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Study of some aspects of reproductive biology of goby, *Parachaeturichthys ocellatus* (Day 1873) from the creeks of Mumbai.

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Abstract

The present study is aimed to assess some aspects of reproductive biology of *Parachaeturichthys ocellatus* (Day 1873) from the creeks of Mumbai. Fish samples were collected every fortnight from the creeks of Mumbai from June 2010 to September 2011. Sexual dimorphism was observed predominantly during breeding months. Overall sex ratio was 1:0.71 for Male: Female indicating dominance of males. The study of maturity stages revealed 56% of matured males and 77% of matured females in the month of February 2011. The length at which fifty percentage of male fish mature (L_{50}) was 91mm while that of female was 94mm. Ova diameter revealed the minimum size of ova was 0.01mm and maximum size was 0.70mm. The frequency polygon of ova diameter in the different stages of maturity showed only one peak suggesting that individual fish spawn only once in the year. Fecundity ranged between 21,635-1,79,334 eggs .Fecundity showed a fairly linear relationship with Total Length, Total Weight, Ovary Length and Ovary Weight and the correlation was highly significant. Gonadosomatic index revealed maximum values in February 2011 and minimum values in November 2010 in both male and female which agrees with maturity stages. The present reproductive study will be useful in enhancing the productivity and successful management of fishery of *P.ocellatus* from the creeks of Mumbai.

Keywords: Sexual dimorphism, Sex ratio, Maturity stages, Fecundity, Gonadosomatic index

Introduction

Reproduction is the sole method through which an organism endeavours to propagate and the evolutionary strategy is to have maximum number of viable off springs for perpetuation of the species. Therefore for studies on biology of an organism, studies on its reproduction are imperative. The secondary sexual characters are the external characters that specially develop during spawning season and/or at maturity and may help identify the sexes by observation of morphology. Sex ratio is the proportion of number of males to the females in a natural population and is an indicator of population behaviour and fecundity (Panthulu, 1961). Mein (1927) introduced gonadosomatic index (GSI) and since then GSI has been widely used as an indicator of relative gonadal development or activity. The high correlation of gonado somatic index with number of matured females and males could be utilised to extrapolate peak spawning season and provide good population level information of reproductive performance

(Durham and Wilde, 2008). Estimation of fecundity is important for acquiring knowledge about different races as different races have characteristic fecundities and egg diameter, which in turn is helpful in recognizing the population whether it is homogenous or heterogeneous type (Shafi, 2012). Fecundity is of central interest in several aspects of fish biology e.g. in recruitment related studies in order to replace spawning stock biomass with total egg production and in studies on life history evolution (Witthames and Marshall, 2008). A thorough review of literature revealed that there is hardly any information on the reproductive biology of *Parachaeturichthys ocellatus*. Therefore the present study was carried out to evaluate various aspects of reproductive biology of *P. ocellatus* from the creeks of Mumbai.

Materials and Methods

Samples of *P. ocellatus* were collected from the creeks of Malad, Vasai, Thane and Mahul creek every fortnight during the period from June 2010 to September 2011. The specimens were cleaned and wiped to remove surface moisture. The total length to nearest millimetre and total weight of the fish to the nearest milligram was noted. The following aspects of the reproductive biology were studied in the samples:

Sexual dimorphism

The morphology of the fish was carefully examined to identify sexually dimorphic characters if any and record was maintained during different months in each fish. The fishes were then dissected and the nature and position of the gonads were recorded.

Sex ratio

The numbers of male and female fish were determined during different months and in different length groups. The Chi-square test was employed to find out the goodness of fit of the observed sex ratio to that of theoretical sex ratio of 1:1 (1male: 1female). The following formula of Bhatnagar (1972) was used for Chi-square test.

$$\chi^2 = \frac{(F-S)^2}{S} + \frac{(M-S)^2}{S}$$

Where χ^2 is the symbol for Chi-Square, F is the observed number of females, M is the observed number of males and S is the expected number of each sex (the hypothetical 1:1 ratio).

Pooled Chi-square (K) was calculated by using the formula of Bhatnagar (1972)

$$K= \frac{(F-M)^2}{F-M}$$

After calculating individual and pooled Chi-square values, the data were tested for heterogeneity.

Maturity stages

The gonads from the dissected fish were excised. The length and weight of the gonads were noted and gonad development stage was determined by visual examination. The stage of maturity and colour of the gonads were recorded. The stages of maturity in fish were determined based on the observation of the gonads. The spawning season was inferred from the observation of matured and ripe gonads in the fish. In all five maturity stages in ovary and testis were considered following the standard laid down by International council for exploration of sea (Lovern and Wood, 1937) and Jayashankar (1991b).

Length at first maturity

The fishes belonging to maturity stage II onwards have been considered as maturing fishes and are used for the purpose of calculating the size at first maturity. A graph was plotted using the percentage of the maturing and mature fish in both sexes in the length group from 66-185mm in males and 66-155mm in females. The size at which 50% fishes were mature was considered as the minimum size for maturity.

Ova diameter

Ova from the preserved ovaries were used for the measurement of ova diameter. An ocular micrometre with capacity of magnification of 0.01mm was used to measure ova diameter. Samples from anterior, middle and posterior region of the ovary were used in all measurements. 500 ova from each ovary were selected as per the methods of June (1953) and Jhingran (1961) for the studies of ova diameter. Spawning frequency was determined based on the peaks in frequency polygon of ova diameter in the mature ovaries.

