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## Male reproductive system and spermatogenesis in the deep water crab *Charybdis smithii* McLeay (Brachyura : Portunidae)

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### ABSTRACT

Morphology of male reproductive tract and spermatogenesis of the potentially commercial deep water crab *Charybdis smithii* were studied in specimens obtained during the cruises of Fishery Oceanographic Research Vessel *Sagar Sampada* in the Indian seas. Male reproductive tract is composed of testis, vasa deferentia, ejaculatory duct and external penis. Vas deferens is classified into anterior vas deferens (AVD), middle vas deferens (MVD) and posterior vas deferens (PVD). The tubular testis comprises numerous acini or seminiferous lobules arranged around central seminiferous duct. The spermatogenesis involves the progressive reduction of cytoplasm and only one developmental stage was found in one seminiferous lobule at a time. The simple ellipsoid spermatospheres are lodged in the AVD. The epithelial cells in the proximal portion of AVD are cuboidal whereas distal portion of AVD has columnar epithelial cells.

### Introduction

The deep water crab *Charybdis smithii* occurs in commercially exploitable quantities in several regions of Indian Exclusive Economic Zone (Silas, 1969; Mohamed and Suseelan, 1973; Sulochanan *et al.*, 1991). The occurrence of this species has often been reported from Indian waters in recent years, yet the published information dealing with its biology, ecology and population structure has remained scant. Aspects of reproductive biology of females and feeding habits have been reported by Balasubramanian and Suseelan (1998a, 1998b). In crustaceans male reproductive biology and physiology is not well understood as

that of females (Aiken and Waddy, 1980) and even less in the case of deep water species. In this paper we report morphology of the male reproductive tract and the process of spermatogenesis.

### Materials and methods

Crabs for the present study were obtained during cruises of Fishery Oceanographic Research Vessel *Sagar Sampada* in the Indian Exclusive Economic Zone and contiguous waters. Live male crabs trawled by the pelagic trawl and bottom trawl (Panikkar, 1990) were used for this study. Gonads and vasa deferentia of 50 male crabs (35-70 mm carapace width) were used for gross morphology and histological studies.

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Morphology of the reproductive tract was studied by dissecting mature crabs. For histological studies parts of testicular lobes and vasa deferentia were fixed in Bouin's fluid for 24–48 h. These tissues were then dehydrated in ascending series of ethanol, embedded in paraffin (melting point 58–62°C) and serially sectioned at 6–8  $\mu\text{m}$  and stained with Harris haematoxylin and eosin.

## Results

Male reproductive system of *C. smithii* is bilateral and composed of testis, vasa deferentia, ejaculatory duct and external penis (Fig. 2). The first and second abdominal appendages are highly modified to function as copulatory organ. In immature crabs the testis is rather inconspicuous and difficult to make out dissection. Mature

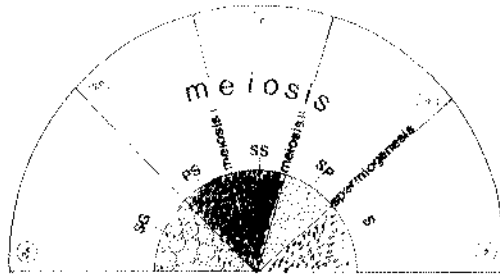


Fig. 1. *Charybdis smithii*. General representation of the stages of spermatogenesis in adult male crab. SG-spermatogonia, PS-primary spermatocytes, SS secondary spermatocytes, SP spermatids, S-sperms, n-haploid, 2n-diploid.

testis has the appearance of slender white convoluted tube (mean width = 2–3 mm). It is sandwiched between the hypodermis of the carapace and the hepatopancreas. The distal portion on either side is bent along the anterior border of the carapace. A short crossbar joins the testis of the two sides, a little

