AMERICAN JOURNAL OF PHARMTECH RESEARCH

Journal home page: http://www.ajptr.com/

Reproductive cycle of the critically threatened Cyprinid fish *Puntius vittatus*

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ABSTRACT

The females were significantly bigger than males at all seasons of the year, and there was a preponderance of females only in spawning months; otherwise, both sexes were found in almost equal numbers. The males matured at a smaller size, 2.5cm SL, than the females, 3.1-3.5 cm SL. The size of the largest mature male was 3.25cm in total length and 2.65 grams in live weight, and that of the largest mature female was 5.22cm in total length and 3 grams in live weight. The maximum GSI of a mature female fish during the spawning season was 3.5%, and that of a mature male was 3.4. The highest value of condition factor of a mature female was 2.9, and that of 0a mature male was 2.6 during the breeding month. The analyses of all these reproductive traits of *P.vittatus* pinpoint the exact spawning season of the fish and also its reproductive capacity to enable one to employ this species in fish farming practice.

Keywords: Puntius vittatus, reproductive cycle, Gonadosomatic index

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Received 01 May 2025, Accepted 25 May 2025

Please cite this article as: Arachi JM., Reproductive cycle of the critically threatened Cyprinid fish Puntius vittatus. American Journal of PharmTech Research 2025.

INTRODUCTION

The reproductive cycle of different species has developed in response to the fish's natural range and habitat. Gonads undergo regular seasonal cyclical changes in size and weight both in males and females (Efizon *et al.*, 2021)[1]. The simplest and most commonly used method for depicting the annual reproductive cycle in fishes is the gonadosomatic index (GSI). (Hasan *et al.*,(2021)[2], Hanida *et al.*,(2021)[3]. GSI provides an idea regarding the pre-spawning, spawning and post-spawning season of the fish (Sidik *et al.*, 2020)[4]. The determination GSI provides information concerning testicular regression and recrudescence (Sidik *et al.*, (2020)[4]. GSI is most often utilized as an indicator of sexual maturation and mating season (Ain *et al.*, 2021)[5], Ardelia *et al.*, (2023)[6], Dwirastina and Marson, 2021), FAO, 2022)[7, 8], Efizon *et al.*, 2021)[1]. The knowledge of condition factor of the species is also necessary to predict the breeding season, as it is an index of fatness of the fish especially during the period of gonadal maturity (Hasan *et al.*,(2021)[2], Hanida *et al.*,(2021)[3]. The sex ratio of the population is analysed to find out whether it deviates significantly from the hypothetical distribution of 1:1 or not (Hasan *et al.*, (2020). The preponderance and synchronized activity of males and females during spawning are also revealed through sex ratio (Tamsil & Hasnidar, 2019, 2024)[9], Ain *et al.*, 2021)[5], Ardelia *et al.*, 2023)[6], Dwirastina and Marson, 2021), FAO, 2022; Efizon *et al.*, 2021)[7,8,5], Yani *et al.*, 2019)[10].

So in the present study, seasonal changes in various reproductive parameters such as gonadosomatic index (GSI), condition factor, relative abundance of fish stages, size at first maturity, of adult fish and sex ratio were analysed to determine the spawning potential and fecundity of *P. vittatus* in relation to the seasonal changes.

MATERIALS AND METHOD

Field sampling and physical conditions

Field sampling was carried out from June 2023 to November 2024. Specimens of *Puntius vittatus* were collected fortnightly by random sampling method using a hand net of 4mm mesh size from a perennial pond fed by Tamiraparani River at Tirunelveli (Tamil Nadu) 8.44°N and 77.44° Each sample consisted of about 300 specimens and was utilized to record data on total length, standard length, body weight, stages of sexual maturity, and length and weight of the gonads. Sex (male or female or immature) was determined either by external appearance or internal anatomy of the gonads.

