiwanica, 14: 23-32.
17. Islam M, 2005. Embryonic and larval development of Thai pangas (*Pangasius sutchi* Fowler, 1937). Development and Growth Diffraction, 47: 1-6.
18. Khan MMR and MFA Mollah, 1998. Embryonic and larval development of African catfish *Clarias gariepinus*. Bangladesh Journal of fisheries, 21: 91-97
19. Rahman MR, MA Rahman, MN Khan and MG Hussain, 2004. Observation of the embryonic and larval development of silurid catfish, Gulsha (*Mystus cavasius* Ham.) Pakistan Journal of Biological Science, 7: 1070-1075.
20. Mollah MFA and ESP Tan, 1982. Effect of incubation temperature on the hatching catfish (*Clarias macrocephalus*) eggs with an illustration on the larval stages. Malaysian National Journal, 36: 123-131.
21. De Graaf GJ and H Janssen, 1996. Artificial reproduction and pond rearing of African catfish (*Clarias gariepinus*) in sub Sahran Africa. FAO Fisheries Technical paper, 362: 1-73.
22. Ogunji JO and RE and Rahe, 1999. Larval development of the African catfish *Heterobranchus longifilis*. 1840 (Teleostei Claridae) and its larval behavior. Journal of Aquaculture Tropics, 14: 11-25.
23. Bagarinao T and TE Chua, 1986. Egg size and larval size among teleost simplications to survival potential. In: Maclean J, Dizon LB, Hossils LV (eds). The First Asain Fisheries Foroum, Asain Fisheries society, Manila. pp. 651-656.