Induced Breeding of Glass Catfish, *Kryptopterus vitreolus* (Ng and Kottelat, 2013)

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ABSTRACT

Induced breeding of glass catfish, *Kryptopterus vitreolus* (Ng and Kottelat, 2013), was carried out at Phitsanulok Inland Fisheries Research and Development Center from September 2018 to November 2018, in order to compare the concentration of human chorionic gonadotropin (HCG) for induced breeding of this species. The experiment consisted of four treatments, in which the female broodstock was injected with HCG at different concentrations to stimulate egg maturation. Then, after 24 h, the females in all treatments were injected with 10 μg·kg^{-1} buserelin acetate (BUS) in combination with 10 mg·kg^{-1} domperidone (DOM) to stimulate ovulation. Treatment 1 females were injected with 0.9 % sodium chloride to serve as a control group. Treatments 2, 3 and 4 females were injected with HCG at 100, 300 and 500 IU·kg^{-1}, respectively. The male fish were injected only with BUS and DOM, at the same concentrations as the females. The results showed that the female brooders in the control group did not spawn, while 100 % ovulation was achieved from females receiving all treatment levels of HCG. Concentration of HCG clearly showed positive influence on most parameters observed, such as readiness of spawning and egg weight. Maximum fertilization rate, hatching rate and survival rate of the fry were found for females injected with 500 IU·kg^{-1} HCG, and showed significant differences from other treatments ($p<0.05$). The eggs of glass catfish were 0.75-0.85 mm in diameter, bright yellow and adhesively attached to substrates. The hatching took approximately 21.30 h at 25.3-26.7 °C. Hatchting larvae were 2.5±0.1 mm in length. It was concluded that 500 IU·kg^{-1} HCG with 10 μg·kg^{-1} BUS and 10 mg·kg^{-1} DOM can result in successful artificial breeding of this species.

Keywords: *Kryptopterus vitreolus*, HCG, Induced breeding, Embryonic development

INTRODUCTION

The glass catfish *Kryptopterus vitreolus* (Ng and Kottelat, 2013) is an Asian glass catfish with elongated body and slightly curved tail. The name of the fish reflects its scale-less, translucent body; thus, the internal organs can be easily seen from the outside. There are two pairs of long barbels; the first pair is attached to the upper jaw, while the second pair is shorter and attached to the mandible. The dorsal fin is small and almost invisible, while the anal fin is long and connected to the caudal fin. The head is small, and most of the internal organs are opaque and positioned at the anterior end. The glass catfish is usually found in groups that can number 100 or more, all facing the current in the same direction. When they are scared, they scatter and then return to schooling again. The average size of adult fish is about 8-10 cm total length. This species is often kept as an aquarium fish. In Thailand, it was found that the glass catfish was mainly captured from Phattalung Province. Captured fish at 2.5-3.0 inches long could be sold at 2-3 Baht per fish, which would

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generate 500-1,000 Baht per day as a fisherman's income. The main countries importing the fish were Singapore and Malaysia (Tongwatanakorn *et al*., 2010). Volume of the glass catfish exported in 2016 was 2,494,033 fish, valued at 10,182,219 Baht. (Suvarnabhumi Airport Fish Inspection Office, 2016). Currently, breeding strategies for this species are not well established, one reason being that there is abundance in the wild. However, there is a risk for populations of this species, which is probably endemic to Thai waters, to become diminished in the future. Thus, this research aimed to develop a strategy for breeding the glass catfish in captivity to cope with the threats that face these fish in their natural habitat. Hypophysation is a technique of breeding manipulation using hormones to enhance oocyte maturation, followed by ovulation and spawning of fish in captivity (Adebayo and Fagbenro, 2004). This strategy, once perfected, used as a knowledge of breeding to conserve increase the number of fish species to natural water and would be beneficial to fish farmers for reducing fisheries from the water sources.

