



Induced Breeding of Asian Striped Catfish, *Pangasianodon hypophthalmus* under Farmer Participatory Mode in Punjab

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ABSTRACT

Asian striped catfish *Pangasianodon hypophthalmus* (Sauvage, 1878) is considered one of the major food fish with huge potential for vertical and horizontal development of aquaculture sector in India. Present study was conducted in a farmer's participatory mode, as off campus trial, with the major objectives to disseminate the breeding and seed rearing technology of Pangas catfish. For the said purpose, 4 yr+ brood stock, reared by GADVASU after 4 yr of over wintering under poly house condition, was provided to the farmers. The fish was induced to breed using carp pituitary gland as an inducing agent. Pituitary hormone extract in female was administered intramuscularly @ 2.5 - 3.0 mg/kg body weight (BW) as priming dose and after 6 hr second resolving dose @10-12 mg/kg BW was injected. While male was injected with pituitary hormone @ 2.5 - 3.0 mg/kg BW at the time of second injection to the female. After 12-17 hr, of hormone injection, female and male were striped for external fertilization through dry method. Relative fecundity ranged between 0.80-0.95 lakh eggs/kg BW, while fertilization and hatching rate ranged between 75-85 and 55–65 per cent, respectively, with fry recovery rate of 18-20 per cent after 15 d of nursery rearing. The said breakthrough could solve the problem of non- availability of Pangas catfish seed in North Indian states and motivate the farmers to adopt the Pangas culture on large scale with locally available seed.

Key Words: Breeding, Farmer, *Pangas*, *Participatory mode*, Punjab, Seed

INTRODUCTION

With increasing population in the developing countries like India, aquaculture diversification is also required to achieve future production targets (food security) for supply of quality animal protein. Aquaculture in Punjab is predominantly carp poly-culture based, with an estimated total fish production of 1.36 lakh tons (t), including 98,556t from aquaculture (DAHDF, 2019). However, carps fetch low price in the market in Punjab due to presence of intra-muscular bones/spines, which is not preferred by the Punjabi people, who generally prefer to consume fish without/less intramuscular spines, like catfishes and murrels.

The Asian striped catfish, *Pangasianodon hypophthalmus* (Sauvage, 1878) is native to

the Mekong and Chao Phraya basins in Indochina. It is an important food fish, recognized as a superior aquaculture species for tropical regions with huge demand in the international market (Pal, 2010). It is now considered as the 3rd most important freshwater fish because of its fast growth, versatile feeding habit and hardiness. It has been introduced in Singapore, Philippines, Taiwan, Malaysia, China, Myanmar, Bangladesh, Nepal and India. In India, it was introduced in West Bengal through Bangladesh during 1997 (Mukai, 2011). After its successful culture in Andhra Pradesh and West Bengal, now it is also becoming a potential candidate species for diversification in northern states (Lakra and Singh, 2010; Singh and Lakra, 2012; Kumar *et al*, 2013), as it has been found to produce 3 times more fish

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in Punjab, with about 2.5 times higher net income over traditional carp culture practices (Datta *et al*, 2016). Hence, besides diversification it also serves an important purpose of producing more fish, without any additional land and water requirement.

Being a cold sensitive species, pangas is most suitable species for culture in southern India throughout the year. However, in northern India, pangas culture is restricted to summer months (April to November). Due to demand for spineless fish, at present Pangas fish is transported all the way from Andhra Pradesh and sold extensively in the fish markets of the northern States, including Punjab. Due to lack of proper preservation facilities, ignorance and long distance transport, the quality of the fish always remains a debatable issue. Hence, efforts have been made in the recent past to introduce pangas culture in Punjab and Haryana. Non availability of seed, restricted culture period and mandatory harvesting before the onset of winters are the major bottle necks in commercial adoption of pangas catfish culture in this region. Presently, farmers are entirely dependent on other States (West Bengal) for the availability of seed. Long distance transportation adds to seed cost, causes mortality (due to handling and transportation stress) and results in delayed stocking, besides procurement hassles from booking to lifting from the nearest located airport. Hence, it's vital to establish pangas hatchery in the region to overcome above listed

constraints, so that quality seed is available to the farmers at a lesser cost without any procurement hardships.