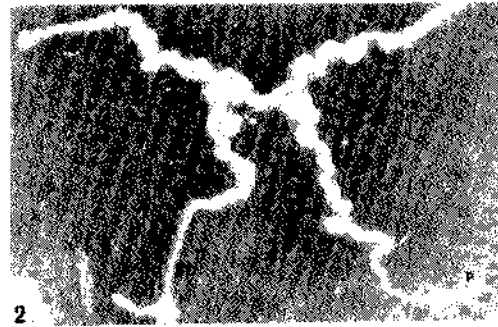
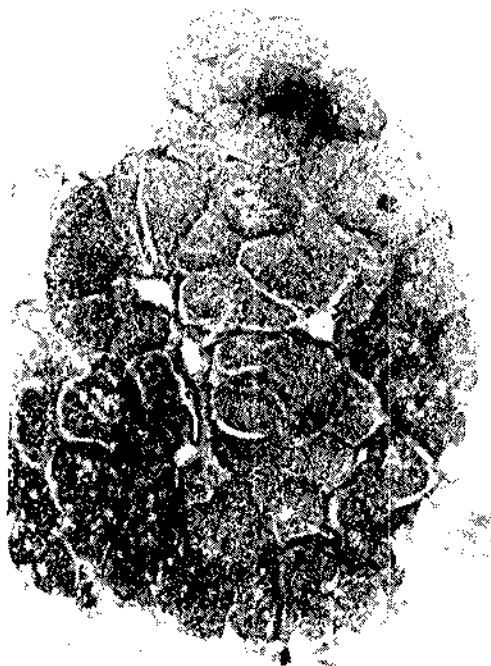


Fig. 2. *Charybdis smithii* - Male reproductive system. T-testis, AVD-anterior vas deferens, MVD-median vas deferens, PVD-posterior vas deferens, ED-ejaculatory duct, P-penis.

ahead of the middle of the carapace. Vas deference is composed of three regions : anterior vas deferens (AVD), middle vas deferens (MVD) and posterior vas deferens (PVD). The AVD are white, tightly coiled and lying on either side of the median line of cephalothorax posterior to the dorsal part of the stomach. The slender translucent and delicate anteriormost coils of AVD is bound by thin but strong membrane and cannot be separated or straightened by dissection. The coils increase in size postero-ventrally and lead into the middle vas deferens. The PVD is massive for its proximal part, but gradually narrow before opening to the ejaculatory duct. The ejaculatory duct arises behind the PVD as narrow tube which passes through the musculature of the fifth walking leg and opens to the base of coxal segment through penis. The penis is slender weak tube with diameter ranging from 1.6 to 1.8 mm and length 6–8 mm. Each penis passes into the anterior proximal foramen of the first pleopod, which serves as the functional intromittant organ. The second pleopod is inserted into the



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Fig. 3. Testis: light micrograph (x 300). Note acini (A) and seminiferous duct (arrows).

posterior foramen of the first pleopod and it forces semen and spermatophore through the first pleopod.

The testis is essentially a tubular organ composed of numerous acini or follicle (= seminiferous lobules) which are arranged around a central seminiferous duct or collecting tubule (Fig. 3). The wall of testis has two layers - delicate surface membrane and a crenated layer of fibrillar connective tissue around most of its periphery but always show confluence of its contents with neighbouring follicle or seminiferous duct. The seminiferous duct varies from 40 to 300  $\mu\text{m}$  in diameter and lined with columnar epithelial cells. This duct empties into the vas deferens.

### Spermatogenesis

The development of male gametes from spermatogonia through primary and secondary spermatocytes to sper-

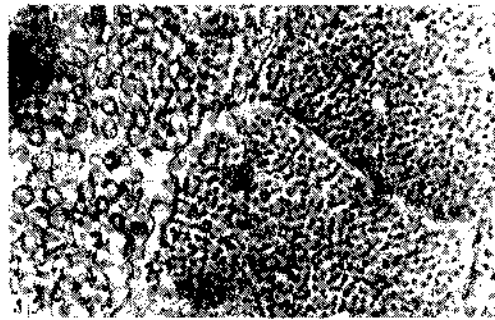


Fig. 4. Acinus showing spermatogonia (SG) and primary spermatocytes (PS) (x 400).

matids takes place in the follicles. The various stages of spermatogenesis are summarised in Fig. 1. In the immature crab, the whole testis is occupied by spermatogonia. In maturing/mature stages of gonad, the spermatogonia can be seen in groups in the periphery of seminiferous lobule (Fig. 4). Spermatogonia either divide mitotically to provide more spermatogonia or enter meiotic prophase and become primary spermatocytes (Fig. 4). Secondary spermatocytes form at the end of first meiotic division (Fig. 5) and spermatids result at the end of second meiotic division (Fig. 6).

