www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 13: 523-527 (2013) DOI: 10.4194/1303-2712-v13_3_17

Induced Breeding of the Stinging Catfish, *Heteropneustes fossilis*: Comparison among Different Inducing Agents

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Received 25 September 2012
Accepted 26 August 2013

Abstract

The present study furnishes the comparison on the performance of different inducing agents in the induced breeding of the stinging catfish, *Heteropneustes fossilis*. During the study two experiments were conducted in two different hatcheries of Bangladesh. In the experiment 1, pituitary gland extract (PGE) was administered at 6 mg/kg body weight of females and 2 mg/kg body weight of males. In contrast, ovaprim was administered at 0.3 ml/kg and 0.1 ml/kg body weight of females and males, respectively. On the other hand, in case of experiment 2, PGE was administered at the same rate as experiment 1 while ovaprim was administered at 0.5 ml/kg and 0.1 ml/kg body weight of females and males, respectively and human chorionic gonadotropin (HCG) was injected at 1000 IU/kg body weight of both male and female fishes. Breeding success was found to be higher in ovaprim treated individuals in both the experiments in all aspects including latency period, ovulation rate, fertilization rate, hatching rate and incubation period compared to that of PGE and HCG induced individuals. In the ovaprim induced individuals, the latency period was within 10 hours while in PGE and HCG induced individuals, the latency period was 15 hours. In addition, the present investigation also revealed that, ovaprim is more efficient in terms of ovulation, fertilization and hatching rates when using at a rate of 0.5 ml/kg body weight of female fishes than using at a rate of 0.3 ml/kg body weight of female fishes. Results of the present study would help the hatchery managers in managing the induced breeding programs of *H. fossilis* and other catfishes.

Keywords: *Heteropneustes fossilis*, induced spawning, pituitary gland extract, ovaprim, ovulation rate, fertilization rate, hatching rate.

Introduction

The stinging catfish *Heteropneutes fossilis* (Bloch, 1974) belongs to the family Heteropneustidae is a commercially important fish species in Bangladesh. This is primarily a fish of ponds, ditches, *beel*s, swamps and marshes, but sometimes found in muddy rivers (Jha and Rayamajhi, 2010; Froese and Pauly, 2012). The air-breathing apparatus of stinging catfish enables it to exist in almost any kind of water. It is also able to tolerate slightly brackish water. Commonly, during the dry season *H. fossilis* lives in semiliquid and semi-dry mud, and even when the mud dries up they take their bodies to the bottom of fissures and crevices formed by the cracking mud. *H. fossilis* can respire aerially by gulping in air at various intervals when the oxygen content of water is low (Munshi, 1993). Whilst it is heavily utilized for food and for medicine in many parts of its range, and it may be threatened by over exploitation and habitat loss and degradation (especially from pollution and dams) and subsequently, it is considered least concern at present (IUCN, 2012). Because of its fast growth, tolerance to high stocking densities, high market value, ability to survive in oxygen-low waters, low fat, high protein and iron content and medicinal values, *H. fossilis* is considered as an ideal fish species for aquaculture (Dehadrai *et al.*, 1985; Alok *et al.*, 1993; Vijayakumar *et al.*, 1998; Haniffa and Sridhar, 2002; Froese and Pauly, 2012). Also, aquaculture of this species will be helpful not only in increasing the overall production but also in the conservation of this important fish species.

Aquaculture of the stinging catfish in Bangladesh is widely spreading. However, constant supply of good quality fingerlings is vital for the culture of any fish species including *H. fossilis*. Although, major sources of fry and fingerlings for aquaculture were mainly the capture fishery due to the limited capacity of the then existing hatchery facilities in the past, nonetheless, induced breeding techniques have continually improving in Bangladesh.

Subsequently, at present, hatchery produced fry/fingerlings become the major sources of seed for the aquaculture industry in the country. While the production of fish seed from hatchery sources has increased dramatically, the quality has not improved owing to poor hatchery management practices resulting deleterious effects such as negative selection, inbreeding depression, indiscriminate interspecific hybridization etc.

Although a few studies on the induced breeding of *H. fossilis* are available including effects of carp pituitary gland extract, human chorionic gonadotropin and synthetic hormone (ovaprim) doses on induced breeding, maturation and ovulation of *H. fossilis* (Alok *et al.*, 1993; Begum *et al.*, 2001; Nayak *et al.*, 2001; Haniffa and Sridhar, 2002; Haniffa *et al.*, 2002), however, detailed studies on the induced breeding of *H. fossilis* are clearly missing in Bangladesh. Subsequently, the present study furnishes information on the comparative performances of different inducing agents on the breeding success of *H. fossilis.*

Materials and Methods

Study Site

The present study was conducted at two fish hatcheries in Feni and Jessore districts of Bangladesh during February 2010 to July 2010 (experiment 1 in Feni) and March 2012 to August 2012 (experiment 2 in Jessore).

