

Reproductive Biology and Histology of Female Bigeye Snapper *Lutjanus lutjanus* (Bloch, 1790) off Madras Coast Along South-East Coast of India.

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Abstract

The present study envisages on the reproductive biology and histology of the female bigeye snapper (*Lutjanus*, Bloch 1790) off Madras coast along south- east coast of India. The samples were collected from the trawl catches of the vessel *M.F.V. Samudrika* (OAL: 28.8m, GRT: 151T, BHP: 650) from January 2007 to December 2008 between the area 12°30' N to 13° 32' N and 80° 12'E to 80°30'E. A total of 634 specimens (283 nos. females and 351 nos. males) of *Lutjanus lutjanus* were examined. The female *Lutjanus lutjanus* reached first sexual maturity at 122 mm with a range of 801-246 mm total length (TL) of the fish and the sex ratio was 1:1.24 (F:M). Five maturity stages were determined according to the maturity of the ovary. The highest value of gonadosomatic index (GSI) was recorded during February and then decreased 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 125 to 246mm TL varied from 2011 to 28860 nos. The fecundity indices i.e. the number of ova per gram of fish body weight varied from 73.39 to 229.67 nos., number of ova per gram of ovary weight varied from 773.46 to 3960.05 nos. and number of ova per mm of fish length varied from 16.09 to 117.06 nos. Monthly distribution of the maturity stages, GSI, egg diameter revealed that this species has a long spawning season extending nearly a year round.

Key words: Snapper, Spawning season, Batch fecundity, Sex ratio, Maturity, Ovary

Introduction

Snappers (Family: Lutjanidae) are one of the highly valued fishery resources along the south-east coast of India and are caught by trawlers, bottom-set liner and other craft from rocky, coral grounds of 30 - 100 m depth. Snappers form 0.6% of the total estimated production (6.6 lakh t) in Tamil Nadu and the fishery comprises mainly of *Lutjanus lutjanus, L. fulvus, Pristipomoides filamentosus, L. ehrenbergii, L. indicus, L. fulviflamma, L. madras* and *L. quinquelineatus.* 64% of the total snappers are landed by trawlers (CMFRI, 2015).

The main objective of studying maturation and spawning of marine fishes is to identify and predict the biological transformation which the fish undergoes. These studies enable to determine the rate of regeneration of the stocks, estimate the spawning season, spawning potential or fecundity of the species, size at first maturity and sex ratio, which are essential to understand the biological changes of the population. 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 (Grimes, 1987).

There is consistent evidence that Lutjanids are batch spawners, distribution of ova diameter from western Atlantic species *L. purpures* (De Moraes, 1970), *L. griseus* (Campos and Bashirullah, 1975), *L. synagris* (Erhardt, 1977) and *R. aurorubens* (Grimes and Huntsman, 1980); and the Indo-west Pacific species *L.kasmira* (Rangarajan, 1971) *P. multidens* and *P. typus* (Min *et al.*, 1977; Kikkawa 1984) and (Everson, 1984) all show marked polymodality, a characteristic generally taken to indicate multiple or serial spawning by individual females during the reproductive season.

Arnold *et al.* (1978) studied the Spawning of red snapper, *L. campechanus* in captivity; Everson (1984), on the spawning and gonadal maturation of the Ehu, *Etelius carbunculus* in North-western Hawaiian Islands;

Maturation, spawning and fecundity of Opakapaka, Pristipomoides filamentosus, in the North-western Patrícia Gonçalves et

al. (2009) studied the estimates of batch fecundity and spawning fraction for the southern stock of horse mackerel (*Trachurus trachurus*) in Northeast Atlantic ocean.

From Indian waters, Majority of works were mainly on fishery (Alagaraja *et al.*; James *et al.*, 1994), diversity and abundance (Murugan *et al.* 2014). Rangarajan (1971), Chowdhary (1978), Kochar (1988) and Acharya (1990) studied the maturation and spawning of fish species occurring in Indian waters such as *Lutjanus kasmira*, *Lactarius lactarius*, *Atropus atropus*, *Carangoides malabaricus* and *Nemipterus japonicus* respectively. Ramachandran *et al.* (2013) studied the age, growth and maturity of *L. vitta* from south–west coast of India. The present studies is emphasised on aspects of reproductive biology and histology of the big eye snapper from southeast coast of India.

