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Early development of brood of *Pangasianodon hypophthalmus* using Green House Technique

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ABSTRACT

An attempt was made to develop broods of Thai Pangas, *Pangasianodon hypophthalmus* earlier than normal spawning season using Green House (GP) Technique during July, 2015 to June, 2020. The study was conducted in four earthen ponds of 0.08 ha each. For Green House technique, two ponds were covered fully with transparent polyethylene sheet fastened in frame, made of bamboo to receive sunlight for increasing water temperature. The other two ponds were kept open and were treated as open pond (CP) as control. Ponds were treated with lime (CaO) @ 250 kg/ha and then filled up with water up to a depth of 1.5 m. Before stocking, ponds were treated with dolomite @ 20 ppm and fertilizer with urea @ 2.5 ppm and TSP @ 1.00 ppm. After growing sufficient plankton, all four ponds were equally stocked with adult and healthy *Pangasianodon hypophthalmus*@ 990 nos/ha corresponding to 12kg/dec in October 2015. Size of male fish ranged from 3.0 to 3.5 kg while female was 4.5 to 5.5 kg. The ratio of stocked fishes (female: male) was 2:1. The stocked fishes were fed with commercial pellet feed containing 35% protein, supplemented with vitamin premix. Cod liver oil was added at 1-2 ml/kg of feed to hasty eggs for early maturation. Feeding rate ranged from 10-3% of total body weight twice daily. Fishes were reared for five months to get suitable broods earlier than the normal spawning season. Stocked fishes were checked monthly by examining secondary sexual characters. In the GP ponds, fully matured females were first observed on 6th February 2016, which was two months earlier than the normal spawning season. About 21% female and 60% male Pangas became fully matured during mid-February in GP. A total of 98% female and 100% male of stocked Pangas became fully matured and ready for inducing during mid-March. Augmenting spawning season than the normal will increase culture period of Pangas, which ultimately will help to increase Pangas production.

INTRODUCTION

Thai Pangas (*Pangasianodon hypophthalmus*) is an indigenous fish species of Thailand, living in the River Mekong (Roberts and Vidthayanon 1991). This species is particularly important for their fast growth, lucrative size and high market demand. This species can also be stocked at a much higher density in ponds compared to other cultivable species. It was introduced in Bangladesh from Thailand in 1989 (Banglapedia 2006) and has become an important source of food fish for the country. After introduced in Bangladesh, Pangas was successfully first bred in captivity by hormone injections in 1993 (Rahman et al. 1993). The estimated total Pangas production in Bangladesh was about 3.71 lakh metric tons in the year 2014 and which contributes 12.57% in the inland fisheries production (DoF 2015). They attain sexual maturity at about 2.5-3 years of age. Captive breeding season of Pangas starts from late April and continues up to August in Bangladesh. Peak spawning season lies between May and June (Hossain et al. 2004). Generally, broods of Pangas become available in last April to May and it needs more than one month to produce seed of Pangas. So, fries of Pangas are available in late May or early June in the private hatcheries. As a result, culture period of Pangas become shortened, that ultimately impacts negatively on Pangas

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production. If supply of gravid Pangas could be ensured in early season in January-February, then seed production of Pangas would be available in late March, that would increase production by extending grow-out period. The main constraint of production of Pangas is the late availability of seed due to low temperature in winter. Water temperature is one of the most important factors, which influences growth, reproduction and other biological activities of fish. Temperature is the main triggering factor for the development of broods. Hence, it become imperative to give emphasis on early production of broods of Pangas under green house technique for extending both maturation, seed production and grow-out period.