Human chorionic gonadotropin (HCG) induces gonads to produce sex hormone, and has been used successfully in hatcheries for induced breeding in catfish (Legendre *et al*., 2000). HCG is a glycoprotein hormone derived from the urine of pregnant women. A study of *Epinephelus fario*, *Liza ramada* and *Wallago attu* Schneider, 1801 breeding indicated that HCG injection could induce egg development (Kuo *et al*., 1988; Mousa, 2010; Phaeviset *et al*., 2016). The fish responding to HCG showed increasing abdomen size and body weight, indicating egg development (Park, 2002; Promprasert *et al*., 2011). When the hormone was injected into the fish, the oocyte diameter increased, resulting in enlarged abdomen and disruption of germinal vesicles in oocytes, which are typical signals of oocyte maturation in fish before ovulation (Mylonas *et al*., 1992). Therefore, the use of HCG to stimulate the eggs is essential in promoting successful breeding. Along with HCG, Buserelin acetate (BUS) (Suprefact™) is also used, in combination with domperidone (DOM, Motilium™) to stimulate ovulation. A benefit of induced breeding is that the larval production is greater than that for fish captured from the wild. This suggests that fish fry could be produced on a commercial scale, and this alternative would reduce pressure on wild populations.

MATERIALS AND METHODS

Experimental design

Completely randomized design (CRD) consisted of four treatments with different levels of HCG (0, 100, 300 and 500 IU·kg^{-1} body weight); five replicates (one female fish as a replicate) were assigned for the experiment. Treatment 1 fish were injected with sodium chloride (0.9 %) as a control group. After stimulation with HCG for 24 h, the female fish in all treatments were injected with 10 µg·kg^{-1} body weight BUS to stimulate ovulation in combination with 10 mg·kg^{-1}body weight DOM, following methods for induced breeding of the small fish species *Botia almorhae* and *Syncrossus helodes* (Galsri *et al*., 2010; Promprasert *et al*., 2011).

Fish broodstock

The glass catfish used in the experiment were collected from natural rivers in Phatthalung Province and were transported by air to the hatchery of Phitsanulok Inland Fisheries Research and Development Center, Phromphiram sub-District, Phromphiram District, Phitsanulok, Thailand. A total of 200 fish were raised in two 1,000 l cement tanks (100 fish per tank) with 32 cm water depth, supplied with aeration through air stones. Fish were fed with *Moina* sp. twice a day at 08.00 and 17.00 hrs during September 2017 to August 2018. The experiment was run from September 2018 until November 2018.

Stimulation of oocyte maturation

To prepare for breeding, females and males at a 1:1 sex ratio were stocked into a rectangular glass aquarium. A total of 20 females and 20 males were weighed and measured for length by two-digit balance and digital vernier caliper, respectively. The male brooders were smaller, with 7.70±0.40 cm average length and 1.88±0.52 g average weight, while females were 8.41±0.28 cm average length

and 3.46±0.24 g average weight. The females were identified by large abdomens and red sex papillae. Similarly sized males and females were selected for breeding experiments. The selected broodstock were placed separately (by sex) in eight glass aquaria (0.50x0.90x0.50 m), with either five females or five males per tank. Females were injected with different levels of HCG as described previously. Oocyte growth was observed by enlarging of the abdomen, and width of the belly was recorded using a digital vernier caliper.

Induction of oocyte maturation and stimulation of ovulation

Twenty-four hours after injection with HCG, the female fish in all treatments were injected with 10 $\mu g \cdot kg^{-1}$ BUS and 10 $mg \cdot kg^{-1}$ DOM to induce ovulation, following reports on induced breeding of *Botia almorhae* and *Syncrossus helodes* (Galsri *et al.*, 2010; Promprasert *et al*., 2011). Ovulation was monitored about 12 h after injections. Time of ovulation was recorded, as well as quantity and total weight of eggs per female. Quality of eggs was measured by the number of fertilized and non-fertilized eggs. Eggs were kept separately for each batch of artificial fertilization.

Artificial breeding

The male fish were injected with 10 $\mu g \cdot kg^{-1}$ BUS and 10 $mg \cdot kg^{-1}$ DOM at the same time as the stimulation of ovulation in females. The 20 male fish were euthanized with 100 ppm clove oil before removing the testis. The testis of 20 male fish were put on a clean white cloth, squashed and mixed with 10 ml of 0.45% normal saline to produce sperm solution. This solution was then subjected to sperm quality observation under a microscope for characteristics such as strength of sperm and sperm movement in order to reduce any variability of the experiment in relation to sperm quality. The sperm solution was stored at 0-4 °C (up to 24 h). A 0.5 ml volume of sperm was mixed with each egg batch using dry method artificial breeding.