Brood Collection

The brood fish for the artificial breeding of *H. fossilis* were obtained from the respective fish hatcheries. A total of 30 brooders in each experiment were stocked in each hatchery collected from the wild stocks. All the brood stocks were acclimatized before the induced breeding procedures and were kept separately in ponds of 14.3 × 8.15 × 1.5 m. in experiment 1 and 15 × 9.65 × 1.5 m. in experiment 2 for four months before the start of the breeding season.

Brood Stock Management

The brood fishes were fed on supplementary diet formulated from 25% fish meal, 20% rice bran, 20% wheat flour, 15% mustard oil cake, 4% molasses and 1% vitamin premix. The brooders were reared for four months with feeding at two times a day at the rate of 5-6% of the body weight in both the experiments. In addition, the ponds were treated with animal manure at 15 days interval at the rate of 1250 kg/hectare. Furthermore, inorganic fertilizers namely Urea and Triple Super Phosphate (TSP) were applied at the rate of 50 kg/hectare and 25 kg/hectare, respectively.

Brood Selection and Conditioning

Brood fish were collected from the rearing ponds using a cast net in the morning between 8:00-9:00 am on the day of the breeding trials and immediately transferred to circular tanks in respective hatcheries. The males and females were kept in separate tanks and continuous water flows were sustained at a rate of 10 l/min. Water quality parameters were found as- dissolved oxygen: 5.2-5.7 ppm; CO_2: 4.6-5.8 ppm; pH: 7.3-8.5; temperature: 27 – 30 °C in experiment 1 while in case of experiment 2 water quality parameters were recorded as- dissolved oxygen: 5.4-6.2 ppm; CO_2: 4.2-6.1 ppm; pH: 7.1-8.2; temperature: 26.5-29°C. However, no supplementary feed were provided throughout the conditioning period.

Brood Stock Injection and Breeding Induced

Commercially available dehydrated carp pituitary gland extracts (PGE) and synthetic hormone ovaprim were used in experiment 1 whereas dehydrated carp pituitary gland extracts (PGE), synthetic hormone ovaprim and human chorionic gonadotropin (HCG) were used in experiment 2. The body weight (g) of each brooder was weighed on an electronic balance (College B204-S, Switzerland) to estimate the required amount of inducing agents.

The brooders were divided into two groups consisting of three females and five males of *H. fossilis* each in both experiments, and then subjected to hormone treatment. In experiment 1, one group was injected using PGE (6 mg/kg body weight of females and 2 mg/kg body weight of males) and the second group using ovaprim hormone (0.3 ml/kg and 0.1 ml/kg body weight of females and males, respectively) in the hatchery situated at Feni. On the other hand, in experiment 2, one group was injected using PGE (6 mg/kg body weight of females and 2 mg/kg body weight of males), the second group using ovaprim hormone (0.5 ml/kg and 0.1 ml/kg body weight of females and males, respectively) and the third group using HCG (1000 IU/kg body weight of both male and female fish) in the hatchery situated at Jessore. For all the treatments, the hormone was administered by intra-muscular injection on muscles beneath the dorsal fin slightly above the lateral line. After injection, the brooders were kept in separate breeding tanks for each treatment.

Breeding and Egg Transfer for Incubation

All the brooders were ovulated after a period of 10-15 hrs after injection in both the experiments. The brooders were then transferred from the holding tanks after the completion of ovulation. Whereas, the fertilized eggs were transferred into mini rectangular hatching trays with taking precaution to avoid damage and fungal/bacterial contamination during the egg

collection. The number of eggs released into each tray was estimated using gravimetric methods adapted from Legender (1986) and reviewed by Lagler (1992). Afterwards, a continuous flow of water was maintained for aeration to guarantee the environmental conditions were optimal for the hatching process.

