

DOI : <http://doi.org/10.22438/jeb/40/3/MRN-768>

# Induced breeding of *Catla catla* carried out at low temperature in FRP carp hatchery of Arunachal Pradesh, India

Paper received: 23.10.2017

Revised received: 07.04.2018

Re-revised received: 03.08.2018

Accepted: 03.09.2018

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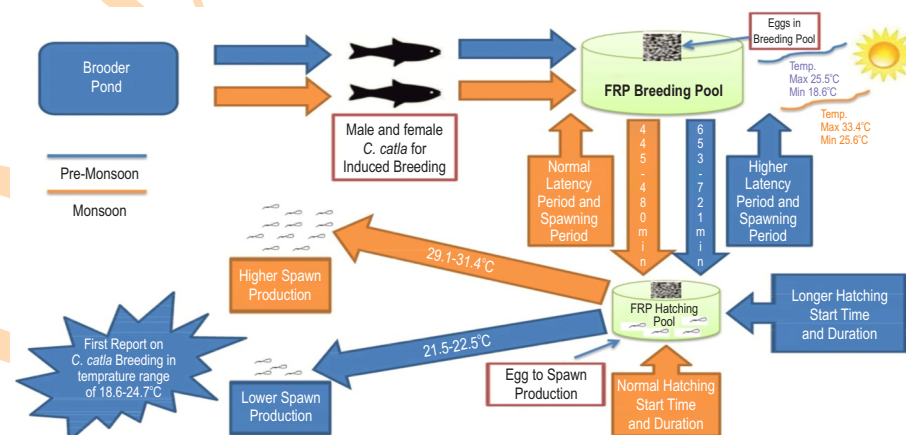
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## Abstract

**Aim :** The present study was undertaken to find out the possibilities of induced breeding of *Catla catla*, at low water temperature using fibre reinforced (FRP) tanks in a carp hatchery.

**Methodology :** Brood fish of *C. catla* was maintained in 0.1 ha pond with proper feeding and pond management. Six induced breeding programmes: 3 in April (pre-monsoon) and 3 in June (monsoon) of 2017 were conducted with *C. catla* using synthetic hormone, Ovasin intra-peritoneally. The relationship between temperature variation and latency period, effective spawning period, fecundity, hatching start time, hatching duration and spawn production with its recovery was observed.

**Results :** The latency period (time gap between injection and first egg release) was very high and ranged between 653.3 and 721.6 min for breeding in the pre-monsoon and ranged between 446.6 and 480 min for monsoon period. In pre-monsoon, the effective spawning period between 86.6 and 116.6 min and in monsoon between 53.3 and 73.3 min; both varied significantly ( $p < 0.05$ ). In pre-monsoon period, the average water temperature during larval incubation in hatching pool ranged between 21.58 and 22.58°C, whereas during monsoon period it ranged between 30.1 and 30.4°C. The spawn production was 0.13 to 0.22; and 0.47 to 0.65 (lakh  $\text{kg}^{-1}$  b. wt. of female) in pre-monsoon and monsoon period, respectively, and showed a significant difference ( $p < 0.05$ ).



**Interpretation :** This report on breeding of *C. catla* in the temperature range of 18.6-24.7°C indicates the possibility of spawn production of Indian Major Carp in the north east hilly region states of India.

**Key words :** *Catla catla*, FRP carp hatchery, Induced breeding, Low temperature, Latency period, Spawn production

**How to cite :** Ghosh, A., B.C. Mohapatra, P.P. Chakrabarti, A. Hussan and A. Das: Induced breeding of *Catla catla* carried out at low temperature in FRP carp hatchery of Arunachal Pradesh, India. *J. Environ. Biol.*, **40**, 328-334 (2019).