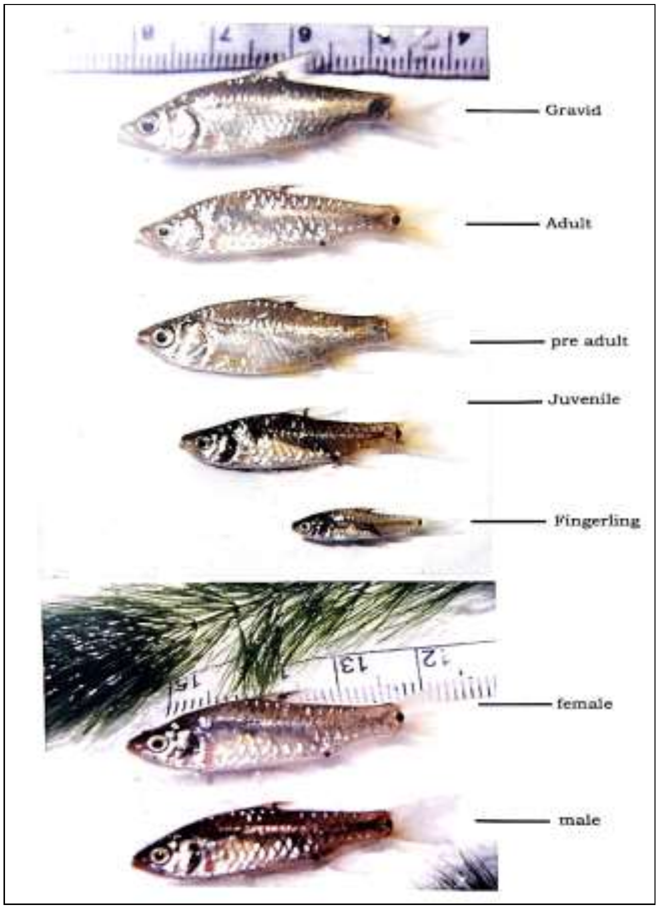

Plate 1: Puntius vittatus: Maturity stages

Gonadosomatic Index (GSI)

The ovaries and testes of *Puntius vittatus* were dissected out from the body cavity and weighed to the accuracy of 0.01g using an electronic balance. The gonadosomatic index (GSI) for each male and female *P. vittatus* was determined by using the following formula (FAO, 2022),

$$GSI = (W_1 / W_2)100$$

Where, W_1 is the wet weight of the gonad in grams and W_2, the wet weight of fish in grams. Changes in GSI values plotted against the sampling months were used to determine the spawning season of *P. vittatus* (Dwirastina and Marson, 2021).

Condition factor

The condition factor (K) of *P. vittatus* was determined using the equation adopted from Hasan *et al.*,(2020).

$$K = 100\ W / L^3$$

Where, W is the body weight in grams, and L is the standard length in cm.

Size at first maturity

The females examined during the spawning season were pooled together to determine the size at first sexual maturity. Size at sexual maturity (Lm_{50}) for *P. vittatus* was determined with the GSI-method by plotting GSI values against the standard length. and validated by the presence of vitellogenic oocytes or mature sperm (Tamsil and Hasnidar, 2024)[9]. Lm_{50} was considered as the standard length class, which comprises 50% of the reproductively active population (Hasan *et al* ., 2020). Specimens were categorized into different standard length groups on the basis of their stages of maturity, to determine minimum size at first reproduction. The length at which 50% of the specimens attain maturity is considered the length at first maturity.