The spermatogonia has large granular nuclei varying in size from 7-10  $\mu\text{m}$  in diameter. Their nucleoli are well developed and cytoplasm of spermatogonia is represented by thin eosinophilic covering. The primary and secondary spermatocytes have more condensed nuclei and they are smaller than the nuclei of spermatogonia. Newly formed spermatids have dense basophilic nuclei and smaller amount of cytoplasm. The nuclei of sper-

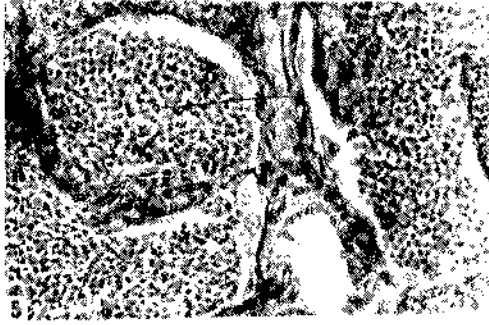


Fig. 5. Section of testis showing secondary spermatocytes (SSS) (x 400). Note each lobule is in a single stage of development.

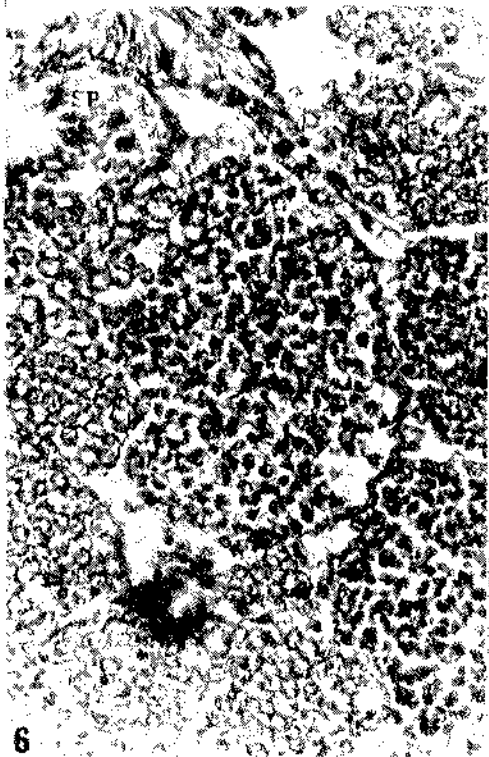


Fig. 6. Section of acinus showing spermatids (SP) and sperm (S) (x 400).

matocytes measured 5–6  $\mu\text{m}$ . Finished sperms are larger than the partly developed spermatids. Appearance of mature sperms depends on the plane of section

(Fig. 6). Since spermatogonia involves progressive reduction of cytoplasm and condensation of chromatin to produce spermatocytes, the spermatogonia cells are found to be larger than the spermatocytes. The spermatocytes in turn are larger than the spermatids. The cells within each follicle appear in the same stage of spermatogenesis and those in adjacent follicle tend to be in closely related phases.



Fig. 7. Cross section of proximal portion of anterior vas deferens. Many portions of vas deferens are apparent (x 100).

A transverse section of coiled mass of AVD is shown in Fig. 7. The different portions of AVD which have come in the plane of section are represented by many circles. Numerous ellipsoidal sperm masses surrounded by thin walled spermatophore are contained in the lumen (Fig. 8 & 9) and this portion



Fig. 8. Section of anterior vas deferens. Note the lumen filled with spermatozoa (SPH). Arrow indicates the cuboidal epithelial cells ( $\times 400$ ).

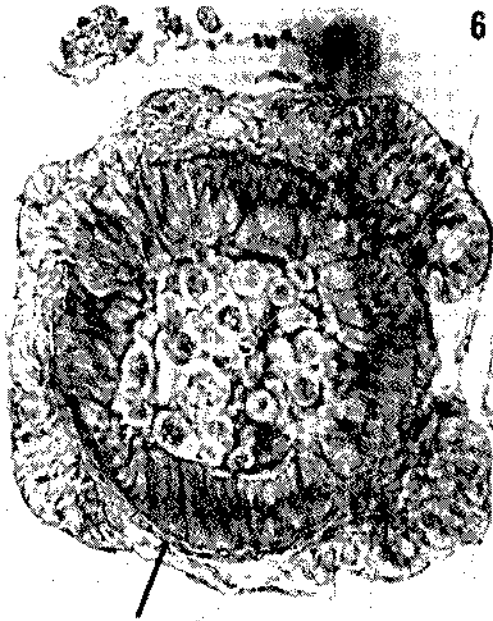


Fig. 9. Cross section through the distal region of anterior vas deferens. Note columnar epithelial cells surrounding the spermatozoa ( $\times 100$ ).

of vas deferens is the only region where spermatozoa are found at the proximal part of AVD, the wall is lined with cuboidal epithelium surrounded by thin strata of muscle and connective tissue whereas distal portion of the AVD is lined with columnar epithelial cells having a central nucleus in each (Fig. 10). The lumen appears to be filled with viscous fluid in which spermatozoa are lodged.