## Introduction

Freshwater fish culture is an important food production activity for human consumption in Asia and throughout the world (Gjedrem *et al.*, 2012). Fish seed is one of the primary inputs for freshwater fish culture and its availability became possible after the first success of induced breeding of Indian major carps during 1957. For faster development and multiplication of aquaculture industry, adequate supply of good quality seeds of particular fish species is required. The seeds collected from natural water bodies are generally mixed with different species and insufficient to meet the regular demand in time. The technology of induced breeding through hypophysation has helped in mass production of quality carp seed and reduced the dependence on natural seed collection. Quality seed production can be achieved through scientific broodstock management, establishment of hatcheries, and refinement of induced breeding techniques and rearing of seed across the country. The long way contribution of several researchers from hapa breeding to cemented eco-hatchery, and then to portable FRP carp hatchery has made easy in availability of stocking material for aquaculture (Mohapatra *et al.*, 2008).

FRP hatchery, developed by the ICAR-Central Institute of Freshwater Aquaculture (ICAR-CIFA) Bhubaneswar, has been installed and successfully operated in 26 states of India (Mohapatra *et al.*, 2016). Breeding among fishes is regulated by environmental factors that trigger internal physiological mechanisms. The final event of the breeding cycle, the release of eggs and milt resulting in spawning can be controlled by placing the fish in an appropriate environment and by changing its internal regulatory mechanism by injecting hormones or other inducing substances. The optimum range of water quality parameters like temperature, pH and dissolved oxygen for carp breeding, spawn rearing, reproduction, mortality and growth are influenced by some abiotic factors as reported in *Cyprinus carpio* (Singh and Das, 2006) and in exotic carp by Mohan (2000). Temperature is the main environmental factor governing the development of fish eggs (Nwosu and Holzlohner, 2000). There are available reports on embryonic and larval developments of different fish species at varying water temperatures, Fox *et al.*, 2003; Coombs and Hibey, 2006). In India, a breeding technique of cold water fish species, with or without hormone injection has been developed for *T. khudree*, *T. putitora*, *T. tor* and hybrid mahseer (Ogale, 2002; Sangma and Basavaraja, 2010). Attempts have been made to breed *T. putitora* (Golden mahseer) in the Kumaon region (Sunder *et al.*, 1993; Ogale, 1997). Induced breeding of *Labeo dyocheilus* has been achieved using ovaprimin cold water condition under captivity (Pandey *et al.*, 2011). In light of the above, the present study was undertaken to investigate the possibilities of induced breeding of *Catla catla*, an Indian Major Carp at low temperature using FRP carp hatchery at Sonajuli, Papum Pare district, Arunachal Pradesh, India.

## Materials and Methods

**Brood stock management :** The brooders of *C. catla* in the 2+ year age group (mean weight  $2.1 \pm 0.1$  kg,  $n = 10$ ) were stocked

during August, 2016 in 0.1 ha (50 x 20 m; 1.5-2 m depth) pond with stocking density of 2500 kg ha<sup>-1</sup> at the Sonajuli farm, Papum Pare District, Arunachal Pradesh, India (27.03°N; 93.73°E). Essentially, maintenance of fishes was undertaken through periodic fertilization of ponds using raw cow dung manure @ 10,000 kg ha<sup>-1</sup> yr<sup>-1</sup>, urea @ 100 kg ha<sup>-1</sup> yr<sup>-1</sup> and single super phosphate @ 200 kg ha<sup>-1</sup> yr<sup>-1</sup> in equal monthly instalments, followed by liming (CaCO<sub>3</sub>) @ 500 kg ha<sup>-1</sup> yr<sup>-1</sup> after 7 days of each fertilization. Water replenishment of 30% was done in ponds during the pre-spawning period (January-March, 2017). Analysis of pond water was conducted every day during operation of induced breeding following the methods of APHA (2012). Brood fish was fed with commercial floating fish feed (GROWFIN™; diameter: 4 mm; crude protein minimum/ crude fat minimum: 28/4 %) @ 2% body weight/day from August 2016-July 2017. Regular observations of brood fishes were done during the pre-monsoon (April-May) and monsoon (June-July) periods at fortnightly basis to assess the gonadal maturation to facilitate for induced breeding operation.

