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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 ellopsid spermatosphores 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 Fishey 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 carpace 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 µm and stained with Harris haematxylin 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

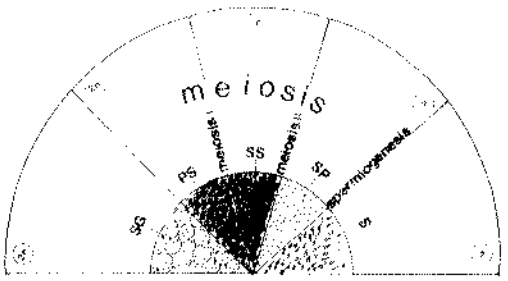

Fig. 1. *Charybdis smithii*. General representation of the stages of spermatogenesis in adult male crab. SG-spermatogonia, Ps-primary spermatocytes, SS secondary spermatocytes, SP spermatids, S-sperms, n-haploid, 2n-diploid.

testis has the appearance of slender white convoluted tube (mean width = 2–3 mm). It is sandwitched 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

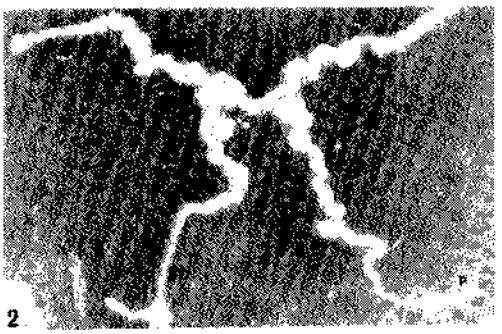

Fig. 2. *Charybdis smithi* - Male reproductive system. T-testis, AVD-anterior, vas deferens, MVD-median vas deferens, PVD-posterior vas deferens, ED-ejaculatory duct, P-penis.

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 transluscent 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 posterio-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 arterior proximal foramen of the first pleopod, which serves as the functional intromitant organ. The second pleopod is inserted into the

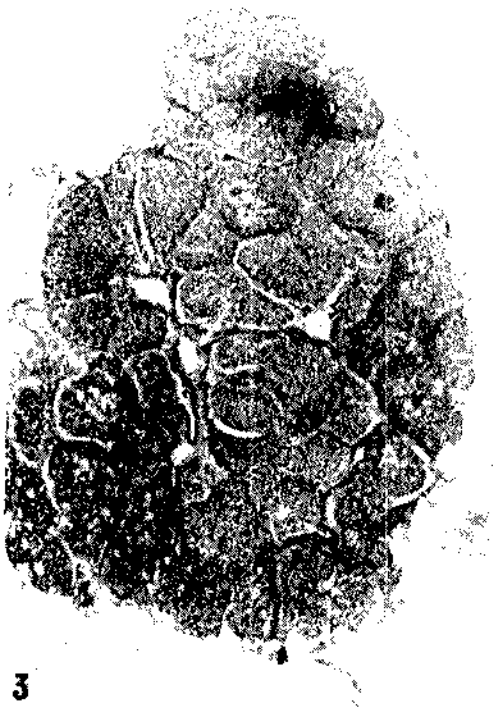

Fig. 3. Testis : light micrograph (x 300). Note acini (A) and seminiferous duct (arrows).

posterior foramen of the first pleopod and it forces semen and spermatophore throgh 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 μm in diameter and lined with columner epithelial cells. This duct empties into the vas deferens.

Spermatogenesis

The development of male gametes from spermatogonia through primary and secondary spermatocytes to spermatids 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).

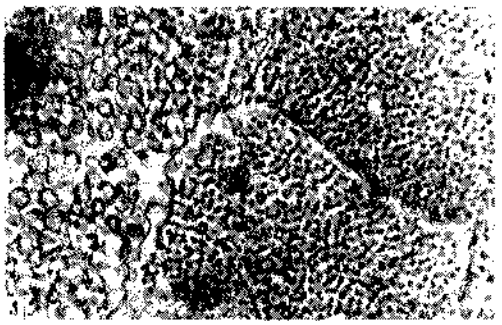

Fig. 4. Acinus showing spermatogonia (SG) and primary spermatocytes (PS) (x 400).

