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Comparative Studies on Gonad Development, Fecundity and Oocyte Maturation of Spotted Snakehead, *Channa punctatus* (Bloch, 1793) in Different Water Bodies

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The present study was carried out to compare gonad development, oocyte maturation and fecundity of the spotted snakehead (*Channa punctatus*) fish during January to November 2015 in different water bodies (such as pond, Joghati baor and Bhairab river) in Jashore, Bangladesh. Mean GSI (gonadosomatic index) values of the female *C. punctatus* during January, March, May, July, September and November were significantly highest in pond, followed by those in Joghati baor and the lowest in Bhairab river. Absolute fecundity during the month of July in pond, baor and river was 30,006±2,027, 23,629±2,356 and 16,659±2,486, respectively, and the relative fecundity was 5064±183, 4865±106 and 4641±138 eggs, respectively. Mean ova diameter in pond, baor and river in March was 0.25±0.04 mm, 0.16±0.02 mm and 0.21±0.03 mm; in May was 0.36±0.03 mm,

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0.29±0.03 mm and 0.23±0.04 mm; in July was 1.06±0.06 mm, 0.95±0.05 mm and 0.87±0.03 mm; and in September was 0.84±0.04 mm, 0.82±0.03 mm and 0.72±0.05 mm, respectively. Mean GSI values of the male *C. punctatus* during January, March, May, July, September and November were significantly higher in pond than those in baor and river, respectively. The mean highest GSI for female was found to be 6.06±0.11 in pond during the month of July, while the lowest GSI of 0.30±0.08 was in Bhairab river during November. The highest GSI value for male was obtained to be 0.70±0.16 in pond during the month of July, while the lowest GSI of 0.12±0.03 was found in Bhairab river during January. However, significant differences ($P<0.05$) were found in GSI values, absolute fecundity, relative fecundity and ova diameter of *C. punctatus* during different months in pond, Joghati baor and Bhairab river. The findings from the present research would immensely be useful for captive breeding and seed production techniques of *C. punctatus* for aquaculture production, sustainable management and species conservation.

Keywords: Channa punctatus; reproductive biology; fecundity; Gonadosomatic index; ova diameter.

1. INTRODUCTION

Spotted Snakehead (*Channa punctatus*) is one of the important air-breathing fish. It is regarded as one of the important freshwater fishery resources in Bangladesh, India, Afghanistan, Pakistan, Sri Lanka, Nepal Myanmar and Tibet [1-3]. In Bangladesh, it is locally known as “Taki” [1] and has been considered as one of the most important fish species in flood plains [4]. The body of this fish is distinctly cylindrical in cross-section, but appears slightly flattened dorsoventrally. *Channa punctatus* normally grows to around 15.0 cm (5.9 inch) in length, but males up to 31.0 cm (12.2 inch) have also been captured [3]. The male has black spots on a yellow under-belly and the female usually has a swollen abdomen and may also be distinguished by diffused black blotches in the ventral region. In adult female, the ventral fins are shorter and never reach the vent, whilst in male, the ventral fins extent to, or a little beyond the vent. It is a high-priced freshwater fish and abundantly found in ponds, beels, canals and rivers of Bangladesh. *Channa punctatus* has a great demand in the market because of its good flavor, high nutritional value and availability throughout the year. It has been used as food as well as aquarium fish for its aesthetic view, cylindrical body shape and erratic swimming behavior in the water body. The fish is delicious, and due to high nutritional and medicinal values, it is recommended by doctor to recover the health of the patients after illness [5]. However, in recent years, *C. punctatus* has become vulnerable and listed as near threatened species due to natural, domestic and anthropogenic activities – leading to water pollution, habitat degradation, declination of natural water body etc. [6,7]. The natural water body contains high level of heavy metals as it gains the metals continuously from industrial and agricultural sources [8].

Fish live in a polluted environment over the course of their life time show reproductive disorder such as lack of gonad development to decreased egg production and abnormality of offspring [9]. As an aquatic organism, fish accumulate the contaminants directly from the polluted environment and indirectly through food chain [10]. Ovarian weight declines significantly and the occurrence of yolky oocytes and histology of ovarian sample reveals the high incidence of atresia in the ovaries of the fishes, which are collected from polluted region. Liquefaction of yolk in the ovaries of fishes in polluted region has also been recorded [11].

High concentrations of pesticides are known to reduce the survival, growth and reproduction of fish. Some pesticides such as organochlorine, organophosphates and carbamates are responsible for the morphological damage of fish testis. Not only the male fish but also the female fish is affected in the similar way. They show delayed oocyte development and also prohibit the synthesis of steroid hormone [12]. The effect of fenitrothion, an organophosphate and carbofuran, a carbamate pesticide was observed on the testicular recrudescence of the fish, *C. punctatus* [13]. Fenitrothion causes reduction in testicular weight, necrosis in spermatids and the presence of emptied lobules. Carbofuran treatment delays the formation of spermatids and sperms.