Materials and Methods

Specimens of Lutjanus lutjanus were collected from the survey vessel M.F.V. Samudrika of Fishery Survey of India operating along the lower east coast of India from January'2007 to December'2008. Further samples from local landings were collected from Royapuram fishing harbour. A total of 634 specimens (283 nos. females and 351 nos. males) of Lutjanus lutjanus were examined. The fishes were brought to the laboratory and identified based on Allen (1985) and 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 (Davis and West, 1993). Testes were also preserved in 10% formalin in sea water solution for histological studies and to determine the different stages of development. The female and male gonads were subjected to histological studies for determining the stages. Lateral sections of the preserved testis and ovaries were taken at 0.4 microns thickness and photos were taken at 10x on a tri-occular microscope fitted with



camera. Five stages of maturity scale for ovaries (Qasim, 1973) were followed in estimating the maturity stages. Ova being spherical in shape, measurement of ovum was taken using stage micrometer where in each microdivision was equal to 0.015mm. The hydrated oocyte method is the best method for batch spawners (Hunter et al. 1985). 30 Nos. of hydrated ovaries which had not lost oocytes collected during the peak breeding season were removed from the prior weighed and measured female 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 dried 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 and the hydrated oocytes were counted with the help of hand counter. Wrinkled appearance was observed due to formalin preservation. Oocyte counts from each weighed sample were then converted into numbers per gram to estimate the number per ovary using the formula of batch fecundity (Hunter et al., 1985):

$$BF_{est} = n_w / WW \times GW$$

where,

 BF_{est} = estimate of batch fecundity.

 n_{w} = number counted per wedge sample.

WW = preserved ovary wedge weight (gm).

GW = preserved gonad weight (gm).

Results

Five maturity stages of ovary have been recognized as follows:

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.

Histological section reveals that in immature ovaries the nucleus is clear and unyolked, lumen is clearly visible, Chromatin nucleolus stage, where in the oocytes are small and spherical with distinct thin basophilic cytoplasmic zone and a relatively large nucleus. The perinucleolus stage wherein the oocytes are oval or polygonal 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.

Histological section reveals the beginning of the 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 nuclear boundary. More accumulation of the yolk in the cytoplasm is observed, 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. J. Andaman Sci. Assoc. 21 (2):2016



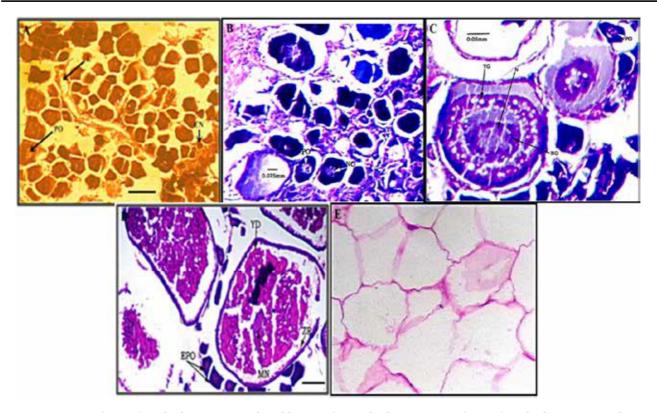
Histological section reveals that furthermore accumulation of yolk is continued. The oocyte becomes larger in size with the increase of yolk globules and oil droplets. The nucleoli were arranged in the nuclear boundary. Many yolk globules are fused together due to yolk liquefaction at the beginning of 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.

Histological section of ripe ovaries reveals the availability of ripe ova showing 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.