Sex-ratio

The sex ratio of *P.vittatus* was calculated from the length wise (SL) occurrence of each sex in the total catch for every month (class I, 25-33mm; class II, 34-42mm and class III 43-51mm) (Hanida *et al.*, 2021). Deviations in sex-ratios from an expected 1:1 ratio were tested using X^2 goodness of fit (Ardelia *et al.*, 2023)[6].

$$\% \text{ males} = \frac{\text{No of males}}{\text{No. of individuals examined}}$$

Data analysis

The relationships between the two sets of variables were analysed by plotting the respective values in a scatter diagram and calculating a regression equation (Arevalo *et al.*, 2023)[11],

$$Y = a + b\,x$$

Where, Y is a dependent variable, *i.e.*, fecundity, x is an independent variable, *i.e.*, fish length, fish weight, ovary length and ovary weight, a and b are the constants;

or in the logarithmic form,

$$\text{Log } Y = \log a + b \log X.$$

The correlation coefficient (γ) was calculated using Stat view 5.0 for each relationship. Data were also subjected to student's t test and one way / two way analysis of variance (ANOVA) (Zar, 1984)[12].

RESULTS AND DISCUSSION

Gonadosomatic Index (GSI)

Mean GSI exhibited a biphasic increase during the reproductive cycle of female *P. vittatus* (Figure 1).

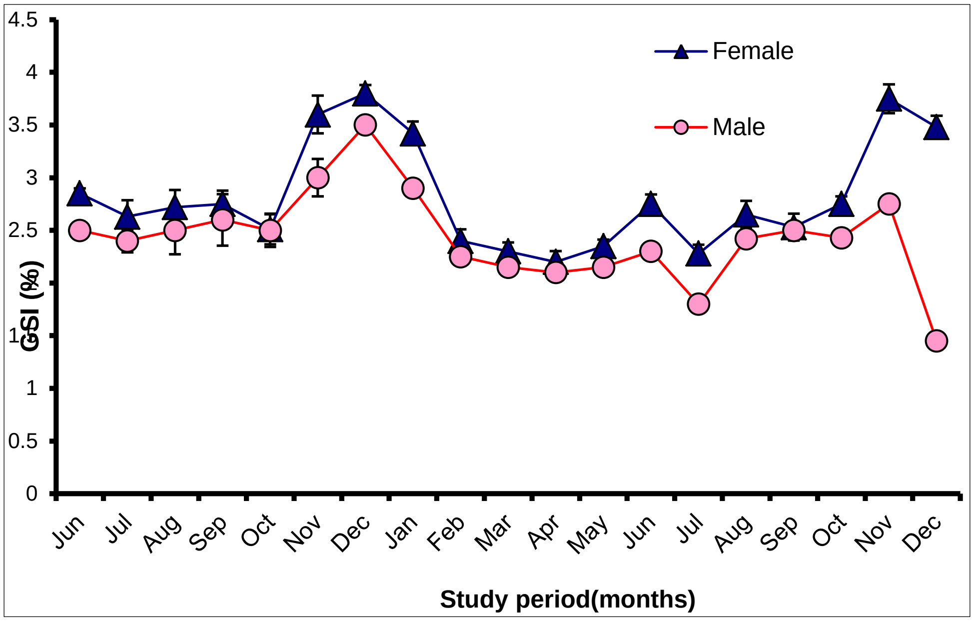

Figure 1: Puntius vittatus.Gonadosomatic indices of females and males plotted against months of the study period from June 2023 to December 2024

The females showed a mean GSI of 2.5% in October (2023) and 3.6% (November) at the threshold of ovulation and spawning. After spawning in December 2023, the GSI declined gradually to 2.57% in January 2024. The GSI again rose to 3.2% during February (2024) and again dropped to 2.2% (March 2024) and in April to 2.1%. The fortnightly values of GSI of females for 18 months are shown in Figure 2a. During June, there was a slight increase in GSI, 2.8 ± 0.41%, indicating a minor spawning peak. The monthly changes in GSI reflect the ovarian activity. The maximum and minimum GSI corresponded to the mature and resting phases of ovarian development, respectively.

The male GSI also showed a biphasic pattern of increase as shown in figure 1. The mean GSI of male was 3.3 ± 0.245% in November, 2.9 ± 0.21% in January (Fig.2b). Closely after the spawning period (February), the GSI got reduced to 2.25 ± 0.215%. During the non breeding months of March (2.2 ± 0.125%) and April the index was very low (2.1 ± 0.115%) which indicative of the complete regression of the testes. In June the GSI of male was slightly higher (2.8 ± 0.135%) indicating a short minor breeding season. From July to September the GSI was low but a little higher than that during the months of February, March, April and May, which suggestive of the pre spawning period. The maximum GSI manifested in an individual male was 3.4%.