MATERIALS AND METHODS

The experiment was carried out at Floodplain Sub-Station, Santahar, Bogura during the period from July 2015 to June 2020. The experiment was conducted in rain fed ponds having an area of 0.08 ha (800 m^2) each with an average depth of 1.5m. All the ponds were similar in size, depth, basin, conformation and contour and bottom type. Among the ponds, two ponds were fully covered with transparent polyethylene sheet fastened in frame, made of bamboo (Figure 1) that was treated as "Green House Pond" (GP_1 and GP_2). The other two ponds were kept open and were treated as Open Pond (CP_1 and CP_2) as control. All the ponds were prepared by drying, bottom soil was excavated to increase depth and the dykes were repaired properly and cleaning aquatic vegetation. All the ponds were made free from undesirable fishes by applying rotenone at the rate of 16 kg/h. After 15 days of poisoning, lime (CaO) was applied at the rate of 125 kg/ha followed by cow dung at the rate of 750 kg/ha and waited for a week. After that ponds were filled up with water up to a depth of 1.5 m. Inorganic fertilizers such as Urea and TSP were applied in the ponds at the rate of 63 and 63 kg/ha, respectively to promote algal growth and waited for another week to allow the water become suitable for stocking.

Figure 1: Photographs of Green house (GP_1 and GP_2) constructed over experimental ponds.

After growing sufficient plankton, all the ponds were stocked with adult and healthy Thai Pangas at the rate of 990 nos/ha corresponding to 12 kg/dec in October 2015. Commercial pellets containing 35% crude protein supplemented with vitamin premix were supplied to stocked fishes. Daily feeding rate was 10 % in the first month, 8% in the second month, 5% in the third month and 3% in the rest of the period. The daily ration was divided into two equal portions and applied in morning and evening. Cod liver oil were added at 1-2 ml/kg feed for augment maturation of eggs. Rearing period of fishes was 5 months from October to February (Table 1).

Table 1: Design of the experiment

Treatments	Replications	Stocking ratio (Female: Male**)**	Stocking density (no./decimal)	Brood development period (Month)
T_1 (GP)	2	2:1	(2+1) = 3	5
T_2 (OP)	2	2:1	(2+1) = 3	5

GP= Green house pond, OP= Open pond

The ratio of stocked fishes (female: male) was 2:1. The size of male Pangas was 3.0-3.5 kg and that of female was 4.5-5.5 kg. After stocking ponds were fertilized with inorganic fertilizer (Urea and TSP) fortnightly at the rate of 63 and 63 kg/ha, respectively. Water quality parameters such as Temperature (^{0}C), pH, total alkalinity, dissolved oxygen, hardness and Ammonia of water were recorded weekly using a commercial kit box (Model: FF-3, USA). Partial water of the ponds was exchanged in every 7 days intervals after stocking of fish but it was not more than 10% at a time. Aerator was used in the ponds to maintained dissolved oxygen. Stone lime was applied fortnightly at a rate of 63 kg/ha to maintain pH and others water quality parameters in suitable range. Depth of water was always maintained at a minimum level of around one meter in all ponds.

After stocking, fishes were checked by observing secondary sexual characters to assess gonadal maturation. Gravid females were identified by their swollen, distended, soft abdomen with reddish and swollen vent while mature males were identified by their protrude, reddish genital opening and oozing of milt with gentle pressure on the abdomen.

Induced breeding programme was undertaken with the broods developed under GP technique to assess the suitability of broods and to ascertain the spawning season. A synthetic gonadotropin releasing hormone analogue (SGnRH) commercially known as "Ovaprim" and Pituitary gland (PG) were used as pre-maturation agent. Pituitary gland (PG) and Human Corionic Gonadotropin (HCG) were used as the inducing agent for induced breeding of Thai Pangas (Table 2).

