ISSN: 2347-5129
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2016; 4(5): 354-358

www.fisheriesjournal.com
Received: 19-07-2016
Accepted: 20-08-2016

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Captive breeding through synthetic hormone induced breeding of an eastern Himalayan hill stream fish, *Barilius barila* (Hamilton, 1822)

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Abstract
Barilius barila, is a vulnerable and high valued edible Eastern Himalayan hill stream fish of Terai and Dooars regions of northern region of West Bengal. The study presents an experimental work on the captive breeding through induced breeding using synthetic hormone in *Barilius barila* carried for over a period of one year. This fish spawned in captivity only in running water system with a dosage of 0.5ml/fish WOVA-FH, a synthetic hormone. The results showed fecundity to range from 1440 to 7050 with an average rate of 5020.The average Gonado-somatic Index of *Barilius barila* for female was 12.77 and for male 6.69. Gonado-Somatic Index was higher in female than male. Gastro-somatic Index ranged from 5.94 to 11.73. The present work contributed to the lacunae in the information on fecundity, Gonado-somatic Index and spawning biology of *Barilius barila.*

Keywords: *Barilius barila*, fecundity, gonado-somatic index, dose standardization

1. Introduction
Barilius barila (Hamilton, 1822) [12], belonging to the family Cyprinidae (Order Cypriniformes), is a vulnerable (IUCN, 2015) [14] and an important edible hill stream fish of Eastern Himalaya, India. In the northern part of West Bengal, India, that is, in the Terai and Dooars regions of Eastern Himalaya, *Barilius barila* is a delicacy and is sold at an exorbitant price. The fish, being a surface feeder, and susceptible to stress, is characterized by a relatively elongated compressed body with blue-black bars or spots on the body, and the dorsal fin being inserted behind the middle of the body (Hamilton, 1822) [12]. They are omnivorous and feed on phytoplankton, zooplankton, insect larvae and algae. Species of *Barilius* are inhabitants of small, clean, medium to fast flowing torrential hill streams of India, Bangladesh, Nepal and Myanmar (Sukham *et al.,*2014) [26]. In captive condition they prefer well oxygenated and clean water. Despite its great economic importance, several wild populations have been suffering drastic reduction. Literature available on genus *Barilius* are very scanty. Information available shows results on food and feeding habits of genus *Barila* (Badola and Singh, 1980) [2] habitat characterization, feeding habits, food of *Barilius bendelisis* (Farswan *et al*.,1989 [11] and Shehgal, 1999 [23]), karyotype structure in *Barilius bendelisis* (Sukham *et al*., 2013) [25], cytogenetical study on *Barilius ngawa* (Sukham *et al*., 2014) [26] and population structure of Indian hill trout, *Barilius bendelisis* (Sah, 2011) [21]. There is, however, no information available as such on the spawning biology of *Barilius barila*. Therefore, the present study investigates the spawning biology and dose standardization for induced breeding of the vulnerable fish *Barilius barila* (IUCN, 2015) [14].

2. Materials and Methods
2.1 Collection and experimental site
Barilius barila, weighing 10 gm to 15 gm, were collected from sampling sites located at Chandan Chowra of Cooch Behar district of West Bengal, India, lying at $26^0$37'North latitude and $89^0$37'East longitude. The work was surveyed over a period of one year (October 2014 to November 2015). After harvesting from the hill streams, the fishes were immediately oxygen packed in sterile polythene bags and kept in cartons for transport to the Wet Laboratory. In the laboratory, the fishes were transferred to suitable aquariums provided with aeration for acclimatization, regular rearing and maturation. Fresh, dechlorinated and well aerated water

was used for domestication of the fish in all the tanks. For rearing and breeding in all the experimental glass tanks pH, Specific conductivity and Total Dissolved Solids (TDS) were determined by using portable meter (Eutech). Total hardness (EDTA method) and Dissolved Oxygen (Winkler's method) were determined by Standard Methods (APHA, 2012) [1]. The most important criteria for this fish is they must be provided with clean and well oxygenated water. Frequent exchange of water of about 50% per day was done for *Barilius barila* to maintain the desired water quality.