**FRP carp hatchery operation:** For induced breeding of carp fishes, specially designed FRP based carp hatchery was installed at the Sonajuli farm. It was composed of 3 units: breeding/spawning pool, spawning/incubation pool and egg/spawn collection tank. The spawning pool was cylindro-vertical in shape having 2.15 m diameter, 0.9 m height, 1:22 bottom slope and could hold 2950 l of water during operation. The system was also provided with water circulation and showering facilities. During operation, water supply came from an overhead tank placed at a height of 8m from the hatchery floor for providing water circulation inside the pool. There were 5 numbers of 15 mm diameters rigid PVC elbows fitted with caring nipples and all were fitted in the same direction at the floor of the pool. There was one single point 25 mm diameter water inlet also fitted at the side wall of the pool at bottom region. For regulation of water flow, all the water inlet pipes were interconnected and fitted with individual full-way valves. At the top of the pool, one shower was provided for better aeration.

Hatching/incubation pool was cylindro-vertical in shape with 1.4 m diameter and 0.98 m height having 1400 l total volume (1200 litre net egg incubation volume). It had 2 chambers: inner chamber (0.4 m diameter and 90 cm height covered with nylon bolting cloth of 0.25 mm mesh to filter excess water into the drain) and outer chamber for rearing of hatchling. It had six numbers of 15 mm diameter duck-mouth for water supply fitted at the bottom of the hatchery at 45° angle. There were two drainage outputs: one at the centre of the inner chamber and the second one at the base of the outer chamber of the pool. The flow rate in the pool during operation was maintained at 0.3-0.4 l sec<sup>-1</sup>. Egg/spawn collection tank was rectangular in size (1x0.5x0.5 m), having water holding capacity of 250 l. During operation, the water level in the tank was maintained at a height of 0.45 m (water volume 225 litres) by fixing a drainpipe of 63 mm at a distance of 38.7 cm from the base. Cotton inner hapa of tank size were used inside the collection tank to collect egg/spawn from the breeding and hatching pool, respectively.

**Induced spawning in FRP carp hatchery:** On the basis of free-oozing milt after gentle pressure in abdomen in male; and a swollen and soft abdomen with pinkish vent in female, the brood fishes (Table 1) were selected as mature ones for the breeding operation. For induced breeding operations, eight brood fishes were selected each time at a ratio of 1:1 of male and female. Ovasis (Salmon GnRH + Domperidone) was injected intra-peritoneally at a dose of 0.25 ml and 0.5 ml per kg body weight of male and female, respectively. The injected fishes were released in the breeding pool for self spawning. After completion of spawning and fertilization, eggs were collected in the collection tank and were transferred to the hatching pool. Fishes were removed from the breeding pool with a scoop net and free swimming spawn was collected from the hatching pool after 72 hrs of hatching. Water circulation and continuous aeration with the shower was maintained and the temperature was monitored hourly during the breeding experiments.

Water temperature was measured directly from the breeding unit before and after spawning, and from hatching unit before and after hatching. Temperature and pH were measured by digital thermometer and digital pH meter, respectively. Three breeding operations during pre-monsoon

and monsoon period were conducted for *C. catla*. Latency period for egg release, relative fecundity percentage of fertilization of eggs, effective spawning period, hatching time, percentage of spawn recovery and spawn production per kg body weight were calculated after each breeding operation. The average of each breeding set is given in Table 1.

