Bulletin of Environment, Pharmacology and Life Sciences
Bull. Env. Pharmacol. Life Sci., Vol 9[2] January 2020 : 08-15

Online ISSN 2277-1808
Journal's URL:http://www.bepls.com
CODEN: BEPLAD
Global Impact Factor 0.876
Universal Impact Factor 0.9804
NAAS Rating 4.95

ORIGINAL ARTICLE **OPEN ACCESS**

Reproductive Biology of Captive reared spotted Snakehead, *Channa punctatus* (Bloch 1793) Channidae

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ABSTRACT
Understanding the reproductive biology of any fish provides a comprehensive synthesis of fish reproduction and in the long run it will be of helpful to conserve a vulnerable species in the wild condition. Yearlong investigations were carried out to estimate gonadosomatic index, fecundity and uncover gonadal histology of Channa punctatus reared in captivity. Adult C. punctatus were stocked at the rate of 4fish/m² in 6 earthen ponds (40 square meters each). Fish were fed twice daily at 9:00 hours and 16:00 hours with commercial feed (Fish meal 45%, Grain and cereals 50%, Vitamins and binders 5%) available in the market. On the basis of monthly sampled fish, spawning season of the fish raised in captivity was disclosed. Relationship between total length and body weight occupied r^2 value of 0.96, while that of body weight and fecundity was 0.779. The peak of GSI value was recorded as 3.68 for ovary, while it was 1.322 in case of testis. Absolute fecundity varied from 1987±123 - 22767±576 with an average value of 9558±230. Absolute fecundity increased with total length, body weight and ovary weight. Histological study revealed five stages of gonad development in both the sexes. The GSI values, fecundity and monthly gonadal changes indicated the spawning season extends from June to September. Presence of more than two stages throughout the entire study period indicated the asynchronous batch spawned oocyte development in captive reared C. punctatus.
Keywords: *Snakeheads, Fecundity, Gonado somatic index, Reproductive biology, Channidae*

Received 29.12.2019 Revised 06.01.2020 Accepted 16.01.2020

INTRODUCTION

The fishes of the family Channidae are economically important for their high nutritional and medicinal value [8]. Spotted snakehead, *Channa punctatus,* one of the commonly available fishes of channidae have been disappearing from the wild in Bangladesh. Increase risk of disease, siltation, pollution, poisoning, and loss of habitat, overexploitation, destructive fishing and invasion of alien species are considered as major cause of their disappearing [17,21]. The *C. punctatus* is also popular for its superior taste, protein content and reduced intramuscular bone, high nutritive and medicinal values [14]. This fish has prospect of being as aquarium fish for its body shape, behavior and attraction [29]. However, the fish has been assessed as least concern recently [16].

It is important to know the reproductive biological information such as gonadosomatic index, fecundity to initiate artificial breeding as well as conserve any fish in wild condition. Gonadosomatic index is indicating parameter of the fish reproductive biology, which provides clue about reproductive status of the species and aid in determination of breeding period [12, 23, 31]. Fecundity is defined as the total number of ripen eggs obtained in spawning season from particular species of female fish [1]. The information of fish fecundity is crucial in respect to fish population studies and in sustainable management and exploitation of the fishery, and aquaculture industry [3]. Information on the induction of spawning and artificial propagation of *C. punctatus* is limited and very few preliminary works were done. Later on, few aspects of the reproductive behavior of *C. punctatus* has been addressed previously [14,26]. The knowledge on reproductive biology i.e., gonadal maturation and annual reproductive cycle is the prerequisite for successful reproduction of captive reared *C. punctatus*. However, little is known about the reproductive biology of *C. punctatus* in captivity. Thus, the present study was conducted to ascertain the breeding biology of *C. punctatus* in captive condition.

MATERIAL AND METHODS

Study site & experimental fish

The study area was Sylhet Agricultural University, Sylhet, Bangladesh and is located between 24°54' North latitude and 91°54' East longitude. The study was conducted in 6 earthen ponds (40sq.m each) of Sylhet Agricultural University, Sylhet, Bangladesh. Water hyacinth was used for the shelter of fish. Adult fish (>125 mm) as described by Haniffa et al., [14] were collected from local fish traders and stocked in the earthen ponds.

