Original Research Article

Assessment of Sexual Maturity and Spawning Biology of *Channa punctata* (Bloch, 1793): Conservation and Reproduction

ABSTRACT

The study was conducted in the freshwater station of the Bangladesh Fisheries Research Institute in Mymensingh between September 2021 and August 2022. Finding the sex ratio, sexual maturity, LWR, reproductive biology, fecundity, and histological examination of gonads of C. punctata were the main objectives of the present study. The correlation between length and weight was determined by analyzing the power function. Both sexes showed positive allometric growth and the correlation between length and weight was TW= $0.011TL^{3.09}$ for males and TW= $0.009TL^{3.11}$ for females. The mean condition factor for males was 1.09 (0.89 to 1.24), whereas the mean condition factor for females was 1.12 (0.95 to 1.25). In both cases, the condition factor was more than 1, indicating that the physical condition of both sexes was excellent. Among the 329 samples, 142 (43.16%) were male and 187 (56.84%) were female (male: female = 1: 1.32). The chi-square test showed a significant departure from the 1:1 ratio between the number of males and females within the sample months (df = 1, $\chi 2$= 0.01, p=0.05).When they reached first sexual maturity (L50), both sexes were of similar size, as evidenced by their lengths of 14.95 cm. The peak spawning season for both sexes was observed in June and July; however spawning season lasted from April to July. According to the study results, July was recorded the highest absolute fecundity (23609), while December recorded the lowest (1998).

Keywords:*Sexual maturity; sex ratio;reproductive cycle; fecundity*

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1.INTRODUCTION

In Bangladesh, the Spotted Snakehead *Channa punctata*, also known as Taki, is a freshwater air-breathing fish with a high nutritional value. *C. punctata* is a small indigenous species (SIS), only growing to a maximum size of 25 cm, and has a head and body that resemble snakes. Several countries, including Bangladesh, India, Afghanistan, Nepal, Myanmar, Sri Lanka, Pakistan, and China may harbour large populations of this species [1]. It is a freshwater fish that can be found in large numbers in lakes, ponds, canals, floodplains, streams, and rivers in Bangladesh [2] as well as in a few locations in Southeast Asia [3]. In addition to being a delicious fish, *C. punctate* is also recommended as a food source because it is high in protein and other nutrients that have therapeutic benefits [4][5]. There are 17.48% protein, 4.92% fats, 1.73% ash, and 75.25% moisture in 100 g of this species' freshly edible flesh [6]. This species is significant as a fish for aquariums and decorations because of its behaviour, body colour, and outward appearance [7]. However, due to habitat loss, spawning site destruction, overexploitation, siltation, pollution, overexploitation, use of small-mesh fishing gear, and aggressive fishing, this species is rapidly disappearing from Bangladesh's natural waterways and is now regarded as being close to being extinct [8]. For the purpose of starting breeding programmes and ensuring the survival of wildlife, it is essential to comprehend length-weight correlations and reproductive biology, including the gonadosomatic index and fecundity. Information on the fish's reproductive health and breeding season is provided by the gonadosomatic index. Another critical element, fecundity, also influences productivity and population estimate [5]. The total number of mature eggs in a female fish's reproductive system serves as a measure of the fish's maturity [9]. Fish fecundity and the gonadosomatic index are used to predict when they will spawn. The length-weight ratio of the fish is an important factor in determining how well the fish grows in various environments. A length-weight ratio is an essential tool in fisheries biology because it establishes both the mathematical relationship between fish length and weight as well as the discrepancy from the expected weight for the length of the fish [10][11][12]. To determine the degree of fish wellbeing, a different measure known as the condition factor is employed [13]. Surprisingly few studies on the GSI, fecundity, length-weight relationship, sex ratio, and condition factor have been conducted in Bangladesh. In order to ascertain the gonadosomatic index, fecundity, length-weight relationship, condition factor, and sex ratio of *C. punctata*, the current study was conducted. These findings will be helpful to researchers and those in charge of formulating fisheries policy with regard to the conservation and management of this species in the wild as well as the start of induced breeding.

