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Reproductive biology of Pharaoh cuttlefish *Sepia pharaonis* Ehrenberg, 1831 along the Gujarat coast, India

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Gujarat is a leading maritime state in the production of marine finfish and cephalopods in India. The cephalopods are one of the major fishery resources along the region having a good domestic and export demand. These resources are exploited majorly by bottom trawls along the region. *Sepia pharaonis* contributes nearly 20 % to the cephalopod landings of Gujarat. However, the fishery and reproductive biology of the cephalopod, *Sepia pharaonis* is poorly known from the region. The specimens for biological analysis along with fishing operations and fishing data using a structured questionnaire were collected from the multi-day trawlers based at the Veraval Fishing Harbour for the period of 2017 – 18. The maximum observed length (Dorsal Mantle Length (DML)) among all specimens was 361 mm with a mean DML of 239.52 mm (sample range DML). Sex ratio revealed that the males are dominant over the females (M: F = 1:0.75) in the catch composition. The size at first maturity was observed at 213.1 mm DML, which is lower than the mean size of the fishery. The month-wise significant variations ($p < 0.05$) were observed in the values of GSI and NSI during the study period and peak values indicate the spawning season of the fishery. The proportion of maturity stages in different months as well as maturity indices indicates perennial spawning season with peak spawning activity during December – March months. The ova diameter studies revealed ovum sizes from 0.99 – 14.30 mm (6.52±3.35). The fecundity varied from 1,358 – 6,005 ova (average = 2,883). The current research study is the maiden attempt along the region and further supports the formulation of management plans for the sustainable exploitation of the resource.

[**Keywords**: Fishery management, Gujarat coast, Ova diameter, Reproductive biology, *Sepia pharaonis*]

Introduction

India's marine fish production was predicted to be 3.05 million tonnes (t) in 2021, an increase of 11.8 % from the year before. In Gujarat, the molluscan resources contribute around 6 % of the total landing, out of which cuttlefish was dominant group sharing nearly 48.15 %. The overall production of cephalopods in 2021 was 1.56 lakh ton[(ref. 1)]. Gujarat is known for the mechanized sector fishing and is a leading contributor to the India's marine fish landings for the year 2021 with 5.76 lakh tones, out of which cephalopods constituted 5.97 %. Cuttlefish fishery was dominated by *Sepia elliptica* followed by *Sepia pharaonis*, *Sepiella inermis*, *Sepia prashadi*, *Sepia omani* and *Sepia kobiensis*. Pharaoh cuttlefish, *Sepia pharaonis* Ehrenberg, (1831) (local name *Makul*) belonging to the family Sepiidae and order Sepiida is a large neritic demersal species, endemic to tropical waters and commonly found in the Indo-West Pacific and the Mediterranean Sea, from the Red Sea to Japan and Australia. The *S. pharaonis* export trade contributes significantly to the India's GDP, due to its high demand and high unit price in East Asian counties. As India is a major exporter of cephalopods, it's very important to study the reproductive biology of these important fish species to ensure their sustainable supply[2].

The biology of *S. pharaonis* is well documented in the Persian gulf[3], Suez Gulf (Red Sea) Egypt[4] and in the Suez Canal[5]. Moreover, species stock assessment study is carried out from the Arabian Sea off Oman[6] and morphological characteristics are reported from the Persian Gulf Bushehr region[7]. Many prior studies on the fishery and biology of *S. pharaonis* have been conducted along the Indian coast[8,9], the south-west coast of India[10,11] and along the east coast of India[12]. However, information on the reproductive biology of

S. pharaonis is limited and no information is available along the north-west coast of India, especially from the Gujarat coast. Hence, the present study aims to explore the maturation, spawning, fecundity and reproductive indices of *S. pharaonis* along the Gujarat coast which is essential to understand the life history, sustainable exploitation, and for formulation of management plans for this important resource.