*Fecundity

To study the fecundity of the fish a small portion of the preserved ovary was excised from the anterior, middle and posterior portion and was weighed to the

nearest 0.1g. The sample of the ovary was then teased out in water with the help of lancet. The matured opaque eggs in each of the three sections of the ovary were counted and the mean numbers of eggs were calculated using the formula:

$$F = \frac{\text{Total weight of ovary}}{\text{Weight of the sub sample}} \times \text{Number of mature ova in the sub sample}$$

A mathematical relationship of fecundity was worked out by the formula proposed by Snedecor (1961) which is as follows: $F= aX^b$ where F is the fecundity, X represents various parameters like total length, total body weight, ovary weight and ovary length, 'a' is the point of intercept and 'b' the regression coefficient. The exponential relationship was transformed into a straight line from the following equation Log F = Log a +b Log X where F is the fecundity, X is the variable and 'a' and 'b' are the two constants.

*Gonadosomatic index (GSI)

The Gonadosomatic index (GSI) was computed by formula based on June (1953) and Yuen (1955) which is as follows

$$GSI = \frac{\text{Gonad weight}}{\text{Total Body weight}} \times 100$$

Results and Discussion

Various aspects of reproductive biology like sexual dimorphism, sex ratio, spawning periodicity, gonado somatic index and fecundity were studied. Various observations were recorded and analysed using appropriate statistical tools.

Sexual dimorphism

The plate no. 1 shows the male and female *P. ocellatus* ventral view. The plate show characters with which the male and female fish could be distinguished by observation of external characters. Sexual dimorphism observed in *P. ocellatus* was prominent during the breeding season. Sexually dimorphic characters noted in *P. ocellatus* during the breeding season were colouration around the pelvic and anal fin, length of the body, depth of the abdomen, length of the second dorsal fin, shape and sturdiness of pectoral fin and pelvic fin, length and shape of urinogenital papillae. The characters like length of the second dorsal fin ray and sturdiness of the pectoral fin were observed throughout the period of study. In *P. ocellatus* males during the breeding season the region around pelvic and anal fins turned reddish in comparison to females. The body of male fish was longer and slender than that of female while the female body was deeper especially the abdomen than that of male. Dorado (2010) and Unito-Ceniza *et al.*, (2012) observed similar body shape in male and female *G. Giuris*. The second dorsal fin ray of the male *P. ocellatus* was longer than that of the female. Gore (2007) observed in *Boleophthalmus boddaerti* that the first, the second, the third and the fourth dorsal fin rays of the male were longer than those of the female. In male *P. ocellatus* the fin rays of second dorsal fin had spots which were darker compared to those on the fin rays of the female fish. The pectoral fins of male *P.ocellatus* were larger and stronger than those of the females. In general the males appeared sturdier than the females. The urinogenital papillae of male *P. ocellatus* were straight, thin, long and pointed while in female they were rounded, short, and fleshy. Similar observations were recorded in urinogenital papillae of *Gobioides broussoneti* (Mata Cortes *et al* 2004), *Glossogobius giuris* (Doha, 1974; Rao and Rao, 2007) and *Boleophthalmus boddaerti* (Gore, 2007). The pelvic fins of male and female are fused together but the anterior end of joint fin in male fish exhibited bifurcation while in female the same was rounded. However the fish were not sexually dimorphic throughout the life span and prominent dimorphic characters were observed during February, March, April, August and September. During the remaining period the sexes had to be determined by observation of gonads. During maturation the testes were white in colour whereas of mature they were creamy white. The ovaries in female *P. ocellatus* were initially yellow in the maturing females and they turned deep orange in the mature state. Thus it can be concluded that *P. ocellatus* exhibited sexual dimorphism during February, March, April, August and September months

Plate no.1: Male and female *Parachaeturichthys ocellatus* (ventral view)

Sex ratio

The variation in sex ratio of *P. ocellatus* is presented in Table no.1. A total of 1174 individuals comprising of 685 males and 489 females were collected in different months during the study period. The overall sex ratio was 1: 0.71, M: F indicating the dominance of males. Sex ratio indicates the proportion of male and female in the population and is expected to be 1:1. Any deviation from equilibrium may be considered to indicate the dominance of one sex over other in the population. Male dominance was recorded in *Glossogobius giuris* from Gosthani estuary (Rao and Rao, 2007) and *Bathygobius soporator* from Badagry creek (Lawson and Thomas, 2010).

During the present study, the dominance of male or female in the overall sex ratio was evident depending upon season. The females dominated males in September, April, May and June 2011 while the males dominated in all the other months from June 2010 to September 2011. In *P. ocellatus* the sex ratio fluctuated around 1:1 and did not deviate significantly at $p \leq 0.05$ during the months of August 2010 at 1:0.83, M: F; September 2010 at 1:1.09,M: F; December 2010 at 1:0.74, M: F; February 2011 at 1:0.76, M: F; March 2011 at 1:0.97, M: F and September 2011 at 1 :0.8, M: F. In February and March 2011 most of the fishes are reproductively mature. In all the remaining months the sex ratio seemed to deviate significantly from the expected ratio 1:1. The deviation from the normal 1:1 sex ratio might be due to differential fishing (Kesteven, 1942), differences in age and size at maturity (Reynolds, 1974), differential behaviour of sexes, differences in morphology and physiological activity (Baglin, 1982), through environmental conditions, (Bal and Rao, 1984) and partial segregation of mature fish through their habitat preference (Parrish *et al.*, 1986). In *P. ocellatus* the average sex ratio was 1:0.71, M: F which was a very narrow range and did not deviate significantly at $p \leq 0.05$. It would be interesting to study the reasons for the fluctuations in sex ratio vis a vis the male and female dominance which varied during different months of the year. Though the near equilibrium of sex ratio was observed during the months from August, September, December 2010, February to June and September 2011 the females were slightly dominant in the population of *P. ocellatus* in the terms of sex ratio during the months of September 2010, April, May and June 2011 whereas the males dominated in the population in all the other months.

