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Diet composition and reproductive biology of spineless cuttlefish *Sepiella inermis* (Orbigny, 1848) from Ratnagiri (Arabian Sea, North-west coast of India)

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Abstract

In the present study, we investigated some biological aspects of the spineless cuttlefish, *Sepiella inermis* (Orbigny, 1848) along the Ratnagiri coast of Maharashtra, India. A study on diet composition and reproductive biology of the species was carried out. Dorsal mantle length (DML) ranged from 35.9 to 93.3 mm for males and 29.3 to 98 mm for females. Feeding intensity was noted to be low during most of the study period. *S. inermis* was found to have a protracted spawning season. The overall male: female ratio was 1:1.02 during the study period. The average fecundity was found to be 358 eggs. Length at sexual maturity was estimated to be 42 mm and 53 mm for males and females respectively. The information obtained from the study will help the fishery agencies to formulate management measures in sustaining the landings of *S. inermis* in the region.

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Introduction

Squids and cuttlefishes belonging to the class Cephalopoda support the largest catches of all molluscan fisheries (King, 1995). The production of cephalopods in India during the year 2019 stood at 217,699 t (CMFRI, 2019) contributing 6.6% to the total marine fish production. In India, around six species of cuttlefish are commonly landed namely, *Sepia pharaonis, Sepia aculeata, Sepia elliptica, Sepia prashadi, Sepia brevimana* and *Sepiella inermis* (Silas *et al.,* 1982; Meiyappan and Mohamed, 2003). They are widely distributed along both east and west coast up to depths of about 40 m. Trawlers are the main gear used to capture *S. inermis.* In Kerala and Tamil Nadu, shore seines and boat seines are also involved in its exploitation (Silas *et al.,* 1982). Along the Mumbai coast, *S. inermis* is caught by the artisanal gear dol net and mini trawls (Sundaram and Khan, 2011). *Sepiella inermis* belonging to the family Sepidae is an important species of cuttlefish captured off the Ratnagiri coast. The cephalopods contributed 31,896 t in 2019-20 to the marine fish landings of Maharashtra which show 16.69% growth over the previous year (Anon., 2020). Though *S. inermis* is an important species in the cuttlefish fishery from the shallow inshore waters, not much work has been carried out on this species from the Ratnagiri coast of Maharashtra, India. Thus, very scanty information is available on the biology of this major species of cuttlefish exploited along the Ratnagiri coast. Therefore, the present study aims to investigate the biological aspects of *S. inermis* along the Ratnagiri coast.

Materials and methods

Sampling

The samples from trawl bycatches were collected from the Mirkarwada fish landing centre (16059'42"N and 730 16'14"E) of Ratnagiri (Fig. 1) at weekly intervals from March 2017 to February 2018 except during monsoon (June and July) due to monsoon fishing ban. The present study was based on a total of 946 individuals of *Sepiella inermis* ranging from 29 mm to 98 mm dorsal mantle length (DML), comprising 468 males, 460 females and 18 indeterminants.

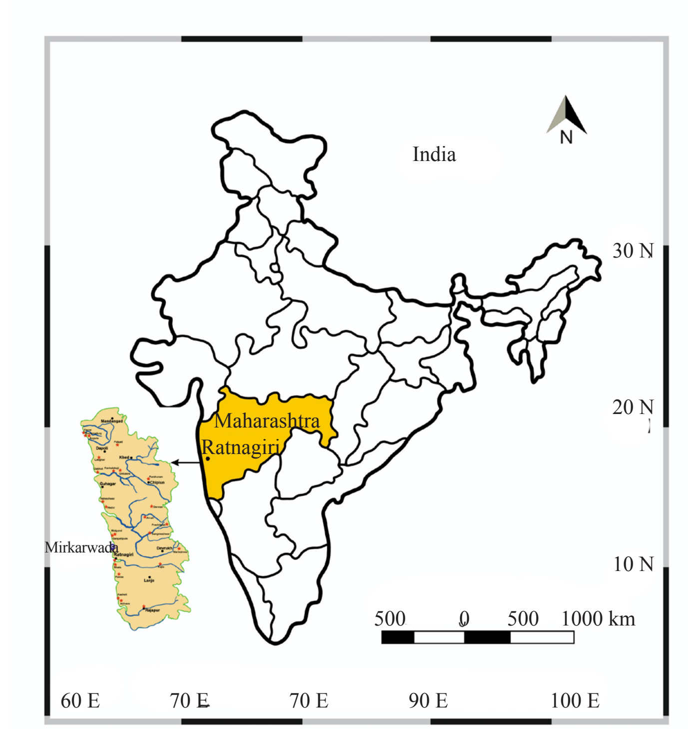

Fig. 1. Study area showing the sampling location of Mirkarwada, Ratnagiri.