2. MATERIAL AND METHODS

2.1 Study Area and Period

The study was carried out from July 2021 to June 2022 over a period of 12 consecutive months at the Bangladesh Fisheries Research Institute, Freshwater Station in Mymensingh. Latitude 24.7214° N north and longitude 90.4212° E east are the coordinates for the research region (Fig 1).

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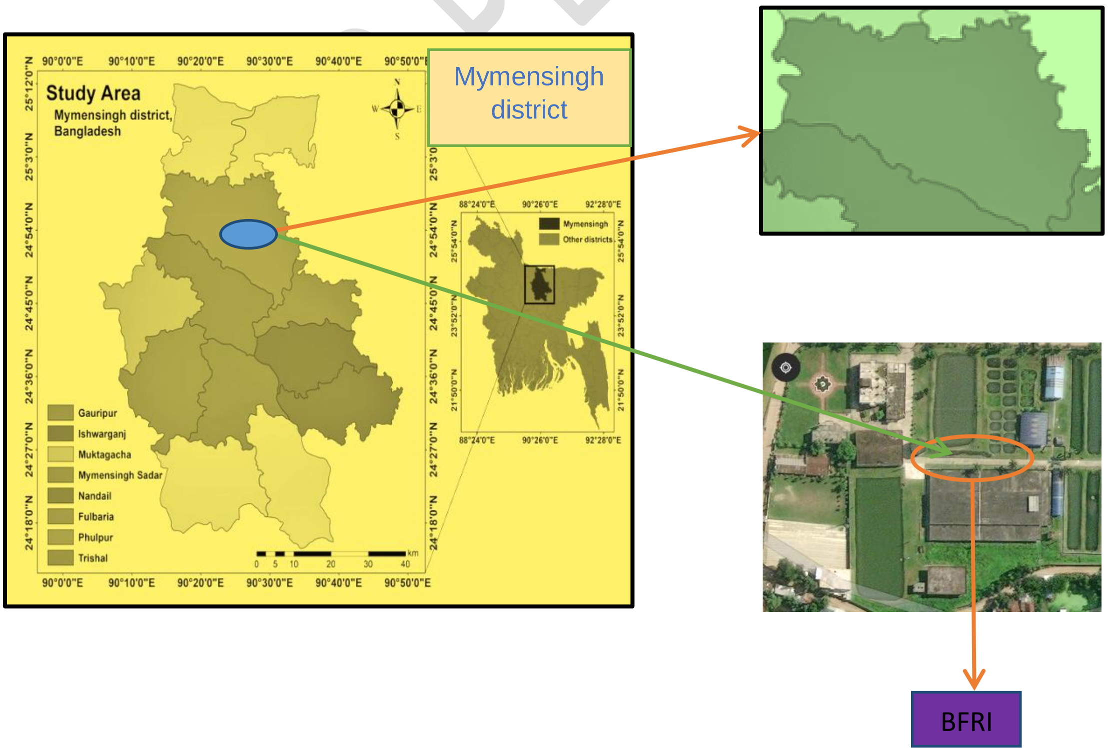

Fig 1. Study area (Source: Google maps and Google earth)

2.2 Collection of Samples

Each month, fish samples were collected from the local market of Mymensingh. After being collection, fish were immediately measured for total length and weight.Total length was determined to the nearest cm on a measuring scale, and weight was determined to the nearest g on an electric balance.

2.3 Sex Ratio

It is very difficult to identify the sex of *C. punctata* based solely on external characteristics, with the exception of spawning. Each fish was dissected as well as the gonads were taken out after collection. Each gonad was examined to determine its sex. By comparing the proportions of the two sexes to one another, sex ratios were calculated. The equation was used to analyse the data using the chi-square test (χ2) with the assumption that the population's sex ratio was 1:1 (p=0.05) [14].

$$X^2 = \frac{\sum(\mathrm{O} - \mathrm{E})^2}{\mathrm{E}}$$

Where, 'O' denotes the observed value and 'E' denotes the expected value

2.4 Size at First Sexual Maturity

The size of both sexes at the time of first sexual maturity was determined by measuring the length of fishes and observing the stages of maturation. Fish with sexual maturity were measured by length frequency, and the first sexual maturity was calculated using the smallest average size that produced 50% of sexually mature individuals [15].