A lot of reports about aquatic pollution suggest that the heavy metals have extensively contaminated the aquatic ecosystem that are released mainly from domestic, industrial and other anthropogenic activities [14-19]. The polluted environment, which is caused by heavy metals affects the breeding behavior and development of fishes in Harike Wetland of the

Punjab state in India [20]. The female fishes, which were collected from polluted sites showed several reproductive alterations such as inhibited gonad maturation, reduced oocyte growth and plasmatic vitellogenin level, and 3-fold lower gonadal aromatase [21]. The gonadosomatic index and fecundity of the species were observed to be less in the specimens from Vizag harbor waters compared to those from the relatively unpolluted Gostani waters [22]. However, such types of information are still lacking in snakehead fishes. Therefore, the current research was undertaken to compare the gonad development, oocyte maturation and fecundity of the spotted snakehead (*Channa punctatus*) fish in different water bodies (such as pond, baor and river) in Jashore, Bangladesh.

2. MATERIALS AND METHODS

2.1 Study Area

The study was conducted in the local freshwater pond, Joghati baor and Bhairab river under Jessore district, which is situated on 23°10'14.3904" N and 89°12'44.7048" E in the southwestern region of Bangladesh.

2.2 Sample Collection

In total, 108 female and 108 male specimens of *C. punctatus* were collected from the local pond, baor and river through bimonthly samplings from January and November 2015. During collection, it was considered that the sample fish was free from disease and any physical damage of the body. Soon after collection, the live specimens were transported to the Laboratory of the Department of Fisheries and Marine Bioscience in Jashore University of Science and Technology, Jashore, Bangladesh, and maintained in aerated glass aquaria before use for the experiment.

2.3 Sexual Dimorphism

Sexes of collected *C. punctatus* were identified according to the criteria followed by Saikia et al. [23].

2.4 Measurement of Fish

Each individual of *C. punctatus* from the bimonthly collections was weighed to the nearest 0.01 g for the total weight (TW) using digital electronic balance. The mean weight of female fishes was 90.56±11.97 g with a range from 75.48 to 104.75 g, while the males were 84.86±13.78 g having a range between 72.67 and 102.71 g, respectively.

2.5 Collection of Gonads (Ovary and Testis)

The fishes were dissected-out with scissors. From the anus to the lower jaw was cut with a scissor and the belly was opened. The whole mass (stomach, intestine and gonad) were removed very carefully and placed on a petri dish. The gonads were first separated from stomach and intestine, and then washed and cleaned with distilled water. Weight of the gonad was recorded. For the purpose of fecundity studies, ovary of fish was preserved in 10% buffered formalin not only for preservation but also served as easier way to separate eggs from the wall of follicles.

2.6 Determination of Gonadosomatic Index (GSI)

Both females and males of *C. punctatus* were collected and weighed. The gonadosomatic index of the female and male fishes were calculated according to the formula used by Hossain et al. [7] as follows:

$$\mathrm{GSI} = \frac{\text{Wet weight of gonad (g)} \times 100}{\text{Total weight of fish (g)}}$$

2.7 Estimation of Fecundity

The fecundity of sexually matured *C. punctatus* was determined, using the formula followed by Bir et al. [24] as below:

$$\text{Fecundity} = \frac{\text{Total gonad weight (g)} \times \mathrm{N}}{\text{Weight of small portion of total gonad (g)}}$$

Where, N = Number of eggs in the small portion of gonad.

2.8. Assessment of Gonadal Maturity

The maturation stage of each specimen was determined microscopically considering colouration, transparency, and for ovaries, the visualization and appearance of the ova. The maturity stages of the male and female gonads were classified according to Prasad [25] and computed bimonthly to ascertain the gonadal maturation and breeding season of *C. punctatus* in different water bodies.

2.9 Measurement of Ova Diameter

Diameter of the ova was measured from each of the anterior, central and posterior portion of the ovaries of the individual fish by using a photographic microscope (Zeiss Primo Star Model, Germany). During the experimental period, diameters of eggs were measured at 10x magnification.

2.10 Statistical Analysis

Qualitative and quantitative analysis of the data obtained were carried out statistically. Graph Pad Prism 5.0 (GraphPad Prism, San Diego, CA, USA) version was used for the presentation of graphs from different types of data. One-way Analysis of Variance (ANOVA), followed by Tukey, Descriptive, and Homogeneity of Variance Test was used for the analysis of GSI value (both male and female), fecundity and ova diameter for female of different sample in pond, baor and river using SPSS Version 16.0. The level for statistical significance was set at 0.05%.

3. RESULTS

3.1 Determination of Peak Breeding Season

The highest mean GSI value of *C. punctatus* female in pond, Joghati baor and Bhairab river during the month of July was found to be 6.06±0.11, 3.99±0.13 and 3.59±0.21, respectively. Similarly, the mean highest GSI value of *C. punctatus* male in pond, baor, and river in July was 0.70±0.16, 0.58±0.10 and 0.33±0.11, respectively. Therefore, July was the peak breeding season of *C. punctatus* in the study areas.