Diet composition

Gut content analysis was carried out qualitatively and quantitatively. Qualitative analysis was done as per the procedure given by Qasim and Jacob (1972) and Biswas (1993). Identification of individual items was not always possible due to the semi-digested condition of food inside the stomach and the advanced state of digestion. Hence the categories of crustaceans, cephalopods and fishes included both identifiable and unidentifiable specimens. Quantitative analysis was carried out by the Points method (Hynes 1950). Each category of food item was allotted a number of points and all the points gained by each food item are summed and expressed as a percentage of the total points. Monthly data were pooled.

Reproductive biology

The cuttlefish were cut open and the sex and the stage of maturity were noted. Gonads were dissected out and the excess moisture was removed using blotting paper. They were weighed using analytical balance to the nearest 0.001 g.

The Gonado-somatic index (GSI) was estimated as per the following equation (Bal and Rao, 1984):

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

The nidamental gland index (NGI) was estimated using the following equation (Sundaram and Khan, 2011):

$$NGI = \frac{\text{Nidamental gland weight}}{\text{Total body weight}} \times 100$$

Data on sex ratio were analysed by χ^2 (chi-square) test to find out whether either sex was dominant. The maturity stages of males and females were identified based on the macroscopic characteristics of the testes and ovaries. To ascertain the different maturity stages, colour of the ovary and testes, size and length of gonads, size of the ova and other characteristics were noted. The female and male maturity stages of *S. inermis* were classified as immature (I), maturing (II), mature (III) and spent (IV). For determining the mean length at first reproduction (L_m), specimens with ovaries and testes in stages II, III and IV of maturation were considered as mature. Samples were collected to estimate the proportion of sexually mature individuals in different length classes (King, 1995). Fecundity was determined as per Sinha (1995). The development of ova from one stage of maturity to another was studied using measurements of ova diameters derived using software tps utility program version 1.74, tps Dig version 2.30 and PAST version 2.17. Frequency polygons were drawn for all stages of maturity.

Results and discussion

Diet composition

In the present study, the stomach contents of *S. inermis* were noted to be crustacean remains, cephalopods, digested matter and fish thereby indicating that *S. inermis* is a carnivore feeder which confirms the findings of Sundaram and Khan (2011), Unnithan (1982) and Parmar *et. al.* (2018).

Month-wise composition of food items

The average proportions of the gut contents for the entire study period were Digested matter 26.43%; Fishes 14.49%; Cephalopods 0.10% and Crustaceans 58.97% (Fig. 2). Crustaceans were observed in the highest quantity during May (76.40%) and least in November (29.80%). The digested matter was recorded with a peak in December (49.58%) and the lowest quantity was found during May (15.40%). Fishes constituted the

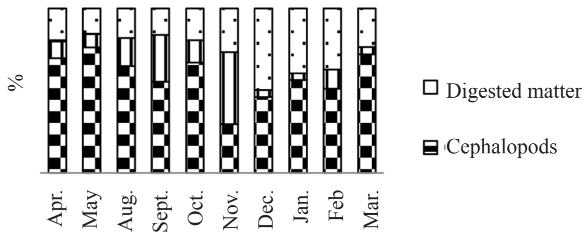

Fig. 2. Percentage composition of food items of *S. inermis* during different months

highest quantity during November (43.60%) and the lowest in January (4.09%). Cephalopods were also recorded in a few months with the least abundance in April (0.83%) and May (0.20%). Sundaram and Khan (2011) observed that crustacean remains, fishes, cephalopods and digested matter were the major constituents with crustacean remains being the dominant food item. The maximum amount of crustacean remains, fishes, cephalopods and digested matter were during January, September, March and November respectively. As per Parmar *et. al.* (2018) crustaceans were found to be the major constituent in all months with the maximum in December whereas the occurrence of fish was dominantly noted during September.