2.5 Length-Weight Relationship

The following equation, which was given by [10], was used to determine the connection between the total weight and total length:

$$W=aL^b$$

Where, W= Weight of fish in grams, L= Length of fish in centimetres, a= intercept of the regression line and b= regression coefficient.

2.6ConditionFactor

Fish condition factor (C_F) was determined by applying the equation of [16].

$$\mathrm{CF} = \frac{\mathrm{W}}{\mathrm{L}^3} \times 100$$

Where W= Weight of fish in gramsand L= Length of fish in centimetres

2.7 Gonadosomatic Index

Each gonad was removed through abdominal dissection, and they were weighed to the nearest grams. According to the following formula [17], the gonad somatic index (GSI) was calculated:

$$\mathrm{GSI}\ (\%) = \frac{\text{Weight of the gonad}}{\text{Weight of the fish}} \times 100$$

The gonad samples were preserved for future use by treating them with 10% buffered formalin and storing them at room temperature.

2.8 Fecundity

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Three ovarian subsamples were taken from the anterior, posterior, and middle regions of the gonad to determine fecundity. Then, in a dish, the oocytes from the three subsamples were counted.The gravimetric method was used to calculate fecundity from the average of the subsamples.

$$F = \frac{n \times G}{g}$$

Where F represents fecundity, n represents the mean number of oocytes in the subsample, G represents the weight of the gonads, and g represents the weight of the subsample.

2.9 Histological Preparation of Gonads

The fixative was used to extract samples of preserved gonads. The gonad centres were then sectioned into cassettes that were each 0.5 cm thick for histological analysis. After that, they were microtome sections 5-7 microns thick, depleted in a graded alcohol sequence, stained with both haematoxylin and eosin, and mounted in DPX mounting medium. There were at least two glass slides created for each ovary. At 10x and 40x magnification, the gonads' developmental stages were observed and visualised under the microscope.

2.10 StatisticalAnalysis

These statistical tests were performed using a one-way analysis of variance (ANOVA) with a 5% level of significance. Statistical regression analysis was performed on the relationship between fecundity and weight, fecundity and weight of fish, condition factor, and length-weight correlation data using the SPSS version 20.0 software and Microsoft Excel 2016.

3. RESULTS AND DISCUSSION

3.1 Sex Ratio

Of the 329 samples of *C. punctata*, 142 (43.16%) were male and 187 (56.84%) were female (males: females = 1: 1.32). Chi-square analysis (df = 1, $\chi 2$= 0.01, p=0.05) was used to examine the total sex ratio for deviations from the hypothesized 1:1 ratio, and significant deviations were found (Table 1).Mian [5] found that the sex ratio of *C. punctata* was 1:1.16, which is relevant to the current study. The results of the current study are essentially consistent with those of [3], who found that the monthly variation in the overall sex ratio (M: F) in *C. punctata* was 1: 1.33. Amzad [2]was determined a sex ratio of 1: 1.28 for males and females of *C. punctata*, which is nearly identical to our results in this study. However, the sex ratio differs between species as well as between years within a population; in the overwhelming majority of cases, it is near one [18].