3.2 GSI Value of Female Fish

Graphical presentation of GSI values of female *C. punctatus* obtained from pond, baor and river during different months in the present study are shown in Figs. 1–3. Mean GSI (gonadosomatic index) values of the female *C. punctatus* during January, March, May, July, September and November were significantly higher (P<0.05) in pond than those in Joghati baor and Bhairab river, respectively. Furthermore, significantly highest (P<0.05) GSI value (6.06±0.11) was found in pond during the month of July, while the lowest GSI of 0.30±0.08 was found in Bhairab river during the month of November.

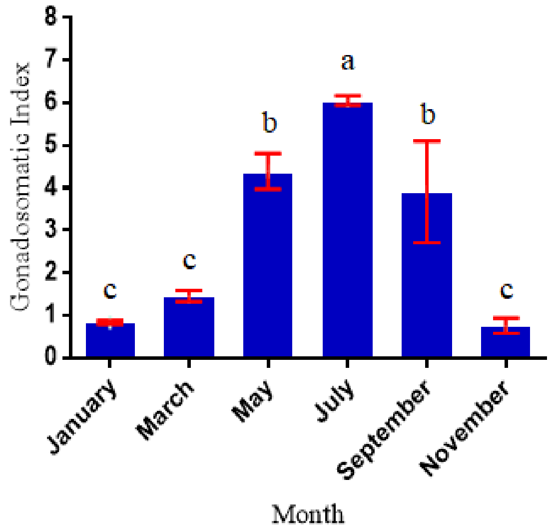

Fig. 1. Mean (±SD) GSI values of female *C. punctatus* in pond during different months. Columns with different letters represent means that are significantly different (P<0.05)

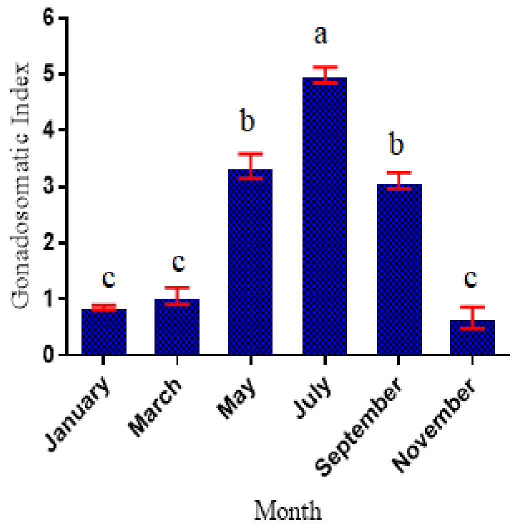

Fig. 2. Mean (±SD) GSI values of female *C. punctatus* in Goghati baor during different months from. Columns with different letters represent means that are significantly different (P<0.05)

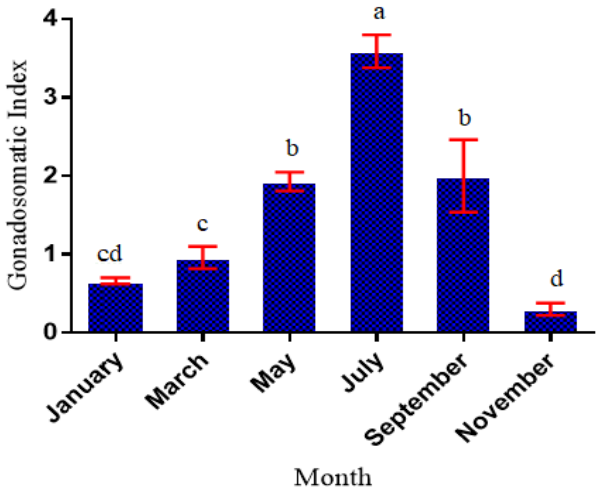

Fig. 3. Mean (±SD) GSI values of female *C. punctatus* in Bhairab river during different months. Columns with different letters represent means that are significantly different (P<0.05)

3.3 Comparisons of Female GSI Values in Pond, Baor and River

Comparisons of the GSI values of female *C. punctatus* among pond, baor and river during different months are depicted in Figs. 4–9. The mean GSI value was the highest in pond, followed by baor and the lowest in river throughout the entire six months sampling period (P<0.05).

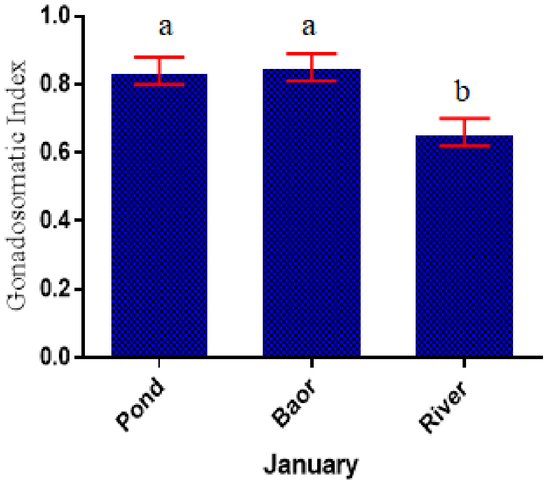

Fig. 4. Mean (±SD) GSI values of female *C. punctatus* in pond, baor and river during the month of January. Columns with different letters represent means that are significantly different (P<0.05)