Pangas catfish is a cold sensitive fish and mortality starts within a few hours of exposure to water temperature < 15°C. Further, pangas fish matures after 3-4 yr, which needs to be overwintered (under poly-house conditions) for at least 4 yr to develop the brood stock for breeding under climatic conditions of Punjab. To address this challenge, brood stock production and induced breeding technology has been standardized by the College of Fisheries, GADVASU, Ludhiana (Punjab) for the first time in the Northern-Western India (Datta *et al*, 2018). With an objective to demonstrate induced breeding of Pangas catfish to the farmers, off campus breeding trials were taken up in a farmer's participatory mode with one of the progressive fish farmer of the State, with a target to mobilize fish farmers into pangas catfish culture for achieving dual benefit of higher productivity and doubling of farmer's income.

MATERIALS AND METHODS

Brood stock development

Juveniles of *P. hypophthalmus* (length 2.93± 0.2 cm and weight 0.25 ± 0.05g) were procured from Kolkata, West Bengal in 2015 and reared in 700 m² earthen pond (@ stocking density of 3/m²) during summer (April to November) and overwintered

Table 1: Physico-chemical parameters of water in the brood stock rearing pond.

Water parameters	Mean ± SE values	Range
Temperature (° C)	27.33 ± 0.18	26.90 - 27.90
pH	7.10 ± 0.06	7.01 - 7.14
Dissolved Oxygen (mg/ l)	7.17 ±0.23	6.48 - 7.86
Alkalinity (mg/l)	315 ± 10.98	286 - 360
Hardness (mg l ⁻¹)	308 ± 4.26	296 - 324
Ammonia - nitrogen (NH ₃ -N (mg/ l)	0.091 ± 0.019	0.039 - 0.127
Phosphate- phosphorus PO ₄ -P (mg/ l)	0.456 ± 0.56	0.018 - 0.944
Nitrate – nitrogen NO ₃ -N (mg/ l)	0.532 ± 0.35	0.198 - 0.75

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during winters (November/December- March) for 4 yr to develop healthy brood stock with 100 per cent overwintering survival, at College of Fisheries, GADVASU (Fig. 1-3). Water temperature range in poly house was maintained $22.5 \pm 1^{\circ}\text{C}$ throughout the overwintering period. Floating feed (28.0 % crude protein on dry weight basis) was formulated using agro-industrial by-products i.e. rice bran (30%), de-oiled Ground nut (30%), de-oiled soybean (25%), fish meal (13 %) and vitamin – mineral mixture (2%). Fish was fed @ 2% of body weight (BW) twice a day (in 2 split doses) at 9.00 am and 5.00pm.

For demonstration and hand holding support, healthy four years old brooders (female 2500 – 3000 g and male 2000-2500 g) were selected and transferred to the fish hatchery of a progressive fish farmer of the State, Mr. Ranjodh Singh (Village Nanoki, District Patiala), aspiring to assess possibilities of developing pangas hatchery in the State. Brooders were acclimatized for a period of 15d on farmer's pond. The breeding trial was conducted during the months of June and August (Fig 4-6). Before induced breeding, the selected mature male and female brooders were kept separately in flow through circular tanks (diameter 3.0 m and depth 0.75 m) under continuous showering for conditioning (1 d) purpose.

Induced breeding

Pituitary glands of Indian major carps preserved in absolute alcohol and stored in the refrigerator were used for preparation of pituitary extract (PE). At the time of breeding females were administered a priming dose of 2.5 - 3.0 mg PE/kg BW. After 6 hr (h), a second resolving dose of 10-12 mg PE/kg BW was given. A single dose of 2.5 – 3.0 mg PE/kg BW was injected to the males at the same time the resolving dose was given to the female. The PE was injected intramuscularly in the region of caudal peduncle above lateral line of fish, with 2 ml syringe (graduated to 0.1 ml division) and 22 no. needle. After injection male and female fish were kept in separate tanks with continuous showering to create natural raining stimulating condition. The

tanks were well covered to avoid fish jumping and an outlet was provided for drainage of excess water. The females were found ready for stripping after 12-17 hr of post injection in different trials conducted during the month of June to August.

