Fishery Technology 53 (2016) : 1 - 8

Seasonal and Moult Related Changes in Spermatogenesis of *Travancoriana schirnerae*

M. K. Smija and A. R. Sudha Devi*
Mary Matha Arts & Science College, Mananthavady - 670 645, Wayanad, India

Abstract

The present study examined the seasonal and moult related changes in spermatogenic process of the freshwater crab *Travancoriana schirnerae* using histological methods. The annual spermatogenic process revived during March/April with gonadal proliferation and division of spermatocytes and attained its peak in May/June with acini containing mature sperms. A decline in activity was detectable during July/August, that corresponded to the mating season with inactive and resting secondary spermatocytes and spermatozoa. From September to February, the testis remained inactive, enclosing pycnotic gonia, spermatocytes and degenerating residual sperms. The premoult and postmoult testes exhibited inactivity with pycnotic gonia, spermatocytes and residual sperms whereas the intermoult testes appeared active with gonial proliferation, meiotic spermatocytes and mature spermatozoa. It has also been observed that there exists an antagonism between growth and reproduction in this species.

Keywords: Acini, spermatogonia, spermatozoa, spermatogenesis, *Travancoriana schirnerae*

Introduction

Spermatogenesis in decapods has long been a subject of interest to a large number of workers; some of them have given excellent descriptions on spermatogenesis of macruran and anomuran groups (Fasten, 1914; Hinsch, 1980). With regard to brachyurans, most important contributions were made by earlier workers such as Fasten (1918) and Binford (1913) in the Pacific coast edible crab *Cancer magister* and the stone crab *Menippe mercenaria*. However, these works revealed only a general pattern of spermatogenesis. Later studies were not decisive and mainly described spermiogenesis in a few marine and estuarine crabs like *Eriocheir japonicus* (Yasuzumi, 1960), *Carcinus maenas* (Pochon-Masson, 1962) and *Uca tangeri* (Medina & Rodríguez, 1992) using electron microscopic techniques. Studies of Minagawa et al. (1994) and others (Simeó et al., 2009; Stewart et al., 2010) in the red frog crab *Ranina ranina*, spider crab *Maja brachydactyla* and the blue swimming crab *Portunus pelagicus* have exposed new features of the brachyuran spermatogenesis. Though pertinent literature is quite voluminous on spermatogenesis of marine and estuarine brachyurans, there have been very few published accounts on spermatogenesis of freshwater crabs (Joshi & Khanna, 1982; Wang et al., 1999).

Decapod crustaceans belonging to different ecological niche are shown to depend on definite patterns for programming growth and reproduction. Programming of growth and reproduction has been expansively researched in female brachyurans (Adiyodi, 1988). But, on perusal of literature, very little information is available with respect to the intricate balancing between growth and reproduction in male brachyurans. Suganthi & Anilkumar (1999) published an account on moult related fluctuations in spermatogenesis of the estuarine crab *Metopograpsus messor*. There had been limited studies on spermatogeneis of freshwater brachyurans. More studies are required to unfold the intricacies of the male reproductive physiology of freshwater brachyurans. The edible freshwater crab, *Travancoriana schirnerae*, inhabiting the wetlands of Wayanad, Kerala forms a cheap source of animal protein to the poor, malnourished local tribes. An understanding of the pattern of spermatogenesis would permit a clear knowledge of their reproductive cyclicities, which is a pre-requisite to develop techniques for aquaculture practices. In view of the above, the present investigation on the seasonal and

Received 10 March 2015; Revised 25 July 2015; Accepted 03 January 2016

* *E-mail: arsudhadevi@yahoo.co.in*

moult related fluctuations in spermatogenic activity of *T. schirnerae* was undertaken.

Materials and Methods

Adult males of *T. schirnerae* (carapace width 4.0-5.0 cm; n=165) were collected during June 2009-June 2011from the paddy fields near Mary Matha Arts and Science college campus, Mananthavady. Prior to dissection, their carapace width (CW), body weight and moult stages were recorded. The moult stages were determined by noticing the changes in the carapace texture and by observing the setal development of the epipodite of the third maxilliped (Anilkumar, 1980). For histological studies, the testes were dissected out and their size, colour and wet weight were recorded. The tissues were then fixed in Bouin's solution, dehydrated in ascending series of ethanol, cleared in xylene and embedded in paraffin wax. Serial sections (5 µm thickness), stained with Heidenhain's hematoxylin-eosin, were examined under a Leica DM 500 Research microscope and photomicrographed.