Materials and Methods

Samples were collected from the selected multi-day trawlers operated along the Gujarat coast with geo coordinate range of 19°06’00” N; 71°18’00” E and 22°09’00” N; 68°24’00” E; between August 2017 and March 2018 (Fig. 1). A total of 179 (102 males, 77 females) individuals were collected for the biological studies. Samples were dissected to determine the sex and maturity stage and measurements were made on Dorsal Mantle Length (DML, to the nearest 1 mm), Total Weight (TW to the nearest 0.1 g), Gonad Weight (GW to the nearest 0.1 g), and Nidamental Gland Weight (NGW to the nearest 0.1 g). Type of sex and maturity stages was also recorded during the same time. The data on fishing operations and catch details for the period of 2017 – 18 was obtained using a structured questionnaire from the multi-day trawlers based at the Veraval Fishing Harbour.

Fishing method

Cuttlefish resources were exploited along the continental shelf by medium-sized trawlers (42 – 58 feet) operating at a depth of 60 – 200 m. Trawl nets operated for cuttlefish fishery is with a cod end mesh size of 18 – 22 mm mesh size and were hauled for a duration of approx. 2 – 3 h/haul with a towing speed of 1.8 – 2.2 knots.

Sex-ratio

Month-wise sex ratio was determined and Chi-square test was performed to test the homogeneity of male and female distribution in the population[13].

Maturity studies

To find out the peak maturation season of the species, the frequency of occurrence of mature females in the catch throughout the period of study was examined. The sex and maturity stages were determined by examining the developmental status of nidamental glands, accessory nidamental glands and the ovary in females. Maturity was studied only for females based on the different maturity stages *viz*: immature (I), maturing (II), mature (III),

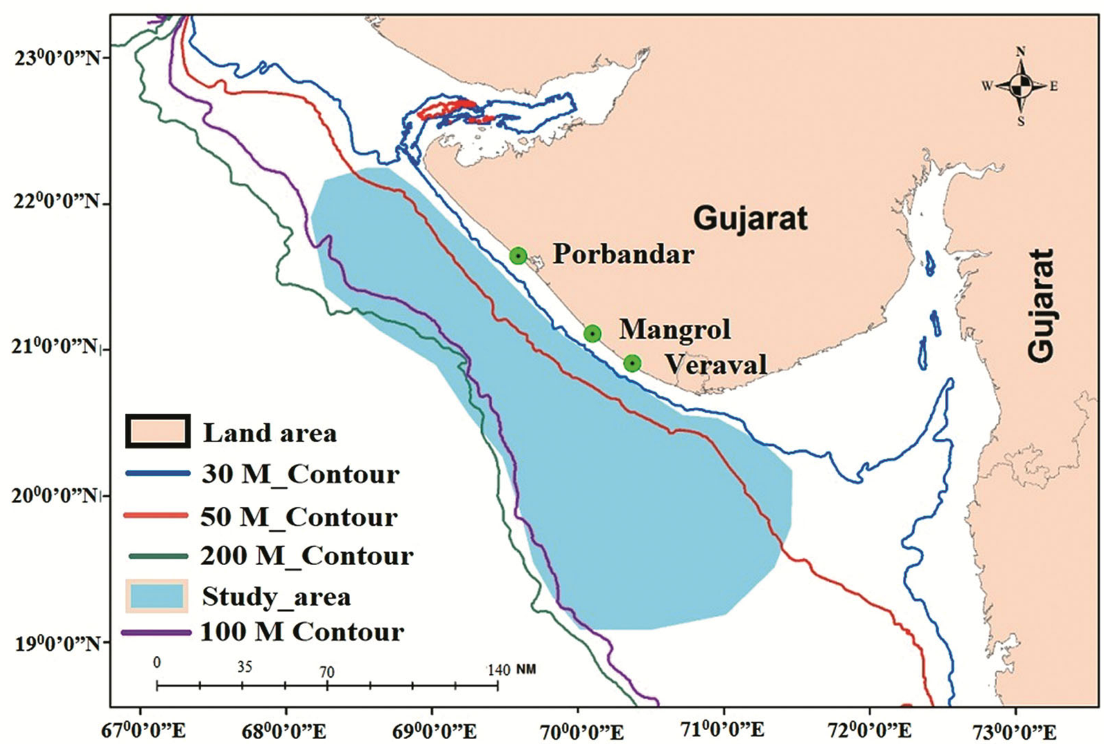

Fig. 1 — Study area along the north-west coast of India

spawning (IV) and spent (V)[8]. The size at first maturity was determined by considering the length at which 50 % of the specimens attained maturity[14] and plotting the cumulative proportion of matured specimen data against the different DML (stage III and above was considered as mature). Both the estimated and the observed proportions were plotted on the same graph to estimate the difference in calculated results and real-time data.