Table 1.Monthly fluctuations in the sexes of the *C. punctata* during the experimental period

Months	N	Female		Male		Chi-square ($\chi 2$)	Sex ratio
		n	%	n	%		F: M
September	27	15	55.56	12	44.44	0.018	1.25:1
October	28	16	57.14	12	42.86	0.001	1.33:1
November	25	14	56.00	11	44.00	0.007	1.27:1
December	26	16	61.54	10	38.46	0.234	1.6:1
January	33	18	54.55	15	45.45	0.071	1.2:1
February	24	13	54.17	11	45.83	0.070	1.18:1
March	26	15	57.69	11	42.31	0.008	1.36:1
April	21	14	66.67	7	33.33	0.827	2.0:1
May	29	16	55.17	13	44.83	0.033	1.23:1
June	31	17	54.84	14	45.16	0.051	1.21:1
July	29	16	55.17	13	44.83	0.033	1.23:1
August	30	17	56.67	13	43.33	0.000	1.31:1

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Total	**329**	**187**	**56.84**	**142**	**43.16**	**0.113**	**1.32:1**

3.2 Size at First Sexual Maturity

The term "size at first sexual maturity" refers to the size at which 50% of fish reach their first sexual maturity (L_{50}). The size at which *C. punctata* first reached maturity was determined by grouping maturing individual data into various length categories with a class interval of 05 cm. The percentage of individuals maturing in each length group is shown in (Figure: 2A&2B). At a mean length of 14.95 cm during spawning, 50% of male and female individuals were sexually mature, indicating their size at initial maturity.According to Chowdhury [19], the early sexual maturity of *C. punctata* for both sexes was 12.67 cm in total length. At sexual maturity, males of *C. puctata* attained a length of 18.5 cm whereas females attained a length of 18.2 cm [20]. According to Biswas [21], the size of a fish during initial sexual development differs not only between species but also within the same species from different habitats.

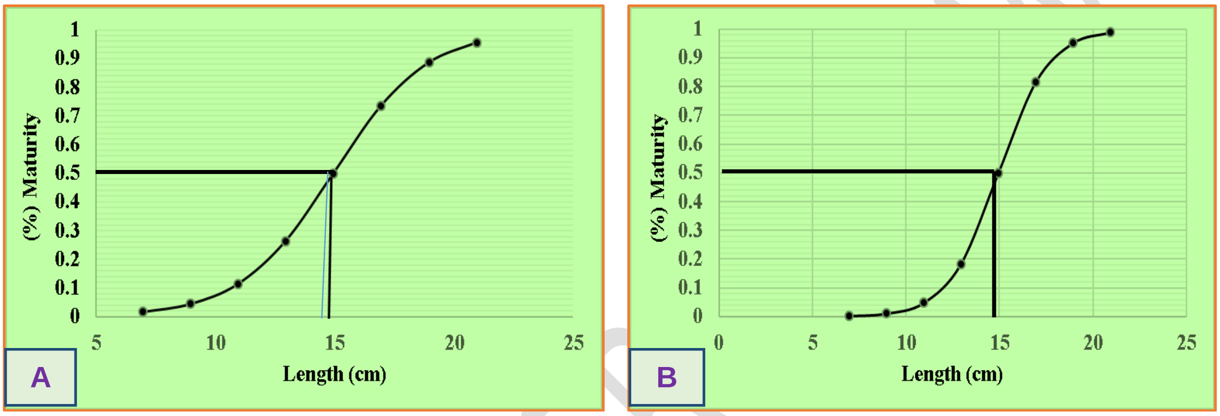

Fig 2. Size at first sexual maturity of the female (A) and male (B) *C. punctata*

