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Sexual Maturity, Spawning Periodicity and Fecundity of Obtuse Barracuda *Sphyraena obtusata* (Cuvier, 1829) along Karnataka Coast, Southeastern Arabian Sea

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ABSTRACT

Background: The success or failure of spawning in any population affects the fishery. Hence, studies on spawning, maturation, fecundity and length at first maturity become essential in drafting management strategies. The detailed information on reproductive traits of *Sphyraena obtusata* will be helpful in sustainable management and conservation in the study area.

Methods: Three hundred and fifty five specimens of *S. obtusata* were collected fortnightly from Mangaluru and Malpe fishing harbors during August 2019 to March 2020. Maturity of gonads was examined by macroscopic and histological investigations. Spawning periodicity was worked out based on maturity stages and seasonal gonadosomatic index. Gravimetric method of analysis was employed to evaluate the fecundity from randomly collected samples of gravid females.

Result: Overall sex ratio revealed that the population had significantly higher ($p<0.05$, χ^2 test) proportion of females than males. The length at 50% maturity (Lm_{50}) for females and males was estimated at 21.1 and 21.5 cm respectively. The gonado-somatic index (GSI) and the availability of matured individuals for both the sexes in various percentage indicated a prolonged breeding period from October to March with peaks in November and December. The absolute fecundity varied from 91,942 eggs (20.0 cm TL) to 1,34,445 eggs (27.0 cm TL) with an average of 1,12,878±2,984 hydrated oocytes per female.

Key words: Fecundity, Gonadal development, Histology, *Sphyraena obtusata*, Vitellogenic.

INTRODUCTION

Reproduction in fishes is an important function in population productivity and its adaptability to fishing and environmental changes. Sex ratio, maturity, fecundity and condition of fish are the elementary factors that influence the productivity of fish population and thus are crucial parameters for estimating their reproductive potential (Morgan, 2009). Reproductive potential of fish is one of the fundamental requisite to designate the individuals of that population in respect to gonadal conditions. Information on the gonadal development and the spawning season of a species enables succeeding studies on population spawning frequency, which is important for its management (Gupta and Banerjee, 2013). The success or failure of spawning in any population affects the fishery. Hence, studies on spawning, maturation, fecundity and length at first maturity become essential in drafting management strategies such as minimum size limit for fishing and assessing fishery resilience (Abraham *et al*. 2011).

The fishes of the family Sphyraenidae are important food and recreational fishes of the tropical, subtropical and at times temperate waters. They are considered as delicious food fishes in India by virtue of their good quality meat. *Sphyraena* is the solitary genus in the family Sphyraenidae encompassing 28 valid species globally. Of these, only 9 species have been recorded in Indian waters (Fricke *et al*. 2021). *Sphyraena obtusata*, a pelagic/neritic species generally known as obtuse barracuda is a commercially important species of barracuda found all along the Indian coast. Globally, the species is distributed in the Indo-Pacific region

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from Red Sea and East Africa to Samoa, North to the Ryukyu Islands and South to the Victoria Island in Australia. It is often found in schools in the marine to brackish water environment at a depth of 5-200 m near coastal areas like bays, estuaries, lagoons, sea grass beds and coral reef areas (Senou, 2001).

The total landings of barracuda in 2019 along the Indian coast were 34,010 tonnes (CMFRI, 2020). Despite the magnitude of the barracuda landings and its commercial importance, the studies on obtuse barracuda is limited to its fishery, length-weight relationship, morphometric measurements and stock assessment along the Indian Ocean (Sivashanthini *et al*. 2009; Shaila Prasad *et al*. 2021; available worldwide on reproductive biology for this species was by Rajesh *et al*. (2021), which is limited to a few basic aspects but lacked the ova diameter studies and detailed histological analysis of gonads. Hence, this study was designed to elucidate the maturation, seasonality of spawning and reproductive potential of *S. obtusata* along Karnataka coast, southeastern Arabian Sea, focusing on sex-ratio, maturity stages with histological examination of ovary, length at first maturity (Lm_{50}), gonadosomatic index (GSI), fecundity and ova diameter.