Condition factor (k)

Condition factor indicates the physiological well being of the fish. Condition factor of female *P.vittatus* ranged from 2.8 to 2.9% . Seasonal changes in condition factor were uniform during the period from October - November which later declined from February to April in both sexes. By late May and early June there was a discernible rise in condition factor which thereafter declined upto September in both sexes.

Dobyrial index

The Dobriel Index (DI) is used to determine the spawning season for each sex. The mean DI in female during June and July was 0.23 ± 0.12%, thereafter the value declined to 0.22 ± 0.01% in August, then value fluctuated during the subsequent months of September and October and reached the maximum 0.245% in November 0.24% in January . A surge was noticed in the DI of females from the basal DI value (0.215%) of the minor spawning month at June.

Size at first maturity

By plotting the SL against the GSI of female of *P.vittatus* during the spawning season it was observed that the smallest female fish with vitellogenic oocytes measured 2.4cm. No female *P.vittatus* smaller than this had GSI values above 2.5 during spawning season. Based on these results, the size at first maturity for female was established in this study as 3.1cm SL. The smallest mature male fish had a length of 2.45cm.

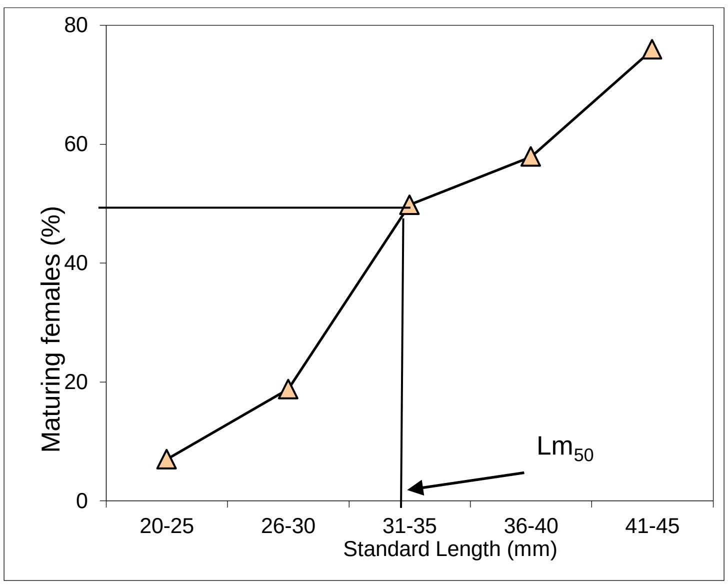

Figure 2: Puntius vittatus Size at maturity females

All female specimens of *P.vittatus* of SL below 2cm were immature . Maturity of *P.vittatus* set in 3.3cm onwards. The length at which 50% of the specimens of *P.vittatus* attained maturity, (the length at first maturity), raned between 3.1cm and 3.5cm SL. The results presented in Table 2 .and Table 3. All male *P.vittatus* below 2.5 cm were immature. Maturity of *P.vittatus* started 2.71cm SL onwards being the length at first maturity, in males, lay between 2.51 and 3cm SL.