Fecundity

The fecundity was estimated by counting maturing and matured ova in the ovaries of stage III and IV. Fecundity was calculated by gravimetric method *i.e.* by preserving ovaries in 5 % formalin and worked out by counting the number of ova in all subsamples of the matured and ripened ovary to the total ovary weight[14].

$$Fecundity\ (F) = \frac{Gn}{g}$$

Where, F = Fecundity, G = Total weight of the ovary, g = Weight of subsample of the ovary, n = Total number of eggs in the subsample of the ovary.

The number of eggs produced per unit body weight of fish is termed as relative fecundity. The Relative Fecundity (RF) was calculated by the following formula:

$$Relative\ fecundity\ (RF) = \frac{Total\ number\ of\ eggs\ present\ in\ the\ ovary}{Total\ weight\ of\ the\ fish}$$

Determination of ova diameter

The ova diameter was measured under the Stereo Zoom Microscope with a digital color camera (resolution 3 megapixels) calibrated using an ocular micrometer (accuracy = 0.001 mm). The average diameter of the ova was obtained by pooling the ova diameter of the representative samples from the all three (anterior, middle and posterior) regions of the ovary[15]. Larger sized ova were measured by Vernier callipers (accuracy = 0.001 mm).

Gonadosomatic Index (GSI)

Gonadal weight expressed as a percentage of body weight is the most popular way to describe how different the body size is from that of the gonad. A one-way analysis of variance (ANOVA) was used to test for differences in GSI values between the months for females. GSI was determined by the formulae given by Olyott *et al.*[16].

$$GSI = \frac{GW \times 100}{[BW - (SW + GW)]}$$

Where, GW is the gonad weight (g), BW is the body weight (g), and SW is the stomach content weight (g).

Nidamental gland–somatic Index (NSI)

To calculate the NSI, the nidamental gland was removed and weighed. Nidamental gland–somatic index (NSI) for the females was calculated by following formula[17].

$$NSI = \frac{NGW \times 100}{(BW - NGW)}$$

Where, BW is the body weight (g), and NGW is the nidamental gland weight (g) (Accuracy = 0.01 g). ANOVA was attempted to know the monthly variation in NSI. The significant difference among different months of NSI values was determined by one-way ANOVA (p = 0.000).

Results

Sex ratio

The monthly variation in the population sex-ratio of *S. pharaonis* is given in Table 1. Among overall

Table 1 — Monthly variations in the sex ratio of *S. pharaonis (August 2017-March 2018)*

Month	Number of individuals			Sex ratio (Male:Female)	Chi-square value	p-value
	Male	Female	Total			
August, 17	5	12	17	1:2.40	2.88	0.090
September, 17	7	9	15	1:1.28	0.33	0.617
October, 17	4	16	19	1:4*	7.63	0.007
November, 17	13	13	26	1:1	0.00	1.000
December, 17	17	5	24	1:0.29**	6.17	0.011
January, 18	22	6	28	1:0.27**	9.14	0.002
February, 18	14	8	22	1:0.57	1.64	0.201
March, 18	20	8	28	1:0.40**	5.14	0.023
Annual	102	77	179	1:0.75	3.49	0.062

* = Female in the population significantly higher (P < 0.05); ** = Males in the population significantly higher (P < 0.05)

samples, around 102 individuals (56.98 %) were males, and 77 (43.01 %) were females. The overall sex ratio was 1:0.75 indicating the dominance of males over females. The annual chi-square value (χ^2 = 3.49) showed non-significant variation in various months in the sex ratio at 5 % level of significance (p > 0.05). Females were significantly (p < 0.05) dominant in October 2017, and males were in December 2017, January 2018, and March 2018 (Table 1).