MATERIALS AND METHODS

Three hundred and fifty five specimens (197 females and 158 males) of *S. obtusata* landed by commercial trawl and purse seine boats were collected fortnightly from Mangaluru (12.853°N, 74.833°E) and Malpe (13.347°N, 74.701°E) fishing harbors during August 2019 to March 2020. The collected specimens were transported in iced condition to the laboratory of Department of Fisheries Resources and Management, College of Fisheries, Mangaluru for further detailed analysis. The total length (TL, cm) and fresh body weight (g) of each individual was measured. Gonads were collected from both males and females, weighed (g) and fixed in Bouin's solution for histological study.

Maturity stages and histology

Maturity stages of gonads were classified following the scale adopted by De Sylva (1963) with suitable modifications. For histological slide preparation, the sections of gonad were collected from the mid-part of the ovary. These sections were fixed in 10% neutral buffered formalin (NBF) for further analysis. Dehydration was done using series of 70%, 90%, and absolute concentration of ethanol and later clarified with xylene. The samples were embedded in paraffin blocks and cut in to 5-6 mm thick sections. These sections were then stained with haematoxylin and eosin. All the procedures *viz.* fixation, dehydration, casting, coding, embedding, microtomy and staining were carried out following Bullock (1989) method.

Sex ratio and size at first maturity

Monthly sex ratio was calculated and chi-square (χ^2) test applied to test whether the observed ratio between males and females differed from the expected ratio (1:1) (Snedecor and Cochran, 1967). Size at maturity (Lm_{50}), elucidated as TL at which 50% of the fishes reached sexual maturity was calculated from the given logistic equation (King, 2007):

$$P = 1/ [1 + \exp -r \times (TL - L_m)]$$

Where,

P is the proportion of mature individuals in a length class, TL is total length, r is intercept and L_m is slope. The r and L_m were estimated using the Non-Linear Regression SOLVER routine from the ratio of reproductive to non-reproductive fish groups.

Gonado-somatic index (GSI)

The gonado-somatic index (GSI) was estimated separately for males and females using the formula:

$$GSI = \frac{\text{Weight of Gonad}}{\text{Weight of fish}} \times 100$$

The significant variation in the GSI values between months and size groups were compared using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test. Spawning periodicity of *S. obtusata* was ascertained by recording the monthly percentage occurrence of gonads in different maturity stages and GSI values during various months. During the annual cycle analyzed, the occurrence of spawning was inferred with both the GSI peaks and the frequency of specimens capable of spawning according to the maturity phases found during different months.

Fecundity and ova diameter

The gravimetric method was employed to evaluate the fecundity (Hunter *et al*. 1992) from randomly collected samples of gravid females. Subsamples from anterior, middle and posterior part of individual ovary were cut and weighed. Thereafter, the number of eggs was counted from subsample and fecundity was calculated using the following formula:

$$\frac{\text{Weight of ovary} \times \text{Number of eggs in subsample}}{\text{Weight of subsample.}}$$

The relationship among fecundity and different variables like total length, fish weight and gonad weight was analyzed using least square method using the equation:

$$F = aX^b$$

Where,

F = Fecundity, a = Constant, X = Variable (fish length and weight or ovary weight) and b = Correlation coefficient.

The exponential relationship was transformed into a straight-line logarithmic form based on the equation as

$$\text{Log } F = \log a + b \text{ Log } X \qquad \text{(Zupa } et\ al. \text{ 2013).}$$

The oocyte diameter was measured following the method of Clark (1934) using an ocular micrometer set on light microscope (one ocular micrometer division = 0.01 mm). All the statistical analysis were performed at significance level $p<0.05$ using IBM-SPSS statistics 21.0 software.