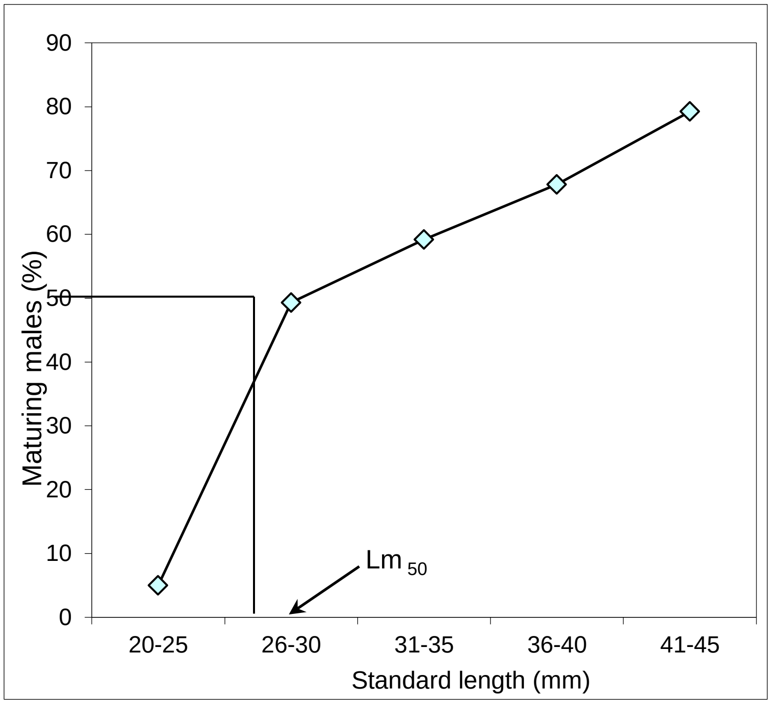

Figure 3: Puntius vittatus Size at maturity males

By another method, the mean size at onset of sexual maturity (L_P 50) in female *P.vittatus* was also estimated as 3.1cm SL in October 2024 (Figure 2). The smallest mature female was found to be 2.9cm SL and the smallest mature male was 2.51cm SL. The females were larger than the males.

Table 1: *P. vittatus* : Gross anatomical features of testicular maturity phases.

Maturity phases	Fish		Testis		GSI		Colour	Macroscopic appearance
	TL(cm)	Weight(g)	Length(cm)	Weight(g)	Range	Mean		
I Immature	0.62 – 2.5	0.32-1.35	0.06 – 1.2	0.007-0.016	0.95-1.55	1.25	Dull white / pale / flesh-coloured	Very thin, slender and thread-like. About half the length of the visceral cavity
II maturing	2 -8	0.3-2.56	0.6-1.4	0.008-0.019	2.18-2.85	2.25	Milky white	Uniformly thickened and enlarged, about three-fourth of the visceral cavity.
III Gravid	3.5-4.4	0.46-1.35	0.7-1.6	0.011-0.021	1.55-2.38	3.0	Milky white	Long, bigger, wide and flat, ribbon-like. Extended full length of the visceral cavity.
IV Spent	3.0-4.2	0.36-0.95	0.5-0.95	0.006-0.009	0.65-1.25	0.95	Coloured	Shrunken, uniformly thin and tubular

Table 2: P. vittatus: Cytomorphological characteristics of different developmental stages of oocytes

Cytological stage	Oocyte stage	Oocyte diameter (µm)	Cytomorphology Nucleus	Cytoplasm
I	Oogonia (OG)	1.5 - 3.00	Large, Spherical	Narrow, Homogeneous
II	Chromatin nucleolar oocyte (CNO)	3.2 – 14.00	Large	Thin layer around nucleus
III	Perinucleolar Oocyte(PNO)	13.2 - 15.11	Large, Oval	Homogeneous, bulky
IV	Cortical alveolar oocyte (CAO)	21.2 - 125.6	Large, Oval	Appearance and dispersion of cortical alveoli and cortical granules dispersed in peripheral ooplasm
V	Early vitellogenic oocyte(EVO)	110 - 315	Irregular periphery	Numerous large acidophilic yolk globules in peripheral ooplasm; lipid bodies near nucleus
VI	Late vitellogenic oocyte (LVO)	297 - 342	GV near animal pole	Increases appearance of yolk granules, densely packed
VII	Germinal vesicle migration (GVM) stage	310 - 362	Migrates to periphery; envelop breaks down	Yolk globules and yolk plates undergo coalescence
VIII	Germinal vesicle break down (GVBD)	345 - 390	Disintegrated nuclear membrane complete nuclear migration	Hydrated
IX	Atretic	395 - 450	Not found	Disorganized vacuolation; oocytes collapsed inward phagocytic granulose cells

Seasonal abundance of maturity stages

The percentage occurrence of female *P. vittatus* with gonads at various stages of maturity Table -3 revealed a distinct periodicity in breeding .