3.3The Length and Weight Relationship

The correlation between the length and weight of *C. punctata* is shown in Table (2). The total length of male and female *C. punctata* in the current study ranged from 16.42 to 19.93 cm and 16.55 to 20.46 cm, respectively. Male body weights in *C. punctata* ranged from 48.23 to 89.85 g, whereas female body weights ranged from 51.28 to 93.54 g. Consistent with the findings, the "a" values for male and female *C. punctata* were 0.009 and 0.011, respectively. Both the male and female of the species had exponent "b" values of 3.09 and 3.11, respectively which is higher than 3. Positive allometric growth patterns were evident in both sexes. From the "b" value, it can be inferred that the fish grow faster than their length.The coefficient of determination (r^2) for females and males were 0.87 and 0.85 respectively (Figure: 3A& 3B), which indicates the length-weight relationship of both sexes was positive and highly significant. The reported relationship shows that weight could explain approximately 87% of length variation in females and approximately 85% of length variation in males. According to (Aung & Sein, 2019) male and female fish had body weights of 32 to 120 g and 38.5 to 140 g, respectively, and total lengths of 14.5 to 21 cm and 15.5 to 22 cm, respectively. Haniffa[22] was reported that the total length of female ranged from 15.9 to 24.4 cm, whereas the total length of males ranged from 15.6 to 25.0 cm. Amzad [2] *was* found a strong positive and significant coefficient of determination (r^2= 0.912) in *C. punctata*, which is broadly compatible with the current finding. Hossain [23]was found a highly positive significant correlation coefficient (r^2=0.912).Similarly, [20] observed that the coefficient of determination "r^2" in *C. punctata* was 0.918, which is positive and highly significant. According to Saikia [7], *C. punctata* in Assam has positive growth as well as allometric growth (>3). A similar result was found by [19] who observed that the growth of *C. punctata* is isometric (b=3) and found that the coefficient of determination (r^2) is 0.97, which is supported our current findings.

Table 2. Relationship between length and weight during the research periodof *C. punctata*

Sex	**N**	**Regression equation**	**a= intercept**	**b=slope**	**r^2**

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Female	143	TW = $0.009TL^{3.11}$	0.009	3.11	0.87
Male	132	TW = $0.011TL^{3.09}$	0.011	3.09	0.85

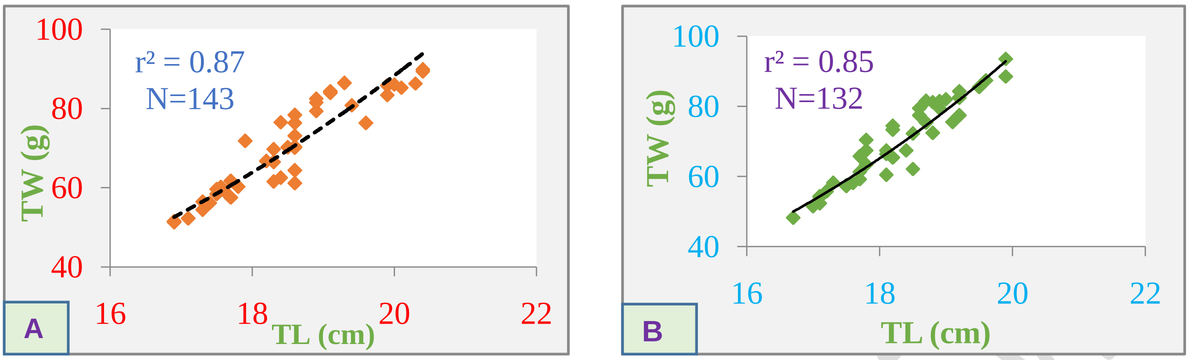

Fig 3. Shows the length and weight relationship of female (A) and male (B) *C. punctata*

3.4Condition Factor

Table (3) displays the average condition factor for males and females. The condition factor for females ranged from 0.89 to 1.24 (1.09±0.06), and for males from 0.95 to 1.25 (1.12±0.07).The results indicate that the condition factor of both sexes were significantly different (p=0.05), indicating that the specimens of both sexes were in an excellent condition. The condition factor reflects current physical and biological circumstances and fluctuates as a result of interactions between feeding conditions, parasite infections, and physiological parameters, indicating changes in food reserves and therefore an indicator of overall fish condition [10].The current findings for both sexes of *C. punctata* are remarkably comparable to those of [24]. Chowdhury[19] was reported that the condition factor for *C. punctata* was 1.05, which agrees with our current findings. Das [25]observed a condition factor value of 1.01 in the Manu River, which is identical to the current study.Kashyap [26] reported that the condition factor value ranged between 0.99 and 1.06 in three different groups from India.