Histological section of the spent area of the ovaries mainly shows the post ovulatory follicles. Follicles are more when compared to immature ovary. (Figure 1E)



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

Figure 1. Lautjanus lutjanus 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 female gonads of *L. lutjanus* in various maturity stages during different months are given in Table.1 and at different length groups are given in Figure 2. It could be seen that the female specimens of *L. lutjanus* of stage I and II were recorded up to 220mm length. Occurrence of III, IV and V stage of ovaries were recorded in the size range 121-140mm and they were recorded up to 260mm length. The larger size ranges i.e. 221-240mm and 241-260mm were dominated by stage IV ovaries. The spent stage i.e. Vth stage ovaries were recorded from a wide range of size range such as 121-140mm and up to 260mm length range suggesting batch spawning of the species. Considering the percentage occurrence of mature gonads (Stage IV) during different

months it appears that L. Iutjanus is having a protracted spawning season and has major spawning season during January to April with peak spawning activity during February. The highest percentage of specimens with ripe ovaries were found during February (39.2%) followed by January (34.3%) and March (32.7%). Percentage of spent ovaries (Stage V) were also found mostly throughout the year but were more during February-May with peak during April (41.9%) followed by May (32.3%) and March (36.5%). Stage III ovaries were mainly found during the months of February (27.5%) followed by January (22.9%) and March (20.4%). Large specimens having immature ovaries (Stage I) stages were found mainly during September (61.5%) followed by August (60.0%) and October (33.3%). The larger number of specimens having stage II ovaries was found during the month of July (55.6%).



Months	Total		Stage-I		Stage-II		Stage-III		Stage-IV		Stage-V	
	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%	Nos.	%
January	35	12.4	01	2.9	08	22.9	08	22.9	12	34.3	06	17.1
February	51	18.0	03	5.9	04	7.8	14	27.5	20	39.2	10	19.6
March	49	17.3	05	10.2	05	10.2	10	20.4	16	32.7	13	26.5
April	31	11.0	00	0.0	03	9.7	05	16.1	10	32.3	13	41.9
May	31	11.0	03	9.7	07	22.6	02	6.5	09	29.0	10	32.3
June	28	9.9	03	10.7	08	28.6	05	17.9	05	17.9	07	25.0
July	09	3.2	00	0.0	05	55.6	01	11.1	01	11.1	02	22.2
August	10	3.5	06	60.0	02	20.0	00	0.0	01	10.0	01	10.0
September	13	4.6	08	61.5	01	7.7	02	15.4	01	7.7	01	7.7
October	12	4.2	04	33.3	03	25.0	01	8.3	02	16.7	02	16.7
November	06	2.1	02	33.3	02	33.3	00	0.0	01	16.7	01	16.7
December	08	2.8	02	25.0	03	37.5	01	12.5	01	12.5	01	12.5
TOTAL	283	100.0	37	13.1	51	18.0	49	17.3	79	27.9	67	23.7

Table 1. Month-wise Numbers and Percentage occurrence of different maturity stages of ovaries of *L. lutianus*.

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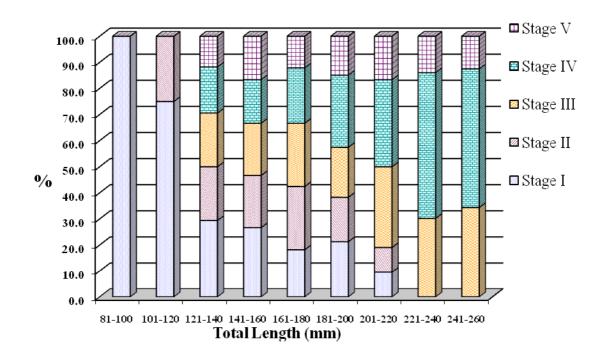


Figure 2. Percentage occurrence of *L.lutjanus* females in different stages of maturity among different length groups.

Spawning Periodicity

Ova-diameter measurements from ovaries at different stages were studied for spawning periodicity of the species. Representative samples of ovaries in different stages of maturity were selected and about 300 ova from each ovary were teased out and their diameter measured. As *Lutjanus* sp. is a batch spawner the ova representing various stages were found present in all the matured ovaries. The frequency distribution of ova diameters taken from different ovaries were grouped at five microdivision intervals and the percentage variation of different sizes of ova in different stages are presented in Figure 3.