Table 3: *P. vittatus:* Percentage occurrence of female size classes in different stages of maturation during the study period from June 2023 –Nov 2024.

Month of Sampling	Total Length(cm)	Immature (%)	Occurrence % Maturing	Mature (%)	Month of Sampling	Total Length(cm)	Immature (%)	Occurrence % Maturing	Mature (%)
Jun – 23	2.0-3.0	65.71	14.29	20.00	Mar -24	2.0-3.0	70.71	14.29	16.00
	3.1-3.5	16.67	55.12	8.30		3.1-3.5	18.75	62.50	18.75
	3.5-5.0	2.56	38.20	54.63		3.5-5.0	6.25	37.50	56.25
Jul – 23	2.0-3.0	60.00	30.00	10.00	Apr -24	2.0-3.0	50.00	25.00	25.00

	3.1-3.5	20.00	75.00	5.00		3.1-3.5	15.00	50.00	35.00
	3.5-5.0	30.00	46.00	24.00		3.5-5.0	45.00	28.60	26.43
Aug-23	2.0-3.0	65.00	29.55	5.50	May -24	2.0-3.0	63.00	16.67	20.00
	3.1-3.5	21.05	2.63	26.32		3.1-3.5	33.33	55.56	11.11
	3.5-5.0	50.00	28.57	21.43		3.5-5.0	40.00	35.00	25.00
Sep -23	2.0-3.0	52.00	35.00	13.00	Jun- 24	2.0-3.0	66.00	15.00	19.00
	3.1-3.5	9.09	68.18	22.73		3.1-3.5	16.67	58.33	25.00
	3.5-5.0	12.00	36.00	62.00		3.5-5.0	4.772	38.10	42.00
Oct-23	2.0-3.0	83.33	16.67	-	July -24	2.0-3.0	68.00	28.00	4.00
	3.1-3.5	13.04	69.57	17.39		3.1-3.5	11.76	52.94	35.29
	3.5-5.0	5.55	33.33	52.11		3.5-5.0	38.00	37.00	25.00
Nov -23	2.0-3.0	55.00	22.22	34.78	Aug -24	2.0-3.0	67.00	25.00	8.00
	3.1-3.5	13.00	12.00	75.00		3.1-3.5	13.33	66.67	20.00
	3.5-5.0	12.60	17.40	70.00		3.5-5.0	50.00	31.82	18.18
Dec -23	2.0-3.0	26.00	33.33	40.64	Sep-24	2.0-3.0	55.00	30.00	15.00
	3.1-3.5	25.00	40.87	34.13		3.1-3.5	12.50	62.50	25.00
	3.5-5.0	9.00	50.00	41.00		3.5-5.0	10.00	30.00	60.00
Jan -24	2.0-3.0	15.00	15.00	70.00	Oct-16	2.0-3.0	62.50	25.00	12.50
	3.1-3.5	5.88	22.14	71.98		3.1-3.5	10.26	35.00	52.10
	3.5-5.0	5.00	20.00	75.00		3.5-5.0	28.00	20.00	52.00
Feb – 24	2.0-3.0	20.00	71.00	8.00	Nov -16	2.0-3.0	14.00	20.00	66.00
	3.1-3.5	13.33	60.00	26.67		3.1-3.5	10.00	14.17	75.83
	3.5-5.0	5.00	30.00	65.00		3.5-5.0	5.00	20.00	75.00

