

Reproductive Biology and histology of the female Brownstripe Red Snapper, *Lutjanus vitta* (Quoy & Gaimard, 1824) off Madras coast along Southeast coast of India

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Abstract

In the present study, 597 specimens (258 nos. females and 339 nos. males) of *Lutjanus vitta* of length range 145-335 mm total length (TL) collected from the trawl catches of the vessel *M.F.V. Samudrika* from January 2007 to December 2008 were utilized. Five maturity stages were determined according to the maturity of the ovary. The major spawning season was during January to March with peak spawning activity during February. The female *L. vitta* reached the first sexual maturity at 160 mm and the sex ratio was 1:1.3 (F:M). The highest value of gonadosomatic index (GSI) was recorded during February and March then decreases slightly in the following months. This slight decrease comes in concomitance with the long spawning season and batch spawning character. The batch fecundity of specimens ranging 160 to 301mm TL varied from 3,717 to 56,090 nos. of ova. The fecundity indices varied from 61.344 to 177.11 nos., number of ova per gram of ovary weight varied from 826.07 to 4217.32 nos. and number of ova per mm of fish length varied from 23.23 to 198.20nos. Monthly distribution of the maturity stages, GSI, egg diameter revealed that this species has a long spawning season extending nearly a year round.

Keywords: Snapper, spawning season, batch fecundity, sex ratio, maturity, ovary

1. Introduction

Snappers (Family: Lutjanidae) are one of the highly valued resources along the southeast coast of India and are caught by trawlers, bottom-set liner and other craft from rocky, coral grounds of 30 - 100 m depth. During 2014, the average landings of snappers was 8,283 t in India, out of 35.9 lakh t of total marine fish landings. Snappers formed 0.6% of the total estimated production (6.6 lakh t) in Tamil Nadu and 64% of the total snappers were landed by trawlers^[1].

In Lutjanids two types of reproductive seasonality are apparent: Continental populations and species exhibit extended summer spawning, and insular populations and species reproduce year round with pulses in spring and fall^[2].

There is consistent evidence that Lutjanids are batch spawners. Distribution of ova diameter from western Atlantic species *L. purpureus*^[3], *L. griseus*^[4], *L. synagris*^[5] and *R. aurorubens*^[6] and the Indo-west Pacific species *L. kasmira*^[7] *Pristipomoides. multidentis* and *P. filamentosus*^[8, 9] all showed marked polymodality, a characteristic generally taken to indicate multiple or serial spawning by individual females during the reproductive season. Marked variations in gonadosomatic indices of fish sampled during spawning season where interpreted to indicate serial spawning season in *R. aurorubens*^[6, 10] and suggested that the low standing reproductive investment of *P. filamentosus* (ovaries of ripe females about 4 % of body weight) made it likely the species was a multiple spawners. Arnold *et al.*^[11] studied the Spawning of red snapper, *L.campechanus* in captivity; Everson^[12] on the spawning and gonadal maturation of the *Etelius carbunculus* in North-western Hawaiian Islands; Maturation, spawning and fecundity of *Pristipomoides filamentosus*, in the North-western Hawaiian Islands^[9].

From Indian waters, Rangarajan^[7], Chowdhary^[13], Kochar^[14] and Acharya^[15] studied the maturation and spawning of fish species occurring in Indian waters such as *Lutjanus kasmira*,

Lactarius lactarius, *Atropus atropus*, *Carangoides malabaricus*, *Nemipterus japonicus* respectively. Bhargava^[16] and Varghese^[17] studied the maturation and spawning of *Upeneus mollucensis* and *Priacanthus hamrur* from west coast of India. Ramachandran *et al.*^[18] studied the age, growth and maturity of *L.vitta* from southwest coast of India. Diversity, occurrence and socio-economic aspects of snappers and job fishes along southeast coast of India were studied by Murugan *et al.*^[19]. The present studies is emphasised on the reproductive biology and histology of the female brownstripe snapper off Madras coast along southeast coast of India.