Table no. 1: Sex ratio of *P. ocellatus* in different months

Months	Males		Females		Total No. of fishes	Expected no. of fishes	Sex ratio	Chi – square (χ^2)	Significance at p≤0.05
	No. of male	%	No. of female	%					
Jun-10	40	63.00	24	37.00	64	32	1:0.60	4	S
Jul-10	36	68.00	17	32.00	53	26.5	1:0.47	6.811321	S
Aug-10	40	55.00	33	45.00	73	36.5	1:0.83	0.671233	NS
Sep-10	23	48.00	25	52.00	48	24	1:1.09	0.083333	NS
Oct-10	80	62.00	49	38.00	129	64.5	1:0.61	7.449612	S
Nov-10	39	64.00	22	36.00	61	30.5	1:0.56	4.737705	S
Dec-10	70	57.00	52	43.00	122	61	1:0.74	2.655738	NS
Jan-11	50	66.00	26	34.00	76	38	1:0.52	7.578947	S
Feb-11	34	57.00	26	43.00	60	30	1:0.76	1.066667	NS
Mar-11	35	51.00	34	49.00	69	34.5	1:0.97	0.014493	NS
Apr-11	17	41.00	24	59.00	41	20.5	1:1.41	1.195122	NS
May-11	8	47.00	9	53.00	17	8.5	1:1.13	0.058824	NS
Jun-11	17	41.00	24	59.00	41	20.5	1:1.41	1.195122	NS
Jul-11	90	64.00	50	36.00	140	70	1:0.56	11.42857	S
Aug-11	81	60.00	54	40.00	135	67.5	1:0.67	5.4	S
Sep-11	25	55.00	20	45.00	45	22.5	1:0.8	0.555556	NS

Critical value of χ^2 at 1 df = 3.84 at p≤0.05

Pooled $\chi^2 = \frac{(F-M)^2}{F+M} = \frac{(489-685)^2}{489+685} = \frac{38416}{1174} = 32.72$ χ^2 at 31df = 44.98

Ratio 1: 0.71, M:F, Not significant

S- Significant, NS- Not Significant, F- female and M- male

Maturity stages

Miller (1984) and Blaber (2000) stated that all gobies are multiple spawners, they breed for most of the year often in wet season. Spawning occurs at a time when environmental conditions are most favourable for larval survival and development.

The percentage occurrence of males and females in various stages of maturity during different months is presented in Figures 1 and 2. Based on the concept of minimum size at maturity, immature males were found in the sample from June 2010 to September 2011 except from February 2011 to April 2011. Maximum number of immature males was present in July and then in again in November 2010. Developing males were present throughout the period of study except in May 2011. Mature males in stage III were present during following months: August to October 2010, January to April 2011 and again from July to September 2011. Ripe males were observed from July to September 2010, predominantly from February to May 2011 and again from July to September 2011. Spent males were observed in the following months: June 2010, September 2010 October 2010, and April 2011 to July 2011. Maximum percentage (56%) of ripe males was found in February 2011.

Immature females were found in sample from July 2010 to August 2010, November 2010 to January 2011 and again from June 2011 to August 2011. Maximum percentage (73%) of immature females was observed in November 2011. Developing females occurred in the sample from July 2010 to January 2011 and again from June 2011 to September 2011. Mature females in Stage III were observed from June 2010 to October 2010 and then from December 2010 to April 2011 and again from June 2011 to September 2011.

Mature females with ripe ovaries were found in June 2010, from August 2010 to October 2010, from February 2011 to June 2011 and again in August 2011 to September 2011. Maximum percentage (77%) of females with ripe ovaries was found in February 2011.

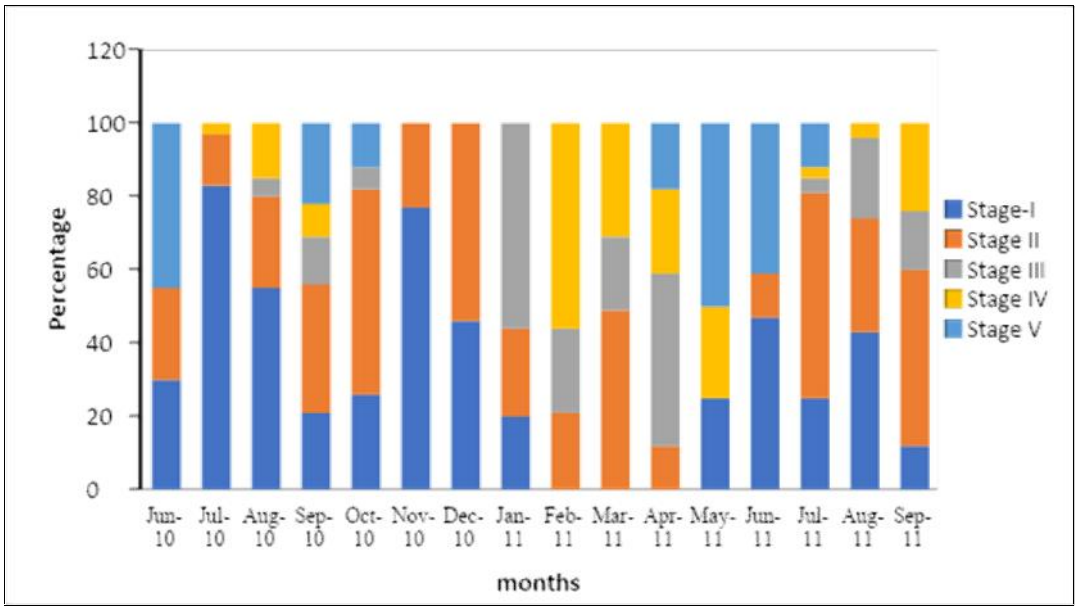


Fig no.1: Maturity stages of testes of male *P. ocellatus* during different months expressed as percentage