Collection, stocking and feeding of fish

The C. punctatus was collected from the natural sources of greater Sylhet. Fish were brought to the on farm in plastic drums equipped with mobile aerators. Before stocking into ponds, fish were kept in the hapa for few hours to adjust them in the new environment. Fish were stocked (6fish/square meter) and fed twice a day at 9 h and 16 h with commercial diet. Chicken viscera and trash fishes were also applied weekly for better growth and development. Water quality parameters were kept under control through regular monitoring and supervision.

Sampling

Samples of C. punctatus were collected fortnightly for one year. The simple random sampling technique was used [10]. A total of 240 samples were collected and sacrificed during the study period. The samples were transported to the laboratory and preserved in a deep freezer at -20°C until examination and analysis. Sampling was done to observe the monthly growth and development of the gonads. To elucidate the breeding season and gonadal cycle of *C. punctatus* 20 fish were sacrificed to collect the gonads for studying gonado-somatic-index.

Morphologoy of Gonads

Previously preserved specimens were dissected to investigate the position and appearance of gonads. The gonads were kept out and placed in physiological saline. They were washed very well to remove the blood, tissues and fats. The record the colour, size, length and weight of the gonads were taken carefully.

Calculation of Gonado-somatic-index (GSI)

The gonads were weighed to the nearest gram using electric balance. The gonado- somatic index (GSI) was obtained according to the following equation:

GSI =GW/ (TW-GW) x 100; Where, GW= gonad weight, and TW= total body weight [11]. Then the samples were preserved in 10 % neutral buffered formalin for further use.

Fecundity

The fecundity was calculated by following methodology described by Muchlisin et al., [43]. Only samples of adult females (~100–120 g) described by Basak et al., [4] were used for fecundity analysis [25,42]. Minimum oocyte size counted for fecundity was greater than 0.79 (±0.18) mm; described by Saikia et al., [29]. About 50% of matured females at final oocyte maturation stages were randomly selected from monthly samples. Three sub-samples of ovaries weighing 0.1 g to 0.2 g were obtained from the anterior, posterior and the middle of gonad and soaked in solution comprising of 60 mL ethanol, 30 mL formaldehyde and 10 mL glacial acetic acid. The solution was used to wash the mucus to prevent the eggs from adhering together, to ease observation. The eggs were placed into a dish and counted under stereo light microscope (Nikon, YS-100). The mean from the three sub-samples were used to calculate absolute and relative fecundity using gravimetric method [5].

Fecundity was calculated by the following formula:

$$F = n \times G / g$$

Where “F” is fecundity, “n” is the average number of eggs in sub-sample, “G” is weight of the gonads and “g” is the weight of the sub-sample.

Histology of gonads

Standard histology procedure was followed described by Vandyk and Pieterse [44]. Monthly preserved gonad samples were taken out from the fixative. Then the samples of the central portion of the gonads of 0.5 cm thick were put into the cassettes separately for histological examination. They were dehydrated in graded alcohol series, embedded in paraffin, sectioned for 5–7µm in thickness using a microtome (MICROM HM355S, Germany) and stained with Haematoxylin and Eosin, then mounted in DPX mountant and photographed with an OLYMPUS-CX41 microscope which was equipped with the SONY DSCW220 camera [11]. At least two glass slides were prepared for each ovary. Gonad development stages were identified by observing under the microscope followed by the description of Navarao et al, [45]. Gonads were visualized in 4X, 10X and 40 X magnifications.

Statistical analysis

Data were analyzed using the SPSS (software version V20.0; Chicago, USA) with the level of significance at $p<0.05$. Correlations and regression between fecundity and other parameters such as total length, body weight, gonad weight, GSI were also determined. Data have been presented as mean ± SEM.