The ready-to-spawn females were taken out and their eggs were stripped on a dry plastic tray by gently pressing their bellies. The males were then stripped and their milt was mixed with the eggs with the help of a clean feather and gentle shaking, following dry method of egg fertilization (Ferosekhan *et al*, 2015). Milt from one male was sufficient to fertilize the eggs of two to three females. A small amount (10–15 ml) of distilled water was added to the mixture and stirred for 1–2 min. After thorough mixing, the tray was rinsed with 100–150 ml of water to remove excess milt and mucus. As the fertilized eggs were adhesive, they were washed with clay mud water for 3 - 4 times before transferring it to Chinese circular hatchery. The incubation period ranged from 30–36 h at a temperature of $26-27.8^{\circ}\text{C}$.

Important parameters of water quality such as water temperature, pH, dissolved oxygen, total alkalinity; hardness, ammonia-nitrogen ($\text{NH}_3\text{-N}$), nitrate-nitrogen ($\text{NO}_3\text{-N}$), nitrite-nitrogen ($\text{NO}_2\text{-N}$) and phosphate-phosphorous ($\text{PO}_4\text{-P}$) were analyzed using standard methods (APHA, 2012). Parameters such as gonado somatic index (GSI), relative fecundity, fertilization and hatching rate were calculated following the formula by Gunasekara *et al* (1996).

$$\text{GSI (\%)} = \frac{\text{Weight of ovary/testes (g)}}{\text{Total weight of fish (g)}} \times 100$$

$$\text{Relative fecundity (No. of eggs/kg BW)} = \frac{\text{Total eggs in the ovary}}{\text{Weight of fish}} \times 100$$

$$\text{Fertilization rate (\%)} = \frac{\text{No. of fertilized eggs}}{\text{Total no. of eggs}} \times 100$$

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

Table 2: Reproductive parameters of *P. hypophthalmus*.

Stripping time after pituitary injection	12-17 hr
Average stripping fecundity	0.50- 0.65 lakh eggs/kg BW
Gonado-somatic Index (GSI)	
Female	10.08± 0.68%
Male	7.024 ±1.60%
Relative fecundity	0.80-0.95 lakh eggs/kg BW
Colour of normal egg	Creamish yellow
Colour of fertilized egg	Transparent
Colour of unfertilized egg	Opaque white
Fertilization rate	75-80%
Hatching rate	55–65%
Egg incubation period	30–36 h (at 27.33 ± 0.18°C)
Diameter of egg before fertilization	1.0 - 1.20 mm
Diameter of egg after fertilization and water hardening	1.10 - 1.30 mm
Brood stock Condition Factor (K- value)	
Female	1.048 ± 0.15
Male	1.051 ± 0.06

RESULTS AND DISCUSSION

Water quality parameters of in brood stock rearing pond viz. temperature, pH and dissolved oxygen were within the suitable range for *P. hypophthalmus* (Table 1), as suggested by Waycott (2015) on *The Fish Site viz*; water temperature 22 to 30°C, dissolved oxygen 2.5- 7.5 mg/l and pH of 6.5 to 9.5. whereas, alkalinity (mg/l) and hardness (mg/l) of water ranged higher (286-360 and 296-324 mg/l, respectively) in present study, as compared to recommended range (alkalinity: 50-150 mg/l and hardness 75-200 mg/l, respectively) suggested by Bhatnagar and Devi (2013).

In Punjab, mid of June to mid of August was found suitable for breeding of *P. hypophthalmus*. Fish matured after attaining 4 years (yr) of age

with an average gonado-stomatic index (GSI) of 10.08±0.68% in female and 7.024±1.602% in male and relative fecundity of 0.80-0.95 lakh eggs/kg BW (Table 2). However, *P. hypophthalmus* has been reported to mature in 3 yr in southern India (Lakra and Singh, 2010). Delayed maturity in Punjab may be attributed to prolong winter and occurrence of late monsoon in Northern India. Generally an incubation time of 12 to 14 hr was required for successful stripping of fish (Ferosekhan *et al*, 2015), but in the present study a minimum of 12 and a maximum of 17 hr were recorded for final ovulation and release of gametes (Table 2). This may be due to the variation in gonadal maturity stages, inducing agent used, dosages given and the agro climatic variation prevailing in particular region over a period of time.