### Discussion

Very few workers have described any well defined maturity stages in male crabs. Baehner (1976), using the testes has described 6 maturity stages of

for males of *Cancer irroratus*. In the present study, however, no such distinct developmental stages were discernible for the testes externally. The gross morphology and histology of male reproductive system of *Charybdis smithii*, in general, is closely agreeing with the structure reported by Cronin (1947) and Johnson 1980 in *Callinectes sapidus*, Estampodor (1949) in *Scylla serrata*, George (1963) and Ryan (1967) in *Portunus sanguinolentus*, McVillie-Smith (1987) in *Chaceon* (= *Geryon*) *marinae* and Hirsch (1988) in *C. fennellii*. In the case of male *P. sanguinolentus* the observed subdivisions of testicular lobe (Ryan, 1967) was only an occasional feature, and whenever noticed, it



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Fig. 10. Columnar epithelium (CEC) of distal region of anterior vas deferens. Nucleus is apparent in the cells (arrow) (x400).

was only incomplete. Joshy and Khanna (1982) could not find any lobation and each incomplete lobe is divided into many acini or seminiferous lobules. The present species showed incomplete lobation and each of the incomplete lobes is divided into many acini. There is a distinct seminiferous duct and all the lobules have access to the same either directly or through other lobules. In *P. koolooense* Joshi and Khanna (1982) could not come across any distinct seminiferous duct, where the seminiferous lobule directly opens into the vas deferens. The seminiferous duct in some case has been reported to be branched as noted by Cronin (1947) in *C. sapidus*. In the present species, however, no indication of branching of

seminiferous duct was observed. Though many portions of seminiferous duct could be seen in a section, it could be due to the looping of whole testis as suggested by Ryan (1967).

Structural and ultrastructural studies of spermatogenesis in crustacean species of different taxonomic group (King, 1948; Aiken and Waddy, 1980; Johnson, 1980) have led to the finding that the process is basically similar in all species. Spermatogenesis of decapod can be synchronous or asynchronous depending on the species (Krol *et al.*, 1992). In synchronous species only one developmental stage occur concurrently in one seminiferous lobule. Present study indicates that *C. smithii* is a synchronous species as in the case of most brachyuran crabs, and penaeid shrimps (Johnson, 1980; Melville-Smith, 1987; Lu *et al.*, 1973). Asynchronous development has been reported in the brachyuran crab *Menippe mercenaria* (Binford, 1913) and penaeid shrimp *Sicyonia ingentis* (Shigekawa and Clark, 1986).

Though the process of spermatogenesis is basically similar in all decapod crustaceans, the formation of spermatophores does not always follow the same in all. In some group of decapods, final maturation occurs after spermatids leave the testis (Krol *et al.*, 1992). In *C. smithii*, however, the development of spermatozoa continues as the cells are transported towards vas deferens. Three general types of spermatophores have been recognised in decapods, i.e. spherical or ellipsoid, tubular and pedunculate (Krol *et al.*, 1992). Comparative morphology of decapod spermatophores suggest their relationship with fertilization (Uma and Subramoniam, 1984). Decapod which carry out external

mating and fertilization developed greater complexity in the formation of spermatophores to protect the sperm mass as effectively as possible (Demestre and Fortuno, 1992). In brachyuran crabs with internal fertilization, the simplest type of spermatophore is found (Subramoniam, 1991). In the present study the ellipsoid spermatophore was observed and Uma and Subramoniam (1984) have also reported similar type of spermatophore for inshore portunid, *Scylla serrata*. The proximal part of the AVD is made up of cuboidal epithelial cells and distal part is made up of columnar epithelial cells. Finding similar pattern in *S. serrata* Uma and Subramoniam (1984) suggested that histological variation between the two regions of AVD is associated with the production of different secretory substances for the formation of spermatophore.

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