RESULT

Morphological features of the gonad of C. *punctatus*

The ovaries of C. punctatus were hollow sac-like, paired and more or less elongated structure lying dorsal to the alimentary canal and ventral to the swim bladder. A posterior extension of tunica albuginae united both the ovaries to form an oviduct which opened to the exterior via the oval shaped urogenital papilla. The left ovary was always slight larger than the right in all the examined fish. The color of the ovary varied from reddish brown in immature ovaries to light yellowish in mature ovaries.

The testes of C. punctatus were white, elongated, paired and ribbed structures. The left lobe of testis was found to be slightly larger than the right one. Two parts of the testis joined posteriorly before entering the cloaca and where they formed the third lobe. They were attached to the body wall by mesenteries. The color of the testis ranged from pinkish to whitish depending on the maturity.

Relationship between and body length and total weight

Total length and body weight found to be strongly correlated with r^2 value of 0.96 (fig.1) which suggest larger fishes tend to have more weight as well as small fishes tend to have decreased weight.

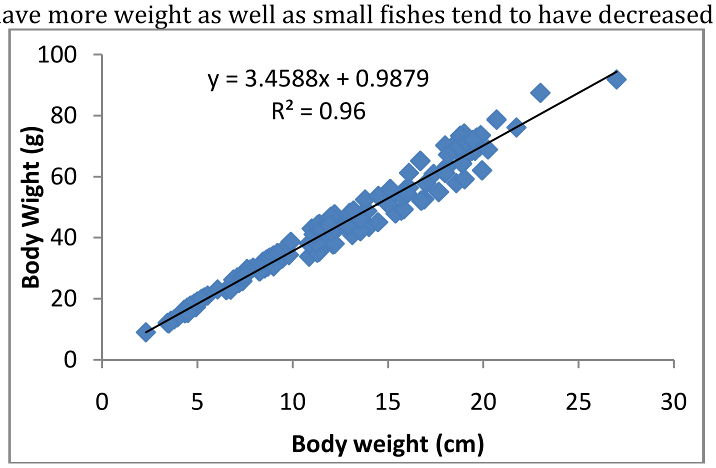


Fig.1 Correlation between body weight and total length of *C. punctatus*

Relationship between body weight and fecundity

There is well positive relation between the body weight and fecundity of *C. punctatus* with a correlation value of 0.902 (fig.2). This means that approximately 80% of the variation in fecundity could be explained by the body weight of C. punctatus.

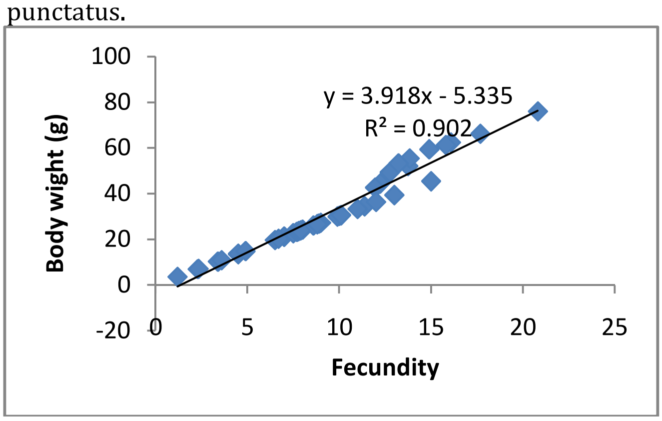


Fig.2 Correlation between body wight and fecundity of *C. punctatus*

GSI Values

Gonado somatic indices were recorded and variations found in different month. The highest GSI value for ovary was recorded in the month of July (3.68) while it was found to be 1.322 in case of testis. Lowest GSI recorded in the month of January and was gradually increasing from February to May and reached a maximum value of 3.68 and 1.322 for female and male respectively. Later in the month of August there was fall in the GSI values for both male and female. This tendency was continued up to December (Fig. 3).

Fecundity of the fishes was showed also variation in different months. Fecundity value found to be lowest in the month of December and recorded from the spent fish.