Hatching and embryonic study

The eggs were hatched in glass aquaria (0.50x0.90x0.50 m) containing 140 l of water. Aeration was supplied through air stones. A total of 100 eggs were simultaneously hatched in an 8 l glass jar containing 4 l water, supplied with aeration through sand stones for estimation of fertilization, hatching and survival rates. Fifty fertilized eggs were sampled in triplicate from glass aquaria and transferred to Petri dish (n=150). Sampled eggs were studied under the stereomicroscope starting from the gastrula stage until hatching. Viable eggs (transparent and showing development) and non-viable eggs (opaque) were counted. Fertilization rate was recorded, hatching rate of fry was calculated. Survival rate was recorded after the fry reached three days old. Photos were taken at each embryonic development stage, and duration of each stage was recorded.

Percent ovulation, fertilization, hatching and survival rates were calculated using the following formulae:

$$\% \text{ ovulation} = \frac{\text{No. of ovulated fish}}{\text{Total no. of injected fish}} \times 100$$

$$\% \text{ fertilization} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of incubated eggs}} \times 100$$

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

$$\% \text{ survival rate} = \frac{\text{No. of 3-days old larvae}}{\text{Total no. of hatchlings}} \times 100$$

Water quality analysis

Water quality monitoring in fish tanks was conducted once a day. Temperature and dissolved oxygen were measured by DO meter (YSI model 550A), while pH was measured by pH meter (YSI model pH100A). Total alkalinity and total hardness were determined by titration method and total ammonia nitrogen was measured by phenate method using a spectrophotometer (HACH model DR/2500) (APHA, 1998).

Data analysis

The data for ovulation rate, fertilization rate, hatching rate, and survival rate were analyzed using analysis of variance (ANOVA) followed by Duncan's new multiple range test. All data were arcsine-transformed before analysis and analyzed to compare the treatment and control groups. Statistical significance of all tests was set at α=0.05.

RESULTS

Induction of oocyte maturation

The average initial lengths of female glass catfish of the control group, and 100, 300 and 500 IU·kg^{-1} HCG treatments were 8.37±0.34, 8.49± 0.14, 8.39±0.33 and 8.37±0.35 cm, and the average initial weights were 3.43±0.31, 3.50±0.14, 3.48± 0.30 and 3.43±0.23 g, respectively. There were no significant differences ($p>0.05$) among groups in average length or weight. Size of abdomen pre- and post-HCG hormone injection was different. The average pre-hormonal injection abdomen widths were 8.30±0.23, 8.34±0.15, 8.32±0.10 and 8.34± 0.23 mm, respectively. There was no significant difference in pre-injection abdominal widths ($p> 0.05$). Twenty-four hours after HCG induction, the average abdomen width increased significantly in females treated with HCG ($p<0.01$). Maximum width of abdomen was recorded from females treated with 500 IU·kg^{-1} HCG (10.70±0.12 mm), followed by 300 IU·kg^{-1} HCG (10.01±0.06 mm), 100 IU·kg^{-1} HCG (9.62±0.06 mm) and no HCG induction in the control group (8.24±0.24). Statistical analysis showed that all treatments were significantly different ($p<0.05$) (Table 1).

Ovulation rate, fertilization rate, hatching rate and survival rate

Ovulation rate was observed following hormonal manipulation. There was no ovulation found in the control group. Ovulation of females injected by HCG in all treatments occurred at about 12-13 h after injection. The average ovulation rate was 0±00, 100±00, 100±00 and 100±00 %, in control, 100 IU·kg^{-1}, 300 IU·kg^{-1} and 500 IU·kg^{-1} HCG groups, respectively. Ovulation in hormone-treated groups differed significantly ($p<0.05$) from the control group. Egg weight/female was 0.05± 0.01, 0.16±0.03 and 0.20±0.13 g and number of eggs/female was 156.80±24.10, 451.60±89.48 and 604.60±82.26 eggs, in 100 IU·kg^{-1}, 300 IU·kg^{-1} and 500 IU·kg^{-1} HCG treatments, respectively. Statistical analysis revealed that the egg weight and number of eggs from females injected with 500 IU·kg^{-1} HCG was significantly different from the other treatments ($p<0.05$). The average fertilization rate ranged from 0±00 to 75.25±1.30 %. Females injected with 500 IU·kg^{-1} HCG showed a significant difference in fertilization rate from other treatments ($p<0.05$), while females injected with 100 and 300 IU·kg^{-1} were not significantly different ($p>0.05$) from each other. The average hatching rates were 49.53±9.53, 64.43±3.43 and 80.29±5.27 %, in 100 IU·kg^{-1}, 300 IU·kg^{-1} and 500 IU·kg^{-1} HCG, respectively. The hatching rate from females injected with 500 IU·kg^{-1} HCG was significantly different from other treatments ($p<0.05$). The average survival rates of fry produced from females injected with normal saline (control), 100 IU·kg^{-1}, 300 IU ·kg^{-1} and 500 IU·kg^{-1} HCG were 0±00, 92.30±0.73, 92.89±1.04 and 94.06±2.09 %, respectively. Survival rate of fry from 500 IU·kg^{-1} HCG injected-females was significantly higher than other groups ($p<0.05$), whereas there was no significant difference in fry survival rate between 100 IU·kg^{-1} and 300 IU·kg^{-1} HCG-injected females ($p>0.05$) (Table 1).