The results of seasonal variations in the sex ratio of *S. pharaonis* indicate seasonal changes from female to male dominance (Table 2). It was observed that females were significantly dominant in post monsoon and males were dominant in winter and summer. The chi-square values showed a significant difference in the sex ratio at 5 % level of significance (p > 0.05) in different seasons.

Length at maturity

Length at first maturity for females was at 213.1 mm of DML (Fig. 2) and the individuals of this size were observed in all the months. All the females above 231 mm DML were in mature condition.

Maturation

Mature females were observed from 206 mm DML onwards. The monthly distribution of the mature specimens (Table 3) shows that ovaries in stages of III

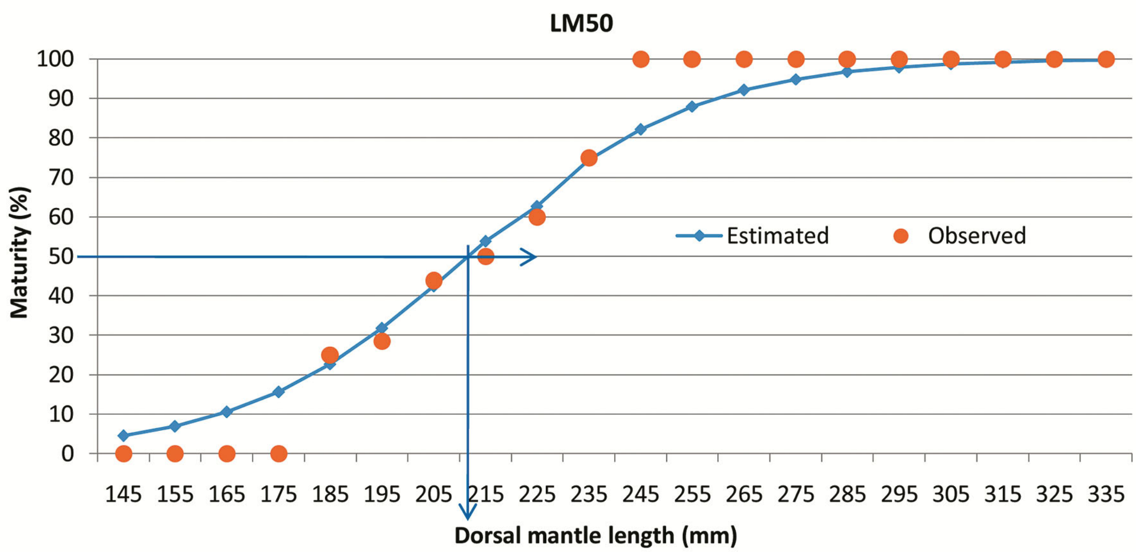

Fig. 2 — Length at first maturity in *S. pharaonis*

Table 2 — Seasonal variations in the sex ratio of *S. pharaonis*

Season	Male	Female	Total	Expected	Chi-square	*df*	*p*-value
Post monsoon	29	50	79	39.5	5.582	1	0.018
Winter	53	19	72	36.00	16.06	1	0.000
Summer	20	8	28	14	5.143	1	0.023

Table 3 — Monthly distribution of Maturity stages of *S. pharaonis (August 2017-March 2018)*

Month	DML (mm)	Weight (g)	Mean DML	Maturity stages				
				I	II	III	IV	V
August, 2017	170-270	447-1474	223	3	5	2	2	0
September, 2017	149-257	323-1426	205	3	6	0	0	0
October, 2017	170-240	450-1198	206	4	7	3	2	0
November, 2017	169-249	400-1238	212	0	7	5	1	0
December, 2017	189-305	511-2125	242	0	0	0	2	3
January, 2018	203-355	623-2834	269	1	0	0	5	0
February, 2018	146-358	518-3009	274	1	1	0	6	0
March, 2018	152-351	279-2789	261	1	0	1	3	3
Annual	*146-358*	*279-3009*	*239*	*13*	*26*	*11*	*21*	*6*

and IV were common in all the months, except September 2017. Stage IV spawning females were predominantly observed from December 2017 to March 2018. However, the *S. pharaonis* population appeared to spawn year-round, with spawning frequency peaking between December 2017 and March 2018.