Fig 1: Matured *B. barila* (female).

Fig 2: Matured *B. barila* (male)

2.2 Dose standardization for induced breeding

Matured male and female fish were injected with synthetic hormone WOVA-FH (Biostadt India Limited, Mumbai) at the base of the pectoral fin with different doses (0.025ml/fish, 0.25ml/fish, 0.5ml/fish and 0.1ml/fish).The dose selection of hormone was done on trial and error basis commencing with the lowest dose (0.025ml/fish) and then gradually increased at high dose (0.1ml/fish). Dose of hormone for induced breeding of *Barilius barila* was started at minimum level. Each dose of hormone was injected to 15 numbers of fishes. The breeding trail was done twice. Breeding tanks of 50 litres capacity with continuous running water system were used. The sex ratio maintained for breeding was 1:2 female and male respectively. Different set-up protocols for breeding were followed are as given in Table1.

Table 1: Summary of the protocols of experimental set-up and design for induced breeding of *Barilius barila* in 50 litres tank with running water system.

Numbers of Set-up	Sex-ratio (Female: Male)	Number of fish in the Set-up	Dose of hormone (WOVA-FH) in ml/fish
Set-up 1	1:2	15	0.025
Set-up 2	1:2	15	0.25
Set-up 3	1:2	15	0.50
Set-up 4	1:2	15	0.10

2.3 Fecundity, Gonado-somatic Index, Gastro-somatic Index, Correlation analysis between length, weight, fecundity, gonad weight, gonad length and stomach weight.

Fecundity was calculated according to the method of Hartman and Conkle (1960) [13] using the expression F=nG/g where, F is Fecundity; n is mean numbers of eggs in all samples, G is weight of ovaries and g is weight of samples. Eggs were sampled from three regions of the gonad namely anterior, middle and posterior.

Gonado-somatic Index (GSI) was expressed according to the method of Vlaming (1982) [28] method for assessing the development of gonads and calculated as GSI = Weight of the ovary/ Total weight x 100. Two lobes of the ovary from each fish sample were removed carefully by dissecting out the abdomen and dried off removing excess moisture with blotting paper.

Gastro-somatic Index (GaSI) was determined by using the formula, GaSI = wx100/W (Desai, 1970) [4]. Here, w = weight of gut; W = total weight of fish.

2.4 Statistical Analysis

To determine statistically, the Coefficient of Correlation and Linear Regression analysis between Gastro-somatic Index and length, weight, fecundity, gonad weight, gonad length and stomach weight respectively, the software MS-Excel was used.

3. Results

Breeding experiments done in aquarium in captivity were conducted successfully for *Barilius barila* during the breeding period October 2014 to November 2015 with the use of the synthetic hormone WOVA-FH. In all the breeding tanks the ranges of physic-chemical parameter maintained were of pH: 7.5 to 8.5; Temperature: 23-32 ^{0}C; Total hardness: 20-35 mg l^{-1}; Specific Conductivity: 110-180 µS; Total Dissolved Solids (TDS): 80-165 mg $l^{-1}$and Dissolved Oxygen: 8.0 – 8.2 mg l^{-1}. Matured female could be easily recognized, by its rounded and swollen abdomen whereas, the matured male by a slimmer and more colourful body. Fertilization was external and spawning occurred once a year during October to November. Reproductive pattern was observed in both the male and female fish during the night. The male was noticed to constantly hit the female on the abdomen with its head while chasing her all around the aquarium.

Table 2: Correlation between the variables total length, body weight, stomach weight, gonad length, fecundity, Gonado-somatic Index and Gastro-somatic Index of the fishes.

