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Reproductive Biology of the Female Deepwater Crab *Charybdis smithii* (Brachyura : Portunidae) from the Indian Seas

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Abstract

Reproductive biology of the potentially commercial deepwater crab *Charybdis smithii* was studied. Samples were obtained during exploratory cruises made in Indian seas by the Fishery Oceanographic Research Vessel *Sagar Sampada*. Samples were obtained from both pelagic and benthic realms. Five stages of sexual development, namely, immature, early maturing, late maturing, ripe and spent, were distinguished based on morphological and histological examination of the ovary. Size at first maturity at 50% level for females was 48.7 mm carapace width (cw). However, the smallest ovigerous female encountered was 45 mm cw. Individual fecundity was relatively low (1,343-42,209) compared to other brachyuran crabs but eggs were larger. The crab population occupying the pelagic realm was exclusively in non-breeding phase, whereas breeding stock were found only at the bottom.

Introduction

The deepwater crab Charybdis smithii Macleay 1838 (Fig. 1) occurs in commercially exploitable quantities in several regions of the Indian seas (Suseelan et al. 1990; Sulochanan et al. 1991; Balasubramanian 1993). Although the occurrence of C. smithii has been very often reported from Indian waters in recent years, published information dealing with the reproductive biology of the species is scant. Della Croce and Holthuis (1965) and Losse (1969). made mention of surface swarming of this species, whereas Silas (1969) reported on its size and fecundity. The present study was prompted by the recognition that biological data on reproduction are required for judicious management and exploitation of this resource. In this paper, we report various aspects of reproductive biology of female crabs: morphology of reproductive tract, ovary development, gonadosomatic index (GSI), minimum size at maturity, fecundity and breeding habitat.



Fig. 1. Dorsal view of the female crab Charybdis smithii.

Materials and Methods

Crabs for the present study were obtained during cruises of the Fishery Oceanographic Research Vessel Sagar Sampada in the Indian EEZ and contiguous waters (Lat. 05° 00' N to 23° 30' N and Long. 65° 00'E to 77° 30'E on the Westcoast. Lat. 05° 00' N to 21° 30' N and Long 77° 30'E to 95° 30' E on the east coast) from February 1985 to December 1991. Three types of nets, namely Isaacs-Kidd midwater trawl (Isaacs and Kidd 1953), pelagic trawl and bottom trawl (Panikkar 1990), were used during the cruises.

In the laboratory, crabs were sexed and carapace width (cw) measured to the nearest 0.1 mm (cw was measured between the sixth anterolateral teeth), and the weight of each animal was recorded to the nearest 0.1 g using an electronic balance. Pleopods and vulva of the crabs were examined to determine if mating/extrusion of eggs had taken place. Presence of eggs or egg remnants or their absence on pleopods, color of egg mass and condition of vulva were noted.

The morphology of the reproductive tract was studied by dissecting mature crabs. The stages of maturation were determined by 1) macroscopic examination of the size, color and turgidity of ovaries after incision of the carapace; 2) analysis of GSI and size of whole oocytes; and 3) histology.

Measurement of whole oocytes and histological examination were made by using 164 females in various developmental stages. The ovaries were dissected just after catch and preserved onboard. Ovaries of 64 of these females were preserved in Bouin's solution and samples processed later, using standard histological techniques. Sections were cut into 6-8 μ m thickness and stained using Harris hematoxylin and counter-stained in eosine. Ovaries of the remaining 100 females were preserved in 4% formaldehyde for analyses of size and number of oocytes. Fifty oocytes were measured along their largest diameter using a binocular microscope with an ocular micrometer calibrated with stage micrometer. GSI was expressed as the percentage of ovarian weight in relation to body weight.

Fecundity was calculated by counting the number of eggs present in the pleopods of 30 ovigerous crabs. Only crabs carrying eggs in the early stages of embryonic development were used for this purpose. Egg-carrying pleopods were first removed from the crabs and immersed in a concentrated solution of sodium hydroxide as suggested by Melville Smith (1987). The eggs came free from the pleopods after 3-6 h. The eggs were then filtered and weighed to the nearest 0.1 g. Three subsamples of eggs were removed from the net filter and accurately weighed and counted. Using these results, the total number of eggs for that crab was estimated.

Results

Morphology of the Female Reproductive Tract

The female reproductive system comprises a pair of ovaries, a pair of spermatheca or seminal receptacle, and a pair of vagina (Fig. 2). The entire ovary is bounded by fibrous connective tissues which separate the organ from the surrounding hemocoel. The ovary is 'H'-shaped and located dorsally just beneath the carapace. The horns of the ovary extend anterolaterally from either side of the gastric mill and lie dorsally to the hepatopancreas. At the posterolateral border of the gastric mill, near the origin of the posterior mandibular muscle bundles, the anterior horns are joined by a commissure. Two posterior horns, which lie ventral to the heart, extend posteriorly on either side of the intestine. The posterior prolongation of the ovary is subequal, one of the horns being larger and extending further 5-6 mm beyond the other. The seminal receptacles arise from the midlateral border of the posterior horns. Each seminal



Fig. 2. Reproductive system of crab. O = ovary, Sp = spermatheca, V = vagina.

receptacle leads into a narrow vaginal tube which further opens outside through a small circular gonopore (vulvae) situated on the sixth thoracic sternite.