RESULTS AND DISCUSSION

The results of the present study impart the first comprehensive report on histological examination of gonads of *S. obtuasta* (Fig 1). Five maturity stages (immature, maturing, mature, spawning and spent recovery) were considered for ovary and testes of *S. obtusata*. The macroscopic and histological descriptions of different developmental phases of ovary are presented in Table 1. During immature stage, the size of the ovary was small and filled with primary growth (PG) oocytes. As the development continues, the changes in the reproductive material have been clearly observed through histological examination (Fig 1). During ovarian generation and succeeding phases, more than two oocyte stages were observed together indicating oocytes released in batches. The matured spawning ovaries were recorded throughout the study (Fig 2a) with maximum during October (40.00%) and minimum during January (6.25%). Similarly, the matured and spawning testes

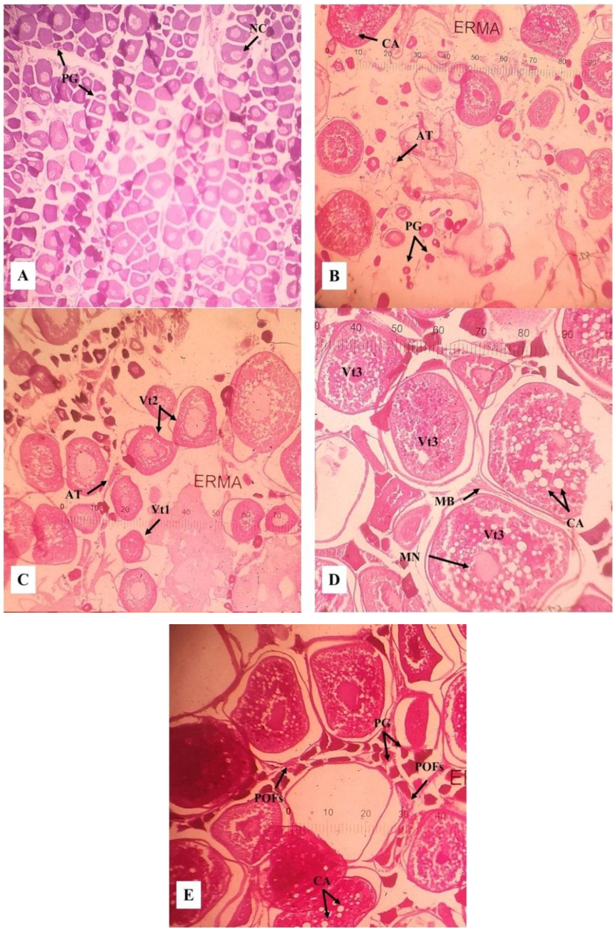

Fig 1: Ovarian developmental phases of *Sphyraena obtusata*: A- Immature; B- Maturing; C- Mature; D- Spawning; E- Spent recovery. Tissues and cellular structures in ovarian stages: PG- primary growth; NC- Nuclear chromatin; CA- Cortical alveolus; AT- Atresia; Vt1- Primary vitellogenic; Vt2- Secondary vitellogenic; Vt3- Tertiary vitellogenic; MN- Migrating nucleus; MB- Muscle bundle; POFs- Postovulatory follicles.

were found throughout the study (Fig 2b) with maximum percentage during September (38.88%) and November (34.78%). The macroscopic and histological examination of ovaries revealed that *S. obtusata* is a batch spawner. Similar gonadal developmental pattern and batch spawning activities was reported in *S. putnamae* from coastal waters of Karnataka (Rajesh *et al*. 2020).

Three hundred and fifty five specimens of *S. obtusata* were analyzed, out of which 55.49% (197) was constituted by females and 44.51% (158) by males indicating a dominance of females in the population. The overall sex ratio 1:1.25 (M: F) differed from the expected 1:1 (χ^2=4.28, p=0.038) ratio (Table 2). The sex ratio varied from 1:0.88 (February) to 1:2.46 (January). No significant difference ($p>0.05$) was observed in sex ratio in different months expect during January ($p<0.05$). Higher proportions of females have been detected in other species of barracuda like *S. putnamae* (Rajesh *et al*. 2020) from southeastern Arabian sea, *S. ensis* (Zavala-Leal *et al*. 2018) and *S. idiastes* (Gonzalez-Acosta *et al*. 2015) from Gulf of California, *S. guachancho* from Mexican waters (Sanchez *et al*. 2011) and *S. chyrsotaenia* from Egyptian Mediterranean waters (Allam *et al*. 2004). The variations in the sex ratio may be due to the difference in the environmental factors, food availability, mortality and spawning activities acting differently for each fishes during their life cycle (Nikolsky, 1963).