Determination of Ovulation, Fertilization and Hatching Rate

Ovulation rate, fertilization rate and hatching rates were calculated using the following formula:

Ovulation rate (%) = (No. of fish ovulated/ Total no. of fish injected) × 100

Fertilization rate (%) = (No. of fertilized eggs/ Total no. of eggs) × 100

Hatching rate (%) = (No. of eggs hatched/ Total no. of fertilized eggs) × 100

Statistical Analysis

Statistical analyses were performed using GraphPad Prism 5 software. Tests for normality of each group were conducted by visual assessment of histograms and box plots, and confirmed using the Kolmogorov-Smirnov test. Only percent data had to be arcsine transformed before analysis. Where the normality assumption was not met, the non-parametric Mann-Whitney U-test and Kruskal-Wallis test were used to compare the length and weight of fishes between groups and experiments. A Chi-square test was used to check the ovulation, fertilization and hatching rates between PGE, ovaprim and HCG treated fishes. All statistical analyses were considered significant at 5% ($P<0.05$).

Results and Discussion

During the present investigation, in experiment 1, male fishes ranged from 15 to 20 cm (17.29±1.95) in total length and 30 to 70 g (55.30±11.58) in body weight while female fishes ranged from 17 to 25 cm (20.84±2.58) in length and 40 to 150 g (90.60±36.56) in body weight. However, Mann-Whitney U-test revealed no significant differences between the two groups of brooders in experiment 1. On the other hand, total length and body weight of males ranged from 14 to 23 cm (18.18±2.15) and 25 to 80 g (62.56±15.46), respectively in experiment 2. In contrary, females used in experiment 2 ranged from 15 to 26 cm (21.82±3.16) in total length and 38 to 161 g (107.1±39.68) in body weight. Kruskal-Wallis test revealed no significant differences in length and weight of 3 groups of brooders in experiment 2. Furthermore, Mann-Whitney U-test exposed no significant differences in length and weight of fishes between the experiments.

Ovulation Rates

Chi square test showed no significant differences in ovulation rates between the treatment groups in the experiment 1. However, ovulation rates were higher in the ovaprim treated fishes (90%) compared to ovulation rates (78.7%) found in the PGE treated fishes (Table 1). On the other hand, chi square test revealed no significant differences among the ovulation rates of ovaprim, PGE and HCG treated fishes in the experiment 2 (Table 1). Though in this case also, the ovulation rates of fishes treated with ovaprim were higher. When compared between the experiments, ovulation rates were highest while using ovaprim at a rate of 0.5 ml/kg body weight of female fish (experiment 2) than using at a rate of 0.3 ml/kg body weight of female fish (experiment 1) (Figure 1). Parallel findings were documented by Begum *et al.* (2001) who described that, the ovulation rates in *H. fossilis* injected with PGE at 75 mg/kg body weight were slightly lower, although they recorded 90% ovulation when the fish were treated with PGE at 100 mg/kg body weight. However, very high doses of PGE hormone often resulted in higher rates of ovulation in the *H. fossils* (Haniffa and Sridhar, 2002). Nonetheless, the latency period was significantly shorter in ovaprim treated fish in contrasting to PGE and HCG injected brooders (Table 1). On the contrary, using same dose of Ovaprim, much longer latency period were recorded by Haniffa and Sridhar (2002) and Kohil and Goswami (1987). However, it is problematic to evidently interpret the instrumental factors for the observed variances. Furthermore, a group of factors are likely to influence biological experiments particularly those involving hormones thereby leading to deviations in the observed latency periods (Gheyas *et al.* 2002).

Fertilization Rates

Fertilization rates were higher in eggs of the ovaprim treated brooders (86.67%) compared to that of PGE treated fish (69.23%) in case of experiment 1. However, chi square test exposed no significant differences between the fertilization rates of ovaprim and PGE treated fishes. On the other hand, in experiment 2, fertilization rates were higher in eggs of the ovaprim treated brooders (90.83%) compared to the fertilization rates of 70.45% and 75.33% in case of PGE and HCG treated fishes, respectively. Similar to the results of experiment 1, in this case also chi square test did not expose any significant differences in the fertilization rates among different inducing agents treated fishes. Finding of this study agrees previous studies indicating the rate of fertilization is generally higher with ovaprim treatments (Nandeesha *et al.*, 1990; More *et al.*, 2010). In addition, earlier studies found the fertilization rate of *H. fossilis* treated with

Table 1. Showing details of induced breeding in Stinging Catfish, *Heteropneustes fossilis* using different inducing agents

Inducing Agent	Experiment 1					Experiment 2				
	LP(hrs)	OR (%)	FR (%)	HR (%)	IP (hrs)	LP(hrs)	OR (%)	FR (%)	HR (%)	IP (hrs)
PG	15	78.67%	69.23%	72.72%	5.0	15	76.51%	70.45%	70.25%	5.0
Ovaprim	10	90.00%	86.67%	76.92%	3.5	10	93.77%	90.83%	82.48%	3.5
HCG	---	---	---	---	---	15	82.67%	75.33%	66.58%	5.0