2. Materials and Methods

The samples of *Lutjanus vitta* for the study were collected during January'2007 to December'2008 between the Lat.12°30' N to 13° 32' N and Long. 80°12'E to 80°30'E from the trawl catches of the survey vessel *M.F.V.Samudrika* of Fishery Survey of India, Chennai operating along the lower east coast of India. Samples were also collected from the local landings at Royapuram Fishing harbour. Out of 597 specimens of *L.vitta* collected 258 nos. were females and 339 nos. were males. The fishes were brought to the laboratory and identified based on Allen^[20]. Their total length and weight were noted down after removing the surface moisture with blotting paper. The ovaries were removed from the female specimens and were weighed accurately using electronic monopan balance. The colour, shape and size of the ovaries were observed before preserving in 10% formalin in sea water solution^[21]. Lateral sections of the preserved ovaries were taken at 0.4 microns thickness and photos were taken at 10x on a trinocular microscope fitted with camera. Five stages of maturity scale for ovaries was followed in estimating the maturity stages^[22]. Ova being spherical in shape, measurement of ovum was taken using stage micrometer where in each micro-division was equal to 0.015mm. The hydrated oocyte method is the

best method for batch spawners^[23]. Thirty nos. of hydrated ovaries which has not lost oocytes collected during the peak breeding season were removed and stored in an individual vial of 10% formalin in sea water solution for batch fecundity studies. The ovaries were removed from the formalin fixative and blot dry with blotting paper, the ovarian membrane was broken and three samples from each lobe were removed i.e. one about one-third of the distance from each end of the ovary to insure that no two samples come from the same portion of the ovary. The samples were weighed to nearest 0.1mg. After

loosening the oocytes by gently tapping the piece of ovary with blunt tip of forceps they were placed on a glass slide and covered with 3-4 drops of glycerine and spread over the slide and observed under the microscope. The hydrated oocytes were counted with the help of had counter. Oocyte counts were taken by following standard procedure and the batch fecundity was calculated by following Hunter *et al.*^[23].

3. Results

3.1 Maturity studies

Table 1: Five maturity stages of ovary were recognized as follows:

Maturity stages	General and Microscopic characteristics of ovaries	Histological characteristics of ovaries
Stage-I (Immature)	Ovaries translucent, colourless, threadlike in appearance and occupy less than one third of the body cavity. Ova are not visible to naked eyes, but under microscope ova are irregular in shape, with a clear nucleus and transparent as yet yolk is not formed.	Nucleus is clear and unyolked, Chromatin nucleolus stage, where in the oocytes are small and spherical with distinct thin basophilic cytoplasmic zone. The peri nucleolus stage wherein the oocytes are oval in shape still having a strong basophilic, homogenous cytoplasm and surrounded by a thin flattened epithelium. The nucleoli started to arrange near the nuclear membrane. (Figure.1A)
Stage-II (Maturing)	Ovaries yellowish white in colour and still thread like in appearance; ova are visible to naked eyes; under microscope ova are spherical in shape, partly opaque in appearance due to commencement of yolk development.	Lipid yolk vesicle characterised by the appearance of lipid and yolk vesicles in the cytoplasm. At the beginning of this stage the cytoplasm appears as two layers. The inner is large dark and more basophilic, while the outer layer is lighter and comparatively less basophilic. The nucleoli were arranged on the boundary of the Nucleus. More accumulation of the yolk in the cytoplasm was observed and hence increasing the oocyte diameter. (Figure.1B)
Stage-III (Mature)	Ovaries light yellowish in colour and enlarged in size; ova are clearly visible to naked eyes; under microscope spherical in shape and opaque in appearance except the transparent periphery.	The oocyte becomes larger in size with the increase of yolk globules and oil droplets. The nucleoli were arranged in the nuclear boundary. Many yolk globules are fused together due to yolk liquefaction at the beginning of this stage as well as many oil droplets fuse together. The follicular epithelium or follicular layer thickens. (Figure.1C)
Stage-IV (Ripe)	Ovaries deep yellow in colour; with maximum size. Under microscope, ova are spherical in shape and opaque due to huge amount of yolk present. In this stage, ova are with their full size and start to become liberated through oviducts on putting light pressure on the abdomen. Hydrated oocytes ready for spawning were found in this stage.	Ova cell wall having two layers of zona radiata. However the ripe ovary shows oocytes of mainly of all stages particularly of second and third stage along with ripe oocytes confirming the batch spawning activity. (Figure.1D)
Stage-V (Spent)	Ovaries are shrunk, slightly reddish and flabby in nature occupying nearly half of the body cavity. Immature & intermediate groups of ova are present, loosely packed. These had ova belonging to stage II followed by stage IV respectively.	Spent area of the ovaries mainly shows the post ovulatory follicles. Follicles are more when compared to immature ovary. (Figure.1E)