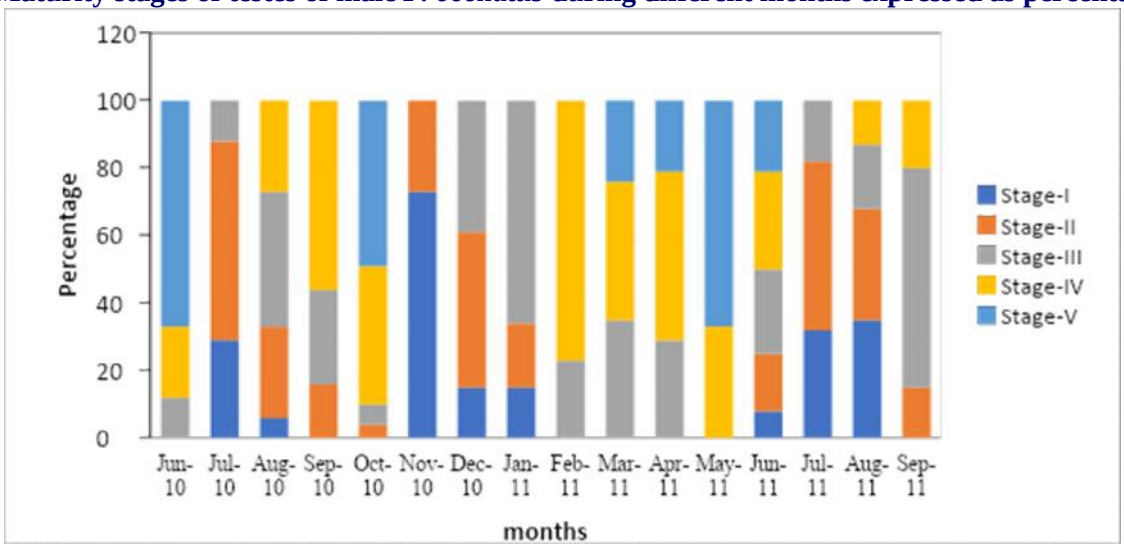


Fig no.2: Maturity stages in ovaries of female *P. ocellatus* during different months expressed as percentage.

Length at which fifty percent of the fish attain maturity

The percentage of mature fishes at each length group was plotted for both male and female. This is shown in Figure no 3 and 4. The size at which 50 percent i.e. L_{50} of the population of *P. ocellatus* mature in male was 91mm whereas in female was 94mm. Thus it can be observed that in *P. ocellatus* males attain sexual maturity at a smaller length group than the females Similar observation have been reported in few other species of gobies by Hoda (1986) in *Gobius pagnellus* L_{50} was 52mm for males and L_{50} for females was 55mm, Engin and Seyha (2009) in goby *Pseudocryptes elongates* L_{50} was 154mm in males and 163mm in females. This may indicate that after attaining maturation females allocate more energy for the production of gametes while males with smaller reproductive effort continue growing (Palazon Fernandz *et al.*, 2001). Bowering (1976) pointed out that differences in growth between sexes are the result of genetics that determine the physiology and behaviour of the fish.