Size at first maturity (Lm_{50}) is an essential feature of life history events necessary for success of fishery management, primitive to establishment of means that avoid exploitation of juveniles and resultant reduction of spawning stock. The present investigation revealed that females matured slightly earlier (Lm_{50}=21.1 cm) than males (Lm_{50}=21.5 cm) (Fig 3). All individuals of females and males were matured after attaining 25.8 cm and 24.5 cm TL respectively. A lower Lm_{50} values were reported for *S. chrysotaenia* by Wadie *et al*. (1988) from south-eastern Mediterranean waters (19.0 cm for males and 20.5 cm for females) and Allam *et al*. (2004) from Egyptian Mediterranean waters (17.3 cm for males and

Table 1: Description of the maturity stages in the reproductive cycle of female *Sphyraena obtusata*.

Stage	Macroscopic	Histological
Immature	Ovaries usually clear, whitish and smaller in size, vascularization indistinct.	Only oocytes in PG stages and nuclear chromatin present. Absence of AT and MB. Ovarian wall thin and less space between oocytes (Fig 1A).
Maturing	Ovaries creamy red in color, inflated and enlarging, vascularization visible.	CA and some AT present. PG oocytes still present (Fig 1B).
Mature	Ovaries orange in color, turgent and bigger, vascularization become more distinct. Oocytes can be observed with naked eye.	Predominance of oocytes in vitellogenesis phases. Vt1, Vt2, AT and PG oocytes present. No affirmation of Vt3 and POFs oocytes (Fig 1C).
Spawning	Ovaries creamy yellow in color, bulging and ovarian wall tight to content. Vascularization prominent on both lobes. Individual oocytes visible with naked eye.	Predominance of Vt3 oocytes. Migrating nucleus within the oocytes visible. MB and CA present (Fig 1D).
Spent recovery	Ovaries flaccid, brown in color. Vascularization visible. Oocytes cannot be visible with naked eye.	Abundance of POFs. Some CA or vitellogenic oocytes (Vt1 and Vt2) oocytes present (Fig 1E).

Oocyte stages code: PG- Primary growth; NC- Nuclear chromatin; CA- Cortical alveolus; AT- Atresia; Vt1- Primary vitellogenic; Vt2- Secondary vitellogenic; Vt3- Tertiary vitellogenic; MN- Migrating nucleus; MB- Muscle bundle; POFs- Postovulatory follicles.