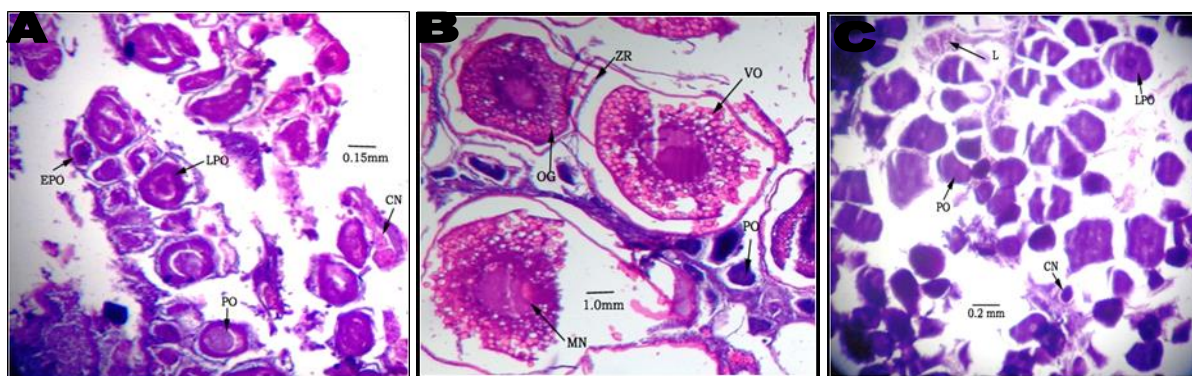


Fig 1: L- Lumen; PO- Perinucleolar oocytes; CN-Chromatin nucleolar oocyte. PO- Perinucleolar oocytes; CN- Chromatin nucleolar oocyte; NO- Nucleolus; VO- Vitellogenic oocyte; YG- Yolk granules; N- Nucleus; YD-Yolk deposition; EPO- Early perinucleolar oocytes; ZR: Zona radiata.

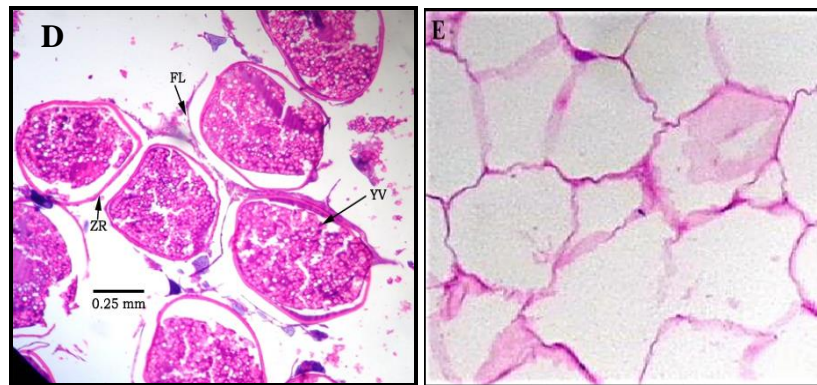


Fig 1: *Lutjanus vitta* ovary, A- Stage-I (Immature); B- Stage-II (Maturing); C- Stage-III (Mature); D-Stage-IV (Ripe); E-Stage-V (Spent).