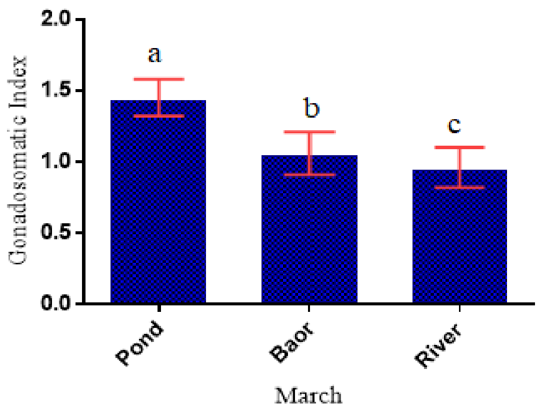

Fig. 5. Mean (±SD) GSI values of female *C. punctatus* in pond, baor and river during the month of March. Columns with different letters represent means that are significantly different (P<0.05)

3.4 Fecundity

Significantly (P<0.05) highest mean absolute fecundity of *C. punctatus* was obtained in pond (30,006±2,027) during the month of July, followed by that in baor (23,629±2,356) and the lowest in river (16,659±2486) (Fig. 10). Relative fecundity (number of eggs per gram of ovary) followed the opposite trends of absolute fecundity, the mean values of which were 5,064±183, 4,865±106 and 4,641±138 in river, baor and pond, respectively (P<0.05) (Fig. 11).

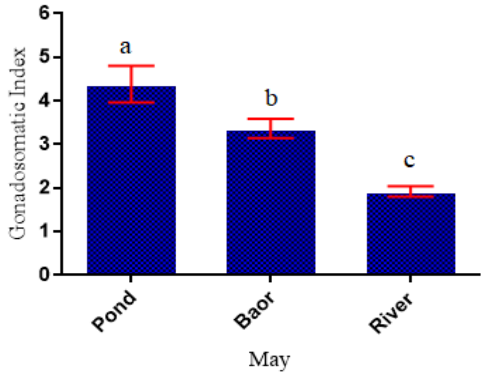

Fig. 6. Mean (±SD) GSI values of female *C. punctatus* in pond, baor and river during the month of May. Columns with different letters represent means that are significantly different (*P*<0.05)

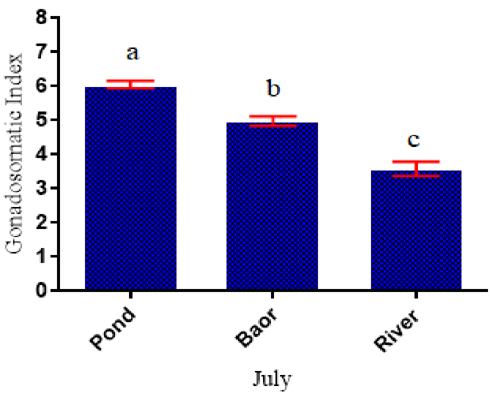

Fig. 7. Mean (±SD) GSI values of female C. punctatus in pond, baor and river during the month of July. Columns with different letters represent means that are significantly different (*P*<0.05)

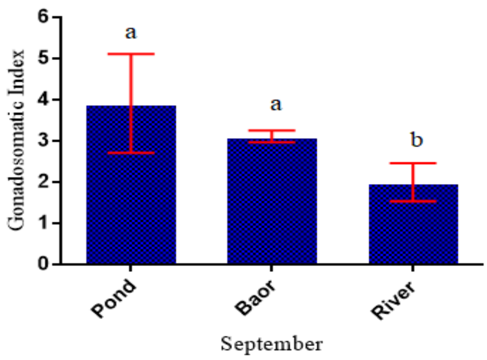

Fig. 8. Mean (±SD) GSI values of female C. punctatus in pond, baor and river during the month of September. Columns with different letters represent means that are significantly different (*P*<0.05)

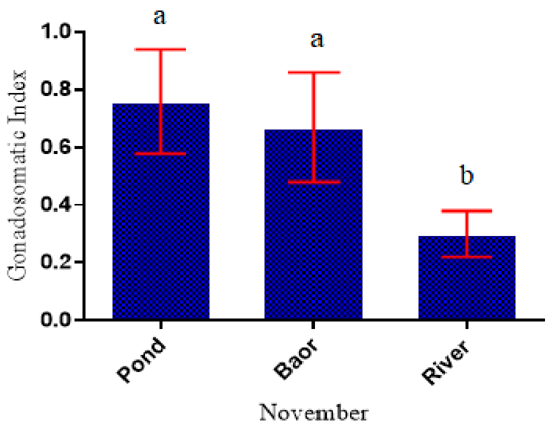

Fig. 9. Mean (±SD) GSI values of female *C. punctatus* in pond, baor and river during the month of November. Columns with different letters represent means that are significantly different (*P*<0.05)