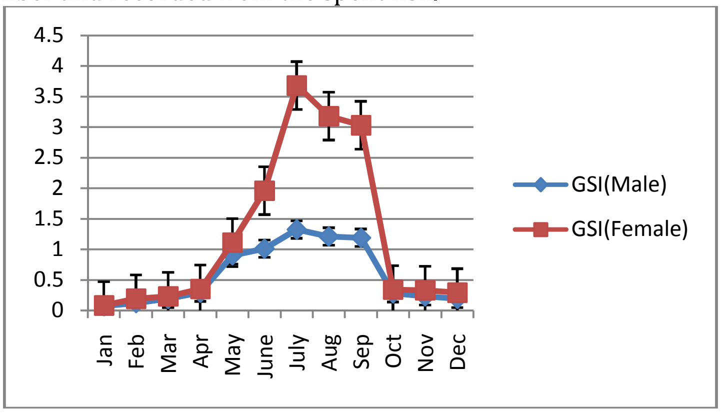


Fig.3 Gonado somatic index (GSI) value changes in male and female *C. puncatus* reared in captivity throughout the entire study

Fecundity

Figure shows the absolute fecundity of mature female *C. punctatus*. In the current study, the highest absolute fecundity (number of oocytes per female) was found to be 22767 in the month of July. Similar trend was also found in the following month i.e., in August (22156). Absolute fecundity value was found to be lowest in the month of January as 1987 with an average value of about 9558 throughout the study period (Fig. 4).

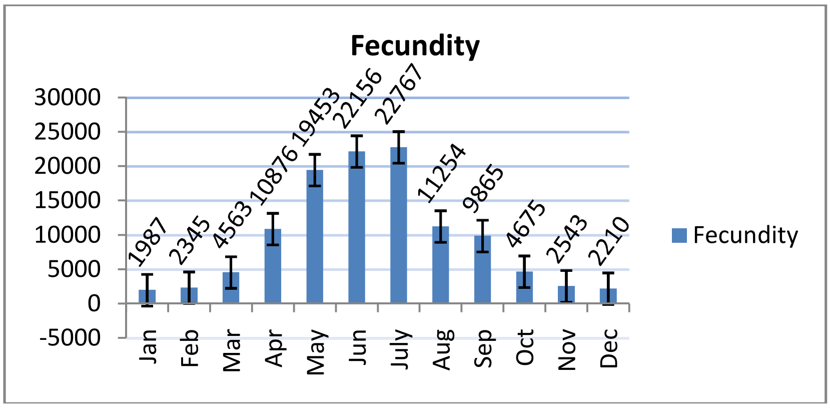
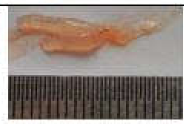
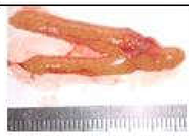
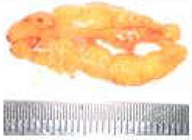
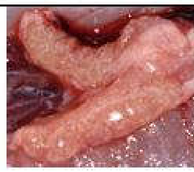


Fig. 4 Absolute fecundity of captive reared *Channa punctatus*

Macroscopic and Microscopic features of gonads (both ovary and testis)

Table 1 Different maturity stages of *C. punctatus* gonads reared in captivity

Stages	Description*	External Features		Observed Month
		Ovary	Testis	
Immature	Sex organs very small and near the spinal column, testicles and ovaries transparent grayish color			December-January
Initial maturation	Testicles and ovaries semi-transparent, gray, half or slightly more than half the abdominal length of enclosure, with a single particle between eggs visible			February-March
Developing	Ovaries and dark testicles, partial capillary blood red and occupies half the yard abdomen, eggs and armed non-eye visible as are scabrous			April through July

Ready to spawning (Gravid) & spawning (spawning)	Sex organs courtyard filled abdominal testicles and white, poured liquid pressure of sperm and eggs completely round and some are semi-transparent eggs and sperm of the current low pressure, semi-transparent eggs with some more egg on the outside of the ovary duct			July-August
spawned (spent)	ovaries soften and Eggs depleted completely abdominal landscaping are discharged			November-December

* Modified after Ghaedi et al., [10]

Macroscopic observation of the gonads (both ovary and testis) revealed different maturity stages of the fish. Five main stages (Primary Growth phase, Yolk vesicle stage, Vitellogenesis, Mature and Spent) were identified throughout the study period according to Gentek et al., [9]. In June, July and August most of the oocytes were in the third and fourth phase while some were also still found to be mature oocytes, yolk vesicles and primary oocytes. This confirms the asynchronous nature ovary of the fish. After decomposition of vitellogenin oocytes are able to go throughout the final maturation stage.