Spawning percentage

Spawning percentage was observed higher (≥ 75 %) from December 2017 to March 2018 (Table 4) with a maximum during December 2017 (100 %). The presence of spawners was observed in all months except in September indicating that *S. pharaonis* spawn all year round.

Gonado-somatic Index (GSI)

The GSI values ranged from 0.84 to 4.45 ($\overline{x}$ = 2.59). Monthly GSI values showed an increasing trend from December 2017 onwards and reached its peak in March, 2018. The lowest values were between August and November 2017 (Fig. 3) with the one-way ANOVA showing a significant variation in GSI values (p < 0.05) [p = 0.033]. The Posthoc comparisons using the Tukey HSD test indicated that the monthly GSI values were significantly different signaling the occurrence of peak spawning between December 2017 and March 2018.

Table 4 — Monthly variations in the spawning percentage of *S. pharaonis (August 2017-March 2018)*

Month	No. of females		% Spawning females
	Observed	Mature	
August, 17	12	4	33.33
September, 17	9	0	0.00
October, 17	16	5	31.25
November, 17	13	6	46.15
December, 17	5	5	100.00
January, 18	6	5	83.33
February, 18	8	6	75.00
March, 18	8	7	87.50
Annual	*77*	*38*	*49.35*

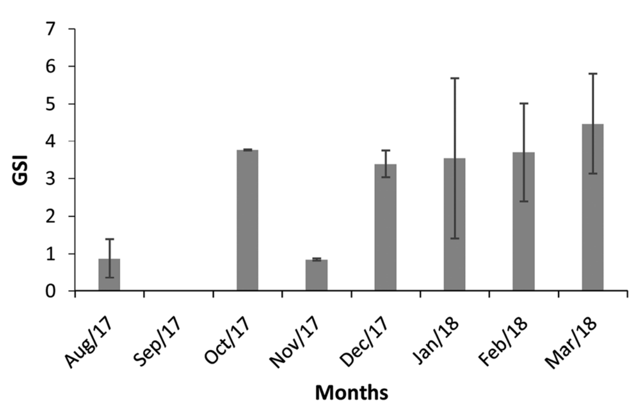

Fig. 3 — GSI of *S. pharaonis*

Nidamental gland-somatic Index (NSI)

NSI is the proxy to indicate the breeding season of *S. pharaonis*. Nidamental glands vary in size and color in correlation with maturation and attain peak size during the spawning period. Month-wise, lower NSI values were observed from August to November 2017 and the higher values were recorded from December 2017 to March 2018 (Fig. 4). One-way ANOVA and Posthoc comparisons using the Tukey HSD test indicated that the monthly NSI values were significantly different showing occurrence of peak spawning activities from December 2017 to March 2018.

Fecundity

Out of the total 77 females, 38 (49.35 %) specimens were mature with countable eggs in 24 females. Matured and ripened ovaries were observed throughout the study period except during September 2017. Fecundity ranged from 1,358 – 6,005 ova with a mean of 2,883 ova (Table 5).

The estimated Relative Fecundity (RF) ranged from one to four egg per gram with an average of 2. The values of RF peaked at DML of 227 mm which is close to the length at first maturity. The RF values showed a gradual decline towards the higher lengths. In the present study, fecundity showed a high correlation coefficient with the DML of the fish. The regression of fecundity and DML can be expressed as Log F = 12.89*Log (DML) – 352.4 with R^2 value of 0.156 (Fig. 5).