A major peak in the abundance of gravid females was recorded in November followed by January which then gradually decreased reaching a minimum in February. The peak of the spawning activity of *P.vittatus* was indicated by the availability of more mature females during November and January followed by post-spawning (spent) females in December and February with shrunken ovaries. From February to April, the number of immature fish was found at higher levels and the maximum was is February and March. The recovery of *Puntius vittatus* was noticed from late November to February. In June, there was a partial maturation among the females. In late October, the number of females with developing ovaries was high, and there were many ripening females too. At the end of October and early November, the fish were gravid and ready to spawn. The percentage occurrence of males with gonads in various stages of maturity is shown in Table 4.

Table 4: *P.vittatus*: Percentage occurrence of male size classes in different stages of maturation during the study period from June 2023 – Nov 2025.

Month of Sampling	Total Length (cm)	Immature (%)	Occurrence % Maturing	Mature (%)	Month of Sampling	Total Length (cm)	Immature (%)	Occurrence % Maturing	Mature (%)
Jun- 23	2.0-3.0	70.00	25.00	5.00	Mar-24	2.0-3.0	52.00	28.57	19.00
	3.1-3.5	57.89	15.78	26.32		3.1-3.5	19.23	57.69	23.08
	3.5-5.0	10.00	65.00	25.00		3.5-5.0	35.00	55.00	10.00
July- 23	2.0-3.0	73.00	20.00	7.00	Apr-24	2.0-3.0	33.33	57.00	10.00
	3.1-3.5	18.18	72.73	9.09		3.1-3.5	10.34	58.62	31.00
	3.5-5.0	8.00	64.00	28.00		3.5-5.0	30.00	58.80	12.00
Aug-23	2.0-3.0	89.00	10.00	11.00	May-24	2.0-3.0	53.00	23.08	23.00
	3.1-3.5	17.24	68.97	13.79		3.1-3.5	29.41	58.82	11.76
	3.5-5.0	20.00	17.65	51.23		3.5-5.0	5.26	47.37	47.37
Sep-23	2.0-3.0	65.00	20.00	15.00	Jun-24	2.0-3.0	65.67	28.33	7.00
	3.1-3.5	18.75	65.63	15.63		3.1-3.5	11.11	62.96	25.93
	3.5-5.0	15.00	60.00	25.00		3.5-5.0	15.00	62.00	23.00
Oct-23	2.0-3.0	30.00	50.00	20.00	July-24	2.0-3.0	66.72	20.00	6.66
	3.1-3.5	20.00	12.00	68.00		3.1-3.5	13.04	47.83	39.13
	3.5-5.0	-	33.33	70.12		3.5-5.0	-	20.00	67.12
Nov-23	2.0-3.0	8.10	12.50	-	Aug-24	2.0-3.0	70.00	20.00	10.00
	3.1-3.5	14.29	30.43	45.00		3.1-3.5	16.67	58.33	25.00
	3.5-5.0	8.00	25.00	67.00		3.5-5.0	15.00	28.57	56.43
Dec-23	2.0-3.0	10.00	60.00	30.00	Sep-24	2.0-3.0	60.00	23.5	16.50
	3.1-3.5	6.89	55.17	37.93		3.1-3.5	12.50	16.68	70.80
	3.5-5.0	10.00	50.00	40.00		3.5-5.0	18.00	55.00	27.00
Jan-24	2.0-3.0	15.00	11.11	74.00	Oct-24	2.0-3.0	52.63	66.84	10.53
	3.1-3.5	7.10	37.71	57.14		3.1-3.5	9.09	15.15	80.45
	3.5-5.0	29.41	47.06	85.11		3.5-5.0	15.00	55.00	27.00
Feb– 24	2.0-3.0	33.00	55.00	12.00	Nov-24	2.0-3.0	20.00	20.00	60.00
	3.1-3.5	15.38	61.54	23.08		3.1-3.5	44.44	55.55	55.00
	3.5-5.0	7.14	50.00	42.86		3.5-5.0	28.57	57.14	95.45

Sex ratio

The percentage frequencies of male and female *P.vittatus* were 56.46 / 43.9. This difference was not statistically significant (P<0.05), indicating that the overall sex ratio was around 1:1. The sex composition of random samples of *Puntius vittatus* examined every month showed that the two sexes are present in more (or) less equal number; the variation in the sex ratio was negligible during most months. In August, September, March and July the males of *Puntius vittatus* were found to be more than the number of females. The female and male ratio of *Puntius vittatus* was almost 1:1.5 during September and October (2024).