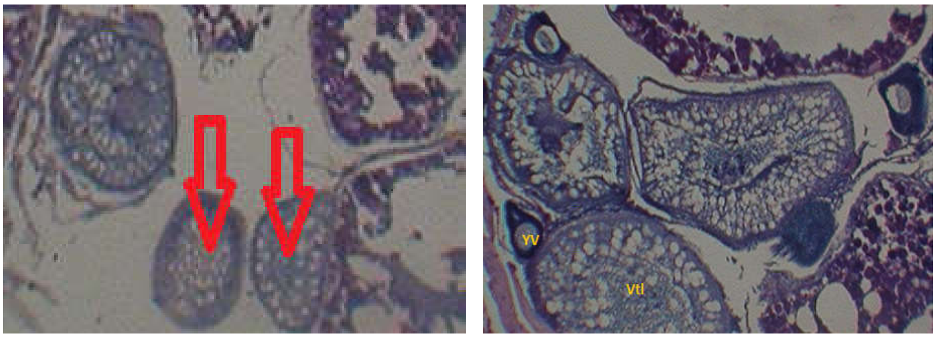


Fig.5 Yolk vesicle stage (left) and Vitellogenesis stage (right)

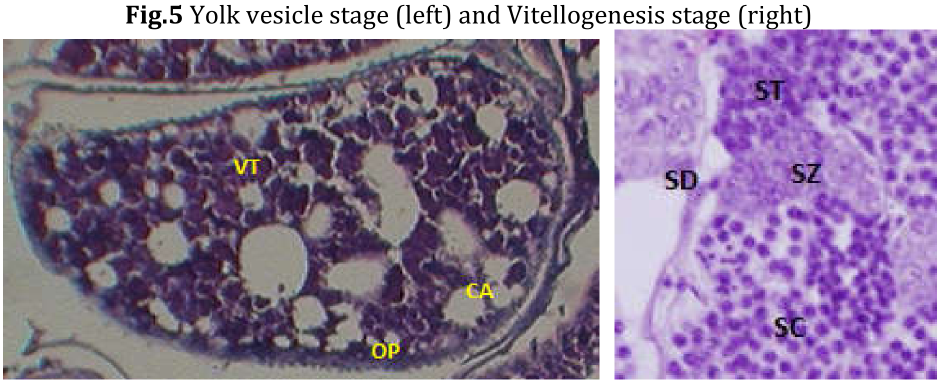


Fig.6 A mature egg (left) and a mature testis showing matured sperm (right)

DISCUSSION

Several studies evident the statistically significant relation between different body parameters likes fecundity and total length; body weight, gonad length and gonad weight [7,18]. The fecundity of the species increased with fish length and body weight [46]. Hossain et al., [15]. observed strong correlation between length and body weight (r2=0.912) in female *C. punctatus* and previous studies on captive-reared *C. striata* by Ghaedi *et al.*, [10] strengthened this finding. The relation between fecundity and weight changes significantly during breeding approaches and may be due to increase in egg size [36]. The descend r^2 value reveals, fish with higher weight seemed to have high number of oocytes compare to the low eight individuals. This finding was more or less supportive with the findings of Hossain et al. [15] for wild *C. punctatus*, and Sultana [49] for captive *C. striata*. Similar trend has been occupied Singh [36]. The present study for the relation between total length and body weight (r^2= 0.96) and body weight and fecundity (r^2=0.902) were found as very supportive and relevant to the previous findings.