The spermatogonia has large granular nuclei varying in size from 7–10/μm 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-

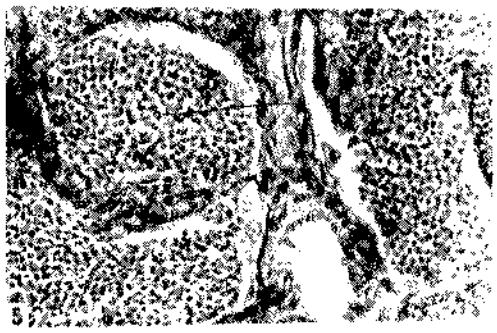

Fig. 5. Section of testis showing secondary spermatocytes (SS) (x 400). Note each lobule is in a single stage of development.

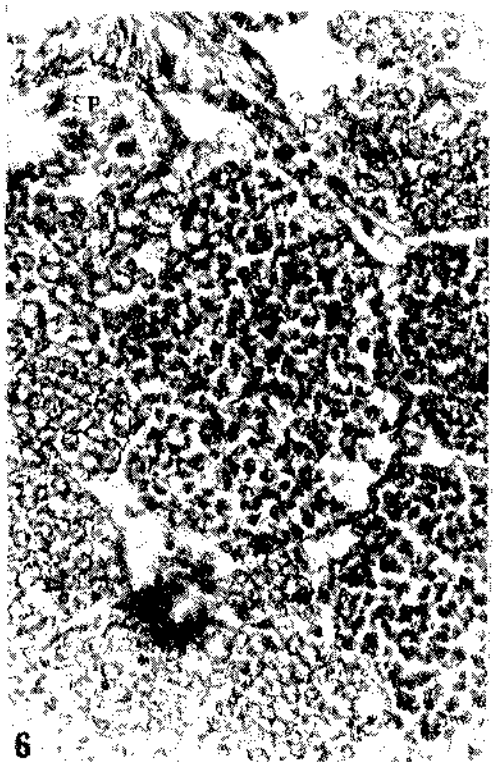

Fig. 6. Section of acinus showing spermatids (SP) and sperms (S) (x 400).

matocytes measured 5–6 µm. 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.

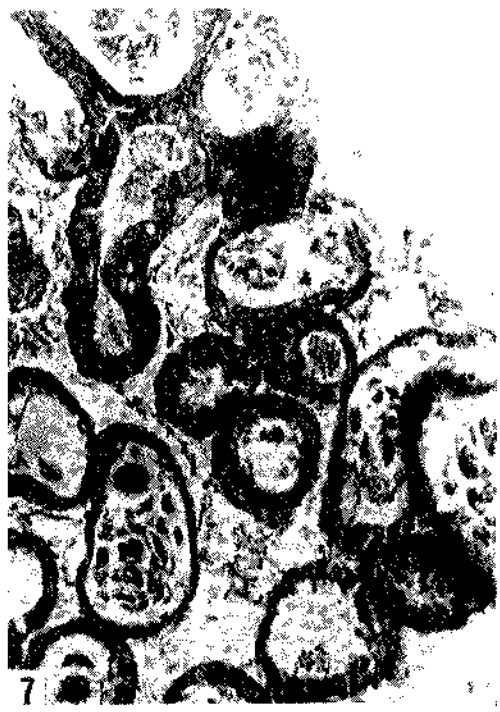

Fig. 7. Cross section of proximal portion of anterior vas deferens. Many portions of vas deferens are apparent (x 100).