Unnithan (1982) reported that stomach contents included ahrimps, fishes, stomatopods, crabs and other crustaceans, and among these, shrimps dominated fishes followed by other items. However, variations in individual food items consumed by *S. inermis* are observed which may be due to the presence of respective food items in different regions. Stomachs were having mixed type of food items such as fish, crustaceans or cephalopod parts in *Sepia aculeata* as per Nalwa *et al.* (2005). The carnivorous-feeding habit of *S. inermis* noted in the present study confirms the findings of Tang and Khoo (1973); Unnithan (1982); Sundaram and Khan (2011) and Parmar *et al*. (2018).

Size-wise percentage composition of food items

In the present study, crustacean remains and digested matter was the dominant food items in juvenile and small-size fishes. Though crustacean remains were found in moderate quantities in all size groups, adults seemed to prefer fishes and cephalopods (Table 1).

Sundaram and Khan (2011) observed that *S. inermis* showed a preference for crustacean remains in all size groups. Fishes were preferred most by size groups 20-39 and 55-69 mm DML. Cephalopod parts were observed in small quantities in higher-size groups, revealing occasional cannibalism. As per Tand and Khoo (1973) shrimps were preferred by juveniles whereas both fish and shrimps were consumed by larger specimens of *S. inermis.* Unnithan (1982) observed that in *S. inermis,* shrimps constituted the common food item and fishes were next in importance whose preference seems to increase towards higher size groups.

The size-wise preference for various food items noted in the present study confirms the findings of Sundaram and Khan (2011), Tand and Khoo (1973) and Unnithan (1982) and it can be concluded that juveniles of *S. inermis* in common prefer crustacean diet while fishes and cephalopods are preferred by adults. Cannibalism as reported by Sundaram and Khan (2011) was not confirmed in the present study as cephalopods observed in the stomachs could not be identified up to the species level.

Feeding intensity

Results on the feeding intensity reveal that 8.32% of the stomachs were noted to be full, 7.30% were three-quarters full (3/4), 20.38% of the stomachs were half occupied (1/2), 34.81% were occupied up to a quarter (1/4) and 29.22% were empty stomachs during the entire study period (Fig. 3). The highest percentage of full stomachs were found in October (24.00%) followed by March (12.00%) and April (8.97%); while the lowest percentage was found in September (4.00%).

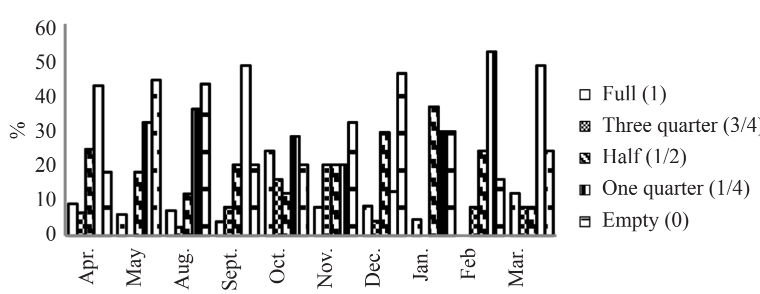

Fig. 3. Month-wise fullness of stomach in *S. inermis*

Table 1. Percentage composition of food items in the stomachs of *S. inermis* in various size groups

Size groups in (mm) DML	Crustacean remains	Fish	Cephalopods	Digested matter
26-35	48.46	0.00	0.00	51.54
36-45	65.54	2.50	0.00	31.96
46-55	60.86	7.17	0.00	31.97
56-65	57.37	14.03	0.10	28.49
66-75	60.50	15.52	0.02	23.96
76-85	59.50	22.37	0.36	17.77
86-95	40.86	45.71	0.00	13.43
96-105	20.00	80.00	0.00	0.00

Sundaram and Khan (2011) observed that *S. inermis* is having the maximum number of 'empty' stomachs in the length group 20-39 mm. '¼ full' stomachs dominated in all size groups. '½ full' ranged between 20-39 mm and 70-84 mm. '¾ full' and 'gorged' stomachs were maximum in the 70-84 mm group.