Spawning season

The percentage occurrence of ovaries of *L. vitta* in various maturity stages during different months are given in Table. 2 and at different length groups are given in Figure: 2. The highest percentage of specimens with ripe ovaries were found during February (31.7%) followed by March (26.2%) and January (25.9%). Percentage of spent ovaries (Stage V) were also found mostly throughout the year but were more during March-May with peak during March(33.3%) followed by April(31.4%) and May (30.8%). Large number of specimens having immature ovaries (Stage I) stages were found mainly during November (57.1%) followed by October (54.5%) and September (50.0%). Stage II ovaries were found more during

the month of June (50.0%). Inferring the percentage occurrence of mature gonads (Stage IV) during different months it appears that *L. vitta* is having a protracted spawning season through the year and has major spawning season during January to March with peak spawning activity during February.

The female specimens of *L. vitta* of stages I and II were recorded up to 231-250 mm length range. III and IV stages were recorded from 151-170 mm and up to 291-310 mm. Dominance of stage IV ovaries was noticed at 271-290 mm length range followed by 291-310 mm length range. Spent stage was noticed at specimens having length range 171-190 mm and 291-310 mm length range suggesting batch spawning.

Table 2: Month-wise Numbers and Percentage occurrence of different maturity stages of ovaries of *L. vitta*.

Month	Total		Stage-I		Stage-II		Stage-III		Stage-IV		Stage-V	
	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%
January	27	10.5	03	11.1	01	3.7	09	33.3	07	25.9	07	25.9
February	41	15.9	01	2.4	03	7.3	13	31.7	13	31.7	11	26.8
March	42	16.3	04	9.5	02	4.8	11	26.2	11	26.2	14	33.3
April	35	13.6	02	5.7	11	31.4	05	14.3	06	17.1	11	31.4
May	26	10.1	02	7.7	10	38.5	02	7.7	04	15.4	08	30.8
June	14	5.4	00	0.0	07	50.0	03	21.4	01	7.1	03	21.4
July	12	4.7	02	16.7	03	25.0	02	16.7	02	16.7	03	25.0
August	07	2.7	03	42.9	01	14.3	01	14.3	01	14.3	01	14.3
September	08	3.1	04	50.0	03	37.5	01	12.5	00	0.0	00	0.0
October	11	4.3	06	54.5	01	9.1	02	18.2	01	9.1	01	9.1
November	14	5.4	08	57.1	02	14.3	02	14.3	02	14.3	00	0.0
December	21	8.1	10	47.6	05	23.8	03	14.3	02	9.5	01	4.8
Total	258	100.0	45	17.4	49	19.0	54	20.9	50	19.4	60	23.3

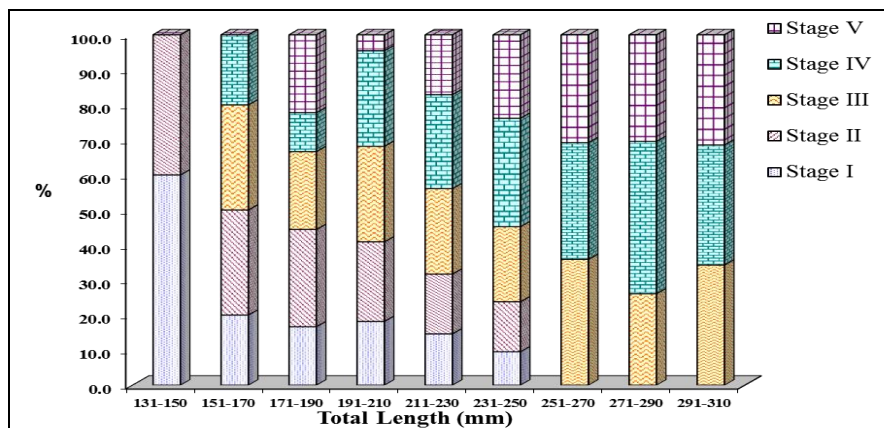


Fig 2: Percentage occurrence of *L.vitta* females in different stages of maturity among different length groups.