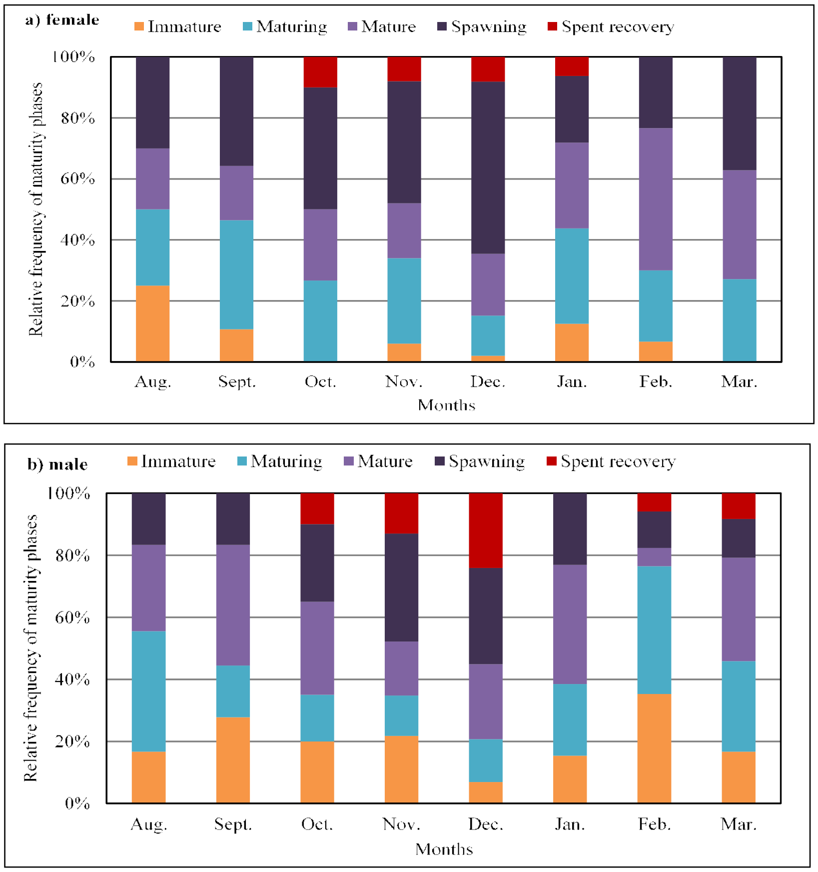

Fig 2: Relative frequency of maturity phases of a) female and b) male *Sphyraena obtusata*.

Table 2: Month-wise sex ratio of *Sphyraena obtusata*.

Months	Male	Female	Sex ratio (M:F)	χ^2	*p-value*
Aug.	14	17	1:1.21	0.29	0.590
Sept.	18	28	1:1.56	2.17	0.140
Oct.	20	30	1:1.50	2.00	0.157
Nov.	23	37	1:1.61	3.27	0.071
Dec.	29	25	1:0.86	0.30	0.586
Jan.	13	32	1:2.46	8.02	0.005*
Feb.	17	15	1:0.88	0.13	0.724
Mar.	24	13	1:0.54	3.27	0.071
Total	158	197	1:1.25	4.28	0.038*

*(p<0.05).

19.3 cm for females). However, higher Lm_{50} values were reported for *S. putnamae* (38.0 cm for males and 39.4 cm for females) by Rajesh *et al*. (2020) from Karnataka coast and *S. guachancho* (27.8 cm for males and 30.3 cm for females) by Akadje *et al*. (2019) from Ivorian coast. The variations observed in Lm_{50} values from different regions could be due to the difference in temperature, availability of food for growth, method used to estimate maturity and difference in genetic potential (Maggio *et al*. 2018).

The average GSI value of female (3.42±0.17) was significantly (p<0.05) higher than male GSI (1.21±0.07) (Fig 4). In females, GSI values were significantly (p<0.05) higher during December (5.24±0.52) followed by March (4.99±0.32), January (3.88±0.61), February (3.87±0.40) and October (3.86 ± 0.22), while lower values were observed during August (1.15±0.12) and September (1.08±0.08). The higher GSI value in the present study coincides with the presence of spawning capable females. The highest and lowest values of male GSI was recorded during December (1.98±0.18) and September (0.53±0.05) respectively (Fig 4). The percentage distribution of different maturity stages of ovary and testes and GSI values of males and females represents prolonged spawning season from October to March with peaks in November and December. Similar prolonged spawning activity of *S. obtusata* was reported from south-west coast of India by Rajesh *et al*. (2021). The spawning season of other barracuda species such as *S. ensis* was reported to be from April to June along Gulf of California (Zavala-Leal *et al*. 2018), *S. guachancho* during January (low cold season) along Ivorian coast (Akadje *et al*. 2019) and *S. putnamae* during April to May and December to January along southeastern Arabian Sea (Rajesh *et al*. 2020). This difference in spawning periods confirms the hypothesis of O'Toole (2008) whereby reproductive activity as well as the growth of barracudas varies with geographical area.

The estimated fecundity ranges from 91,942 in a fish of 20.0 cm TL to 1,34,445 eggs in a fish of 27.0 cm TL with an average of 1,12,878±2,984 (mean±SE) hydrated