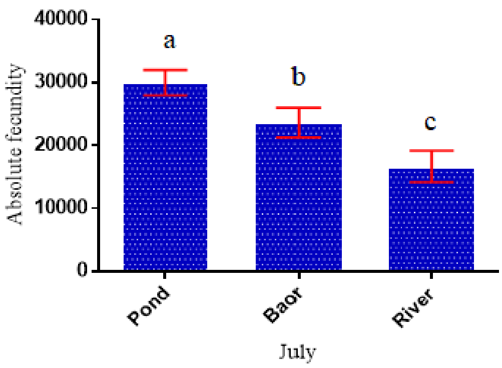

Fig. 10. Mean (±SD) absolute fecundity of *C. punctatus* in pond, baor and river during the month of July. Columns with different letters represent means that are significantly different (*P*<0.05)

3.5 Ova Diameter

Comparisons of ova diameters of female *C. punctatus* among the pond, baor and river in different months are shown in Figs. 12–15. Regardless of months, ova diameters of *C. punctatus* were always larger in pond than those in baor and river, respectively (*P*<0.05), and in all sampling sites, significantly (*P*<0.05) largest ova diameter was found during July, followed by September and May, while the smallest ova was obtained in March.

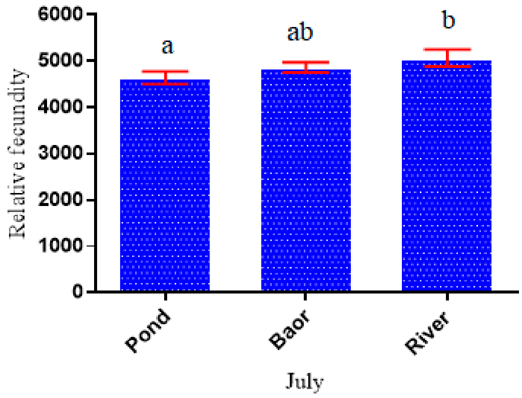

Fig. 11. Mean (±SD) relative fecundity of *C. punctatus* in pond, baor and river during the month of July. Columns with different letters represent means that are significantly different (*P*<0.05)

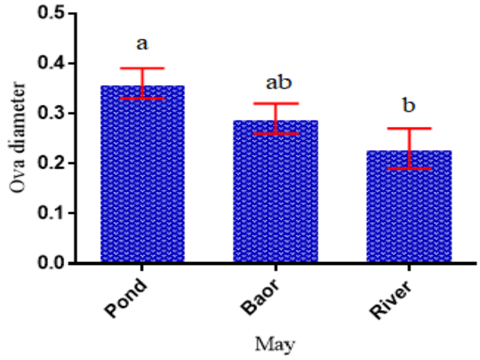

Fig. 13. Mean (±SD) ova diameter (mm) of *C. punctatus* in pond, baor and river during the month of May. Columns with different letters represent means that are significantly different (*P*<0.05)

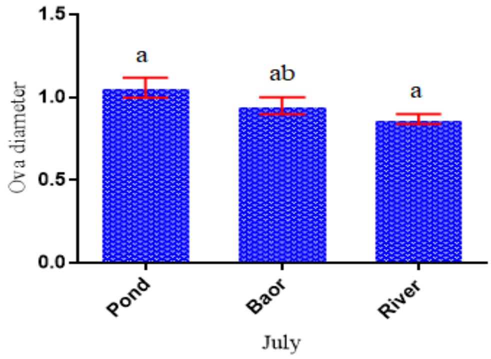

Fig. 14. Mean (±SD) ova diameter (mm) of C. punctatus in pond, baor and river during the month of July. Columns with different letters represent means that are significantly different (*P*<0.05)

3.6 GSI Value of Male Fish

GSI values obtained from the male *C. punctatus* in pond, baor and river during different months are graphically presented in Figs. 16–18. Mean GSI (gonadosomatic index) values of the male *C. punctatus* were significantly higher (*P*<0.05) in pond than those obtained in Joghati baor and Bhairab river during January, March, May, July, September and November in this order. Similar to the trends of female GSI, the significantly highest male GSI value of 0.70±0.16 was found in pond during the month of July and the lowest value of 0.12±0.03 in Bhairab river during the month of January.