The smallest observed fecund female measured 208 mm mantle length with 915 g total weight,

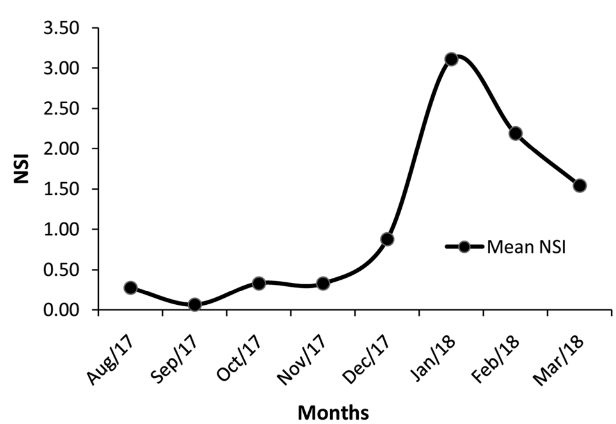

Fig. 4 — Monthly variations in NSI values of *S. pharaonis*

Table 5 — Fecundity of *S. pharaonis* with respect to mantle length and body weight

Sr. no	Mantle length (mm)	Weight (g)	Fecundity	Relative fecundity
1	208	824	1358	2
2	208	915	1933	2
3	215	869	2165	2
4	216	925	1471	2
5	225	999	1743	2
6	227	932	6005	6
7	229	983	3573	4
8	231	944	3266	3
9	235	1150	3007	3
10	235	1005	1375	1
11	235	974	2568	3
12	240	1198	3595	3
13	242	1111	3561	3
14	250	1200	1680	1
15	255	1396	2272	2
16	257	1229	2869	2
17	261	1452	3582	2
18	266	1509	3130	2
19	271	1483	3836	3
20	273	1568	2389	2
21	288	1638	2632	2
22	290	1719	2911	2
23	328	2789	3520	1
24	336	3009	4744	2
Min	*208*	*824*	*1358*	*1*
Max	*336*	*3009*	*6005*	*6*
Mean	*250.875*	*1325.88*	*2883*	*2*

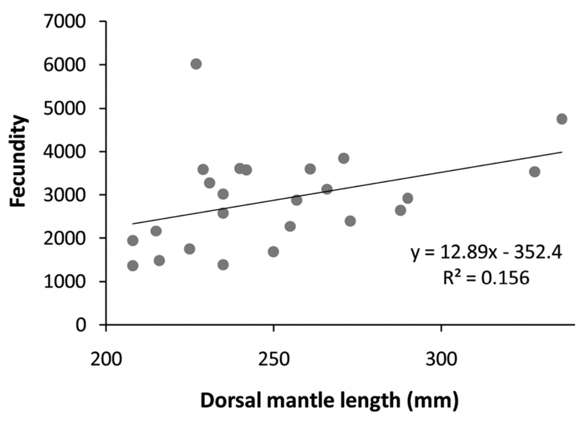

Fig. 5 — Relationship of mantle length with fecundity

which had a gonad of 38.3 g with 1,933 eggs. The largest observed fecund female had 336 mm mantle length with 3,009 g total weight. Its gonad weighed 84.5 g, with 4,744 eggs. The minimum number of eggs (1,358) was observed in a specimen with 208 mm mantle length and 824 g total weight, and its gonad weighed 7.66 g. The highest numbers of eggs (6,005) were observed in a female having 227 mm mantle length and 932 g total weight. Its gonad weighed 13.67 g. Results indicated that individual fecundity could be a function of body mass.

Table 6 — Monthly variations in the average diameter of ova *(August 2017-March 2018)*

Month	No. of ova	Average ova diameter (mm)	Ova diameter range (mm)
August, 2017	30	3.31±1.81	0.99 – 7.48
September, 2017*	-	-	-
October, 2017	30	4.77±2.24	1.43 – 9.79
November, 2017	30	5.43±2.38	1.65 – 10.45
December, 2017	30	6.44±2.39	2.31 – 10.45
January, 2018	30	7.44±3.25	2.64 – 14.3
February, 2018	30	9.12±2.54	2.64 – 14.3
March, 2018	30	9.16±3.78	2.53 – 14.3
Annual	*210*	*6.52±3.35*	*0.99 – 14.30*