Stages of Maturity

Based on color change, external morphology and histology, the ovary was divisible into five maturity stages, namely, immature, early maturing, late maturing, ripe and spent. Table 1 shows the details of the morphological changes taking place in different maturity stages of the ovary.

IMMATURE

The ovary is thin, tubular, translucent and extremely difficult to locate macroscopically. It is usually less than 2 mm thick and without pronounced lobation. Ova are small, between 25 and 45 μ m, with a mean of 30 μ m.

EARLY MATURING

The ovary is easily visible macroscopically, ivory or light yellow in color, and occupies about one half of the volume of hepatopancreas dorsally. At this stage, the ovary has a lumen and well developed germinal strand with oogonial cells. These oogonial cells are characterized by large nuclei and small amounts of ooplasm (Fig. 3).

LATE MATURING

The volume of the ovary is subequal to the hepatopancreas in size. Ova are conspicuous when the ovary is viewed macroscopically. The color of the ovary varies from yellow to yellowish orange. Oogonial cells develop into primary oocytes. The nuclei of these oocytes continue to be large with uniformly distributed chromatin (Fig. 4).

Maturity stages	No. of Observations	Color	Horn width range (mm)	Weight (g [.])	GSI	Ova diameter (µm)
Immature	12	Translucent	1.1-1.5	0.34±0.04	0.92-1.38(1.23)	25-45(30)
Early maturing	12	lvory to light yellow	2.0-3.5	0.62±0.14	2.4-2.9(2.62)	51-119(70)
Late maturing	20	Yellow to yellowish orange	3.5-7.0	1.10±0.43	3.26-5.1(3.91)	85-357(177)
Ripe	05	Dark orange	5.5-10.0	4.10±0.34	6.0-8.3(6.6)	102-374(233)
Spent	15	Translucent	2.0-4.0	0.37±0.03	2.6-3.5(2.82)	34-340(102)

Table 1. Color, size, gonadosomatic index (GSI) and ova diameter in different maturity stages of the ovary of *Charybdis smithii*. Mean values of GSI and ova diameter are given in parenthesis.



Fig. 3. Histological section of early maturing ovary (H & E x100) GZ = germinal zone, oo = oogonia, Po = primary oocyte.



Fig. 4. Histological section of late maturing ovary (H & E x 400).

RIPE

The ovary is the dominant visible organ obscuring the hepatopancreas dorsally with dark orange coloration. The enclosing fibrous connective tissue is highly stretched, often to the point of bursting during dissection. The nucleus is solid and centrally placed, with small yolk droplets appearing in the peripheral region of the ooplasm (Fig. 5).



Fig. 5. Histological section of ripe ovary. Note the solid and centrally placed nucleus (N) and yolk droplets (Y) (H & E x400).



Fig. 6. Histological section of spent ovary (H & E x 100). Do = developing oocyte, P = phagocytic cells, Uo = unspawned ova.

SPENT

The ovaries appear flaccid, translucent and greatly reduced. Unspawned ova are visible through outer fibrous connective tissue. The germ strand is well defined, with oogonial and developing oocytes radiating out from this region (Fig. 6). The greatest part of the ovary consists of fibrous connective tissue and hemocoel space containing blood cells and phagocytes. Mature unspawned ova undergoing resorption are often present and surrounded by phagocytes.

The mean GSI was 1.23 in immature crabs with a gradual increase to 3.91 in late maturing. The highest GSI value of 6.6 was observed in the ripe stage, followed by a steep decline in the spent stage with a mean value of 2.82.

Minimum Size at Maturity

The smallest berried female encountered during the present study measured 45 mm cw. From an analysis of incidence of ovigerous females at every 1 mm interval among 322 females over a size range of 40-62 mm cw, the size at first maturity at 50% level was 48.7 mm cw (Fig. 7).

Ovigerous Females

After extrusion of eggs during oviposition, the eggs attach to the setae present on the endopod of the pleopods. Based on color change, increase in size, and change in shape of eegs (berry), three different stages of egg development were observed, and a summary is given in Table 2. Mean egg size was 300 μ m in stage 1, increased to 340 μ m in stage 2, and attained its highest value of 476 μ m in stage 3. The ovaries of egg-bearing females showed various phases of maturation and their development was almost completed when the berry was in the third stage of embryonic development. This would suggest that ovarian maturation is a continuous process after attainment of maturity.

Fecundity

The 30 ovigerous females used for calculation of fecundity ranged in size from 48 to 61 mm cw. Individual fecundity ranged from 1,343 to 42,209, the average being 16,168.

Stages of	No. of	Color o f	Shape	Size of eggs (µm)	
development	specimens		of		
	examined	berry	eggs	Range	Mean
Stage 1	15	Red orange	Spherical	233-340	300
Stage 2	10	Light yellow	Spherical	310-425	340
Stage 3	5	Deep brown to	Elliptical	415-595	476
		black			

Table 2. Colors of berry, shapes and sizes of eggs in different stages of embryonic development.