Table 1. Effect of different levels of HCG on female glass catfish broodstock performance. Total length and weight, size of abdomen pre- and 24 h post-injection with HCG, ovulation rate, fertilization rate, hatching rate and the survival rate of fry in each group are presented as mean±SD.

Data	HCG (IU•kg^{-1})			
	Control	100	300	500
Total length (cm)	8.37±0.34^{a}	8.49±0.14^{a}	8.39±0.33^{a}	8.37±0.35^{a}
Weight (g)	3.43±0.31^{a}	3.50±0.14^{a}	3.48±0.30^{a}	3.43±0.23^{a}
Pre-injection, abdomen size (mm)	8.30±0.23^{a}	8.34±0.15^{a}	8.32±0.10^{a}	8.34±0.23^{a}
After 24 h, abdomen size (mm)	8.24±0.24^{d}	9.62±0.06^{c}	10.01±0.06^{b}	10.70±0.12^{a}
Time to ovulation (h)	0	12-13	12-13	12-13
Ovulating fish (no.)	0±00^{b}	5±0.00^{a}	5±0.00^{a}	5±0.00^{a}
Ovulation rate (%)	0±00^{b}	100±00^{a}	100±00^{a}	100±00^{a}
Egg weight/female (g)	0±00^{d}	0.05±0.01^{c}	0.16±0.03^{b}	0.20±0.13^{a}
Eggs/female (no.)	0±00^{d}	156.80±24.10^{c}	451.60±89.48^{b}	604.60±82.26^{a}
Fertilization rate (%)	0±00^{c}	34.75±4.76^{b}	34.90±5.31^{b}	75.25±1.30^{a}
Hatching rate (%)	0±00^{d}	49.53±9.53^{c}	64.43±3.43^{b}	80.29±5.27^{a}
Survival rate (%)	0±00^{d}	92.30±0.73^{bc}	92.89±1.04^{ab}	94.06±2.09^{a}

Different superscript letters in the same row indicate significant differences ($p<0.05$).

Embryonic development

The glass catfish eggs were 0.75-0.85 mm in diameter, yellowish and adhesive. The development of the embryo after fertilization is summarized as follows: The cleavage period was completed at 2 h and 20 min post fertilization. Morula stage ended at 3 h and 50 min. blastula stage at 7 h, and then gastrula stage at 10 h. Somite development started at 13 h and 30 min and the 20-somite stage was completed at 15 h 5 min. At 25.3-26.7 °C water temperature, larvae took 21.30 h to develop (Table 2 and Figure 1). The newly-hatched larvae were 2.5 mm in length with large yolk sac. The early stage larvae generally swam to the bottom of the glass aquaria.

6-hours larvae

The total length of larvae at six hours post-hatch was approximately 2.8 mm. A pair of barbels was found at the mandible. Abdomen was round with pigmentation. The body was transparent. Fins were not separated. Head was free from yolk sac (Figure 2a). The fry generally stayed at the bottom of the aquarium.

1 day-old larvae

The total length of the larvae was about 4.7 mm. The mouth was opened, the yolk sac appeared to be smaller than the previous stage. The tailbone was straight. The eyes were black. The fins were not separated (Figure 2b).

3 days-old larvae

The larvae at three days of age had a total length of approximately 5.7 mm. The head was relatively larger than the previous stage. A connection appeared between dorsal and caudal fins. Caudal bones suggesting good growth appeared to be triangular in shape, as shown in Figure 2c. A pair of long barbels was observed, starting from the maxillae and extending to the base of pectoral fin. At this stage, the anal opening was clearly seen along with decreasing size of the yolk sac. Body was transparent and black spots were found at the opercula, one on each side.