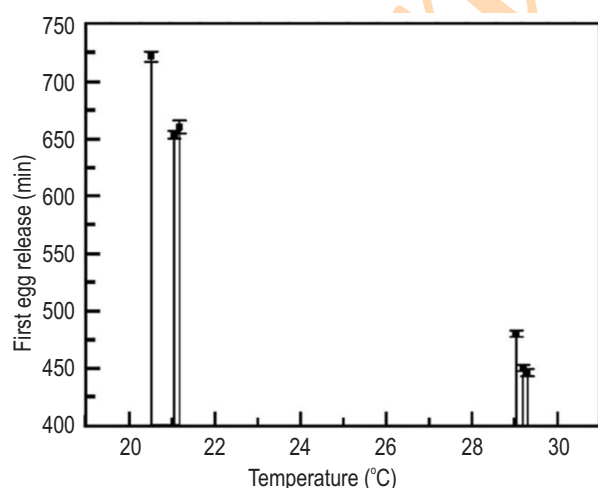
**Statistical analysis:** The results are presented as mean  $\pm$  standard deviation. Statistical analysis followed by Pearson's correlation test, to examine the relationship between latency period and effective spawning period in relation with temperature. The degree of association was measured by a correlation coefficient, denoted by  $r$ . The t-test was done for statistical significance, whether or not the difference between the mean of two groups exist.

## Results and Discussion

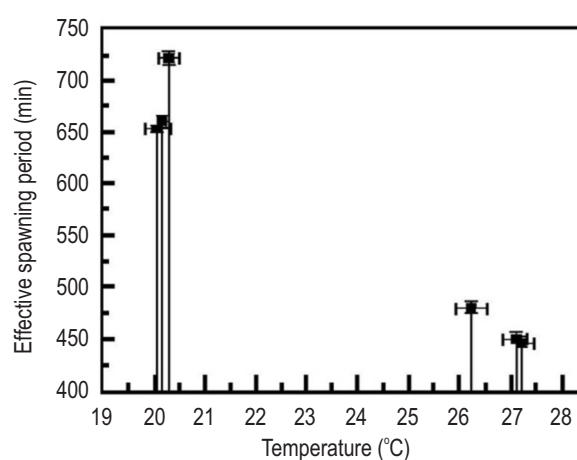
The present study indicated that during pre-monsoon period of 2017, the temperature of the experimental area ranged between 18-25°C and during monsoon it ranged between 25-33°C. The variations in response of fish during induced breeding, such as latency period and effective spawning period are

**Table 1:** Response of *C. catla* during induced breeding

Season	Expt. No	Water temp. (Min/Max) °C	Average weight of female brooder (kg)	Average weight of male brooder (kg)	Percentage of female/male fish responded	Latency period in min (A)	Completion time of egg release in min (B)	Effective spawning period (B-A)
Pre-monsoon	1	18.6-24.7	2.025 $\pm$ 0.11	1.85 $\pm$ 0.09	50/50	721.6 $\pm$ 4.4	838.3 $\pm$ 4.4	116.6 $\pm$ 8.8
	2	19.6-25.1	2.05 $\pm$ 0.08	1.725 $\pm$ 0.07	50/50	653.3 $\pm$ 3.3	758.3 $\pm$ 1.6	105 $\pm$ 2.8
	3	19.8-25.5	2.05 $\pm$ 0.02	1.8 $\pm$ 0.01	50/50	660.0 $\pm$ 5.7	746.6 $\pm$ 3.3	86.6 $\pm$ 3.3
Monsoon	4	25.6-32.2	2.075 $\pm$ 0.1	1.8 $\pm$ 0.07	75/75	480.0 $\pm$ 2.8	553.3 $\pm$ 1.6	73.3 $\pm$ 4.4
	5	26.3-33.4	1.975 $\pm$ 0.1	1.8 $\pm$ 0.14	100/100	446.6 $\pm$ 3.3	501.6 $\pm$ 1.6	55 $\pm$ 2.8
	6	26.2-33.3	2.125 $\pm$ 0.1	1.8 $\pm$ 0.07	100/100	450.0 $\pm$ 2.8	503.3 $\pm$ 4.4	53.3 $\pm$ 1.6