Correlation between Characters	X	Y	r
Total length and body weight	Total length	Body weight	0.81*
Body weight and fecundity	Body weight	Fecundity	0.54*
Total length and fecundity	Total length	Fecundity	0.52*
Body weight and gonad length	Body weight	Gonad length	0.81*
Gonado-somatic index and Gastro-somatic index	GSI	GaSI	0.37*
Body weight and stomach weight	Body weight	Stomach weight	0.91*

*Show significance at $p \leq 0.01$

Four different doses of WOVA-FH, a synthetic hormone, namely 0.025 ml/fish as 1st dose; 0.25 ml/fish as 2nd dose; 0.5ml/fish as 3rd dose and 0.1 ml/fish as 4th dose were used (Table 1). The best response to reproduction was obtained from the dosage of 0.5 ml/ fish. The higher fertilization,

hatching and survival rates were found in fish injected with 0.5 ml/fish as in Set-up-3. Same dose of WOVA-FH hormone was injected to both male and female. Injected fishes were released in tanks and it was observed that after 6.30-7.00 hours of injection they started spawning simultaneously. Spawning was observed only in Set-up-3 but not in the other Set-ups. All fishes died in Set-up-4 within 2days. In Set-up-1 and Set-up-2 the fishes were most active and consumed feed properly but spawning was not observed. The results thus revealed that WOVA-FH at 0.5ml per fish was sufficient to induce spawning in *B. barila*.

The study of fecundity of *B. barila* revealed 1440 to 7050 numbers with an average fecundity rate of 5020.The average Gonado-somatic Index of *Barilius barila* for female was 12.77 and for male 6.69. Gonado-somatic Index was higher in female than male. The Gastro-somatic Index ranged from 5.94 to 11.73 with an average of 8.69. To establish the mathematical relationship between total length, body weight, stomach weight, gonad length, fecundity, Gonado-somatic Index (GSI) and Gastro-somatic Index (GaSI), Coefficient of Correlation (r) was done using MS- Excel (Table 2). The scatter diagram of body weight and total length (Fig.5a), fecundity and body weight (Fig.5b), fecundity and total length (Fig.5c), gonad length and body weight (Fig.5d), gastro-somatic index and gonado-somatic index (Fig.5e) and stomach weight and body weight (Fig.5f) showed a linear relationship by using the linear regression equation Y = a+ bx, where, 'a' and 'b' are constants and X and Y are the variables. The Coefficient of Correlation(r) showed significance at $p \leq 0.01$.Correlation between the different parameters of *Barilius barila,* total length and body weight, body weight and gonad length and body weight and stomach weight relation was more significant than other parameter. Length – weight relationship of *Barilius barila* was Log W =35.50 + 4.453 log L in figure 5a. The theoretical value of b (regression coefficient) in length-weight relationship is reported as 4.453. The growth of fish was positive allometric growth.

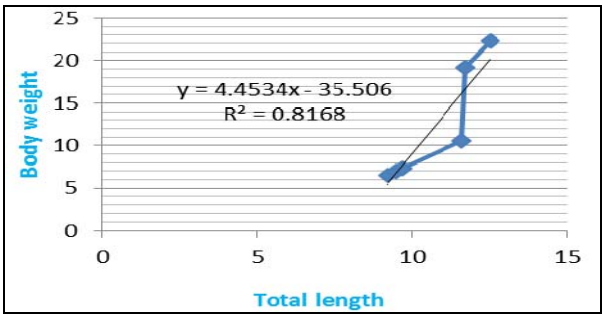

Fig 5a: Linear Regression and Coefficient of Correlation between the body weight and total length

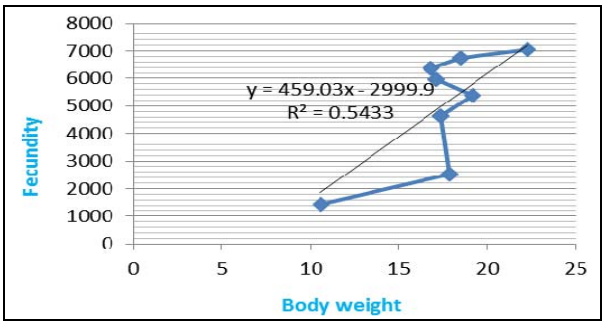

Fig 5b: Linear Regression and Coefficient of Correlation between the fecundity and body weight