As per Unnithan (1982), a considerable percentage of *S. inermis,* 50-76.4% of males and 23.5-31.2% of females, showed empty stomachs. Gorged stomachs were noticed only in females. Feeding intensity was generally poor in the two sexes, 5.8 to 25% of males and in females the stomachs were 1/2 full or full. The intensity of feeding was highest during January to April. Tand and Khoo (1973) reported that juveniles of *S. inermis* fed heavily in May and June whereas mature males showed heavy feeding in all months except April and mature females fed heavily in August. Nalwa *et al.* (2005) reported that 26.42% of the stomachs were found empty, 19.81% had traces of food, 11.32% were quarter full, 9.43% were half full, 7.55% were three quarters full and 25.47% completely full.

Feeding intensity was noted to be low during most of the study period and also coincided with a prolonged spawning period. Individuals with full stomachs were observed during October. This may be due to increased feeding during the recovery phase after spawning. Major spawning is noted in September which is discussed elsewhere.

Gonado-somatic index (GSI)

The GSI of females and males were recorded and given in Fig. 4 and 5. GSI was highest for females during August (12.00) and February (11.01)

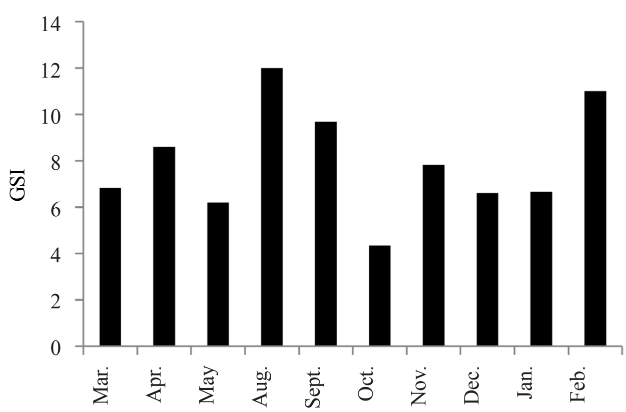

Fig. 4. Monthly GSI trend in females of *S. inermis.*

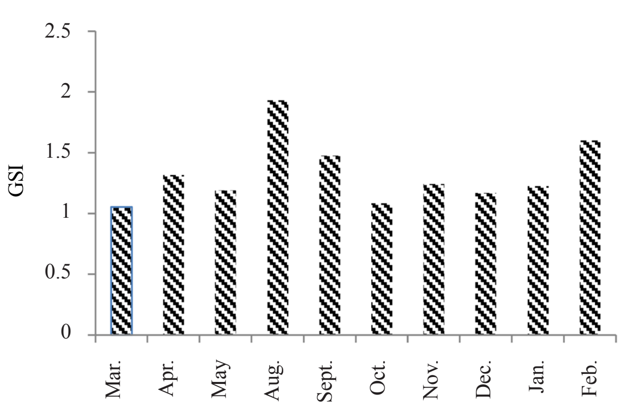

Fig. 5. Monthly GSI trend in males of *S. inermis*

followed by small peaks during April and November also, showing the occurrence of more ripe individuals. For males, GSI was highest during August (1.9317) and February (1.60) with minute peaks during April and November.

The GSI values for females indicated an increasing trend from May onwards and reached a maximum in August (12.00), which further dipped and gained in November a little, followed by slender depression and again reached a maximum in February (11.01). Thereafter a steep decrease was evident up to March with a slight increase in April. The GSI for males showed an increasing trend from May to August which declined thereafter and again reached a maximum value in February with a slight rise, during November. The GSI value again dipped after February and gained a small up during April.