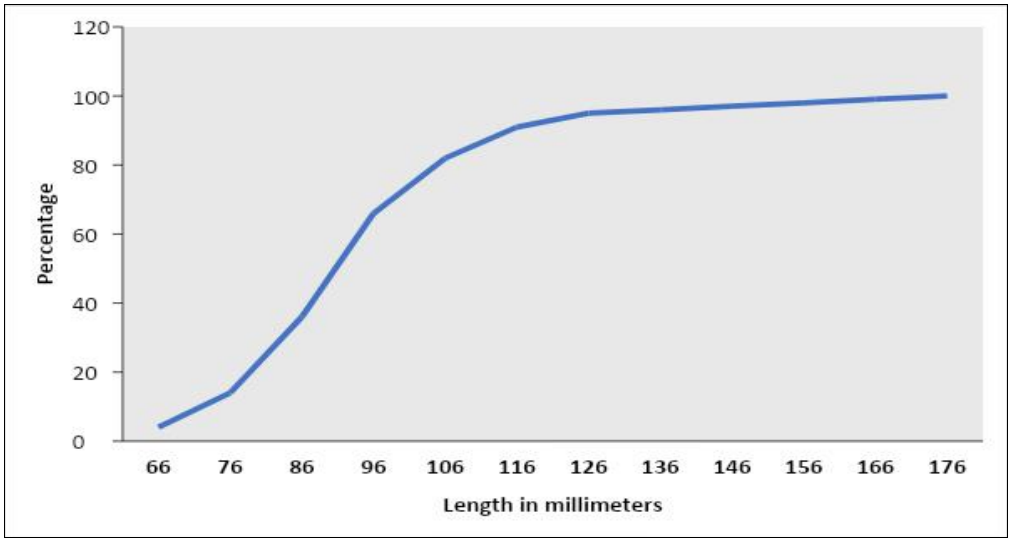


Fig no.3: Minimum size at which 50% of male *P. ocellatus* attain maturity

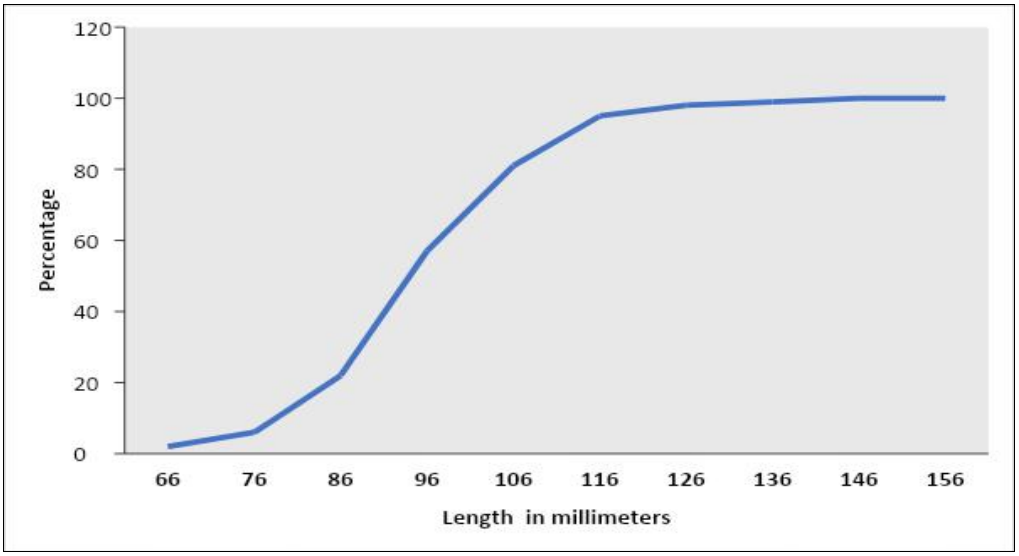


Fig no.4: Minimum size at which 50% of female *P. ocellatus* attain maturity

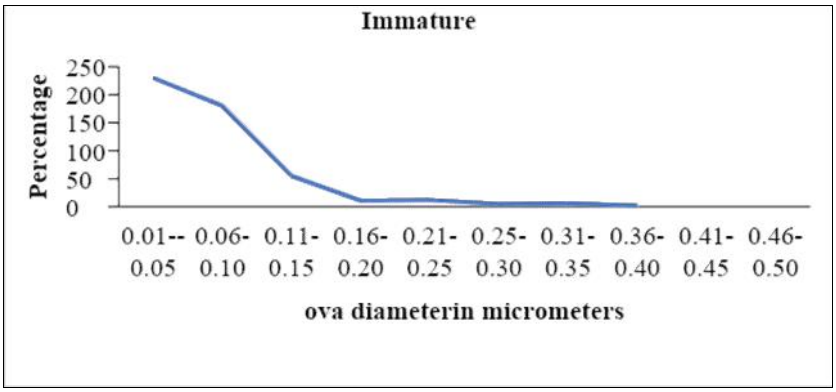
Ova diameter

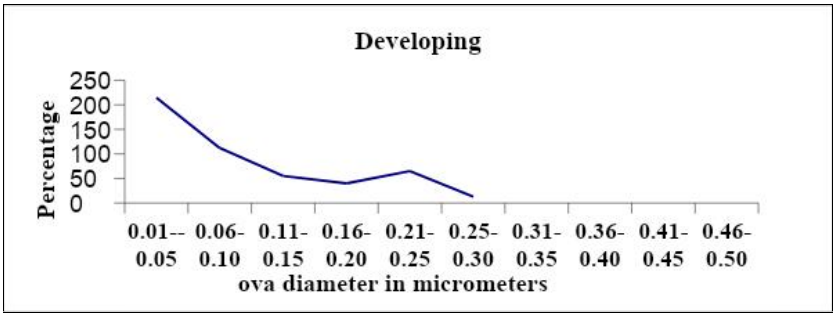
Table no.3 shows the ova diameter in various stages of maturity. The ova diameter of *P. ocellatus* ranged in size from 0.01mm to 0.70mm.

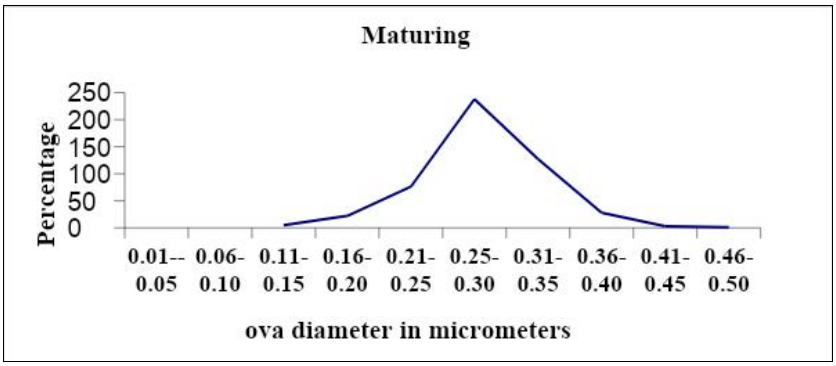
Table no.3 Ova diameter in various stages of maturity.

Stage	Nature of maturity	Size of the ovum
Stage I	Immature	0.01-0.25mm
Stage II	Developing	0.06-0.35mm
Stage III	Mature	0.11-0.55mm
Stage IV	Ripe	0.5-0.70mm
Stage V	Spent	0.06-0.35mm

Ova diameter frequency polygons of different stages were plotted by measuring the diameter of ova of all stages of maturity. Ova diameter frequency polygon at different stages of maturity are presented in Figure no.5. The frequency polygon of ova diameter in different stages of maturity in *P. ocellatus* showed only one peak. A single peak in frequency polygon was also observed in all months except November and December. According to Hickling and Rutenberg (1936) the number of peaks of ova in a frequency polygon in a mature ovary gives a clue to the number of spawning per year. Measurement of ova diameter and their frequency polygon distribution at different times of the months in a year was a common method in determining the maturity cycle of the fish (Macer 1974). During the present study the frequency polygons of ova diameter showed a single peak in each stage suggesting that in *P. ocellatus* individual fish spawns once in the year.