DISCUSSION

The Gonadosomatic index (GSI) is defined as the proportion of the gonad relative to the total weight of the fish and can be used as an approximation of the maturity stage. The results of monthly changes of GSI values showed that *P. vittatus* had one major spawning peak in north-east monsoon period (November-January) and another spawning peak coincided with south-west monsoon (June). It was found that the period during which GSI was the highest corresponded to the breeding season of the fish. (Hanida *et al*., (2021))[3]. The peak values of testicular weight, lobule diameter, the GSI and sperm concentration in the testes, and the number, size and amount of secretary material in the cytoplasm of the gonodotrophs, coincided with the time of maximum environmental temperature and the longest days in *P .sarana*, (Hasan *et al*.,(2021). Gonadosomatic index (GSI) had been employed many cases to determine or deduce the spawning season of fish stock (Hanida *et al*.,(2021), and thought to be a reliable criterion, especially when supported by other evidences (Arachi and Sumaiya, 2025)[19]. It was assumed that in fish gonads, the maturation of the germ cells was concomitant with the increase of gonad weight, and this increase being expressed by the GSI (Yanaurita *et al*.,(2020)[14]. The GSI values corresponded closely with the developmental morphology of the gonad (Ardelia *et al.*, 2023)[6]. Changes in water temperature and photoperiod correlated with changes of GSI and plasma steroid levels in annual reproductive cycle of fishes (Hasan *et al*.,(2021), Hanida *et al.*,(2021), Mehanna & Farook (2021), and Pratini *et al*.,(2021)[15].

Condition factor is related to nutritional status of fish (Sidik *et al.*, 2020), Sukarman, 2022), Yanaurita *et al*.,(2020)). Condition factor was positively correlated with GSI values in *Clupisoma garua* (Hasan *et al*., 2021). *Hemichromis bimaculatus* (Indira *et al.*, 2013). In the case of *Puntius puntius* (Bhatnagar, 1983) and *Aplocheilus lineatus* (Arachi, 2023)[18], the condition factor decreased at the start of the spawning period due to very high metabolic rates. There was a gradual increase in the condition factor of *Puntius vittatus* during the reproductive period and normalization occurred immediately afterwards.

The size at first reproduction has an important role in the understanding of the life-history adopted by a species during its evolution (Yanaurita *et al*., 2020). Nonetheless, it is accepted that the optimum size for the first maturation depends upon many factors, including the relative allocation of food energy between somatic and gonad growth (Indira *et al.*, 2013) [16]. In *P.vittatus,* the two sexes appear to mature at different sizes, that is 2.51cm in males, and 3.1 to 3.5cm in females. The phenomenon of occurrence of males and females in *P. vittatus* was approximately the same in proportion as reported in a number of fish species. In *P.vittatus,* the sex-ratio did not deviate significantly from the hypothetical distribution of 1:1 Indira *et al.,* (2013)[16] reported in *Hemichromis bimaculatus* that the two sexes were present in more or less equal numbers. The overall sex-ratio approximated unity in five *Barbus species*, but in *P. vittatus* it deviated (Ain *et al.,* 2021).

CONCLUSION

It was observed from the present reproductive studies that *P.vittatus* was a biannual monsoon breeder and an iteroparous species. It had a major spawning season coinciding with the north-east monsoon during November and January, with a spawning peak in January and another spawning season synchronized with the south-west monsoon in June.

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