**Fig. 1:** Response between temperature variations and latency period.



**Fig. 2:** Variation of effective spawning period with temperature.

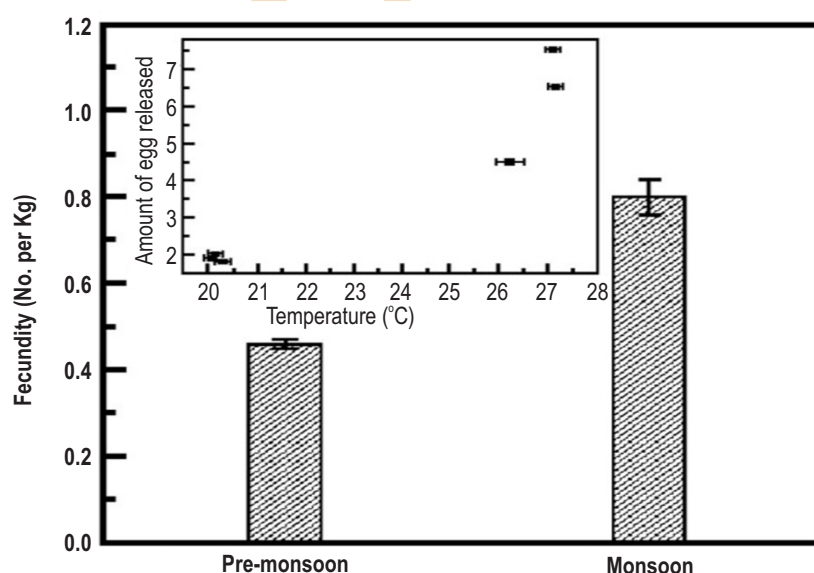
**Table 2:** Spawning and larval development after induced breeding of *C. catla*

Season	Expt. No	Total eggs released (lakh)	Spawning fecundity per kg body weight of female (lakh)	Time of first hatchling observed from spawning time (min)	Duration of hatching (min)	Spawn recovered after hatching (lakh)	Spawn survival (lakh)	Spawn production (lakh kg <sup>-1</sup> b.wt. of female)
Pre-Monsoon	1	1.8±0.05	0.44±0.02	1560±17.3	540±2.88	0.75±0.02	0.55±0.01	0.13±0.01
	2	1.9±0.17	0.46±0.03	1320±11.54	480±5.77	1.05±0.05	0.8±0.02	0.2±0.01
	3	2.0±0.17	0.48±0.01	1380±5.77	370±4.61	1.1±0.05	0.9±0.04	0.22±0.02
Monsoon	4	4.5±0.28	0.72±0.04	660±2.88	300±8.66	3.15±0.08	2.9±0.11	0.47±0.01
	5	6.5±0.23	0.82±0.02	720±8.66	300±5.77	4.5±0.05	4.38±0.21	0.57±0.02
	6	7.5±0.05	0.88±0.01	690±11.54	310±2.88	5.5±0.11	5.33±0.29	0.65±0.02

presented in Table 1. During pre-monsoon season both female and male fishes responded to induced breeding, but only 50% success was achieved (Table 1). The latency period was very high and ranged between 653.3 and 721.6 min for pre-monsoon period as compared to monsoon period, which ranged between 446.6 and 480 min. It was observed that due to change in water temperature the latency period changed significantly ( $p < 0.05$ ) in pre-monsoon and monsoon period. The variation of latency period with change of temperature is shown in Fig. 1. Chaturvedi *et al.* (2015) reported that in case of *C. catla* the egg release took place after 10-12 hrs of hormone administration under optimum temperature range (28-29°C). Whereas, Chakrabarti *et al.* (2016) reported that the optimum temperature range of 29-32°C for spawning of *C. catla* after a latency period of 5-6 hrs. Crandell *et al.* (1995) stated that the female brood stock is to be kept after hormone injection at suitable temperature, as it affects the latency

period. In this study, a negative relationship was found for change in temperature with the latency period. When temperature increased, the latency period decreased, *i.e.*, during lower temperature latency period increased. Temperature and latency period were strongly and negatively correlated ( $r = -0.98609$ ) which means the latency period decreased with the rise in temperature and *vice-versa* (Fig. 1). During the period of study, varied response in fish also observed. In the pre-monsoon period, the percentage of response of male and female fish to induced spawning was 50% however, during monsoon season the percentage of response increased to 75-100%.