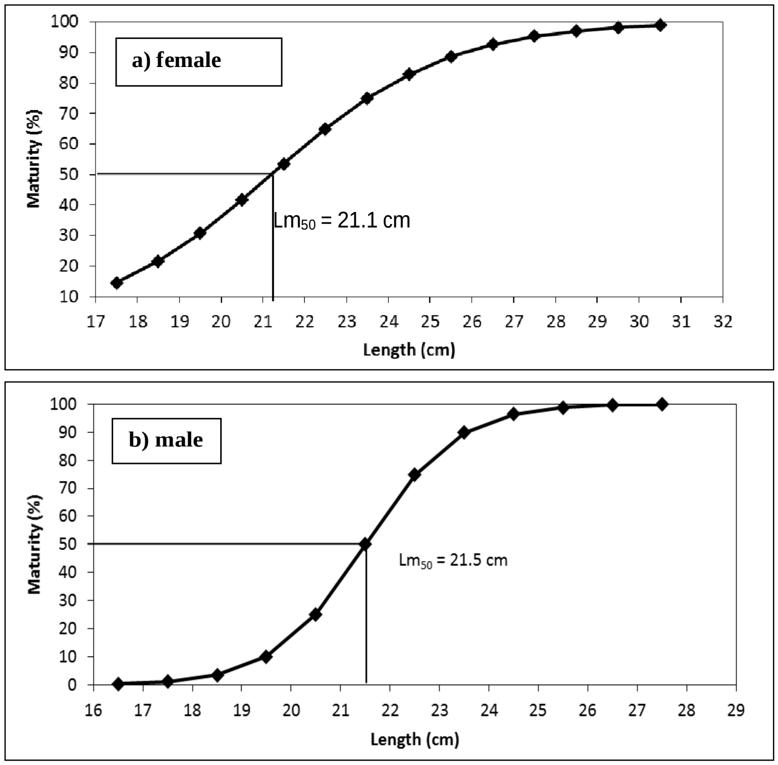

Fig 3: Size at first maturity (Lm_{50}) of – (a) female and (b) male *Sphyraena obtusata.*

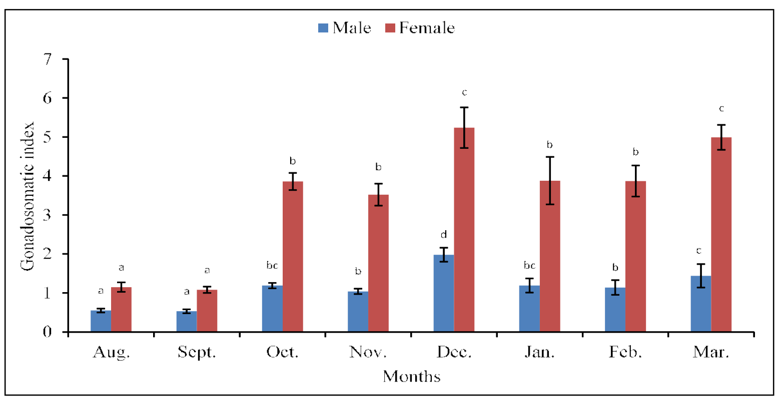

Fig 4: Month-wise gonadosomatic index (GSI) (±SE) of *Sphyraena obtusata*. Means with different superscripts are significantly ($p<0.05$) different.

oocytes per female. In comparison with other *Sphyraena* species, the absolute fecundity of *S. obtusata* was lower than that of *S. putnamae* (69,689 to 9,44,793 for female of length range 30.4-73.0 cm FL) (Rajesh *et al*. 2020) and *S. barracuda* (5,60,000 to 6,70,000 for female of length range 89.5-101.1 cm FL) (De Sylva, 1963). Allam *et al*. (2004) recorded the absolute fecundity of *S. chrysotaenia* as 74,399 to 2,41,853, *S. flavicauda* as 84,197 to 2,60,549 and *S. sphyraena* as 46,778 to 1,03,453 eggs from Egyptian Mediterranean waters of Alexandria. The difference in fecundity may be due to variation in genetic and ecological potential, existing environmental factors, nutritional status, and methodologies utilized for estimation of fecundity (Massuti and Morales-Nin, 1997; Assana *et al*. 2021). Fecundity was fluctuating over the spawning season and positively correlated with total length, body weight, and gonad weight of fish (Fig 5). This shows that the large sized females produce larger number of eggs compared to smaller ones. The increase in fecundity with total length, body weight, and gonad weight of *S. obtusata* in the present investigation is comparable with other barracudas reported by Allam *et al*. (2004) and Rajesh *et al*. (2020).