LP, Latency period; OR, Ovulation rate; FR, Fertilization rate; HR, Hatching rate; IP, Incubation period

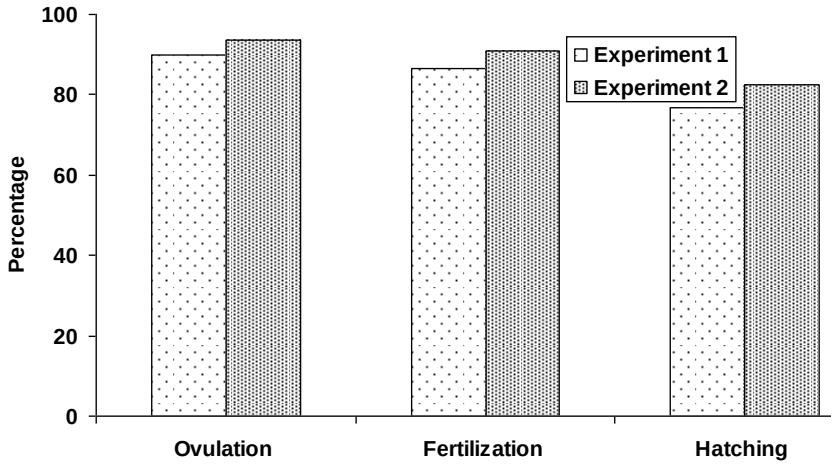

Figure 1. Comparison on the performance of different doses (Exp. 1: 0.3 ml/kg body wt offemale; Exp. 2: 0.5 ml/kg body of female) of the synthetic hormone ovaprim in the induced breeding of *Heteropneustes fossilis*.

ovaprim at 0.3 ml/kg and 0.5 ml/kg body weight as 70% and 75%, respectively (Haniffa and Sridhar, 2002). Furthermore, Begum *et al.* (2001) reported the highest rate of fertilization (98%) in *H. fossilis* injected by PGE at 75 mg/kg which is much higher than that found in the present study of PGE injected fishes. Such deviations in the fertilization rate can be attributed to the huge differences of hormonal doses, size of the brood fish, seasonal variation (Gheyas *et al.*, 2002; Haniffa and Sridhar 2002; Nwokoye *et al.*, 2007), environmental factors, water quality parameters (alkalinity, DO, pH, hardness) (Khan *et al.*, 2006). The quality of the PGE hormone may also have influencing impact on the fertilization rates.

Hatching Rates

In case of experiment 1, the hatching rates were found to be slightly higher (76.92%) for eggs in the ovaprim treated fishes compared to that of PGE treated fishes (72.72%) (Table 1). However, chi square test showed no significant differences in hatching rates between ovaprim and PGE treated fishes. Moreover, the incubation period for eggs in the PGE treated fish was more than 1.5 h longer than the ovaprim treated fish. On the other hand, when considering the experiment 2, fertilization rates were found to be higher in ovaprim treated fishes (82.48%) compared to that of PGE and HCG treated fishes (Table 1). While comparing between experiments it is quite clear that hatchling rates were higher when ovaprim was used at a rate of 0.5 ml/kg body weight of female fish (experiment 2) (Figure 1). Nonetheless, Nayak *et al.* (2001) reported a hatching period of 10-12 h in *H. fossilis* treated with ovaprim treatment at 27±1° C and obtained higher hatching rate of 96% using ovaprim at the rate of 0.4 ml/kg body weight. Haniffa and Sridhar (2002) reported a hatching rate 50.5% and 60% for *H. fossilis* injected with ovaprim at a rate of 0.3 ml/kg and 0.5 ml/kg body weight, respectively. However, in terms of hatchling rate, ovaprim treated fish yielded better results compared the PGE treated fish (Nandeesha *et al.*, 1990; More *et al.*, 2010). All these studies to some extent support the findings of the present study.

In conclusion, ovaprim treated brooders of *H. fossilis* showed better performance during induced breeding in both the experiments during the study. However, ovaprim was found to be more efficient in terms of ovulation, fertilization and hatching rates when using at a rate of 0.5 ml/kg body weight than using at a rate of 0.3 ml/kg body weight of female fishes. Results of the present study would be beneficial for apposite management of induced breeding programs of *H. fossilis* and other catfishes.

Acknowledgements

We would like to give special thanks to (i) the office staffs of the studied fish hatcheries for their

cooperation during the present study, and (ii) anonymous referees for their useful and constructive comments.

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