The effective spawning period varied significantly ( $p < 0.05$ ) for pre-monsoon and monsoon periods and this might be due to change in water temperature. During pre-monsoon season, the effective spawning period ranged between 86.6 and

**Fig. 3:** Fecundity (lakh kg<sup>-1</sup> b.wt.) variation during pre-monsoon and monsoon season and number of eggs released (lakh) at varied temperature (inset).

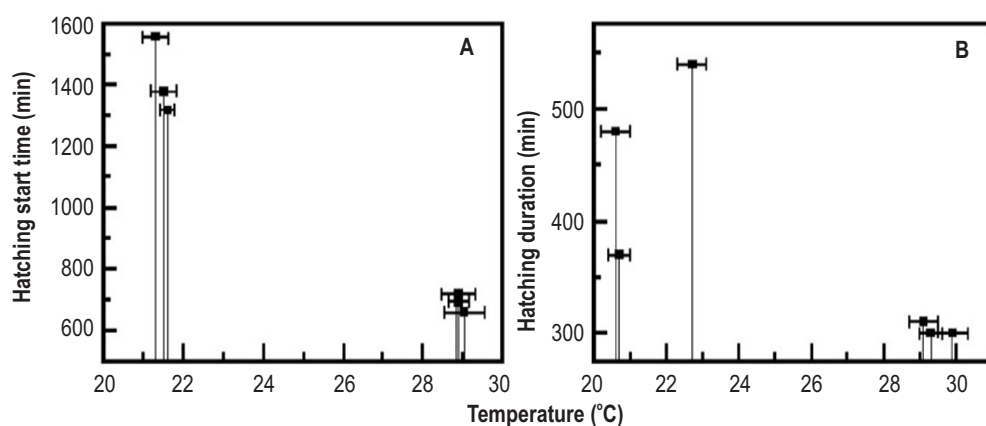


Fig. 4 : Variation of hatching time from egg release and hatching duration with varied temperature.

116.6 min whereas during the monsoon period, it was between 53.3 and 73.3 min. Chakrabarti *et al.* (2016) reported that the latency period during breeding of *C. catla* was 60 min at Bali Island, Sundarban, West Bengal and 55 min at Potaspur, East Midnapore, West Bengal (Chakrabarti *et al.*, 2017) when temperature ranged between 27.5-32.6°C at both the places. The results of the present study revealed that the temperature was inversely proportional to the effective spawning period in *C. catla* (Fig. 2). Correlation analysis in between effective spawning period and temperature showed a strong and negative relationship ( $r = -0.92304$ ), which indicates that the effective spawning period got shortened with higher temperature and *vice-versa* (Fig. 2).

The data shown in Table 2 was compared with the change in water temperature during breeding operations. The amount of eggs released during the pre-monsoon and monsoon season varied significantly ( $p < 0.05$ ). During the pre-monsoon period, when average water temperature was low (18.6-25.5°C) total eggs released were 1.8 to 2.0 lakh from 2 females each weighing 2.025 to 2.05 kg whereas during monsoon season it varied between 4.5 and 7.5 lakh from 3-4 female weighing about 1.975-2.125 kg female, when the average water temperature was comparatively higher (26.23 - 27.2°C) (Fig. 3). The fecundity of *C. catla* varied significantly between 0.44 and 0.48 lakh  $\text{kg}^{-1}$  b.wt. during pre-monsoon season and 0.72 to 0.88 lakh  $\text{kg}^{-1}$  b.wt. during monsoon season. According to Nandi *et al.* (2007), the fecundity of *C. catla* was 0.91 to 0.96 lakh  $\text{kg}^{-1}$  b. wt. with control diet and 1.43 to 1.53 lakh  $\text{kg}^{-1}$  b.wt. using a formulated diet with PUFA supplementation. Chakrabarti *et al.* (2016) also reported fecundity of *C. catla* 0.95 lakh  $\text{kg}^{-1}$  b. wt. from the Bali Island, West Bengal. Though percentage of fertilized of eggs did not differ significantly ( $p > 0.05$ ) with season change.