Table 2: Design of the experiment

Treatment	Hormone injection for female (mg & IU/kg bw)	Hormone injection for male (mg /kg bw)	Month
T_1	1st dose: 2 mg PG+200 IU HCG	-	February
	2nd dose: 12 mg PG+250 IU HCG	1st dose: 2mg PG	
T_2	Pre-maturation: 2 mg PG	Pre-maturation: 1 mg PG	December
	Single dose: 0.8 ml (ovaprim)	Single dose: 0.2 ml (ovaprim)	February

Development pattern (%) of the gonadal maturity (Phillips1969), fecundity (Hossain et al. 1991) and (Shrivastava and Prakash 1979) was estimated by gravimetric methods. Gonadosomatic Index (GSI) is the percentage of gonad weight to the total weight of the fish (Von Vayer, 1910). The gonadosomatic index of the samples collected during the experimental period were calculated in the laboratory and determined for each female separately.

RESULTS AND DISCUSSION

Brood stock management

Under Green House Technique, fishes were reared easily and found suitable to get broods earlied then the normal spawning period. Water quality parameters such as, temperature (0 C), pH, dissolved oxygen (mg/l), Alkalinity(mg/l), Hardness (mg/l) and Ammonia (mg/l) were found suitable for rearing Pangas fishes under Green House Technique (Table 3). The mean value of

water temperature (^{0}C) in GP ponds ranged between 24.83±1.63 and 28.56 ± 2.15 while it was ranged from 17.34 ±1.48 to 23.64 ±2.42in Controlled Ponds (CP) during the Pangas rearing period from October to February (2015-2016) (Table 3). It is remarkable to note that water temperature of GP was always higher to some extent than that of CP. This might be due to use of polyethylene sheet (Thickness 0.6 mm) in GP. Water temperature is one of the most critical factors, that influences growth, reproduction and other biological activities of fish.

Table 3: Water quality parameters in different Pangas rearing ponds (GP, CP) during the study period

Parameters	October		November		December		January		February	
	GP	CP	GP	CP	GP	CP	GP	CP	GP	CP
Temp. (^{0}C)	28.56 ±2.15	23.64 ±2.42	27.24 ±1.39	22.24 ±1.56	25.57 ±1.53	19.14 ±3.01	24.83 ±1.63	17.34 ±1.48	26.35 ±2.01	20.62 ±2.48
pH	8.1± 2.2	7.9± 2.05	8.0± 4.12	8.2± 0.50	7.9± 4.01	8.5± 1.33	7.9± 1.38	8.0± 3.40	8.0± 2.21	8.2± 1.02
DO (mg/l)	6.87± 0.52	6.19± 0.30	6.14±0. 32	6.27± 0.23	5.85± 0.33	7.23± 0.39	6.01± 0.37	6.42± 0.26	7.21± 0.32	7.66± 0.32
Alkalinity (mg/l)	148.62 ±3.67	164.28 ±5.04	143.38 ±3.59	152.83 ±2.16	143.57 ±5.51	154.51 ±3.10	144.18± 7.80	151.35± 3.52	136.04 ±9.08	162.82 ±4.20
Hardness (mg/l)	240 ±4.12	260 ±5.07	225 ±2.16	275 ±1.88	328 ±5.0	355 ±2.31	305 ±4.17	315 ±6.01	315 ±2.75	296 ±3.61
Ammonia (mg/l)	0.28 ±1.60	0.25 ±2.04	0.33 ±0.6	0.30 ±2.11	0.39 ±0.65	0.40 ±1.33	0.42 ±3.15	0.44 ±4.12	0.46 ±2.32	0.47 ±1.07

Temperature is the main triggering factor for the development of broods. During the study period water temperature fluctuated between 24.83 to 28.56 ^{0}C in GP which was suitable for gonadal development of tropical fishes including Pangas (Jhingran & Pullin 1985, Hossain et al. 2007). pH and dissolved oxygen ranged from 7.9-8.5 and 5.85-7.66 (mg/l), respectively. pH of water of all ponds was always alkaline and variation in pH among different ponds was very insignificant. It was shown that the concentration of Do was always higher in GPs than those of CPs. No remarkable variation in alkalinity, hardness and ammonia between two types of ponds was observed. Depth of water was always maintained at a level of around one meter in all ponds. These results are in agreement with those of Daniels et al. (1994), Aktas et al. (1992) and Saha & Alam (2014) where they also reported that thewater quality parameters have direct impact on reproductive biological activities for gonad development of fishes.