3.2 Spawning Periodicity

The frequency distribution of ova diameters taken from different ovaries were grouped at 0.75mm intervals and the percentage variation of different sizes of ova in different stages are presented in Figure.3. In the immature stage, ova diameter ranging from 0.151-0.225 mm were dominant with frequency percentage 64.2% followed by <0.150 mm diameter with 25.5% and 0.226-0.300 mm with 9.4%. In the stage II ovaries the dominance of ova was noticed from 0.226-0.300 mm diameter with 39.8% followed by ova diameter ranging from 0.151-0.225 mm with 28.2% and 0.301-0.375 mm with 23.2%. In ovaries belonging to Stage III, the major mode was at 0.526-0.600 mm with 23.7% followed by 0.451-0.525 mm with 19.3% and 0.601-0.675 mm with 14.4%. In ripe ovaries, the ova diameter ranged from 0.676-0.750 mm were dominant with 23.9% followed by 0.751-0.825 mm 20.5% and 0.601-0.675 mm with 18.3%. In the spent stage i.e., the Vth stage the ovaries were having the ova of different size range among them the ova diameter ranging from 0.301-0.375 mm with 30.0% were dominant followed by 0.226-0.300 mm with 24.0% and 0.676-0.750 mm with 14.0%.

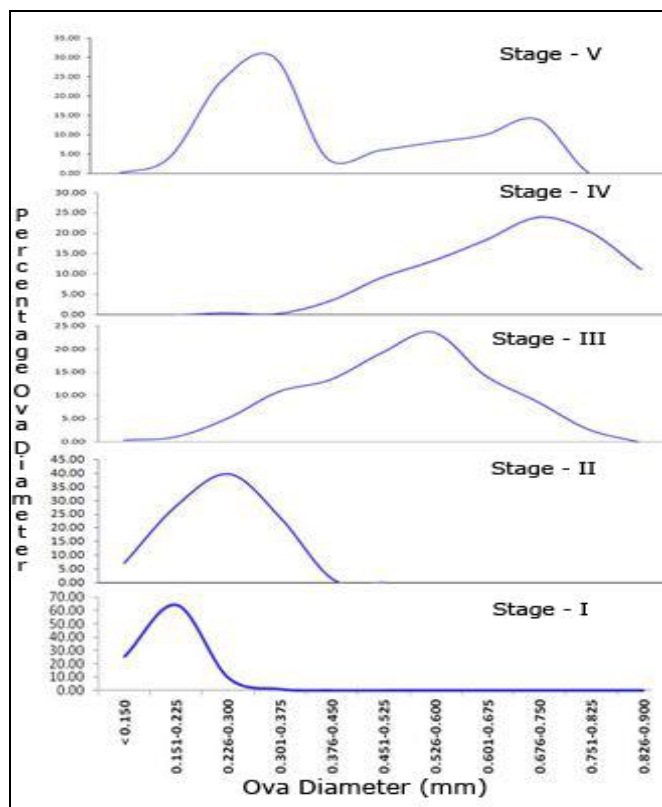


Fig 3: Percentage distribution of Ova diameter in Different stages of *L.vitta* ovaries.

3.3 Batch Fecundity

Thirty nos. fully ripe ovaries collected during the peak breeding season were used for the estimation of batch fecundity. In order to determine the most appropriate sites in the ovary from which to take subsamples to determine batch fecundity of *L. vitta*, the number of eggs/mg tissue was determined for six regions of the ovary of five fishes. Subsamples were taken from anterior, middle and lower part of the left and right ovary. There were no significant differences between anterior, middle and lower (ANOVA, $F=0.169$, $df = 2$, $p = 0.845$). Further, there was no significant difference in numbers of eggs between each lobe. The paired t-test indicated that there was no significant difference between left and right ovaries ($t=0.000094$, $df = 2$, $p=0.99$). The batch fecundity of *L. vitta* varied from 3,717 to 56,090 ova and the length ranged from 160 mm to 301 mm (TL). The mean batch fecundity was 21,016 ova. The smallest mature female was of 160 mm TL which was very near to length at first maturity. The fecundity increased exponentially with fish length. The relationship between the mean batch fecundity and fish length (TL) of *L. vitta* during the peak spawning period is given Figure 4.