Table 3.The average condition factor for *C. punctata* in Mymensingh, Bangladesh

	Sex	N	Mean	Minimum	Maximum
C_F	Male	132	1.12±0.07	0.95	1.25
	Female	143	1.09±0.06	0.89	1.24
	Combined	275	1.11±0.07	0.92	1.24

3.5 Gonadosomatic Index (GSI)

Data from the monthly gonadosomatic index were used to determine the peak spawning season of the *C. punctata*. The highest GSI values were found between March and July for both sexes. Between June and July, when both sexes spawned and July had the highest GSI value (8.32 for the female and 0.42 for the male). Between September and December, GSI values decreased for both sexes (Figure: 4A& 4B). After July, the GSI value dropped, and it peaked in October (0.40 for the female and 0.14 for the male). In the sample, there were significant monthly differences in the GSI value ($p < 0.05$).According to Aung [20], the maximum GSI value was 7.14 in females and 0.30 in males in June for *C. punctata*. Farid [27] was observed that the maximum GSI values for females and males were 6.06 and 0.70, respectively, in July, whereas the lowest values were 0.70 in females and 0.12 in males, which is remarkably similar to our observations. Choudhary [28]was showed that *C. punctata* had the highest GSI values from July to August. According to [3] the months with the minimum GSI values both in males and females were November to February, and the months with the maximum

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were August. As per [2], the maximum GSI value for *C. punctata* was 5.64 in July. Mian [5], in July, the maximum GSI value for *C. punctata* females was 3.68 and 1.32 for males. The decrease in GSI implies energy transformation as a result of increased adjustment for stresses [29].

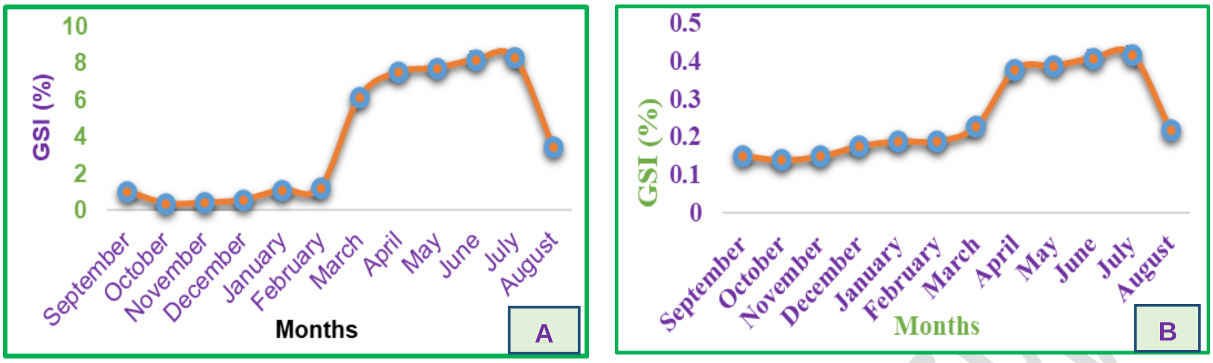

Fig 4. The monthly discrepancy of GSI (%) of female (A)and male (B) *C. punctate*

3.6 Fecundity

The fecundity of sexually mature females is depicted in Figure (5). The highest absolute fecundity in the present study was 23609, which was measured in July. As the fish entered its resting period, a trend decline was observed in the following months, reaching its lowest point in October. October was the month with the lowest absolute fecundity (1998). Contrarily, fecundity increased until it peaked in July and then showed an increasing trend. Throughout the study period, the mean value of absolute fecundity was 9597. The results of the current study are similar to those of [5], who observed that maximum fecundity in July was 32987. In *C. punctata*, [20] reported that fecundity varied from 3854 to 8723 ova, while relative fecundity varied from 60 to 169 ova. In Aligarh, fecundity varied between 2300 and 29600 oocytes, which compares relatively well with the current data [30]. In *C. punctata*, absolute fecundity varied from 2423 to 6466 ova, whereas the relative fecundity varied from 104 to 216 ova. Prasad [3] was observed total fecundity values ranging from 3678 to 27853 ova, which supports the current results. The maximum absolute fecundity of *C. punctata* in July was 26295, which is consistent with the current findings [23]. Fish fecundity varies between species and even among species due to variables such as age, length, body as well as gonad weight, biological parameters of the aquatic environment, and so on [31].