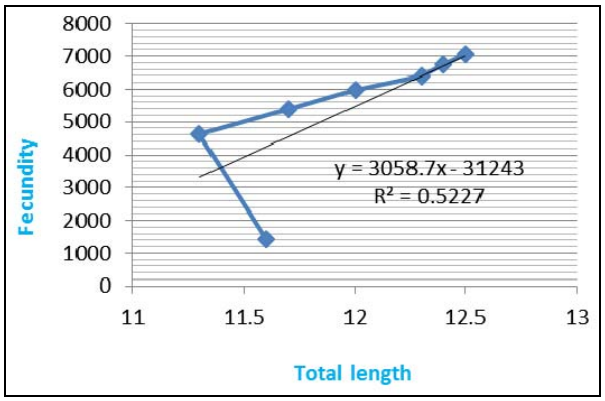

Fig 5c: Linear Regression and Coefficient of Correlation between the fecundity and total length

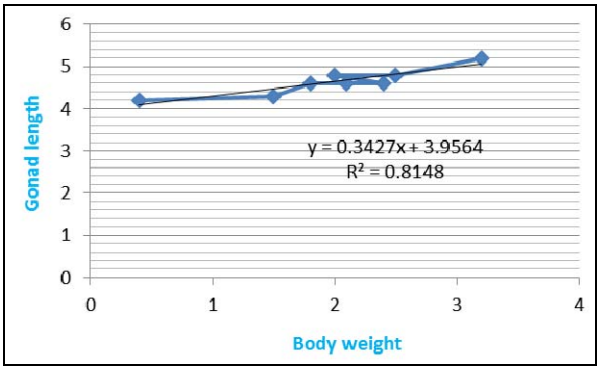

Fig 5d: Linear Regression and Coefficient of Correlation between the gonad length and body weight

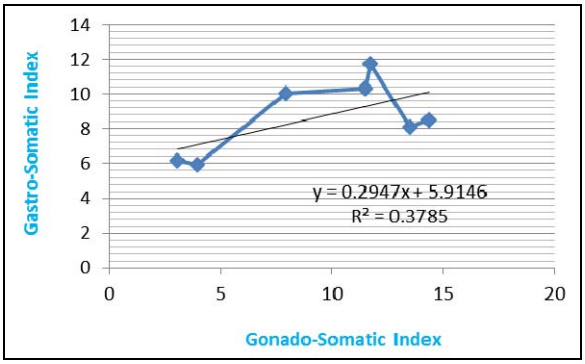

Fig 5e: Linear Regression and Coefficient of Correlation between the GaSI and GSI

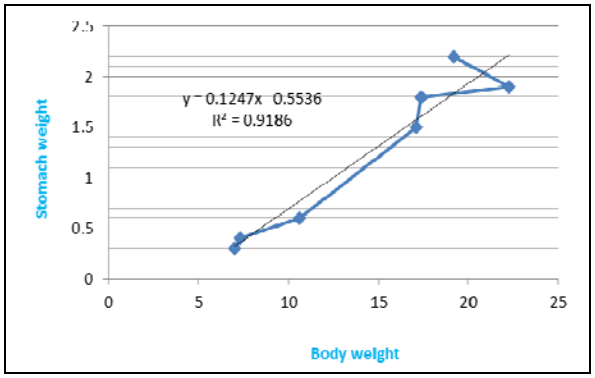

Fig 5f: Linear Regression and Coefficient of Correlation between the stomach weight and body weight