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The eggs released during stripping of female varied between 0.5 to 0.65 lakh eggs/kg BW. Before fertilization, diameter of ova ranged between 1.0 to 1.2 mm. The ovulated or fertilized eggs were round, adhesive and transparent. Stickiness of eggs was removed by treating it with clay mud water. The diameter of eggs after fertilization and water hardening increased and ranged between 1.1 to 1.3 mm. Ferosekhan *et al* (2015) also reported swelling of *P. hypophthalmus* eggs from 1.09-1.28 mm to 1.20- 1.45 mm in size after incubation of fertilized eggs. Variation in diameter in water hardened eggs may be attributed with variation in brood stock weight, age and change in agro-climatic condition.

Good percentage of fertilization (75-85%) and hatching (55–65%) were achieved during the present trials (Table 2). Almost similar results have been reported for the induced breeding of *P. pangasius* by Ferosekhan *et al.* (2015).

Larval feeding

Diluted boiled egg-yolk enriched with multi-vitamins was found suitable as the starter feed (at 3-4hr intervals) at around 40h after hatching. Gradual administration of freshly filtered zooplankton along with diluted boiled egg-yolk at 3-4 hr intervals from 60 to 96hr after hatching revealed better growth of larvae. Diluted boiled chicken liver enriched with multivitamins were provided from 4th day onwards till 12th -14th day of fry rearing (Fig 6-9). Hossain and Rahman (2014) also reported that diluted boiled egg-yolk was well accepted by the larvae and hence, facilitated weaning process of the yolk-sac absorbing larvae to external feed at around 30hr after hatching in *P. sutchi*. After 15 d of nursery rearing 18-20 per cent fry recovery was recorded (Fig. 10), indicating successful larval rearing with the help of feeding regime followed in the present study.



Fig. 1: Pangas Brood stock pond (April to November)



Fig. 2: Pangas overwintering poly-house for brood stock production (December to February)



Fig. 3: Inside view of Poly-house with underwater heating system



Fig. 4: Four year+ Pangas brood stock



Fig. 5: Pangas brood stock pond at farmers Farm



Fig. 6: Selection of brood stock



Fig.7: Pangas seed produced at farmer's pond



Fig. 8: Pangas nursery pond



Fig. 9: Pangas seed harvest



Fig. 10: Fifteen day old Pangas seed

CONCLUSION

Under agro-climatic condition of Punjab, *P. hypophthalmus* attained maturity at the age of 4 yr with an average GSI of 10.08 ± 0.68 per cent in female and 7.024 ± 1.602 per cent in male, with relative fecundity of 0.80– 0.95 lakh eggs/kg BW, stripping fecundity of 0.5- 0.65 lakh eggs/kg BW,

fertilization rate 75-80 per cent, hatching rate 55–65 per cent and fry recovery of 18-20 per cent after 15d of nursery rearing. Successful culture and brood stock development of pangas, through overwintering under poly house condition and successful induced breeding, clearly indicate the possibility of establishing of new species under

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agro-climatic conditions of Punjab. Outcome of the results could help in establishment of pangas hatcheries in North-Western region and hence, solve the problem of non-availability of Pangas seed in Punjab and its neighboring states (Haryana, Rajasthan, Uttarakhand, Himachal Pradesh, Jammu & Kashmir), consequently promoting carp culture diversification for higher productivity and income per unit land holding.

ACKNOWLEDGEMENT

The authors are thankful to the Dean, College of Fisheries, Guru Angad Dev veterinary and Animal Sciences University, Ludhiana for keen interest and providing facilities for this study.

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Received on 22/06/2020

Accepted on 17/08/2020