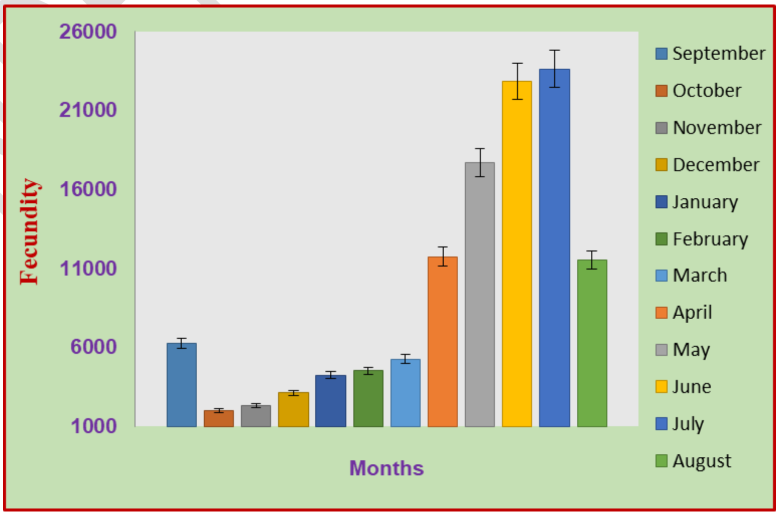

Fig 5. The monthly discrepancy of the fecundity of *C. punctata*

3.7 Microscopic Observation of Gonad

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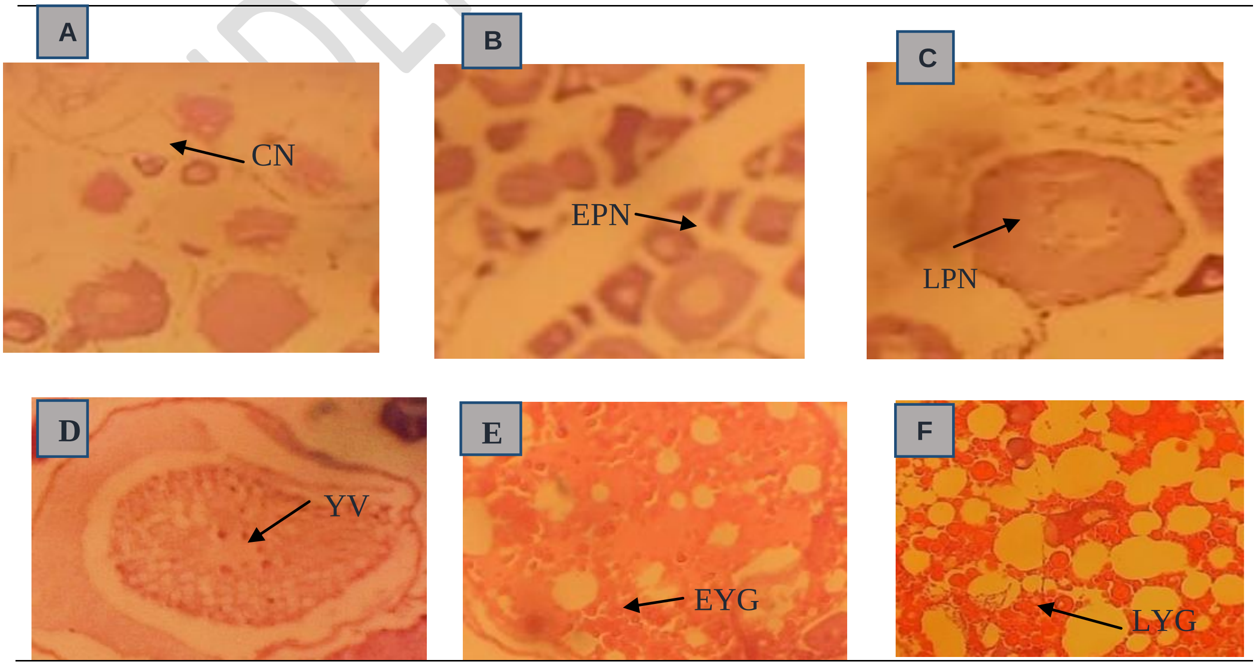
Microscopic analysis of gonad sections from *C. punctata* revealed four different stages of maturation: the immature stage, the maturing stage, the mature stage, and the spent stage (Table: 4).Based on the histopathological evidence, the seven different stages of oocyte maturation in *C. punctata* were identified (Figure: 6). Histopathological examination of the gonads provides more accurate and reliable indicators of the reproductive status of the fish. Gonadal microscopy revealed the different maturation stages of the fish. To identify the development of oocyte stages, the chromatin nucleus stage, early yolk granule stage, late yolk granule stage, yolk vesicle stage, early yolk granule stage, and spent stage were used. Oocytes at different maturation stages were examined, such as chromatin nuclear stage in December, early and late perinuclear oocytes in January, yolk vesicle stage in March, and early yolk granule stage in April-May, late yolk granule stage in July, and spent stage in August. A large percentage of the oocytes were in their third stage in May, June, and July.Histological investigations on gonads deliver more precise and straight signals of an animal's reproductive condition, providing them more trustworthy for reproductive cycle analysis [32]. Amzad [2] was identified four distinct phases in *C. punctata* based on histological examination, which is consistent with our findings.Aung [20] was identified four maturity phases in *C. punctata* based on the overall morphology of their gonads. Hossain [23] was found dissimilar results, identifying six distinct phases of gonadal maturity in *C. punctata*. Five different maturity stages were found by [26] in *C. punctata*.This species' maturity stages varied because the immature and adult phases were difficult to discern.