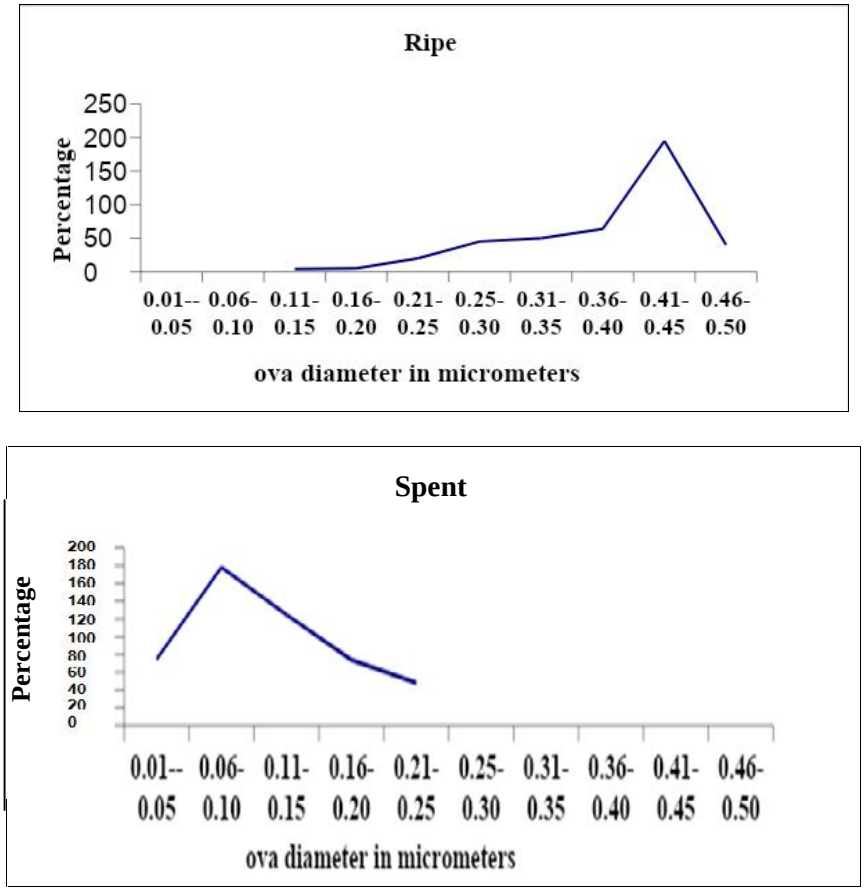


Fig no.5: Ova diameter frequency polygon of *P. ocellatus* in different stages of maturity.

Fecundity

A total of 33 mature females were examined to determine fecundity. The length of the female fishes ranged between 100-153mm and their weight ranged between 10.072-38.383g. The fecundity for the sample of 33 matured female *P. ocellatus* ranged between 21,635-1, 79, 334 eggs with a mean of 48,973 from June 2010 to September 2011. The logarithmic relationship between fecundity and total length of fish was linear and is presented in Figure no.6. The calculated relationship was: Log F = -4.3523+ 4.3671 log total length. The correlation 'r' at 0.9628 indicated a significant relationship at $p< 0.01$.

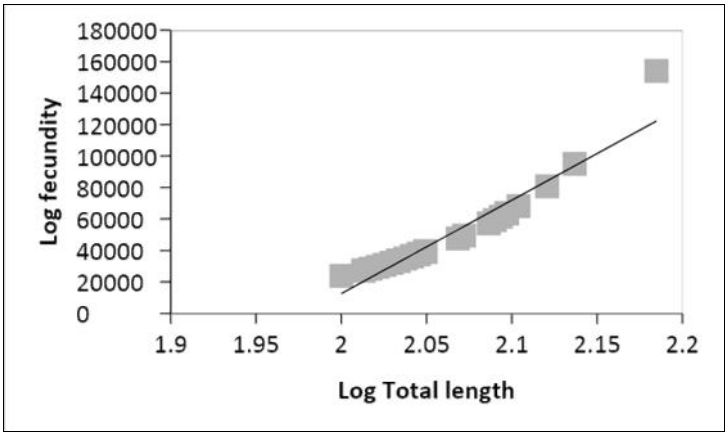


Fig no.6: Fecundity in relation to total length in *P. ocellatus*

The fecundity and body weight also showed a linear relationship and is presented in Fig no.7. The regression equation of fecundity on body weight is expressed as: Log F = 2.7859 + 1.5195 log total weight. The correlation 'r' at 0.9756 indicated a significant relationship between two variable at $p<0.01$.

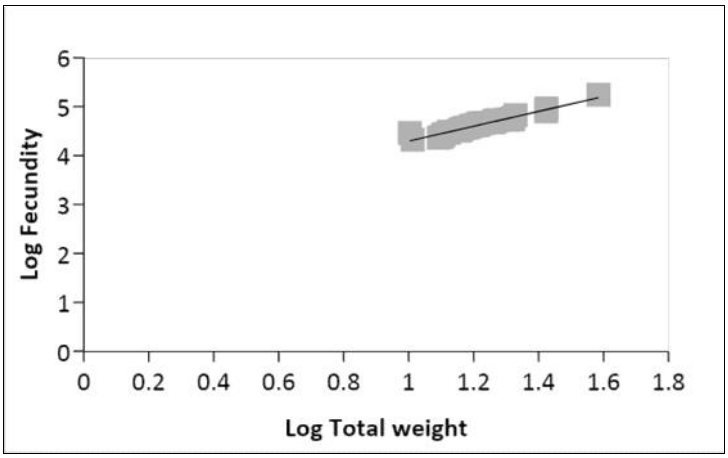


Fig no.7: Fecundity in relation to total weight in *P. ocellatus*

Relationship between fecundity and ovary length and ovary weight were linear and is presented in Figure 8 and 9. The equations that relate fecundity to length and weight of the ovary are:

Log F = -1.7967 + 4.3385 log ovarian weight and Log F = 4.2452 + 1.5182 log ovarian length

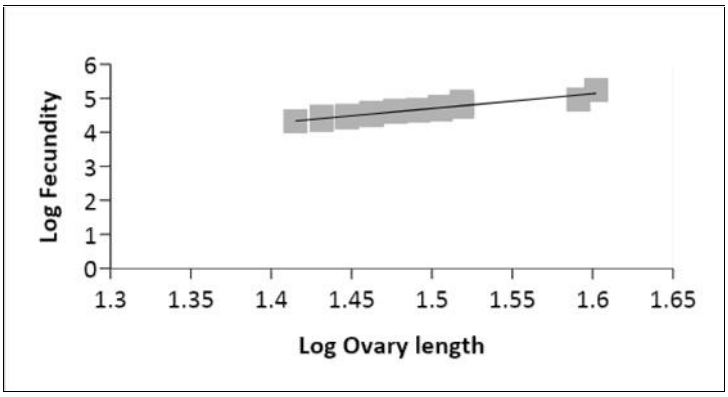


Fig no. 8: Fecundity in relation to ovarian length in *P. ocellatus*

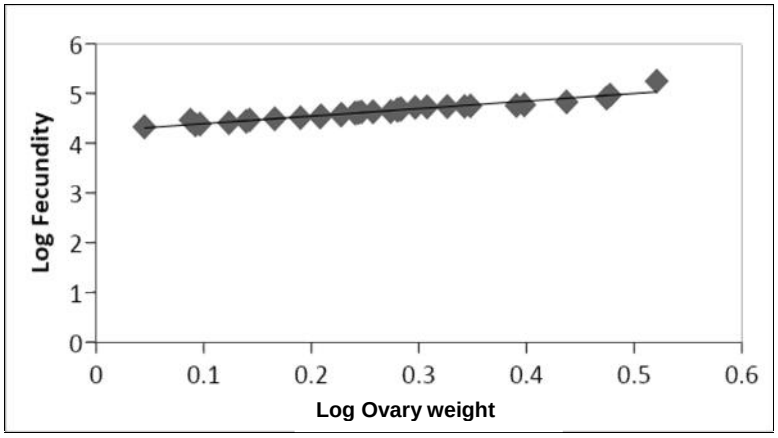


Fig no. 9: Fecundity in relation to ovarian weight of *P. ocellatus*

The correlation 'r' was 0.9705 for the relationships between fecundity and ovary length while it was and 0.9633 for fecundity and ovary weight indicating a high significance at $p<0.01$.

Table no.4 The t values for various parameter

Parameters	t=b-3/Sb	p<0.05	Parameters	t=b-1/Sb	p<0.05
F vs TL	189.99	significant	**F vs TL**	24.85	significant
F vs OL	183.34	significant	**F vs OW**	25.03	significant

The t values for various parameters are shown in Table no.4. It indicates that the regression coefficient 'b' deviates significantly from the value 3 in case of total length and ovary length while it deviates from unity or 1 in case of total weight and ovary weight. The deviations of 'b' values were analysed against 3 in the case of total length and ovary length and against unit or 1 in case of total weight and ovary weight as adopted by Sebastian (2011).

In *P. ocellatus*, the fecundity showed a fairly linear relationship with total length, total weight, ovary length and ovary weight. The positive linear relationship between fecundity and various body parameters was observed by many workers in goby fishes like *Boleophthalmus dussumeiri* (Mutsaddi, 1964), *Glossogobius giuris* (Rao & Rao, 2007), *Stenogobius gymnopomus* (Lekshmi *et al*, 2010. Assessment of fecundity has paramount importance in fisheries management as it provides knowledge about the number of offspring produced in a season and the reproductive capacity of the species (Qasim and Qayyum, 1963).

Gonadosomatic index (GSI)

The GSI for both male and female *P. ocellatus* in different months are presented in Figure 10 and 11. GSI is the ratio of gonad weight to the body weight. The cycle of maturation and monthly variation of GSI are good indicators of the extent of development of gonad with respect to the time of year (Sebastian 2011). In *P. ocellatus* in August 2010 high value of GSI was observed in both male and female fish. The lowest GSI was observed in November 2010 in both male and female fish. The GSI increased progressively from December 2010 to February 2011 with a maximum value in February 2011 in both male and female *P. ocellatus*. The GSI then decreased progressively till May 2011 in both male and female and then the GSI showed an upward trend in June 2011 and again in August 2011 with a dip in July 2011 in both the sexes. Low GSI values in November 2010 and May 2011 are concomitant with a period of early development of gonads when the fish are in a resting period during the gonadal cycle. Thus it can be concluded from the GSI values that *P. ocellatus* may probably breed thought out the year except November and December. The high GSI value from February to April indicates peak spawning which is in agreement with the maturity stages.

The female GSI was consistently higher than males. The consistently high GSI in female *P. ocellatus* probably point to the fact that most of the body reserves may be allocated for the development of ovary. Similar aspects are recorded in many gobiid fishes like *Gobius paganellus* (Miller, 1961), *Gobioides rubicundus* (Kader *et al.,* 1988b), *Boleopthalmus boddaerti* (Ravi, 2000; Gore, 2007), *Stenogobius gymnopomus* (Lekshmi *et al.,* 2010) and *Gobius paganellus* (Hajji *et al.,* 2012). Buxton (1990) pointed out that low value of GSI in case of male is due to low energy investment in gamete production as compared to the female.