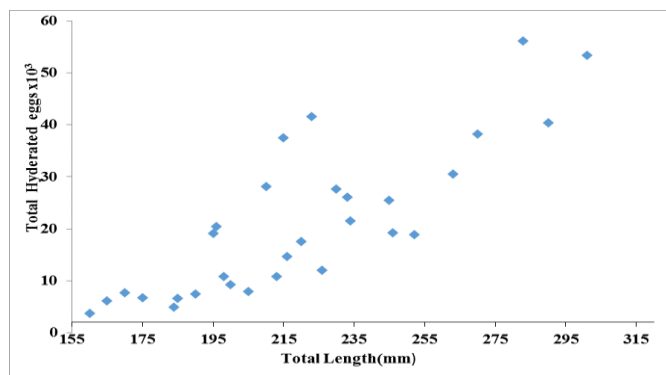


Fig 4: Relationship between batch fecundity and fish length of *Lutjanus vitta* during peak spawning periods.

The fecundity indices were calculated for 15 specimens of total length varying from 160 to 301 mm. The number of ova per gram of fish body weight varied from 61.344 to 177.11nos, number of ova per gram of ovary weight varied from 826.07 to 4217.32 and number of ova per mm of fish length varied from 23.23 to 198.20. The fecundity indices of *L. vitta* is given in Table. 3.

The relative batch fecundity or average number of ova per gram of fish body weight was high for *L.vitta* (122.24), Average No. of ova per gram of ovary weight was 2547.47. Average no. of ova/mm length of fish was 102.50.

Table 3: Fecundity indices of *Lutjanus vitta*.

S. no	TL	Wt.	Ovary Wt.	Total Batch Fecundity	body weight (gm)	No. of ova/gm body Wt.	No. of ova/gm ovary Wt.	No. of ova/mm length of fish
1	160	65.00	4.50	3717	60.50	61.44	826.07	23.23
2	170	80.00	5.80	7659	74.20	103.22	1320.52	45.05
3	195	105.50	8.10	18778	97.40	192.79	2318.24	96.30
4	210	155.00	8.60	24787	146.40	169.31	2882.21	118.03
5	226	180.00	7.80	12040	172.20	69.92	1543.59	53.27
6	234	206.00	8.00	18130	198.00	91.57	2266.25	77.48

7	245	210.00	8.60	25429	201.40	126.26	2956.90	103.79
8	250	223.00	9.80	24615	213.20	115.45	2511.73	98.46
9	252	209.00	8.00	18908	201.00	94.07	2363.54	75.03
10	255	235.00	10.00	25935	225.00	115.27	2593.53	101.71
11	263	240.50	10.20	30256	230.30	131.38	2966.31	115.04
12	265	266.00	11.50	29956	254.50	117.71	2604.87	113.04
13	270	276.00	12.10	38279	263.90	145.05	3163.55	141.77
14	283	330.00	13.30	56090	316.70	177.11	4217.32	198.20
15	301	448.00	14.50	53322	433.50	123.00	3677.36	177.15
Average	215.27	9.39	25860	205.88	122.24	2547.47	102.50	

3.4 Size at first Maturity

The stage I & II were treated as immature and stage III, IV & V were considered as mature for calculating the size at first maturity. The percentage occurrence of females various stages of maturity were grouped in 200mm (TL) group.

In *L. vitta* the length at maturity where 50% of the females are mature (L_{50}) was 160mm (TL) and was observed that the fishes of above 251mm (TL) all were mature followed by 231-250 mm where 76.2% of the specimens were mature. The percentage of mature fishes at different length groups of female *L. vitta* are given in Figure 5.