Table 2. Observations of embryonic development of glass catfish, *Kryptopterus vitreolus* (Ng and Kottelat, 2013) (n=150)

Stage	Time	Remarks
Fertilized egg	1 min	Yellowish and round eggs, average diameter is 1.37±0.05 mm.
1 Cell	15 min	Fertilized eggs begin mitosis. The animal pole is clearly marked with a blastodisc.
2 Cell	30 min	First cleavage, resulting in 2 blastomeres.
4 Cell	45 min	Second cleavage, resulting in to 4 blastomeres.
8 Cell	1 h	Third cleavage, resulting in 8 blastomeres.
16 Cell	1.20 h	Fourth cleavage, resulting in 16 blastomeres.
32 Cell	1.50 h	Fifth cleavage, resulting in 32 blastomeres.
64 Cell	2.20 h	Sixth cleavage, resulting in 64 blastomeres.
Morula	3.50 h	The final cleavage, resulting in 128 blastomeres. Blastomeres stacked into thick and group of cells like a hat. There is no hole in the blastocoel.
Early blastula	4.45 h	Cells start moving and a hollow cavity is formed.
Middle blastula	5.50 h	The shape of cell movement is relatively upward. Blastocoel is formed and appears to widen.
Late blastula	7 h	Thicker cells in blastodisc are found. The central cavity is filled with liquid called blastocoel, which is vital at the blastocyst stage. The layer of cells surrounding the blastocyst is called the throphoblast.
Early gastrula	8 h	Germ ring appears from thickening blastodisc surrounding the yolk.
Middle gastrula	9.05 h	Embryo tail appears as part of the germ ring by a clump of cells forming the origin of the embryo.
Late gastrula	10 h	Cells move downward and entirely cover the yolk at the end of gastrula stage. When the cells cover the yolk completely, the blastopore is closed.
Early neurula	11.10 h	This is the beginning of the embryo stage. Early embryo is long cylindrical and attached to extra embryonic yolk which develops into somite later.
Late neurula	11.50 h	The front and rear of early embryo is lifted to form a head bud and a tail bud.
10 somite	13.30 h	There are 10 somites on the lateral body fold of the embryo. There are optic vesicles which later develop into eyes appearing at head area. This stage is the optic bud stage.
20 somite	15.05 h	There are 20 somites on the lateral body fold of the embryo. At this stage, heart and muscle begin to operate. The tail bud separate from the yolk.
Hatch out	21.30 h	Hatching stage is determined when the tail of larvae first emerges. Head of a newly-hatched larva is still attached to the yolk. The mouth is unopened. The average length of the larvae is 2.5 mm.