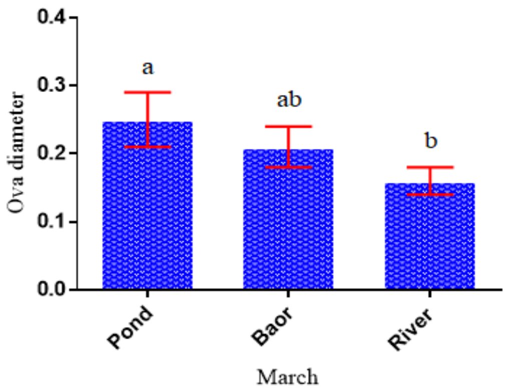

Fig. 12. Mean (±SD) ova diameter (mm) of *C. punctatus* in pond, baor and river during the month of March. Columns with different letters represent means that are significantly different (*P*<0.05)

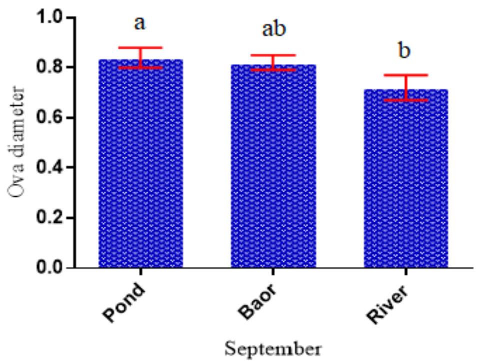

Fig. 15. Mean (±SD) ova diameter (mm) of *C. punctatus* in pond, baor and river during the month of September. Columns with different letters represent means that are significantly different (*P*<0.05)

3.7 Comparisons of Male GSI Values in Pond, Baor and River

Comparisons of the GSI values of male *C. punctatus* in pond, baor and river during different months are shown in Figs. 19–24. Similar to the female *C. punctatus*, significantly highest mean GSI values were found in pond, followed by those in baor and the lowest values in river throughout the whole experimental period.

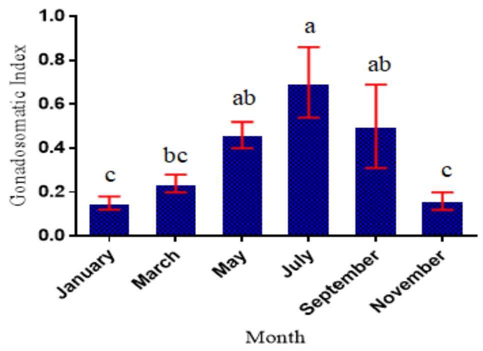

Fig. 16. Mean (±SD) GSI values of male *C. punctatus* in pond during different months. Columns with different letters represent means that are significantly different (*P*<0.05)

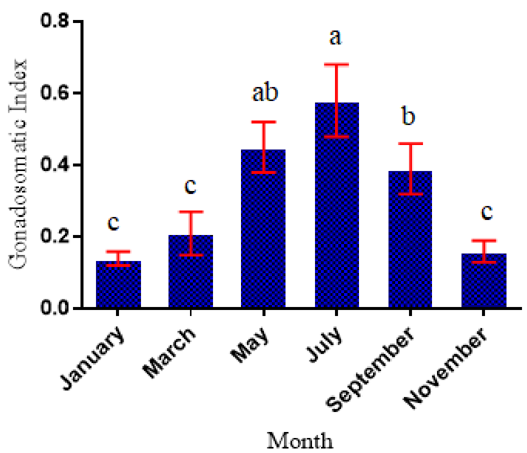

Fig. 17. Mean (±SD) GSI values of male C. punctatus in Goghati baor during different months. Columns with different letters represent means that are significantly different (*P*<0.05)

4. DISCUSSION

The peak breeding season of the spotted snakehead (*Channa punctatus*) in the present study was determined on the basis of the highest gonadosomatic index (GSI) value. Bucholtz et al. [26] suggested that GSI is an important index for the study of sexual maturation of any fishes. In this study, the highest GSI value of *C. punctatus* was found in pond, Goghati baor and Bhairab river during the month of July. Several researchers reported that the breeding season and sexual maturation of *C. punctatus* occurred during: June to October [7,25], The highest GSI value of *C. bleheri* was found during the months of April to July [27].

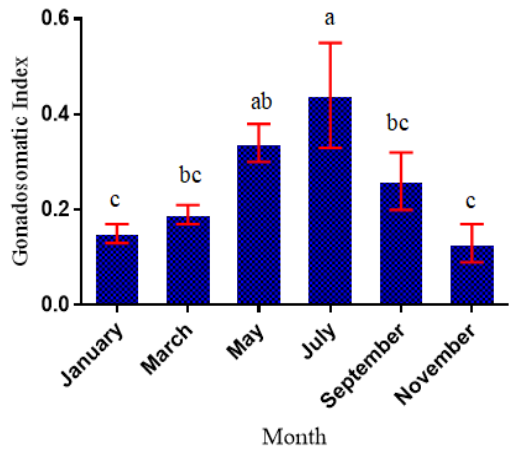

Fig. 18. Mean (±SD) GSI values of male *C. punctatus* in Bhairab river during different months. Columns with different letters represent means that are significantly different (*P*<0.05)