Female C. punctatus shown quite significant variation on the GSI value throughout the study period and peaked at July as 3.68. Fluctuation in GSI was consequence of gonadal maturation which drive liberation of energy from the liver in form of vitellogenine to the gonad prior to spawning season. Gonadosomatic index is a good indicator to define the spawning season of fish [13]. Decline in GSI indicates the transformation of energy due to increase in compensation of stressors [37]. More or less similar trends was recorded in *C. punctatus* [15], *C. marulius* by Siddiquee [35] and captive-reared *C. striata* [10]. Such results are also obtained in *Puntius filamentosus* [20], *Barbus longiceps* [38]. Fecundity of fish varies with the season, size and weight and geographic position. The present research obtained highest absolute fecundity as 22767 in the month of July followed by 22156 in August and lowest of 1987 in January. Present findings are lined with earlier observation of Hossain et. al. [15], Lalta et al., [19] and Marimuthu et al., [22] for the same species. Sultana [39] reported fecundity ranges between 10632 and 22140 in captive *C. striata*, average (33949±3388) of absolute fecundity was occupied in captive *C. striata* from Malaysia [10]. Ali [2] recorded the absolute fecundity ranged 4326.0-9016.0 in *C. striatus* and Jannatul *et. al*, [47] recorded highest absolute fecundity of *C. striata* as (22783.56±3913.10) in the months of June. Except slight changes current finding is very similar to the works of former scientists associated with same species.

The spawning season identified in the present study was between the months of July to September which also supports the findings of others subcontinent researchers. Bhuiyan and Rahman reported a single annual spawning between April and August in Bangladesh and spawning in southern Nepal occurs from June until August [33]. The monthly distribution of oocyte stages of *C. punctatus* was reported based on the maximum number of advanced oocytes in the ovary sections. The results showed the presence of more than two stages throughout the entire study period which indicated the asynchronous oocyte development in captive reared *C. punctatus*. Several authors' works with C. punctatus documented spawning period between Februarys to May. Siddiquee [34] also observed very similar spawning behavior in *C. marulius* and *C. striatus*. Developmental stages of spermatogenic phases are spermatogonia, spermatocytes, spermatids and spermatozoa [41]. Several researchers found six or seven growing stages of oocytes in the ovary of fish ([28, 32,40], while Sarma *et al.,* [30] found five developmental stages in the ovary of fish. Oogonia were used as indicators of the first stage of oocyte development in most fishes [6, 27]. Oocytes grew and developed to reach the vitellogenic stage during reproduction [24]. In the current study majority of described stages were noted for both ovary and testes of *C. punctatus*.

CONCLUSION

The study provides fundamental information on the reproductive pattern of *C. punctatus* to assist in understanding useful biological processes that may be responsible for maintaining the underlying stock structure for proper conservation and management in its natural habitat. The observations from this study with regard to ovarian development in *C. punctatus* will contribute towards developing strategies for brood stock development and management in hatcheries of Bangladesh. Further research needs on food and feeding habits, artificial breeding trial and molecular level study on reproductive biology.

ACKNOWLEGEMENT

The authors are highly thankful to University Grants Commission (UGC) of Bangladesh for providing financial support to conduct the research activities. Thanks, are also due to SAURES, Sylhet Agricultural University for continued support and guidelines. Special thanks to Dr. Harunur Rashid, Professor, Dept. of Fisheries Management, Bangladesh Agricultural University, Mymensingh and Dr. Nazrul Islam, Professor, Department of Anatomy and Histology, Sylhet Agricultural University, Bangladesh for providing the facilities for histological works.

CONFLICT OF INTEREST

No conflicts of interest have been arrived during this research.

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CITATION OF THIS ARTICLE

Mian, S., Shah, A. W., Hossain, M. A., Hossain, M. S., Iqbal, M. M. & Debnath, P. (2019). Reproductive biology of captive reared spotted snakehead, *Channa punctatus* (Bloch 1793) Channidae. . Env. Pharmacol. Life Sci., Vol 9[2] January 2020 : 08-15