Sundaram and Khan (2011) reported highest GSI for females of *S. inermis* in October, January and April from Mumbai waters. These peaks coincide with the spawning period. The GSI values in *S. inermis* ranged from 2.48 to 3.90 for testis while this value ranged from 4.35 to 9.30 for the females. The contribution of gonads to the total weight of the animal was comparatively higher in females than males along Thoothukudi coast (Neethiselvan *et al.,* 2002). The GSI values in males and females in the present study along the Ratnagiri coast correspond to the GSI values of *S. inermis* along Thoothukudi coast (Neethiselvan *et al.* 2002). As per Salman (2015) relative values of GSI mirrored the relative abundance of mature animals and spawning in *S. elegans* from the Aegean Sea. The higher GSI values in the males and females in the present study correspond to the spawning period of *S. inermis* along the Ratnagiri coast. The higher GSI values for both sexes were observed during the spawning season. Whereas higher GSI values for females noted in the present study confirms the findings of Neethiselvan *et al.* (2002). The findings in the present study agree with the earlier studies reported by Sundaram and Khan (2011) and Neethiselvan *et al.* (2002) for *S. inermis* and Salman (2015) for *S. elegans.*

Nidamental gland index

The nidamental gland index (NGI) was calculated for females and averaged for each month (Fig. 6). NGI showed wide variations throughout the sampling period. NGI was highest during February (8.9150) with another small peaks in April, August and November.

The NGI values in the present study for females showed an increasing trend from May to August and declined thereafter which again raised to its maximum value in February. The NGI values as per Sundaram

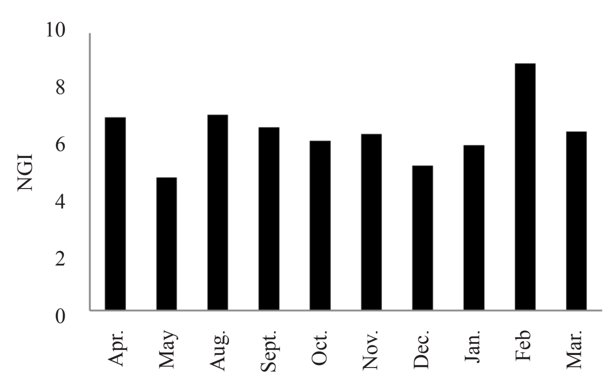

Fig. 6. Monthly NGI trend in females of *S. inermis*

and Khan (2011) indicated an increasing trend during spawning when higher GSI values and a higher proportion of mature specimens were also noted.

The nidamental gland is responsible for secreting the gelatinous egg capsule. After fertilisation of eggs in the coelomic cavity of females, the eggs are embedded in the egg capsules and released into the environment. Higher NGI values are thus noted during spawning season coinciding with higher GSI values. A similar trend was observed in the present study and confirm the earlier findings of Sundaram and Khan (2011).

Sex ratio

The average sex ratio of the females and males was found to be 1:1.02 for the entire study period (Table 2). Females were the dominant sex in all the months except May, September and January. The chi-square test indicated no significant difference between sexes during most of the study period ($p<0.05$).

The present work revealed the average sex ratio of the females and males to be 1:1.02 for the entire study period. Females were the dominant sex in all the months except May, September and January. The chi-square test indicated no significant difference between sexes during the study period ($p<0.05$).

Sundaram and Khan (2011) showed that females of *S. inermis* were dominant throughout the year. The sex ratio ranged between 1:1.10 (March) to 1:2.36 (June). The sexes were significantly different ($p<0.05$) throughout the year except in March. Whereas Unnithan (1982) found that in *S. inermis* females were proportionately more than males during all the months except in November when the two sexes were in equal proportion. As per Neethiselvan *et al.* (2002), sex ratio of *S. inermis* from Thoothukudi, Tamil Nadu did not differ significantly from the expected 1:1 ratio ($p<0.05$).

The findings of the overall sex ratio observed in the present study conform to the findings of Neethiselvan *et al.* (2002). While the findings on the overall sex ratio indicate the dominance of females as reported by Unnithan (1982) and Sundaram and Khan (2011). The dominance of either sex in catches might be possibly related to the size reached at a particular time, the selectivity of the gear and the vulnerability of either sex to capture (Kende *et al.* 2018).

Table 2. Results of the Chi-square test applied to test the significance of observed differences in the sex ratio in monthly samples of *S. inermis*.