Hatching time and duration of hatching depends upon the temperature and varies among different fish species. Kikko *et al.* (2015) reported that lower incubation temperature resulted in smaller hatchling size and longer time to hatch. Thirkgiilli (2000)

stocked gravid journals of *A. leptodactylus* at 14, 16, 18 and 20°C water temperature, and obtained the highest hatching rate at 16°C. Similarly, Pandey *et al.* (2017) found that optimum temperature range condition for egg incubation in coldwater condition for *Labeo dero* was 18-22°C within incubation period of 20-29 hrs, however, Prasad *et al.* (2009) reported shorter period (16-18 hrs) in the same fish at 24-26°C. In this experiment, it was found that the hatching period was extended in *C. catla* during pre-monsoon period due to low temperature, however, the time between first hatching and completion of hatching also extended (Table. 2) significantly ( $p < 0.05$ ) (Fig. 4).

In the present study, the highest survival rate of *C. catla* hatchling was found during monsoon season when the average temperature was 29.14°C and spawn recovery was 5.33 nos. from 7.5 lakh of egg released, which revealed that about 71.06%

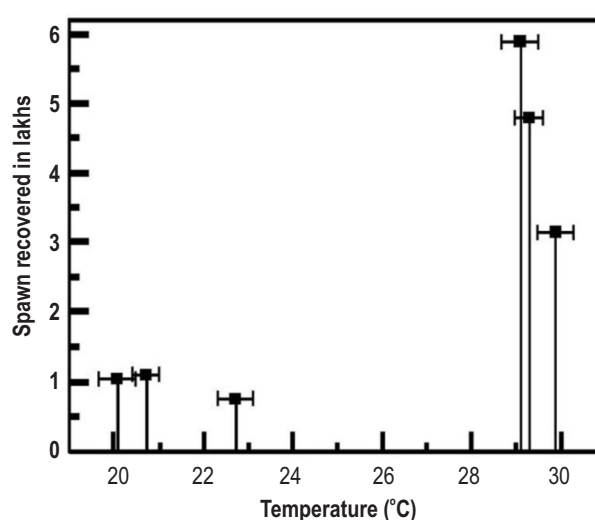


Fig. 5 : Spawn recovered after hatching at varied temperature.

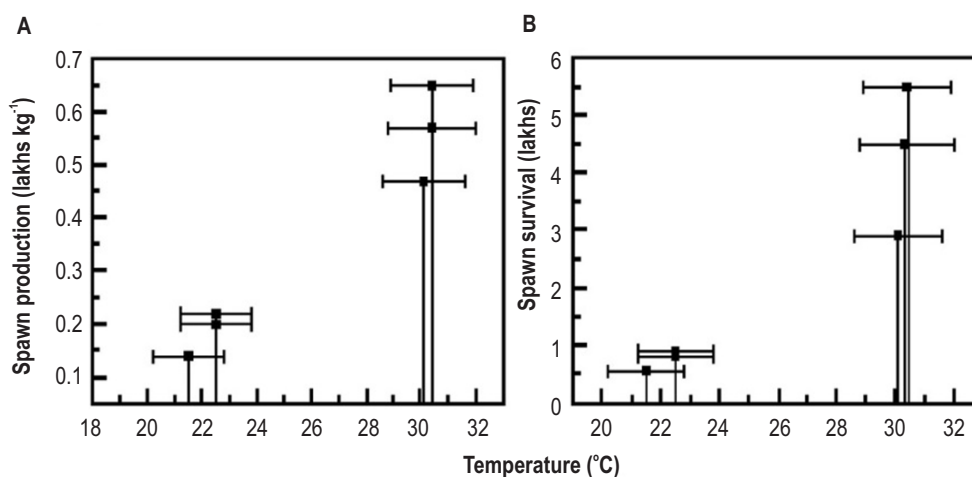


Fig. 6 : Spawn production (lakh kg<sup>-1</sup> b.wt.) and spawn survival at varied temperature.