Gonadosomatic Index (GSI) of Pangas

Gonadosomatic Index (GSI) for both GP and CP Pangas was assessed to ascertain spawning season of Pangas under Green House Technique. In the present study the mean values of GSI of Pangas ranged from 4.46±0.29 to 9.66±0.39 in the month from November to March. The highest mean GSI value was calculated in February and lowest mean GSI value was found in November (Table 4). Similar observations were also reported by Aktas et al. (2003), Dnniels et al. (1994) and Mustafa & Ansari (1983) where they found that off-season ovarian development of female fish is influenced by temperature and photoperiod.

Estimation of fecundity

Fecundity (Lagler 1949) and egg diameter of *P. hypophthalmus* were estimated with the fishes reared under Green House Technique (Table 4).The number of eggs varied from 65,500 to 2,20,000 for the Pangas of 64.4 cm to 67.7 cm length and 4.5 to 5.5 kg body weight. The average number of eggs per g of body weight was 79 and the average number of eggs per g of ovary was 975 for the Pangas reared under Green House Technique. The diameter of ripe ova ranged from

1.20 to 2.10 mm with a mean of 1.97 ± 0.037 for the Pangas reared under Green House Technique. The study indicated that *P. hypophthalmus*is a high fecund fish. These findings were in line with the findings of Rahman *et al*. (1993) and Tsadek et al. (2007).

Induced breeding, fertilization, hatching and survival rates

Standard protocol was followed to induce to breed the Pangas reared under Green House Technique. For the study, three types of hormones viz., Carp Pituitary Gland (cPG), Human Chorionic Gonadotropin (HCG) and a Synthetic Gonadotropin Releasing Hormone Analogue (SGnRHa) commercially known as "Ovaprim" were used (Table 2). After inducing, fertilization rate (%), hatching rate (%) and survival rate (%) were calculated accordingly (Table 4). Early maturation of Thai Pangas is related to the gonado somatic index, length-weight relationship of gonad, ova diameter and fecundity of fishes (Shrivastava and Prakash 1979 and Goswami et al. 2007). Similar results were recorded in present study. In case of Thai Pangas, the fertilization, hatching and survival rate of eggs were reported by 59-96%, 63-82% and 57-72% respectively which was similar to the present study (Rahman et al. 1993 and Kabir et al. 2013).

Table 4: Mean total length, body weight, GSI, fecundity, ova diameter, fertilization rate, hatching rate, and Survival rate of Pangas during the study period

Sampling Parameters	Sampling Month				
	November	December	January	February	March
	Mean± Standard error	Mean± Standard error	Mean± Standard error	Mean ± Standard error	Mean± Standard error
N0. of fish examined	5-6	5-6	5-6	5-6	5-6
Total length (cm)	64.4±1.38	65.4±0.33	66.5±3.01	67.6±1.52	67.7±1.03
Body weight (kg)	3.3±0.47	3.8±0.16	4.2±0.53	4.6±0.34	4.6±0.26
GSI	4.46±0.29	5.95±0.96	8.16±0.35	9.66±0.39	9.23±0.17
Fecundity	66500±9275	87200±13456	148000±16287	220000±34974	210100±28249
Ova diameter (mm)	-	-	-	1.47±0.03 (1.20 to 2.10)	1.49±0.04 (1.20 to 2.10)
Fertilization rate (%)	-	-	-	84.46 ± 4.11	84.31±4.08
Hatching rate (%)	-	-	-	71.20 ± 1.69	73.42±1.71
Survival rates (%)	-	-	-	60.40 ± 0.59	65.69±2.23