*Spawners were absent in the samples

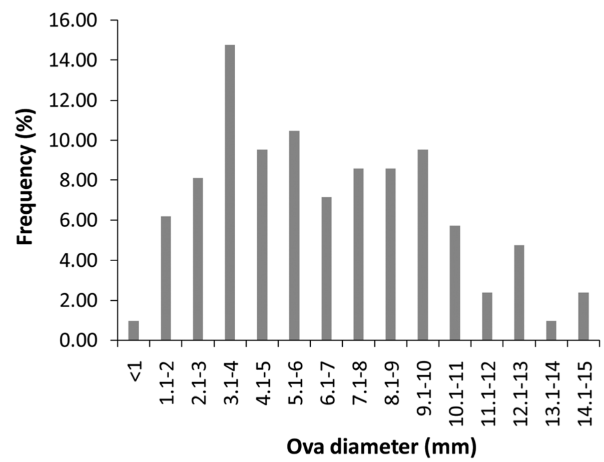

Fig. 6 — Frequency distribution of ova

Ova diameter

The annual average ova diameter was observed as 6.52±3.35 mm and the monthly average ova size was highest in March 2018 and lowest in August 2017 (Table 6). Different sized eggs were found in the same ovary, with the smallest eggs measuring 0.99 mm diameter in August 2017 and the largest eggs measuring 14.3 mm in March 2018. The frequency distribution of eggs under different size categories (Fig. 6) showed that the most frequent eggs size category was 3.1 – 4 mm (14.76 %) followed by 5.1 – 6 mm (10.24 %).

Discussion

In the present study, the estimation of the sex ratio from the pooled data depicted that the males were dominant in the population over females (M: F = 1:0.75). The seasonal ratio of the male: female population was not similar throughout the year. Males were dominated in the overall population, conforming earlier studies[3,9]. Aoyama & Nguyen[18] reported that the sex ratio of smaller individuals of *S. pharaonis* with mantle length less than 30 cm is 1:1; whereas, only males have been observed in large-size population (mantle lengths over 30 cm) along the Yemeni coast. Females outnumbered males in the trawl fishing from the Bay of Bengal and in the hook and line fishery in the Arabian Sea[8]. Sundaram[19] reported that the females dominated over males with sex ratio 1:1.24 from Mumbai waters. Whereas, Riad *et al.*[4] reported that the sex ratio of male-female was 1:4.7 which significantly differ from the findings of the present study. The reasons for the dominance of males may be due to spawning migration, post-spawning mortality and cannibalism. During the post-monsoon, females were significantly dominant and declined in winter and summer as these are major spawning season for *S. pharaonis*. Studies from Yemeni waters[15] revealed that most of the females apparently die after spawning at the age of two years and some males survive to rejoin the reproductive activities in the following year. The natural mortality (M) values for females are higher in Indian waters[9].

Male dominance correlated with peak spawning season in the current study. Spawning migration and post-spawning mortality owing to lack of energy, cannibalism, and predation may be the most important factors affecting the female populations. Variances in sex ratios may also be attributable to dissimilar fishing and variation in fish migration patterns to and from fishing regions. However, more studies is required to understand the true pattern of spawning migration in terms of spatial and temporal features, as well as its impact on the fishery.

The length at first maturity for females was estimated at 213.1 mm DML in the present study conforming[6,20,21] and also contradicting to earlier studies[4,5,8,19,22]. Variation in length at first maturity may be due to the difference in environmental parameters, the abundance of food and fishing methods. Changes in L_{m50} have been attributed to changes in water temperature, which cause changes in habitat preferences, resulting in changes in the species' distribution patterns[23]. The Arabian Sea along India's west coast experiences distinct temperature cycles connected with the South West (SW) monsoon[24] and these temperature changes could possibly account for some of the increased maturity size[25].