The ova diameter for different maturity stages ranged from 0.02-0.48 mm. The ova diameter in different maturity stages ranged from 0.02-0.05 mm (immature), 0.06-0.19 mm (maturing), 0.20-0.28 mm (matured) and 0.29-0.48 mm (spawning). Allam *et al*. (2004) mentioned that the egg size of *S. chrysotaenia* ranged from 0.05-0.85 mm, *S. flavicauda* from 0.05-1.20 mm and *S. sphyraena* ranged from 0.05-1.50 mm. Size of immature eggs were small, alecithal and

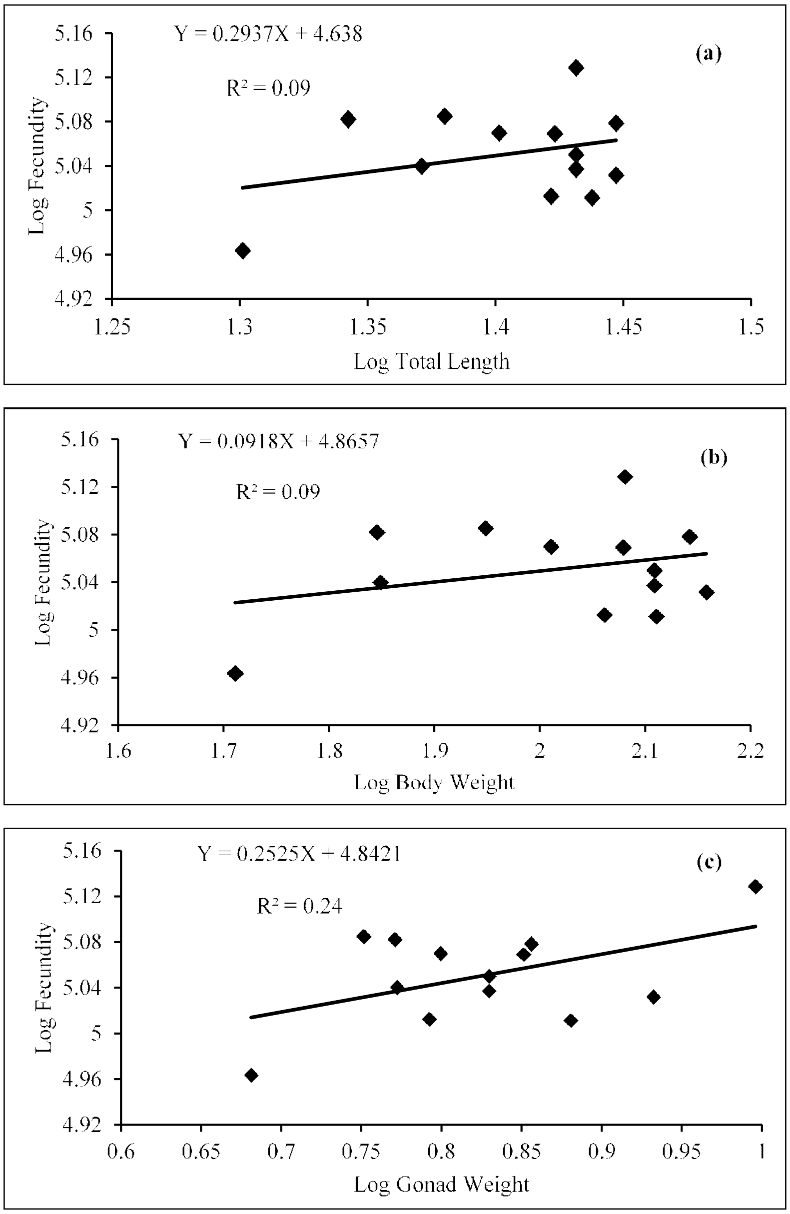

Fig 5: Logarithm relationship between fecundity to (a) total length, (b) body weight and (c) gonad weight of *Sphyraena obtusata.*

whitish translucent in color, while maturing eggs were partially opaque with oligolecithal and ripe eggs were completely transparent with macrolecithal. The presence of ova in different developmental stages represents that the species is a multiple spawner releasing eggs in batches.

CONCLUSION

This study supplements the detailed information on reproductive aspects of *S. obtusata* along the Karnataka coast, southeastern Arabian Sea and provides number of variables for revamping future stock assessments of this species from this region. A clear justification of gonadal development and maturation is essential for egg production and hence is vital for management of fish stocks. The results could be useful to generate advice and development of management strategies to maintain the stock of obtuse barracuda at a sustainable level.

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