Month	Total	Male	Female	df	Chi-square	
April	78	38	40	1	0.05	NS
May	96	52	44	1	0.67	NS
August	84	42	42	1	0.00	NS
September	100	52	48	1	0.16	NS
October	100	48	52	1	0.16	NS
November	100	48	52	1	0.16	NS
December	92	40	52	1	1.57	NS
January	86	48	38	1	1.16	NS
February	92	44	48	1	2.00	NS
March	100	48	52	1	0.16	NS
Total	928	460	468	1	0.32	NS

SS = Significant., NS= Non-significant

Maturity stages

Maturity stages were examined in 468 ovaries and 460 testes. The month-wise maturity stages of females and males are given in Fig. 7 and 8. The maturity in the case of females for stage I (Immature) was observed dominantly in the month of May and August. However, stage II (Maturing) was mostly observed during October and January. Maximum specimens in Stage III (mature) were noted in February, August, November and April. Stage IV (Spent) specimens were observed dominantly in September, March and December. Whereas, stage I (Immature) was observed dominantly in the month of May and December in males. On the other hand, stage II (Maturing) was mostly observed during January, November and March. Specimens in Stage III (mature) were mostly noted in August, November, February and April. Stage IV (Spent) specimens were observed dominantly in September and March.

Sundaram and Khan (2011) stated that a primary peak of mature females was observed in October, followed by three secondary peaks in January, April and August, which overlaps with the peaks in other indices (GSI, NGI) indicating that the species had spawning seasons during respective months.

As per Neethiselvan *et al.* (2002) in *S. inermis,* mature specimens were abundant in November and March during which the GSI values were also maximum for both males and females. Spent specimens were recorded throughout the year with a peak in December and April. Salman (2015) reported that in *S. elegans* two peaks of spawning were observed once in July and in October, during these months relative abundance of mature animals mirrored relative values of GSI and spawning.

Findings in current work revealed that mature (stage III) ovaries were noted maximum during the months of February and August, in the same months' other reproduction indices (GSI, NGI) were also high indicating the spawning period of *S. inermis*. Similar observations on the occurrence of mature individuals in advanced stages coinciding with higher GSI values were noted by Neetheselvan *et al.* (2002) and Sundaram and Khan (2011) for *S. inermis* along the coast of Mumbai and Thoothukudi respectively.

Length at sexual maturity (L_m)

The length at sexual maturity for females was estimated to be 53.80 mm and 42.34 mm for males (Fig. 9 and 10). The length at sexual maturity was estimated to be 53 mm and 42 mm for females and males respectively in the present work. As per Sundaram and Khan (2011) L_m

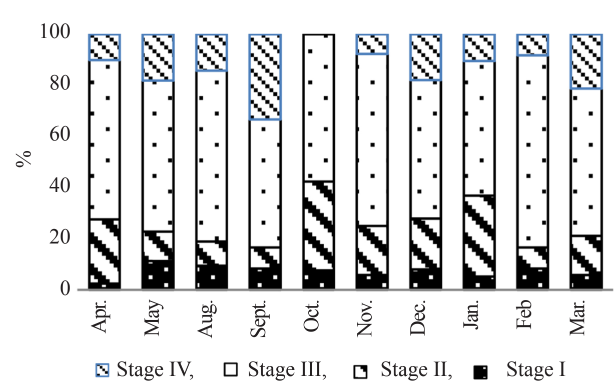

Fig. 7. Monthly variation in maturity stages in females of *S. inermis*.