spawn was produced after the completion of hatchery operation. Lowest result 0.55 was seen during the pre-monsoon season, when the average temperature range was 20.62 to 22.71°C (Fig. 5). A significant difference was found ( $p < 0.05$ ) for spawn recovery during pre-monsoon and monsoon season due to increase in average temperature. Final spawn production (lakh kg<sup>-1</sup> b.wt.) ranged from 0.47 to 0.65 lakh kg<sup>-1</sup> b.wt. female fish during monsoon period whereas 0.13 to 0.22 lakh kg<sup>-1</sup> b.wt. female fish during pre-monsoon period, showing significant ( $p < 0.05$ ) difference.

On 4<sup>th</sup> day after completion of egg release, spawn was collected in a hapa in collection tank by opening the gate valve connected to the outer wall of the hatching pool (Mohapatra *et al.*, 2008). The final spawn production was dependant on the water temperature of the incubation period holding *C. catla* hatchling. During pre-monsoon period, the average water temperature during larval incubation ranged between 21.58 and 22.58°C whereas average temperature during the monsoon period ranged between 30.1 and 30.4°C respectively. The amount of spawn production was 0.13 - 0.22 and 0.47 to 0.65 lakh kg<sup>-1</sup> b.wt. during pre-monsoon and monsoon period, showing a significant difference ( $p < 0.05$ ) between them. Mohan *et al.* (2010) reported that spawning, hatching and seed production was influenced by change in temperature during *Catla carpio* breeding.

The water quality parameters of hatchery is shown in Table 3. The results reveal that during pre-monsoon and monsoon season the average pH was 7.3 and 7.13, respectively, which was very close to the neutral value of pH scale. This range comes within the optimum limit of pH as opined by Gupta *et al.* (2000) and Das *et al.* (2006). The average hardness of water was 111.2 mg l<sup>-1</sup> - 91.73 mg l<sup>-1</sup>, respectively, in pre-monsoon and monsoon season and insignificant variation was recorded between the two seasons. According to Mateen *et al.* (2004) water hardness is more favourable and has no negative effects on

Table 3 : Variation in the average value of abiotic parameters during *C. catla* breeding

Water quality parameters	Monsoon	Pre-monsoon
pH	7.3 ± 0.11	7.13 ± 0.16
Dissolved oxygen*	9.06 ± 0.66	9.06 ± 0.35
Alkalinity*	88.67 ± 2.42	108.53 ± 5.97
Total hardness*	91.73 ± 4.05	111.2 ± 4.77
Free CO <sub>2</sub> *	1.33 ± 0.13	1.86 ± 0.06

\* All values are in mg l<sup>-1</sup>

the growth of *Labeo rohita* and its hybrid. The amount of free CO<sub>2</sub> varied a little and alkalinity was reduced during monsoon season, however this variation was insignificant. Dissolved oxygen is another important factor during the hatchery operation and in this study it was 9.06 mg l<sup>-1</sup> during pre-monsoon and monsoon season. Gupta *et al.* (2000) stated that 4.0 ml l<sup>-1</sup> DO is optimum for hatchery operation of Indian major carps. It may be concluded from the present study that breeding of *C. catla* can be carried out a temperature range of 18.6-24.7°C. Through proper brood stock and hatchery management, spawn production of Indian major carp is possible in the north east hilly regions of India.

### Acknowledgments

The authors acknowledge the financial support from NEH Programme of ICAR-CIFA and facilities provided by the Director, ICAR - Central Institute of Freshwater Aquaculture, Bhubaneswar, India for carrying out this study. The authors are thankful to Mr. Rabindra Kumar Nath, SSS, ICAR-CIFA, Kalyani for his active support during fish breeding programmes and Mr. Tana Nekam Tara, Sonajuli, Papum Pare District, Arunachal Pradesh for farm facilities.

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