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

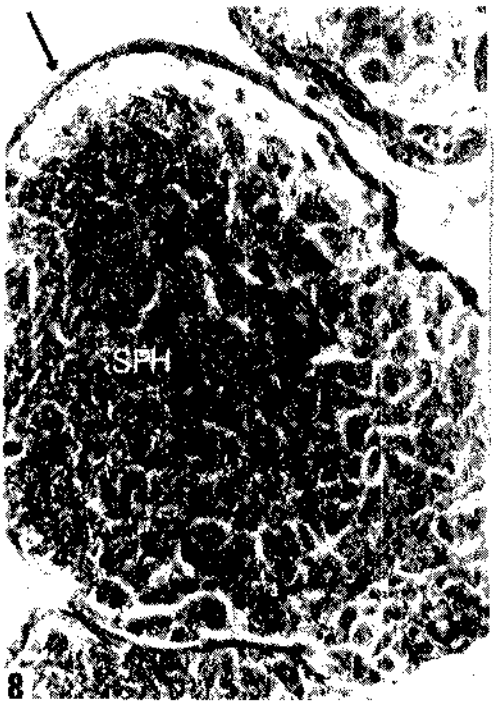

Fig. 8. Section of anterior vas deferens. Note the lumen filled with spermatophores (SPH). Arrow indicates the cuboidal epithelial cells (x 400)

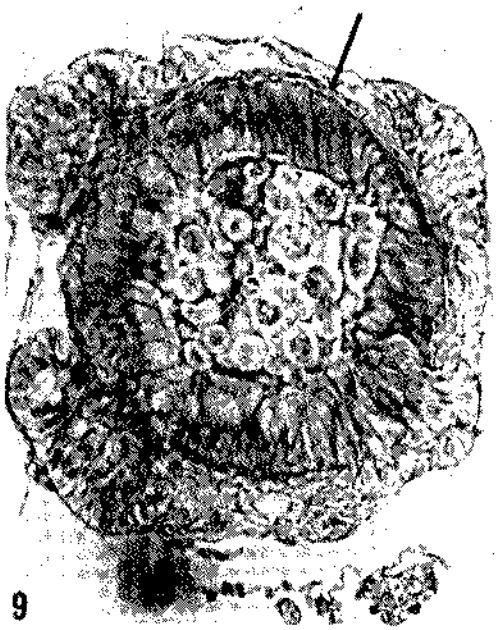

Fig. 9. Cross section through the distal region of anterior vas deferens. Note columnar epithelial cells surrounding the spermatophores (x 100).

of vas deferens is the only region where spermatophores 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 spermatophores are lodged.

Discussion

Very few workers have described any well defined maturity stages in male crabs. Haefner (1976), using the small variations in opacity and size of testes has described 6 maturity stages 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 serrate*, George (1963) and Ryan (1967) in *Portunus sanguinolentus*, Melville-Smith (1987) in *Chaceon* (= *Geryon*) *maritae* and Hinsch (1988) in *C. fenneri*. In the case of male *P. sanguinolentus* the observed subdivisions of testicular lobe (Ryan, 1967) was only an occasional feature, and whenever noticed, it

Fig. 10.Columnar epithelium (EC) of distal region of anterior vas deferens. Nucleus is apparent in the cells (arrow) (x 400).

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. kooloocense* 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 asynochronous 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 ellopsid, 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 ellopsid 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.

Acknowledgments

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References

Aiken, A.D. and S.L. Wady 1980. Reproductive biology. In: *The Biology and Management of Lobsters*, p. 215-276. J.S. Cobb and B.F. Philips (Eds.), Academic Press, New York.

Balasubramanian, C.P. and C. Suseelan 1998a. Reproductive biology of the female deep water crab *Charybdis smithii* (Brachyura : Portunidae) from the Indian seas. *Asian Fish. Sci.*, **10** : 211-222.

Balasubramanian, C.P. and C. Suseelan 1998b. Natural diet of the deep water crab *Charybdis smithii* McLeay (Brachyura : Portunidae) of the seas around India. *Indian J. Fish.*, **45**(4) : 407-411.

Binford, R. 1913. The germ cells and the process of fertilization in crab, *Menippe mercenaria*. *J. Morph.*, **24** : 147-202.

Cronin, L.E. 1947. Anatomy and Histology of male reproductive system of *Callinectes sapidus* Rathbun. *J. Morph.*, **81**(2) : 209-239.

Demestre, M. and J.M. Fortuno 1992. Reproduction of the deep-water shrimp *Aristeus antennatus* (Decapoda : Dendrobranchiata). *Mar. Ecol. Prog. Ser.*, **84** : 41-51.