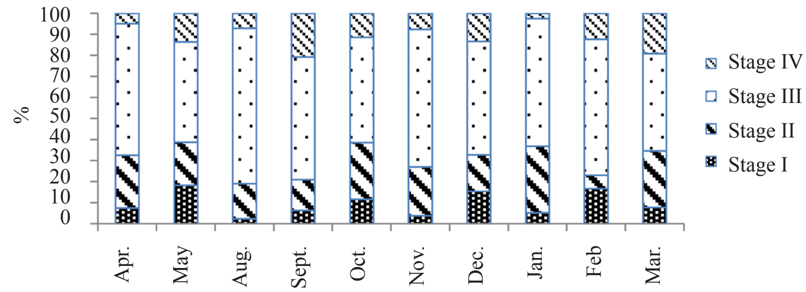

Fig. 8. Monthly variation in maturity stages in males of *S. inermis*

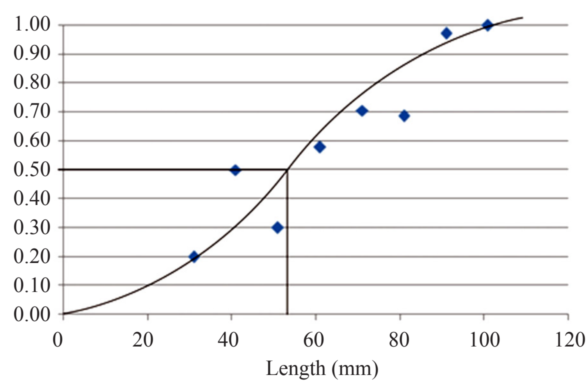

Fig. 9. Length at sexual maturity in males of *S. inermis* along the Ratnagiri coast

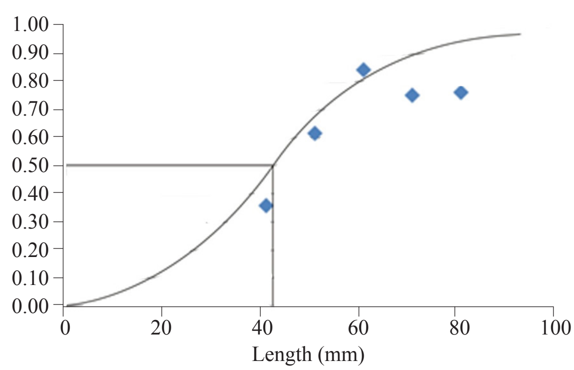

Fig. 10. Length at sexual maturity in females of *S. inermis* along the Ratnagiri coast

was 48 mm for males and 55 mm for females in *Sepiella inermis* along Mumbai waters. Unnithan (1982) reported L_m to be 51 mm for males and 31 mm for females in *S. inermis* off Mandapam and Rameswaram coasts. Whereas as per Neethiselvan *et al.* (2002) the figures were 55 mm for females and 62 mm for males of *S. inermis* from Thoothukudi, Tamil Nadu. Males were noted to attain L_m earlier in the present study which confirms to the findings of Sundaram and Khan (2011).

The difference in size at first maturity and within the sex of *S. inermis* from different regions may be possibly due to the variation in growth rate and maximum size reached by different species under the influence of varying environmental conditions and food availability

Fecundity

Fecundity for *S. inermis* was estimated by considering the stages prior to spawning. The study was undertaken on randomly selected 34 ovaries (Table 3). The fecundity of *S. inermis* ranged from 221 to 608 eggs with an average of 358 eggs. Matured ovaries were taken into consideration for fecundity estimation which ranged from 221 to 608 eggs with an average of 358 eggs.

Sundaram and Khan (2011) stated that the fecundity of *S. inermis* ranged from 216 to 354 ova from Mumbai waters. The fecundity of *S. inermis* varied between 470 and 850, off Mandapam and Rameswaram coasts (Unnithan, 1982). Nabhitabhata (1997) reported the fecundity of *S. inermis* in Bang Pa-kong, Thailand to be 500. Neethiselvan *et al.* (2002) stated that the fecundity ranged from 437 to 684 in the size range of 65 to 100 mm DML of *S. inermis* and the number of ova increased with an increase in DML. The fecundity varies from species to species and within species from region to region in accordance to the reproductive potential of the stocks (Kende *et al.* 2018).

Ova diameter study

Typical ovaries belonging to the four maturity stages were selected and the ova diameter frequency polygons were drawn (Fig. 11). The ova in stage I of maturity ranged from 0.185 to 1.827 mm with modes at 0.45 and 1.05 mm. The modes progressed to 0.75, 1.65 and 1.95 mm, 3.15 mm in stages II and III respectively, with range of 0.324-2.809 mm for stage II and 0.387-3.843 mm for stage III. Ova in stage IV ranged from 0.314 mm to 3.418 mm with a modal value of 2.25 mm.