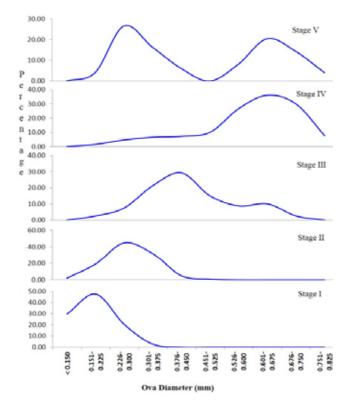


Figure.3. Percentage distribution of Ova diameter in different stages of *L. lutjanus* ovaries.

The ova diameter in the immature ovaries (stage I), ova with 0.101-0.15mm diameter dominated with

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frequency percentage of 47.3%. In stage II ovaries, ova diameter of size range 0.151-0.20mm were dominant with 43.3%. In stage III, ovaries 0.251-0.300mm ova dia were dominant with 30.2% and 0.201-0.250mm (22.0%). The ova diameter of the ova belonging to mature gonads (Stage IV) were at 0.401-0.450mm with 36.1% and 0.451-0.500mm (29.8%). The spent ovaries were having ova of different size range among which the ova diameter ranging 0.151-0.200mm were dominant with 26.5% followed by 0.451-0.500mm (20.4%) and 0.201-0.250mm (16.3%).

Batch Fecundity

30 Nos. fully ripe ovaries 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 *Lutjanus lutjanus*, the number of eggs/mg tissue was determined for six regions of the ovaries of five fishes. Sub samples were taken from anterior, middle and lower part of the left and right ovaries. There were no significant differences between anterior, middle and lower (ANOVA, F= 0.0104, df= 2, p= 0.98). 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= 0.000638, df= 2, p= 0.99).

The batch fecundity of *Lutjanus lutjanus* in specimens of total length ranging 125 to 246mm varied from 2011 to 28860 Nos.. The mean batch fecundity was 10487 Nos. The smallest mature female was of 125mm in TL which was very near to length at first maturity. The relationship between the mean batch fecundity and fish length (TL) of *Lutjanus lutjanus* during the peak spawning period is given in Figure.4. The fecundity increased exponentially with fish length.

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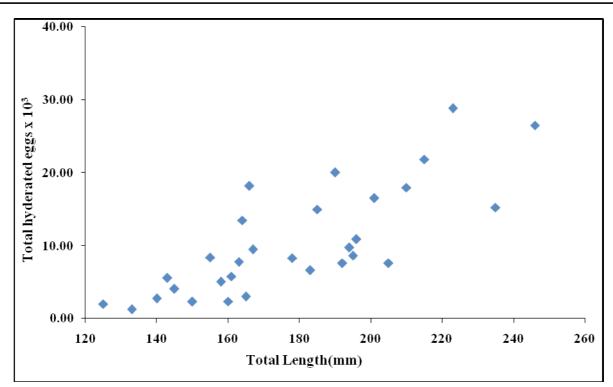


Figure.4. Relationship between batch fecundity and fish length of *Lutjanus lutjanus* during peak spawning periods.

The fecundity indices were calculated for 15 nos. of specimens of total length varying from 125mm to 246mm. The number of ova per gram of fish body weight varied from 73.39 to 229.67 nos., number of ova per gram of ovary weight varied from 773.46 to 3960.05 nos. and number of ova per mm of fish length varied from 16.09 to 117.06 nos. The fecundity indices of *Lutjanus lutjanus* is given in the Table.2.

The relative batch fecundity or average number of ova per gram of fish body weight was high for *L. lutjanus* (115.64), Average nos. of ova per gram of ovary weight was 2025.21. Average no. of ova/mm length of fish for *L. lutjanus* was 50.61.