Estampador, E.P. 1949. *Scylla*. 11. Comparative studies on spermatogenesis and oogenesis. *Phil. J. Sci.*, **78**(3) : 301-353.

George, M.J. 1963. The anatomy of the crab *Neptunus sanguinolentus* (Herbst). Part IV. Reproductive system and embryological studies. *J. Madras Univ.*, **33**B(3) : 289-304

Haefner, P.A. 1976. Distribution, reproduction and moulting of the rock crab, *Cancer irroratatus* Say 1917 in the mid Atlantic Bight. *J. Nat. Hist.*, **10** : 377-397.

Hinsch, G.W. 1988. The morphology of the reproductive tract and seasonality of reproduction in the golden crab *Geryon fenneri* from the eastern Gulf of Mexico. *J. Crustacean Biol.*, **8**(2) : 254-261.

Johnson, P.T. 1980. *Histology of the Blue Crab,* Callinectes sapidus, *a Model for the Decapoda*, 440 pp. Praeger Publishers Division of Greenwood Press, Inc., New York.

Joshi, P.C. and S.S. Khanna 1982. Seasonal changes in the ovary of freshwater crab, *Potamon kooloense* (Rathbun). *Proc. Indian Acad. Sci. (Anim. Sci.)*, **91** : 451-462.

King, J.E. 1948. A study of the reproductive organs of common marine shrimp, *Penaeus setiferus* (Linnaeus). *Biol. Bull.*, **94** : 244-262.

Krol, R.M., W.E. Hawkins and R.M. Overstreet 1992. Reproductive components. In: *Microscopic Anatomy of Invertebrates, Decapod Crustacea*, 10, p. 295-343. F.W. Harrison and A.G. Humes (Eds.), Wiley-Liss, New York.

Lu, C.C., W.H. Clark and L.E. Franklin 1973. Spermatogenesis of the decapod *Penaeus setiferus*. *J. Cell Biol.*, **59** (Part 2) : 202 A.

Melville – Smith, R. 1987. The reproductive biology of *Geryon maritae* (Decapoda, Brachyura) off south-west Africa / Namibia. *Crustaceana*, **53**(3) : 259-275.

Mohamed, K.H. and C. Suseelan 1973. Deep-sea prawn resources of the southwest coast of India. *Spl. Publ. Cent. Mar. Fish Res. Inst. (India)*, p. 614-633.

Panikker, P.A. 1990. FORV *Sagar Sampada* and development of demersal trawls for Indian EEZ – a status paper on prospects and constraints. *Proc. First Workshop Scient. Resul. FORV* Sagar Sampada, 5–7 June, 1989, Cochin, India, K.J. Mathew (Ed.), Cochin, CMFRI, p. 427–434.

Ryan, E.P. 1967. Structure and function of the reproductive system of the crab, *Portunus sanguinolentus* (Herbst) (Brachyura : Portunidae). The male system. *Proc. Symp. Crustacea.* Mar. Biol. Assoc. India, p. 506-521.

Shigekawa, K. and W.H. Clark Jr. 1986. Spermiogenesis in the marine shrimp, *Sicyonia Ingentis*. *Dev. Growth Dift.*, **28** : 95–112.

Silas, E.G. 1969. Exploratory fishing by R.V. *Varuna*. *Bull. Cent. Mar. Fish Res. Inst.*, No. 12, p. 1-86.

Subramoniam, T. 1991. Chemical composition of spermatophores in decapod crustaceans. In: *Crustacean Sexual Biology*, p. 309-321. R.T. Bauer and W.M. Joel (Eds.), Columbia University Press, New York.

Sulochanan, P., K.N.V. Nair and D. Sudarsan 1991. Deep-sea crustacean resources of the Indian Exclusive Economic Zone. *Proc. Nat. Workshop. Fish. Resour. Data Fish Indus*, p. 98-107.

Uma, K. and T. Subramoniam 1984. A comparative study on the spermatophore in *Scylla serrata* (Forsskal) (Decapoda : Brachyura) *Clibanarius longitarsus* (De Haan), Decapoda : Anomura). *J. mar. biol. Ass. India*, **26** (1 & 2) : 103-108.