In the present study, population of *S. pharaonis* found to spawn throughout the year along the Gujarat coast of India. Spawning percentage for this species was observed higher from December 2017 to March 2018. The higher GSI values recorded during the months of December 2017 (3.381) and March 2018 (4.459) also confirms the spawning season. *S. pharaonis* appears to be an intermittent multiple spawner[7]. *S. pharaonis* spawning occurs from October to April along both the coasts of India; occasionally up to August[8]. The peak of male spawning (Stage IV) coincided with the peak of female spawning in April – June in the Suez Canal[5]. Marzouqi *et al.*[6] reported that *S. pharaonis* spawns two times in a year with peak spawning activity between August and December and a secondary spawning during April – May from the Arabian sea of Oman. *S. pharaonis* spawning in Eastern Arabian[21] waters have been reported to peak in October – November (Length at first maturity = 214 mm) and in February – March[11]. Studies on *S. pharaonis* from Persian Gulf[3] revealed that the spawning period covers all months, with two maximum peaks in May and June. Further, Sundaram[19] reported that in Mumbai waters this species exhibit minor spawning season during October – December and a major one during February – May, which is in conformity with the results of the present study. In Egyptian waters, the spawning season was observed during spring and summer and extends till autumn[4]. Overall, large spatial variations are observed in spawning season of *S. pharaonis.*

Monthly GSI and the NSI values varied significantly and were higher from December 2017 to March 2018 coinciding with the peak percentage of spawners. This suggests that the ovaries mature and ripen continuously from December to March. It is inferred that the NG were in the ripe condition in the December 2017 to March 2018 months coinciding with the peak spawning period and lowest maturity indices were between August and November 2017. Taken together, these results suggest that peak spawning season of *S. pharaonis* is between December and March. Studies from the Suez Canal[5] revealed that female maturity indices increased in

March and peaked in April, May, and June; whereas, monthly GSI values were higher during September – December and April – May at Arabian Sea of Oman[6]. Parallel to NSI, the GSI increased in winter and peaked during spring[4]. The mean GSI in females increased from January to March, highest values (~5 %) between March and May, and decreased in June – July in *S. officinalis* in the Mediterranean waters while males did not show any changes[26] similar to the present study. From Karnataka waters[27], higher GSI and NSI were observed in the trawled cuttlefish in November, coinciding with the higher proportion of mature individuals in this month[27].

The fecundity range of 1358 – 6005 with a mean of 2883 ova recorded in the present study is comparable with those of some earlier studies[3-5,10,19]. The RF is highest at 227 mm DML, approximately coinciding with the length at first maturity.

Depending on the size, ova diameter, ova maturation, and distribution range, cuttlefish are known to exhibit significant variations in fecundity, even among individuals of the same species. Date of sampling, stage of maturity, and modifications in environmental variables has also been noted to have an impact on fertility both within and between populations of the same species[28]. Ova diameter frequency analysis in the study showed that the medium size ova (3 – 10 mm) were more frequent throughout the year. The results showed that there were no older or younger individuals in the spawning population of this species. Due to the fact that larger ova sizes indicate populations of early-maturing spawners and smaller ova sizes indicate populations of very old spawners. This conclusion demonstrates that spawning populations of the species are long-term viable.

Conclusion

The judicious exploitation and sustainable management of stocks of the *S. pharaonis* depend on understanding their biology. The outcome of biological studies would be used in determining the size at first capture, net mesh size, and selecting the fishing grounds. Present study reports peak spawning activity between December to March months along the Gujarat coast. The information could be useful in the proper regulation and fishery management of *S. pharaonis*. This species' reproductive potential is demonstrated by its modest fecundity and year-round spawning. Such data is essentially the basis for fishery control, and season-wise and region-wise data will help to understand the environment or region specific changes in the fishery. The goal is to gather information that will aid in boosting yield whilst conserving the natural regeneration properties of the fish population. The current study is critical in order to utilise resources in a sustainable manner and to develop resource management plans.

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Conflict of Interest

There is no known conflict of interest between authors.

Ethical Statement

No animal testing was performed during this study.

Author Contributions

MP & HKK: Conceived and designed research; field work, collection and analysis of samples; and manuscript writing. MP, HKK, VS & VKV: Data analysis. VS: Assisted in field collection. All authors read and approved the manuscript.

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