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Table: 2. Fecundity indices of Lutjanus lutjanus.										
SI No.	. TL Wt. Ovary Wt. Wt.			Total Batch Fe- cundity	body weight (gm)	weight ova/gm		No. of ova/ mm length of fish		
1	125	30.00	2.60	2011	27.40	73.39	773.46	16.09		
2	145	45.60	3.00	4067	42.60	95.48	1355.78	28.05		
3	150	46.00	3.60	3616	42.40	85.28	1004.44	24.11		
4	155	58.00	3.80	8359	54.20	154.23	2199.74	53.93		
5	158	61.00	3.40	5006	57.60	86.90	1472.25	31.68		
6	160	60.00	3.00	3692	57.00	64.78	1230.78	23.08		
7	161	58.00	3.50	5787	54.50	106.18	1653.43	35.94		
8	163	70.00	4.00	8792	66.00	133.22	2198.08	53.94		
9	165	89.50	5.40	19315	84.10	229.67	3576.85	117.06		
10	167	70.00	4.30	10522	65.70	160.15	2446.90	63.00		
11	178	88.00	4.00	8276	84.00	98.52	2069.00	46.49		
12	183	92.00	5.00	6599	87.00	75.85	1319.80	36.06		
13	192	80.00	4.20	7591	75.80	100.15	1807.38	39.54		
14	201	118.00	5.00	16551	113.00	146.47	3310.20	82.34		
15	246	220.00	6.70	26532	213.30	124.39	3960.05	107.86		
Aver		79.07	4.10	9114	74.97	115.64	2025.21	50.61		

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 and males in various stages of maturity were grouped in 200mm (TL) group.

In *Lutjanus lutjanus* the length at maturity where 50% of the females are mature (L_{50}) was 122mm (TL) for female and it was observed that the fishes of above 221mm (TL) were all mature followed by 201-220mm where 81.0% were mature. The percentage of mature fishes at different length groups of female *Lutjans lutjanus* is given in Figure.5.

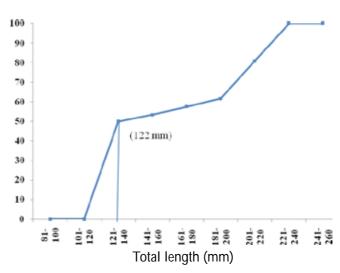


Fig. 5. Percentage of mature fishes at different length groups of *Lutjanus lutjanus* females.



Sex Ratio

A total of 634 species were considered to determine sex ratio in *Lutjanus lutjanus* (Table.3). Out of 364 species examined, 283 were females and 351 were males with a sex ratio of 1:1.24 (44.6% females and 55.4% males). The Chi Square (χ 2) value was 16.339, P = 0.000052 and significance difference was at 1% level. The total length ranged from 108 to 246mm. The month wise sex ratio varied from 1:1 during February and rose to 1:1.9 during September. In all the moths the males outnumbered the females except during the month of February wherein both were of equal ratio. While observing the sex ratio against total length of the fish it is seen that the sex ratio was 1:1 in the length range 81-100mm (TL) and later increased to 1:1.4 in the length range 241-260mm (TL) which infers that the male dominates after 241mm of length in *Lutjanus lutjanus*.

Table. 3. Sex- ratio of Lutjanus lutjanus during different months.

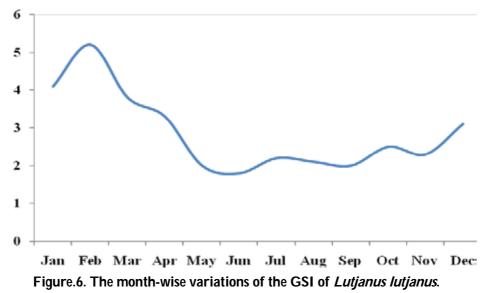
Size range (in mm)	Total number of observa-	No. of observa- tions		Percentage		Sex-ratio	Chi.Sq	Probability	Remarks
	tions	Female Male		Female	Male	F/M	- (χ2)		
81-100	4	2	2	0.7	0.57	1:1.00	0.00	1.000	NS
101-120	46	20	26	7.1	7.41	1:1.30	1.800	0.179	NS
121-140	79	34	45	12.0	12.82	1:1.32	3.559	0.059	NS
141-160	62	30	32	10.6	9.12	1:1.07	0.133	0.715	NS
161-180	74	33	41	11.7	11.68	1:1.24	1.939	0.164	NS
181-200	106	47	59	16.6	16.81	1:1.26	3.064	0.080	NS
201-220	91	42	49	14.8	13.96	1:1.17	1.167	0.280	NS
221-240	94	43	51	15.2	14.53	1:1.19	1.488	0.222	NS
241-260	78	32	46	11.3	13.11	1:1.44	6.125	0.013	S*
Total	634	283	351	100.0	100.00	1:1.24	16.339	0.000053	S**