*Figures in parentheses indicate ranges of different parameters

Assessing development of gonad of Pangas

Induced breeding was started on mid February, in the hatchery complex of the Sub-Station, Santahar. After hormone administration eggs were collected by stripping method. The stocked immature adult female Pangas started to be matured after stocking in GP ponds (Table 5). Augmenting maturity of male and females were noticed throughout the culture period in the GP ponds. In the GP ponds fully matured females were first observed on 6th February 2016. About 21% female and 60% male Pangas became fully matured during mid-February in GP. A total of 98% female and 100% male of stocked Pangas became fully matured and ready for induced breeding during mid-March. However, no Pangas became fully matured in the CP ponds throughout the culture period. Poor development of gonads of a few female Pangas (2%) and male Pangas (5%) was observed in the CP ponds. About

80% of eggs were come out easily from female brood when stripped. The average number of eggs per g of body weight was 79 and the average number of eggs per g of ovary was 975 for the Pangas reared under Green House Technique. The diameter of ripe ova ranged from 1.20 to 2.10 mm for the Pangas reared under Green House Technique (Table 5).

Table 5: Pattern of development of gonad of *P. hypophthalmus* in GP and CP ponds

Date	"Green house" pond (GP)				Open (controlled) pond (CP)			
	No visible development		Fully Matured		No visible development		Fully Matured	
	Female	Male	Female	Male	Female	Male	Female	Male
01/11/2015-20	100	100	0	0	100	100	0	0
16/11/2015-20	100	100	0	0	100	100	0	0
01/12/2015-20	100	100	0	0	100	100	0	0
16/12/2015-20	100	100	0	0	100	100	0	0
01/01/2015-20	100	100	0	0	100	100	0	0
16/01/2015-20	100	100	0	0	100	100	0	0
01/02/2015-20	100	100	0	0	100	100	0	0
16/02/2015-20	79	40	21	60	100	100	0	0
01/03/2015-20	24	09	76	91	100	100	0	0
16/03/2015-20	2	0	98	100	100	100	0	0
01/04/2015-20	0	-	100	-	99	95	1	5
16/04/2015-20	-	-	-	-	98	80	2	20
01/05/2015-20	-	-	-	-	73	55	27	45
16/05/2015-20	-	-	-	-	32	19	68	81

Being an ectothermic, Thai Pangas obtain their heat from the water in which they live. Temperature affects the chemical and biological processes of ectothermic organisms in the water. At low temperature, Pangas does not take sufficient feed (Goswami et al. 2007). Hossain & Rahman (2004) measured assimilation efficiency of feed of adult Thai Pangas at temperature range from 22-31^0 C and found that significantly less feed was ingested by Thai Pangas at water temperatures below 23^0 C than at higher temperatures. In the present investigation, feed was uniformly used depending on the Pangas biomass in both types of ponds. In the controlled ponds, morning temperature of water was less than 18^{0}C and evening temperature was less than 22^0 C for the rearing period. Throughout this period there was sufficient (30-40%) left over feed in the pond indicating that interest of Pangas for feeding was poor in the controlled pond. In the green house pond, very less (2-3%) left over feed was observed in the pond throughout the rearing period. As the temperature of water was well enough for the normal biological activities, Pangas ate supplied feed up to throughout this period. In the green house pond, very less (2-3%) left over feed was observed in the pond throughout the rearing period. As the temperature of water was well enough for the normal biological activities, Pangas are supplied feed up to satiation and received sufficient energy for normal biological activities which ultimately help in developing gonad of Pangas in the green house ponds. These results are in agreement with Saha and Alam (2014) where they also used Green House Technique to increase temperature of water for rearing of *M. rosenbergii* in ponds. They also reported that increase in temperature can accelerate the growth and gonadal development of over-wintering shrimp and stimulate the coupling and egg-laying.

CONCLUSION

Increase in water temperature for enhancing the gonadal development of Thai Pangas through application Green House Technique is a new approach in Bangladesh aquaculture. The findings of the study will be very much helpful for the present Pangas production system. Further research is needed for the standardization of the technique.