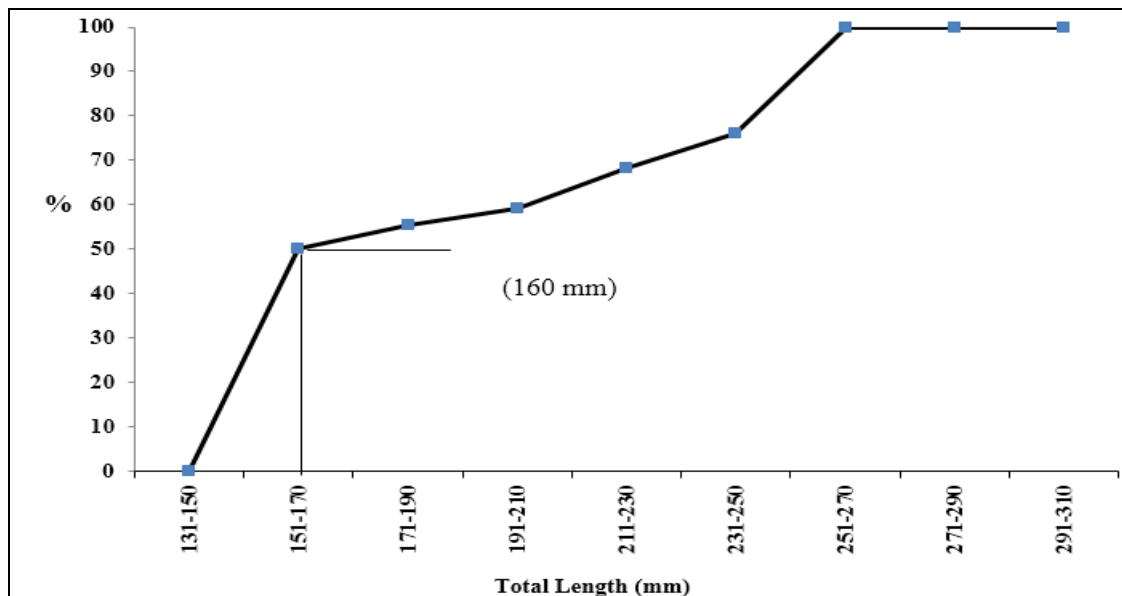


Fig 5: Percentage of mature fishes at different length groups of *Lutjanus vitta* females.

3.5 Sex Ratio

A total of 597 specimens were considered to determine sex ratio in *L. vitta* (Table. 4). Out of 597 species examined, 258 were females and 339 were males with a sex ratio of 1:1.3 (43.2% females and 56.8% males). The Chi. Square (χ^2) value was 25.43, $P = 4.6e-7$ and there was no significant difference. The total length ranged from 131 to 310mm. The month wise sex ratio varied from 1:1.1 during April and rose to 1: 2.1

during November. In the all the months the males outnumbered the females except during the month of April wherein both were of nearly equal ratio. While observing the sex ratio against total length of the fish it is seen that the sex ratio was 1:1.1 in the length range 171-190 mm (TL) and later increased to 1:1.7 in the length range 291-310 mm (TL) which infers that the male dominates after 291mm of length in *L. vitta*.

Table 4: Sex- ratio of *Lutjanus vitta* during different months.

Size range (in mm)	Total number of observations	No. of observations		Percentage		Sex-ratio	Chi. Sq (χ^2)	Probability	Remarks
		Female	Male	Female	Male	F/M			
131-150	11	5	6	1.94	1.77	1:1.20	0.200	0.655	NS
151-170	22	10	12	3.88	3.54	1:1.20	0.400	0.527	NS
171-190	38	18	20	6.98	5.90	1:1.11	0.222	0.637	NS
191-210	52	22	30	8.53	8.85	1:1.36	2.909	0.088	NS
211-230	93	41	52	15.89	15.34	1:1.27	2.951	0.085	NS
231-250	94	42	52	16.28	15.34	1:1.24	2.381	0.122	NS
251-270	83	39	44	15.12	12.98	1:1.13	0.641	0.423	NS
271-290	111	46	65	17.83	19.17	1:1.41	7.848	0.005	S**
291-310	93	35	58	13.57	17.11	1:1.66	15.114	0.000	S**
Total	597	258	339	100.00	100.00	1:1.31	25.430	4.6e-7	S**

NS- Non Significant. S** - Significant at 1% level. S* - Significant at 5% level.