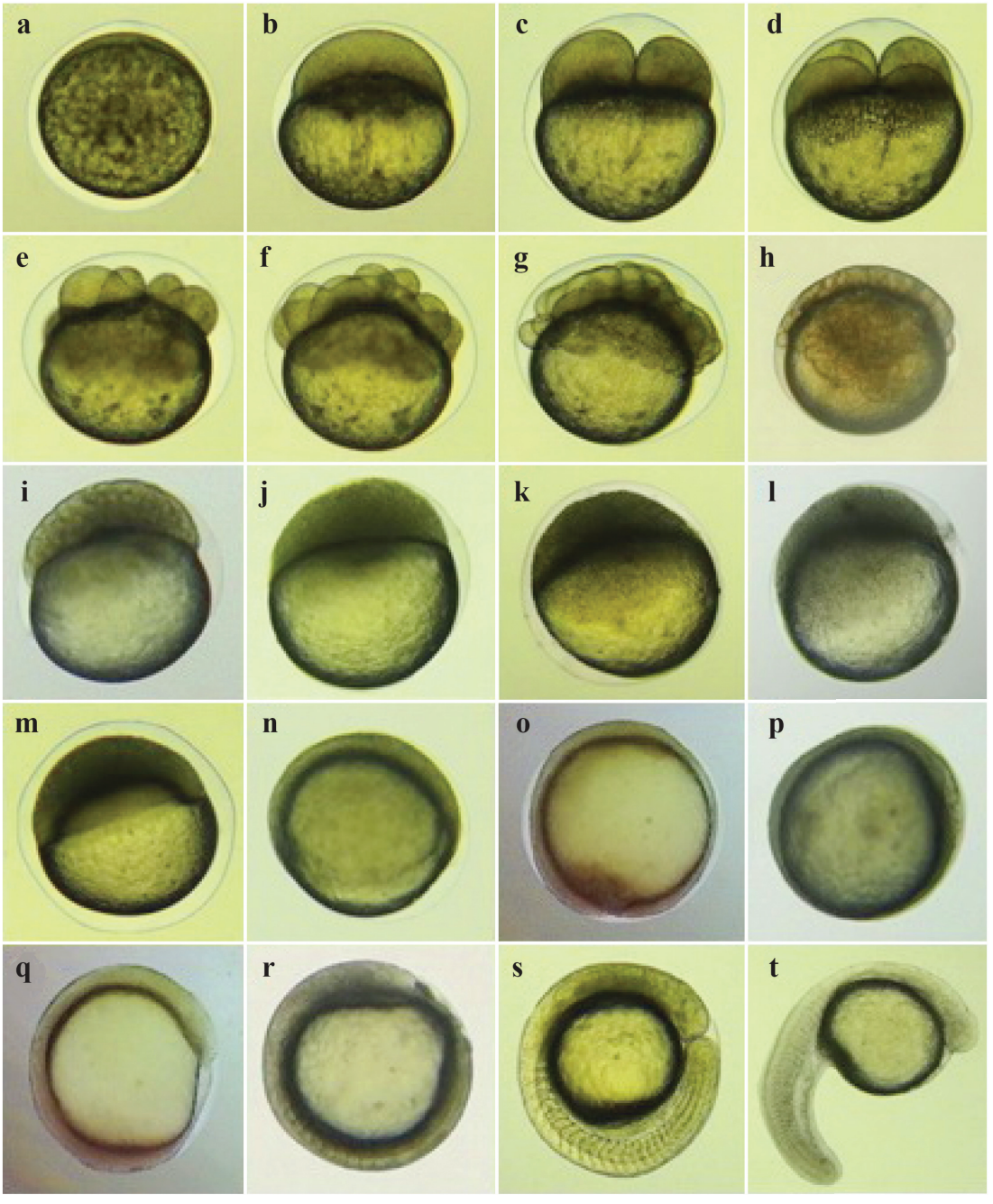

Figure 1. Embryonic development stages of glass catfish, *Kryptopterus vitreolus* (Ng and Kottelat, 2013): (a) Fertilized egg; (b) 1 cell; (c) First cleavage; (d) Second cleavage; (e) Third cleavage; (f) Fourth cleavage; (g) Fifth cleavage; (h) Sixth cleavage; (i) Morula; (j) Early Blastula; (k) Middle blastula; (l) Late blastula; (m) Early gastrula; (n) Middle gastrula; (o) Late gastrula; (p) Early neurula; (q) Late neurula; (r) 10 somite; (s) 20 somite; (t) Hatch-out.

Figure 2. Development of glass catfish larva; (a) 6 hours; (b) 1 day-old; (c) 3 days-old.

Water quality

During the study on egg and larval development period, overall water quality values ranged: temperature ranged from 25.3 to 26.7 °C, pH from 7.5 to 7.9, dissolved oxygen from 4.4 to 5.3 $mg \cdot l^{-1}$, alkalinity from 94 to 110 $mg \cdot l^{-1}$, hardness from 117 to 120 $mg \cdot l^{-1}$ and total ammonia nitrogen from 0.001 to 0.024 $mg \cdot l^{-1}$. Water quality parameters were within ranges of suitable levels for health and growth of freshwater fish (Boyd, 1979).

DISCUSSION

It was demonstrated in this study that stimulation of female glass catfish spawning can be successful by injecting HCG at 100, 300 and 500 $IU \cdot kg^{-1}$ followed 24 h later by 10 $\mu g \cdot kg^{-1}$ BUS and 10 $mg \cdot kg^{-1}$ DOM. Results from this experiment showed 100 % spawning with 34.75-75.25 % fertilization rate. The fertilization rate would indicate successful inducement, since it reflects the completion of oocyte maturation. It is well