REFERENCES

Aktas, M Kumlu M and Eroldogan OT (2003). Off-season maturation and spawning of *Penaeus semisulcatus* by eyestalk ablation and/or temperature- photoperiod regimes. Aquaculture, 228 (1-4): 361-370.

Banglapedia (2006). Banglapedia, national encyclopedia of Bangladesh. *http:// banglapedia. org* (accessed on July 2006).

Daniels WH, Abramo LRD and Harmin KF (1994). Ovarian development of female red swamp crayfish (*Procambarus clarkii*) as influenced by temperature and photoperiod. Journal of Crustacean Biology, 14 (3): 530-537.

DoF (Department of Fisheries), 2015. Fishery wealth of Bangladesh (in Bengali). *Jatio Matshya Shaptahow, 2015 (Shonkalon),* Department of Fisheries, Dhaka. pp.13-14.

Goswami MM, Hazarika R and Saud BJ (2007). Induced spawning, embryonic and larval development of *Pangasius pangasias* (Hamilton-Buchanan). Journal of Inland Fisheries Society. India, 39(2): 1-9.

Hossain MD, Hossain MK, Rahman MH (2007). Water quality parameters and augmentation of brood development in some Water bodies in Natore, Bangladesh. Journal of Life and Earth Science, 2(2): 27-30.

Hossain, A. R. M., B. M. S. Rahman, 2004. Thai Pangus (*Pangasius hypophthalmus*) in Bangladesh-A Review. International Journal of Business and Social Science, 1(2): 98-106.

Hossain MA, Taleb A and Rahman MH (1991). Reproduction and reproductive periodicity of *N. notopterus* (Pall.) Bangladesh Journal of Aquaculture, 11-13: 19-22.

Jhingran VG and Pullin RSV (1985). Hatchery Manual for the common, Chinese and Indian Major Carps. ICLARM Studies and Reviews 11. 191 P. Asian Development Bank, Manila, Philippines.

Kabir AM, Ghaedi A, Talpur AD and Hashim R (2013). Effect of dietary protein levels on reproductive development and distribution of amino acids in the body tissues of female *Pangasianodon hypophthalmus* (Sauvage, 1878) broodstock in captivity. Aquaculture Research, 46(7): 1736–1747.

Lagler KF (1949). Studies in freshwater fishery biology. Ann Arbor, Michigan, 119 p.

Mustafa S and Ansari AR (1983). Fecundity of *Gudusiachapra* from Baigul and Nanaksagar reservoirs (Nainital, India). *Z. angew.* Zoology, 11:133-144.

Phillips, G.L. 1969. Accuracy of fecundity estimation for the minnow, *Chrosomuse rythrogaster* (Cyprinidae), Transactions of the American Fisheries Society, 98(3): 524-526.

Rahman, MK, Akhtar JN and Halder GC (1993). First record of induced breeding of Thai Pangas, *Pangasius sutchi* (Fowler) in Bangladesh. Journal of the Inland Fisheries Society of India, 25(2): 26-30.

Roberts TR and Vidthayanon C (1991). Systematic revision of the Asian Catfish familyPangasiidae, with biological observations and descriptions of three new species. In proceedings of the academy of national science of Philadelphia, 97- 144.

Saha SB and Alam MJ (2014). Giant freshwater prawn *M. rosenbergii* brood development in winter season. Bangladesh Journal of Zoology, 42(2): 153-159.

Shrivastava SS and Prakash MM (1979). Length-weight relationship in a freshwater teleost *N. notopterus.* Journal of Shivaji University, **7**:48-53.

Tsadik GG, Bart AN (2007). Effects of feeding, stoking density and water-flow rate on fecundity, spawning frequency and egg quality of Thai Pangas, *P. Hypophthalmus* (L.) Aquaculture, 272(1/4): 380-388.

Von Vayer H (1910). A method of measuring fish eggs. Bulletin of the Bureau of Fisheries, 28:1011-1014.