3.6 Gonadosomatic index (GSI)

The GSI varied from 2.3 to 6.8. High GSI values due to major spawning season were observed from January to March with peak during February (6.8) followed by March (6.2). Low values of GSI were found during April, June, July, August, September and October. The month-wise variation of the GSI of *L.vitta* are given in Figure.6.

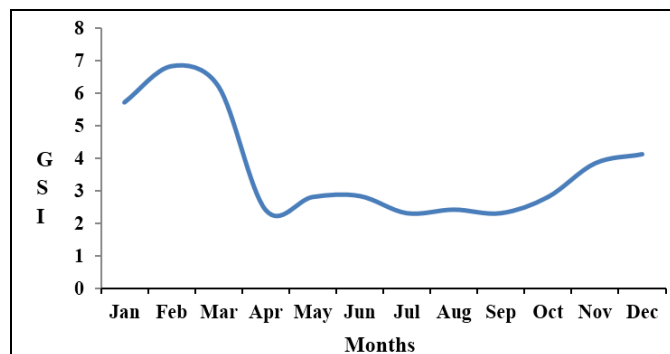


Fig 6: The month-wise variations in the GSI of *L. vitta*.

4. Discussion

Snappers (Lutjanids), like most other species grow to large sizes and are very important for fishery. They are gonochoristic. Following sexual differentiation, sex remains fixed throughout the life. Although studies utilizing histological techniques to examine the gonads are not numerous [24, 25, 26], but only normal gonochoristic testicular and ovarian tissue features are reported. In the present study histological procedure was done for different stages of female gonads of *L.vitta* were made and no evidence of hermaphroditism was observed. The present histological studies where in female gonads were fixed to five stages goes well with the studies of (Davis and West [21] on *Lutjanus vitta* from the Northwest coast of Australia.

The continental *L.vitta* in New Caledonia spawning period is between July to April with peak spawning activity during October to February [27] and the same species in North West Shelf of Australia mainly spawns during September- April with peak spawning activity during September-October even though the species spawns throughout the year [21]. The results of the present study reveals that *L.vitta* though have a protracted spawning period throughout the year the major spawning season is from January to March with peak spawning activity during February.

Striking variation in ovary length and weight of *L.griseus* during reproductive season indicated batch spawning [28]. Similarly, marked variations in GSI of fish sampled during the spawning season were interpreted to indicate serial spawning in *R.aurorubens* [6]. Ralson [10] suggested that the low standing reproductive investment of *P.filamentosus* (Ovaries of ripe females about 4% of the body weight) made it likely the species was a multiple spawners. Everson [24] found spawned-out ovaries only at the end of the spawning season, indicating batch spawning in *E.carbunculus*. Grimes and Huntsman [6] and Everson [12] also found ovaries containing ova in various developmental stages in *R.aurorubens* and *E.carbunculus*, which further suggested batch spawning. From the GSI of *L. vitta* it can be inferred that though they spawn throughout the year one peak spawning activity is observed in the first half of

the year mainly during January to March with peak during February.

The major spawning season of the species under investigation also coincides with spawning duration of *Lethrinus letjan* from December-February and June-August [29]; *Scomberomorus guttauts* from April- May [30]; *Therapon puta* from February-March and August- September [31]; Clupeoid fishes like *Sardinella fimbriata* from January to April [32] and *S.gibbosa* from February-May [33]. In the present studies the ovaries containing the ova of all the stages and Histological studies clearly indicating the batch spawning activity in *L. vitta* and the trends comply with Grimes [2] generalization that the continental species, regardless of latitude, have a restricted spawning season. The mean batch fecundity was 21,016 and mean length was 231mm. Relative ovary weight increases slightly with increasing body size and there is evidence that larger fish spawn more frequently.

Though the present study gives details of the sex maturity, sex ratio, batch fecundity, etc of *L.vitta* off Madras coast, there is a need to study the detailed reproductive biology of all the snappers species occurring along Tamil Nadu coast, as the state lands a major quantity of perches. This will help us to manage the snapper stocks and harvest them in a sustainable way.

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