established that after vitellogenesis, oocyte maturation, ovulation and spawning can take place under natural circumstances or by hormone treatment (Bhattacharya *et al*., 2018). Oocyte maturation is marked by eccentric nucleus and germinal vesicle breakdown, which is called the post-vitellogenic ovarian stage (Adebayo and Fagbenro, 2004). Thus, if more mature oocytes are ovulated, an increased fertilization rate could be attained. Appropriate HCG hormonal levels had to be determined because it influences oocyte maturation in the ovary (Zairin *et al*., 2001). This experiment suggested that HCG at a level of 500 IU·kg^{-1} fish would induce most of the oocytes to maturation followed by ovulation and spawning. The lower doses of HCG at 100 and 300 IU·kg^{-1} fish could induce ovulation; however, smaller egg weight and lower fertilization rate were clearly observed. This indicates that these lower doses of HCG provided insufficient gonadotropin, and hence were unable to induce oocytes to full maturation, resulting in lower success in ovulation and fertilization (Sahoo *et al*., 2008). Hatching rate ranged from 49.53 to 80.29 % and survival rate was recorded at 92.30 to 94.06 %. This result is in line with a study of *Pangasius bocourti* (F1), in which HCG was applied at 500 IU·kg^{-1} for 24 h to females to achieve oocyte maturation prior to stimulating ovulation by injection with 20 µg·kg^{-1} BUS and 10 mg·kg^{-1} DOM (Khuncharoen *et al*., 2006). Cacot *et al*. (2002) reported a study of *Pangasius bocourti* that induction of egg maturation with 500 IU·kg^{-1} HCG for 24 h followed by stimulating ovulation using the same hormone but at 1,500 to 2,500 IU·kg^{-1} successfully induced 66 % ovulation. Range of doses stated here would depend on species.

HCG induced oocyte maturation, which was marked by the breakdown of germinal vesicles. Another signal of induction success was abdominal size, which was enlarged in proportion to the increase in oocyte size. The results indicated that maximum abdomen size was found in females injected with the highest dose, 500 IU·kg^{-1} (increase of 28.42 %), while the lowest dose (100 IU·kg^{-1}, increase of 15.36 %) resulted in significantly smaller abdomens than the other two groups treated with HCG. Promprasert *et al*. (2011) reported on induced breeding of *Syncrossus helodes* with 500 IU·kg^{-1} HCG, and found increases in abdominal size and successful induction of ovulation. At this dose (500 IU·kg^{-1}), with the same concentrations of BUS and DOM, Galsri *et al*. (2010) reported 100 % ovulation in yoyo loach (*Botia lohachata* Chaudhuri, 1912). These studies indicated that 500 IU·kg^{-1} HCG was a commonly used dose in fish breeding protocols. Other work using 500 IU·kg^{-1} on *Pangasius bocourti* reported 66.66 % ovulation rate, but more HCG (750 IU·kg^{-1}) resulted in a decrease in ovulation to 33.33 % (Piasoongnoen and Srisakultiew, 2011). This suggests that using excessive HCG would not increase successful breeding, but instead might reduce the ovulation rate.

Glass catfish eggs were yellowish and adhesive. The larval hatching took about 21.30 h at 25.3-26.7 °C water temperature. The newly-hatched larvae were 2.5±0.1 mm in length. This is similar to larvae of Indian catfish, *Heteropneustes fossilis*, which were found to be 2.5±0.2 mm in length (Puvaneswari *et al*., 2009). However, the glass catfish larvae were smaller in length than the larvae of *Kryptopterus bleekeri* Günther, 1864 (3.88 mm), *Wallago attu* Schneider, 1801 (3.64 mm), *Wallonia micropogon* (4.62 mm), *Pangasius sutchi* Fowler, 1937 (2.9-3.1 mm), *Hemibagrus nemurus*, Valenciennes, 1840 (3.0 mm), *Mystus Montanus*, Jerdon (3.0 mm) and *Horabagrus brachysoma* (3.0-4.0 mm) (Leesa-nga *et al*., 1994; Arockiaraj *et al*., 2003; Islam, 2005; Prompakdee *et al*., 2007; Soontornvipat *et al*., 2007; Adebiyi *et al*., 2013; Phaeviset *et al*., 2016; Sahoo *et al*., 2017).

In conclusion, glass catfish hypophysation was successfully accomplished by injecting HCG at a dose of 500 IU·kg^{-1} female for oocyte maturation for about 24 h, followed by injections of 10 µg·kg^{-1} BUS and 10 mg·kg^{-1} DOM to induce ovulation. Raising glass catfish broodstock in captivity can delay or pause egg maturation. As a result, hormonal induction, followed by artificial breeding is necessary. However, the handling process could injure the fish. Therefore, persons with experience in handling broodstock are required for stripping the eggs. Moreover, research regarding feed formulations for glass catfish gonad maturation is required, so that the natural process of breeding in this species can be accomplished.

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