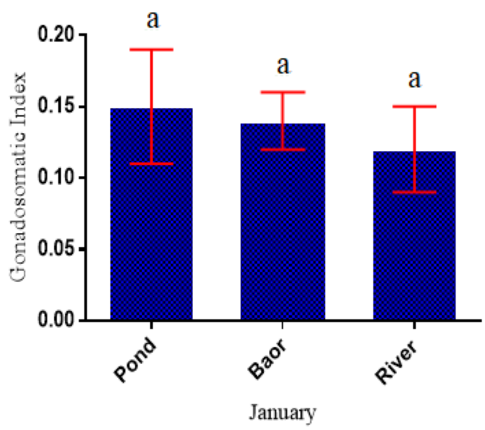

Fig. 19. Mean (±SD) GSI values of male C. punctatus in pond, baor and river during the month of January. Columns with different letters represent means that are significantly different (*P*<0.05)

The present study revealed that *C. punctatus* possesses only one spawning in a whole year and the time was intense in the month of July. Similar finding was reported by Prasad [25], who found in the Varuna river (India) that the spawning of *C. punctatus* occurred only one time in a year during June to October and the peak spawning was in August as more than 80% of ripe gonads were recorded during this month, while April to July was found to be the spawning period in the Punjab province [28]. Only one spawning season was reported for *C. punctatus* during April to August in Bangladesh [29] and June to August in Nepal [30]. Renuka and Joshi [31] confirmed that this fish is a seasonal breeder, the gonads mature at spring and spawning usually coincides with the onset of monsoon.

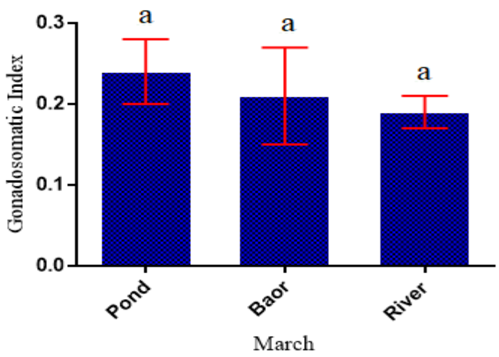

Fig. 20. Mean (±SD) GSI values of male C. punctatus in pond, baor and river during the month of March. Columns with different letters represent means that are significantly different (*P*<0.05)

GSI value of male in pond, baor and river in the month of March

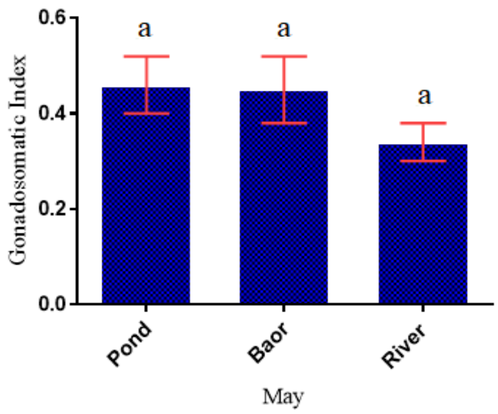

Fig. 21. Mean (±SD) GSI values of male *C. punctatus* in pond, baor and river during the month of May. Columns with different letters represent means that are significantly different (*P*<0.05)

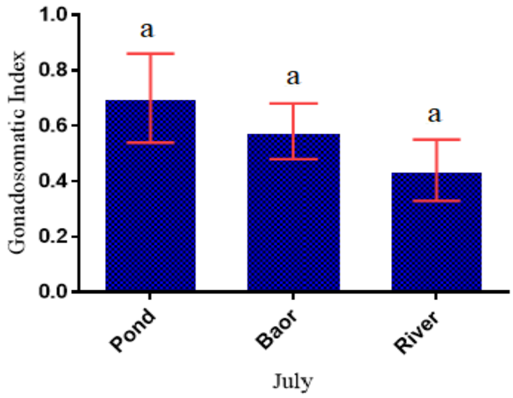

Fig. 22. Mean (±SD) GSI values of male *C. punctatus* in pond, baor and river during the month of July. Columns with different letters represent means that are significantly different (*P*<0.05)

GSI value of male in pond, baor and river in the month of July

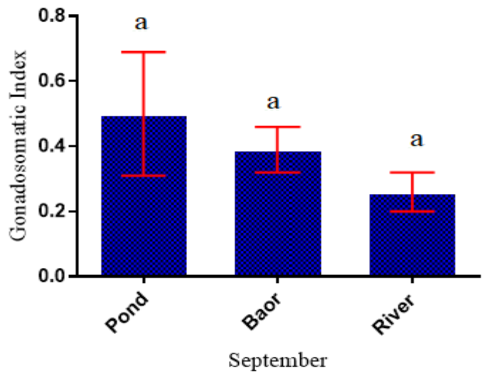

Fig. 23. Mean (±SD) GSI values of male *C. punctatus* in pond, baor and river during the month of September. Columns with different letters represent means that are significantly different (*P*<0.05)

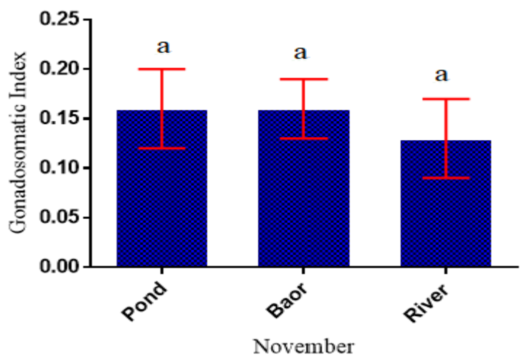

Fig. 24. Mean (±SD) GSI values of male *C. punctatus* in pond, baor and river during the month of November. Columns with different letters represent means that are significantly different (*P*<0.05)

Female *C. punctatus* showed the highest (6.06±0.11) and the lowest (0.76±0.18) GSI values in pond during July and January. Similar results were also found in the same species by Hossain et al. [7] and Prasad et al. [25].