4. Discussion

The study which was conducted for the first time in the fish, *Barilius barila,* revealed that continuous flowing water was essential for induced spawning. The dissolved oxygen level should be high (above 8mgl^{-1}) due to it being a high level hill stream fish and a water temperature of 23^{0}C-32^{0}C should be maintained. In captive condition they remain in shoal and consume feed after two days of acclimatization. The present study showed that *Barilius barila* are migratory in nature and move out after spawning from their site similar to anadromous fish. *Barilius barila* are surface feeders. In captive condition they prefer well oxygenated and clean water. Despite its great economic importance, several wild populations have been suffering drastic reduction. The spawning behaviour of *Barilius barila* was found to be similar to loach and the Indian Major Carps (Dey *et al.,* 2015b) [7]. The latency period was 6.30 to 7.00 hours in fish injected with 0.5ml WOVA-FH per fish. The latency period of *Barilius barila* was higher than other hill stream fish studied, namely loach, *Botia almorhae* (Dey and Barat, 2015) [5], *Botia rostrata* (Dey *et al.,* 2015a) [6], *Botia lohachata* (Dey *et al.,* 2015b) [7] and *Botia dario* (Dey *et al.,* 2015c) [8] but lower than the latency period of *Puntius sarana* (Udit *et al.,* 2014) [27] which was 8 to 9 hours and latency period of *Anabas testudineus* (Sarkar *et al*., 2015) [22] was 8-10 hours after administration of inducing agent. The latency period of *Ompok pabda* (Purkayastha *et al*., 2012) [20] was 6 to 8 hours after administration of Ovatide, a synthetic hormone.

The Gastro-somatic Index of *Barilius barila* which ranged from 5.94 to 11.73 revealed that GaSI was higher than other hill stream fish like GaSI of *Tor putitora* (Kumar *et al.,* 2009) [18] which ranged from 1.32-3.52. The average Gonado somatic Index of *Barilius barila* for female was 12.77 and for male 6.69. The GSI value of female *Barilius barila* was lower than *Botia almorhae* (Joshi and Pathani 2009) [16] which was 16.0. The GSI value of female *Barilius barila* was higher than *Schizothorax plagiostomus* (Jan *et al.*, 2014) [15] and ranged from 1.87 to 12.66. Mirghiyasi *et al*., 2016 [19] have reported that female invest more in gonads than males in *Oxynoemacheilus persa*. These findings were similar to the present study.

Correlation studies between the variables total length, body weight, stomach weight, gonad length, Gonado somatic Index and Gastro somatic Index of the experimented fishes revealed that there was strong significant correlation ($p \leq 0.01$). The correlation between the total length versus body weight, body weight versus gonad length and body weight versus stomach weight were the highest indicating that these parameters are the essential characters for success of spawning. The present study also showed that fecundity increased when gonad weight, body weight and total length of fish increased. Fecundity increases with the body size as studied by Bagenal (1978) [3] and Wootton (1979) [29]. Gonado-somatic Index was higher than Gastro-somatic Index of *Barilius barila.* This finding indicates that when fecundity was high then Gastro somatic Index, meaning weight of whole gastric tract, was lower than gonad weight of fish. The length-weight relationship of fish had significant importance in the study of the growth, gonadal development of a fish population.

5. Conclusion

As conclusion, being reported for the first time, *Barilius barila* can be easily matured and bred successfully under captive conditions in a flow through system. *Barilius barila* is a low fecundity fish and highly sensitive to temperature and water pollution. The impact of climate change, pesticides and siltation may cause the decline of fish population in the natural environment. It is suggested, brood stock management and hatcheries should be established for conservation, and ranching initiated for sustained natural recruitment of the species. Establishment of proper sanctuaries in selected areas of Terai and Dooars rivers, floodplain and reservoirs is recommended for conservation of this species. This work is useful for fish breeders, aquarium keepers and those involved in or interested in the study of indigenous fish.

6. Acknowledgement

The authors are thankful to the University of North Bengal for providing necessary funding in the form of a research grant (No.4356/R-2015 dated 23.12.2015).

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