It is found in the present study that the typical ovaries belong to the four stages of maturity. The ova in immature females (stage I) ranged from 0.185 to 1.827 mm with modes at 0.45 mm and 1.05 mm. The modes progressed to 0.75 mm, 1.65 mm and 1.95 mm, 3.15 mm in maturing (stages II) and mature (stage III) females respectively. Ova in spent females (stage IV) ranged from 0.314 mm to 3.418 mm with a modal value of 2.25 mm. As two modes are evident in mature females (stage III), it can be inferred that *S. inermis* probably spawns twice along the Ratnagiri coast in an extended spawning season.

Though there are no reports on the frequency of spawning of *S. inermis* based on ova diameter studies elsewhere from India albeit the ova diameter values in different maturity stages have been reported (Unnithan 1982; Sarvesan 1996; Sundaram and Khan 2011). The observed ova diameter values from Ratnagiri are parallel with those reported by Sundaram and Khan (2011) (0.38-0.79 mm for immature and 0.38-3.57 mm for mature specimen), Unnithan (1982) (0.67-2.24 mm for immature while 2.56-3.84 mm for mature) and Sarvesan (1996) (0.4-1.0 mm for immature and upto 3.81 for mature). However, based on ova diameter studies, the frequency of spawning for *S. pharaonis* and

Table 3. Number of mature ova in individuals of *S. inermis*

Sl. No.	Total length(mm)	Weight of fish (g)	Weight of ovary (g)	Total No. of mature ova in ovary
1	76.5	66.2	4.85	223
2	65.7	55.8	4.22	360
3	60	44.3	4.1	279
4	74.3	91.65	5.76	537
5	61	44.1	1.81	231
6	77	70.62	4.63	221
7	86	29.45	7.96	483
8	68	64.62	3.7	259
9	68	44.79	3.14	278
10	67	54.8	5.82	247
11	91	111.21	7.23	325
12	78	97.45	6.52	470
13	98	176.53	12.12	548
14	87	92.25	7.78	447
15	68	68.6	5.8	437
16	64	62.5	6.38	281
17	57	38.8	2.44	289
18	53	43	4.87	243
19	64	56.2	5.09	344
20	62	54.5	6.82	314
21	62	46.63	4.61	230
22	80	88.93	7.31	452
23	66	65.83	5.67	330
24	67	73.7	6.56	608
25	77	82.09	3.42	532

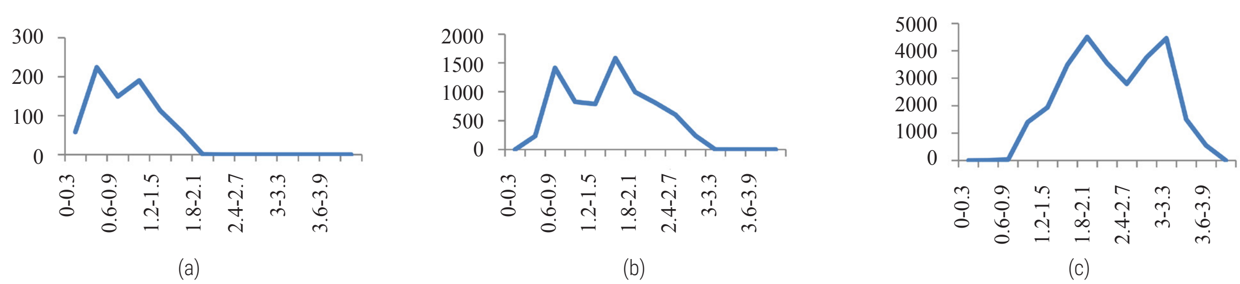

Fig. 10. Ova diameter trend in *S. inermis.* (a) Stage I, (b) Stage II and (c) Stage III

S. elegans has been reported by Gabr *et al.* (1998) and Salman (2015) has reported that *S. pharaonis* spawns twice in an extended spawning season from the Seuz Canal, Egypt and *S. elegans* spawns twice from Aegean Sea (Salman, 2015).

The present investigation provides basic information on the diet composition and reproductive biology of *S. inermis*, which will be useful to understand the relationship of this species with its food organisms and reproductive aspects. The biological data generated during the present study may be useful in the management of this commercially important species along Ratnagiri coast.

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