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

Gonadosomatic index (GSI)

The relative weight or the Gonadosomatic index can be calculated to ascertain the spawning season of a fish. In *Lutjanus lutjanus* the GSI varied from 1.8 to 5.2. High spawning activity was observed from January to April S*- Significant at 5% level.

with peak during February (5.2). Low values ranging from 1.8 to 2.8 were observed mainly during the second half of the year, June being the lowest with 1.8. The month-wise variation of the GSI of *lutjanus lutjanus* is given in Figure.6.



Discussion

Snappers (Lutjanids), 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 (Rodriguez Pino 1962; Alves 1971; Futch and Bruger 1976), normal gonochoristic testicular and ovarian tissue features are reported. Standard histological evidence for hermaphroditism including persistence of an ovarian lumen in testis or testicular tissue embedded in the ovarian mass (Smith 1965; Fishelson 1975), has not been noted. In the present study histological procedure was done for different stages of female gonads of L. lutjanus were made and no evidence of hermaphorditism was observed. The present histological studies where in female gonads were fixed to five stages goes well with the studies of Davis et al. 1993) on Lutjanus vitta from the North-west coast of Australia, Longenecker et al., 2013) on L. fulvus from Papua New Guinea.

The results of the present study reveals that *L. lutjanus* though having a protracted spawning period throughout the year has its major spawning activity during January to April with peak spawning activity during February.

There is consistent evidence that lutjanids are batch spawners. Distribution of ovum diameters from the western Atlantic species L.synagris (Erhardt 1977), L.purpureus (De Moraes 1970), L.griseus (Campos and Bashirullah 1975) and *R. aurorubens* (Grimes and Huntsman 1980); Indo-west Pacific species *P.multidens* and *P.typus* (Min et al. 1977; kikkawa 1984), L.kashmira (Rangarajan 1971), and E.carbunculus (Everson 1984) all show marked polymodality, a characteristic generally taken to indicate multiple or serial spawning by individual females during the reproductive season. Striking variation in ovary length and weight of L.griseus during reproductive season indicated batch spawning to Starck and Schroeder (1970). Similarly, marked variations in GSI of fish sampled during the spawning season were interpreted to indicate serial spawning in R. aurorubens (Grimes and Huntsman 1980), Ralson (1981) suggested that the low standing reproductive investment of P.filamentosus (Ovaries of ripe females about 4% of the body weight) made it likely

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the species was a multiple spawner. Everson (1984) found spawned-out ovaries only at the end of the spawning season, indicating batch spawning in *E.carbunculus*. Grimes and Huntsman (1980) and Everson (1984) also found ovaries containing ova in various developmental stages in *R.aurorubens* and *E.carbunculus*, which further suggested batch spawning. The GSI of *Lutjanus lutjanus* 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 April and later decreases slightly.

The major spawning season of the species under investigation also coincides with spawning duration of Lethrinus letjan from December-February and June-August (Toor 1964) and also Scomberomorous guttatus from April- May(Krishnamoorthi 1958), Therapon puta from February-March and August- September (Prabhu 1956) and clupeoid fishes like Sardinella fimbriata from January to April (Radhakrishnan 1964) and S.gibbosa from February-May (Ganapati & Rao Srinivasa 1957). The ovaries containing the ova of all the stages clearly indicating the batch spawning activity also was further confirmed by Histological studies. In the present study *L.lutjanus* tends to comply with Grimes (1987) generalization that the continental species, regardless of latitude, have a restricted spawning season. The batch fecundity of Lutinaus fulvus from Papua New Guinea varied from 35,305±29141 (mean±SD) and the length varied from 178 to 217mm FL (mean 19.4) (Longnecker et al., 2013). In the present study the mean batch fecundity was 10,487 and mean length was186mm. Relative ovary weight increases slightly with increasing body size and there is evidence that larger fish spawn more frequently.

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

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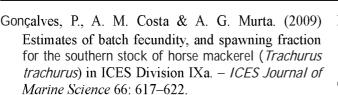
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