It is interesting to note that the GSI values in both male and female *P.ocellatus* follow the same trend in different months. The minimum value of GSI in November 2010 and December 2010 agrees with the stages of gonad maturity that the fishes are in resting phase. Maximum GSI value for both male and female was observed in February 2011 which is in agreement with the stages of maturity indicating that maximum numbers of fishes were in peak spawning period. The drop in values of GSI in May 2011 indicates the termination of spawning season. Thus it can be concluded in general that though high GSI is an indicator of spawning period, for predicting spawning period in *P. ocellatus*, GSI alone is not enough.

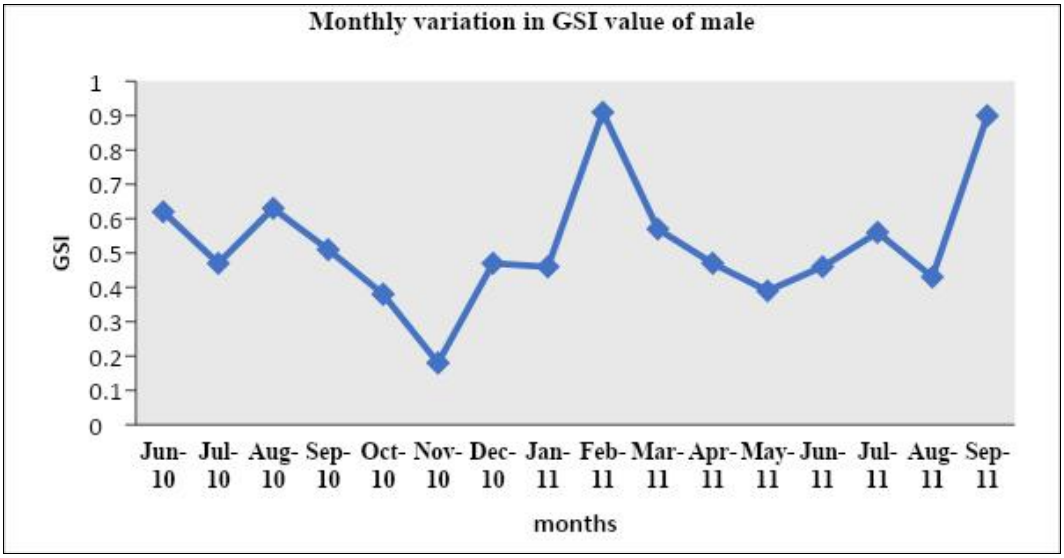


Fig no.6: Variations in gonadosomatic index of male *P. ocellatus* during different months

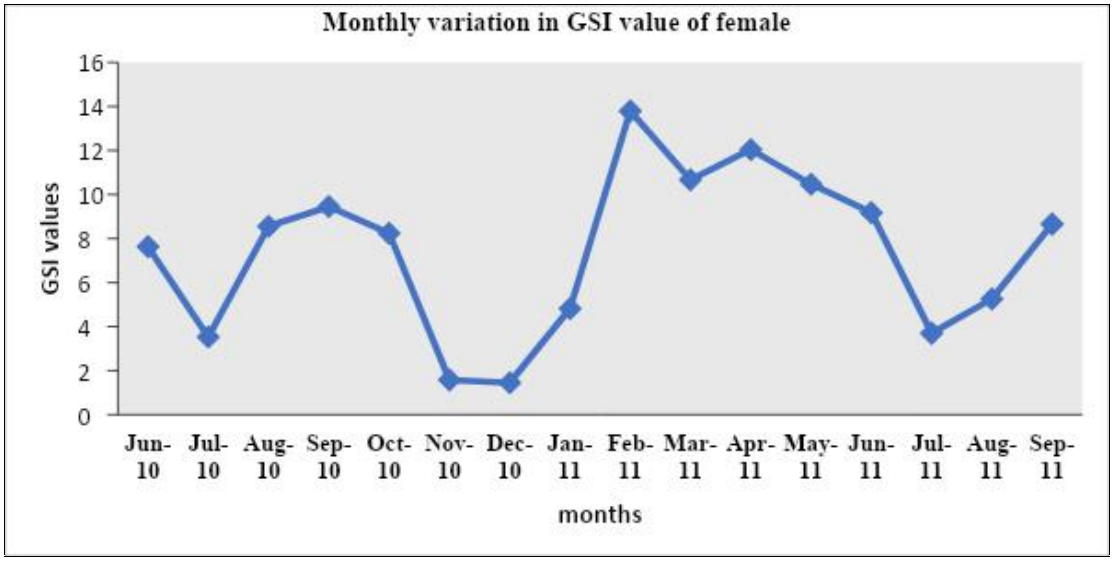


Fig no. 7: Variation in gonadosomatic index of female *P. ocellatus* during different months

Conclusion

The various aspects of reproductive biology like sexual dimorphism, sex ratio, maturity stages, ova diameter, fecundity and gonadosomatic index were studied. The secondary sexual characters observed in fish were more prominent during the spawning season. The overall sex ratio was 1M: 0.71 F indicating the dominance of males. Study of maturity stages showed the percentage occurrence of ripe males and females were 56% and 77% in the month of February 2011.The size at which 50% of the males attained maturity was 91mm and the while value for females was 94mm. Ova diameter was in the range of 0.01mm to 0.70mm.The fecundity values observed in the females with total length between 94-153mm was found to be in the range of 21,635-179,334 with a mean value of 48,973. The relationship between fecundity and total length, total weight, ovarian length and ovarian weight showed a straight line. The monthly GSI value varied in male between 0.18-0.91 and in case of the female fish between 1.58-12.67 and was high in the month of February 2011 which agrees well with maturity stages.

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