Table 4. Several stages of the gonad of *C. punctata* during the research period

Immature	Oocytes with oogonia, chromatin nuclear, and early perinuclear development were seen. Large nuclei were visible in the perinuclear nuclei of the oocytes. Several maturational phases were observed between late December and early January.
Maturing	There were both somewhat yolked and late perinuclear oocytes present. Yolk vesicles can also be found inside oocytes that have a significant amount of yolk. Between late February and early March, we have seen several maturational stages.
Mature	Many of the yolked oocytes were developed. The cytoplasm was filled with granules of the egg. A number of maturational phases were visible from late April to July.
Spent	Empty follicles, a small number of oogonia, and perinucleolar oocytes were found, and these indicators of further spawning were present. The spent gonads began to show up in August and September.



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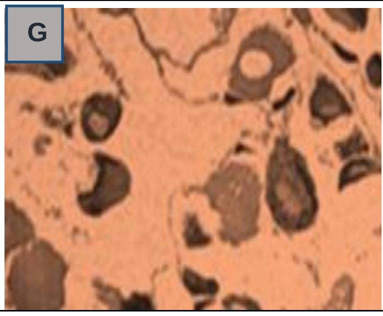

Fig 6. A) Chromatin nuclear oocyte, B) Early perinuclear oocytes, C) Late perinuclear oocytes, D) Yolk vesicle phase E) Early yolk granule phase F) Late yolk granule phase and G) Spent stage

4. CONCLUSION

In accordance with the findings obtained, *C. punctata*spawned during the months of April to July, with July representing the most prolific month. Depending on the reproductive biology findings, scientists, fisheries policymakers, and the general will be able to create an impactful management and preservation plan for the species. Information on the reproductive biology of *C. punctata* would be used to establish induced breeding for mass production in the days ahead. The current study's findings will help with better conservation and management of *C. punctata*, but much more research is required to gather more detailed information on reproductive biologyof *C. punctata*.

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