Fig. 7. Minimum size of oviposition at 50% level in charybdis smithii.

Breeding

A total of 591 female crabs in the size range 11-69 mm cw, obtained from the pelagic trawl and Isaacs-Kidd midwater trawl, and 233 females in the size range of 31-62 mm cw, obtained from bottom trawl catches, were examined for ovarian changes and ovarian condition. In the pelagic collections, the ovaries of all specimens were immature. Furthermore, no crabs in the ovigerous stage could be seen nor did they reveal any indication of egg carriage. Out of 591 females studied, 178 crabs above the minimum size of maturity at 50% level did not show signs of maturity externally or internally. On the other hand, samples drawn from bottom trawl operations showed a high incidence of crabs in breeding condition. About 83% of crabs examined were in the ovigerous state and the remaining had their ovaries in different stages of maturation (Fig. 8).

Discussion

Female Reproductive System

The female reproductive system of *C. smithii* conforms to the general pattern in portunid crabs, closely agreeing with the structure observed by Estampador (1949) in *Scylla serrata*, George (1963) and Ryan (1967) in *Portunus sanguinolentus*, and Johnson (1980) in *Callinectes sapidus*. In varia-



Fig. 8. Relative abundance of ovigerous and non-ovigerous females of *Charybidis smithii* in the pelagic and benthic habitats by size.

tion from other species, a recognizable oviduct has not been observed in this species between ovary and spermatheca. Pearson (1909) observed in *Cancer pagurus* that the posterior prolongations of the ovary are connected at the posterior end. A similar condition has also been reported by Estampador (1949) in species of genus *Scylla* in mature condition. In the present species, however, the posterior extremities of the ovaries are permanently separated. In *P. sanguinolentus*, George (1963) noticed the posterior prolongation on the right side of the ovary to be shorter and narrower than on the left side. In *C. smithii*, there was no consistency in the relative lengths of the lobes of the ovary on the two sides as posterior prolongation on one side was either shorter or longer than on the other side.

In many species of brachyurans, fresh mating of female crabs is indicated externally by a hardened mass of spermatozoa, called sperm plug, together with the associated secretions protruding from the vulva (Hartnoll 1969). No such sperm plug was encountered during the present study as also reported in the deepsea red crab *Chaceon (=Geryon) quinquedens* by Haefner (1977) and Elner et al. (1987).

Maturation

Morphological and histological examinations of ovaries confirmed the distinction of five stages of maturation for *C. smithii*. Among different workers who have studied the maturation of ovaries in brachyuran crabs, there is little consistency as to the number of maturity stages recognized. Haefner (1976, 1977) recorded six stages in rock crab *Cancer irroratus* and five stages in *Chaceon quinquedens*. Dhas et al. (1980) came across five maturity stages in *Portunus pelagicus*, whereas Sukumaran et al. (1986) recognized four maturity stages, excluding the spent stage, in *P. sanguinolentus*. Erdman and Blake (1988) divided the developmental stages in the deepsea golden crab *Chaceon fenneri* into six.

Breeding

Most of the bottom trawl samples were in an advanced stage of maturation or in ovigerous condition, and from this it would appear that the crabs could breed actively at bottom. Fecundity estimated for this crab by Silas (1969) varies between 11,363 and 29,154, with a mean value of 21,956. However, the present results indicate a wide variation in fecundity (1,343-42,209; mean 16,168).

The size of eggs found in the berry was comparatively larger in this species, the maximum diameter recorded being 595 μ m, as compared to the size reported for related species occurring in the coastal waters of India (George 1949; Prasad and Tampi 1953; Naidu 1995). In the case of the congeneric species *C. feriatus*, Padayatti (1990) recorded the maximum size at 430 μ m. Many authors have also reported larger yolky eggs for deepsea brachyuran crabs (Haefner 1978; Hines 1982, 1988; Hinsch 1988). According to Hines (1988), the larger egg size for deepsea brachyurans helps provide nutritional flexibilities to their larvae, compared to smaller eggs of species inhabiting the neritic regions of the seas, where larvae get a more predictable source of food than in the deep-oceanic region.

The present study clearly showed that the benthic population represented the breeding stock of this species, whereas the pelagic population did not form a part of the reproductive population. Observations of Della Croce and Holthuis (1965) and Losse (1969), who also reported the absence of mature/ovigerous crabs of this species in their pelagic collection, agree with the present observations. Further studying a closely related deepwater crab, *Portunus* (Archelous) *affinis*, Jerde (1967) reported the absence of spawning stock in the pelagic habitat. Pelagic stock of *C. smithii* found in oceanic waters might have developed from the larvae, drifted out to sea and subsequently developed into young crabs as suggested by Jerde (1967) for *P.* (A) *affinis*. Presence of clearly distinct breeding and non-breeding stocks of the species will enable the selective commercial exploitation of non-breeding pelagic stock without damaging benthic reproductive stock.

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