The GSI value of female *C. punctatus* was significantly higher (*P*<0.05) in pond than in baor, while the lowest value was obtained in river during the entire study. It may probably be happened due to the pollution in the river. Water quality was very critical in the Bhairab river during summer season due to low discharge volume of fresh water, effect of tidal flow and waste water runoff from densely populated area [32]. A study conducted by Schulz and Martins-Junior [33] revealed that the reproductive parameters including gonado-somatic index and gonadal-somatic relationship of banded astyanax (*Astyanax fasciatus* were decreased with the increase of pollution. Fish that was collected from the heavily contaminated site having less availability of food showed significantly smaller oocyte diameter, lower gonadal index, and gonadal-somatic relationship. Zuber and Jacob [34] found that the GSI of fish from upstream (U) site was significantly higher than fish from the downstream (D) site (U mean = 4.76, D mean = 2.30, *P*≤0.01). A great deal of variation in gonado-somatic index was found in the teleost fishes, which were collected from different locations of India [35]. GSI values were found to be diminished in the fishes, which were collected from polluted sites compared to those from other sites [21,36-38]. Dragun et al. [39] reported that the gonadosomatic index of European chub (*Squalius cephalus*) was 2.4% in less polluted and 0.6% in highly polluted sites of the Sutla river in Croatia.

According to Eyo et al. [40], fecundity is necessary to evaluate the reproductive capacity of individual fish species. In the present study, absolute fecundity was found to be the highest in pond and the lowest in river. Water pollution may have adverse effect on the water quality and food availability, which might be responsible for lowering the fecundity and reproductive output [9]. Fish exposed to low doses of pollutants for the long time showed no apparent effects but it may have considerable impact on reproductive organs, reducing the population of the next generation and later by making the population slowly disappear [41]. The fecundity of *Liza parsia* was observed to be less in the specimens of Vizag harbor waters than those of the relatively unpolluted waters [42]. Variation in fecundity between two different populations was very common affairs and also found that the total number of eggs by an individual female fish was dependent on different factors such as age, size, space, food availability, season, climatic conditions, environmental factors, habitat changes, nutritional status and genetic potential [43,44]. In our study, insignificant result was found in case of fecundity for the female *C. punctatus* with same body weight in same water body but significant variation was found in different water bodies. Insignificant variation in the total fecundity even with same length and same body weight was also revealed in *Mystus bleekeri* [45] and *Anabas testudineus* [46].

In the present study, ova diameter of *C. punctatus* was varied from 1.06±0.06 mm to 0.21±0.03 mm. However, ova diameter was the highest in pond and the lowest in river. Snani et al. [47] reported that the diameter of oocytes was about 250 µm (43.62–282.47 µm and 39.76–270.25 µm) for females collected from El-Kala and Annaba that were polluted sites, whereas about 200 µm (37.76–224.37 µm) for females collected in Skikda but the difference was insignificant. On the other hand, fish collected from the most contaminated water showed significantly smaller oocyte diameter [33].

The GSI value of male fish was significantly highest (*P*<0.05) in pond and the lowest in river during the study. Hassanin et al. [48] reported that the gonadosomatic index and testis weight in the fishes collected from the Ishizu river were significantly lower (*P*<0.05) than in control fishes all over the phases of gonadal cycle and it was lower than in Wada river fish during the time of pre-breeding and post-breeding seasons of the year. A significant decrease of the gonadosomatic index was found in male catfish (*Clarias batrachus*), which were exposed to the selected sub-lethal concentrations of mercuric chloride [49].

5. CONCLUSION

The findings of the present study revealed that *C. punctatus* spawned in the freshwater pond, Joghati baor and Bhairab river and the spawning was intense during the month of July. The development and weight of the ovary differs during different months of the year in pond, baor and river. The GSI value, ova diameter and fecundity were the highest in pond, followed by those in baor and the lowest in river. The lowest values of these reproductive parameters of *C. punctatus* in river waters may be due to the fact that the Bhairab river is continuously polluted from many anthropogenic and Industrial sources. Although further research would need to be undertaken to explore more comprehensive information on the reproductive biology, gonad development and water pollution, the findings emerged from the present research would immensely be helpful towards the successful breeding, larval rearing, seed production, culture as well as conservation and sustainable fishery management of *C. punctatus* in its natural habitat to a greater extent.

DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

ETHICAL APPROVAL

As per international standard, written ethical permission has been collected and preserved by the authors. Animal Ethic committee approval has been collected and preserved by the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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