Results and Discussion

The male reproductive system of *T. schirnerae* was found to consist of four distinct regions: the testis, vas deferens, ejaculatory duct and external penis (Fig. 1). The testis was an elongate, highly coiled, creamy bilobed organ (length: 16 to 26 mm; width: 1.0 to 2.5 mm), situated dorsally in the cephalothorax, abutted to the hepatopancreas. The posterior part of the testis and anterior portion of the vas deferens were joined together to form a commissure, which gave an H-shape to the system. The vas deferens consisted of three regions: a narrow and highly coiled anterior vas deferens (AVD), a thick and less coiled middle vas deferens (MVD) and a narrow posterior vas deferens (PVD). The PVD narrowed to form the ejaculatory duct which entered the musculature of the fifth walking leg and opened to the base of the coxa through external penis.

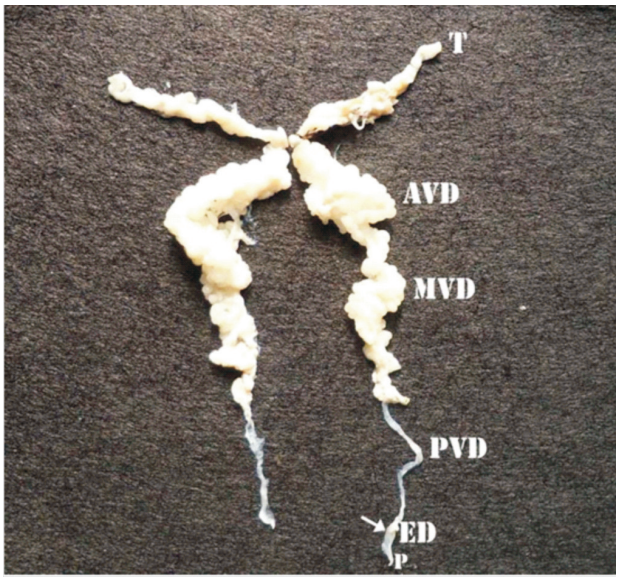

Fig. 1. Male reproductive system of adult *Travancoriana schirnerae*. Testis (T), anterior vas deferens (AVD), median vas deferens (MVD), posterior vas deferens (PVD), ejaculatory duct (ED), penis (P), androgenic gland (arrow)

The gross morphology of the male reproductive system and the testicular structure in *T. schirnerae* were similar to those described for other brachyurans (Minagawa et al., 1994; Balasubramanian & Suseelan, 2000; Garcia & Silva, 2006; Sherkhane et al., 2010). However, in *M. brachydactyla*, the testis comprising of a single seminiferous tubule, was divided into germinal, transformation and evacuation zones (Simeó et al., 2009). In *P. pelagicus*, the testis was composed of anterior and posterior regions containing numerous lobules and each lobule sectioned in to three zones (Stewart et al., 2010). In *T. schirnerae*, the vas deferens was divided into three different structural zones similar to those described for *Armases rubripes* (Santos et al., 2009) and *M. brachydactyla* (Simeó et al., 2009). On the other hand, the vas deferens was composed of two regions in *Chionoecetes opilio* (George, 1963), four in *Scyllarus chacei* (Hinsch & McKnight, 1988) and eight in *Diogenes pugilator* (Manjón-Cabeza & Raso, 2000). Generally, in crustaceans the vas deferens is divided into three to ten regions having different functions (McLaughlin, 1983).

The testis of *T. schirnerae* was composed of innumerable acini of varied shape, size and arrangement, bound externally by a thin fibrous connective tissue sheath. Each acinus was surrounded by a thin basal lamina upon which the germinal epithelium rests. The acini were composed of germinal and accessory cells. The accessory cells (6.0 to 7.5 µm in width), located towards the acinar periphery, exhibited oval to elongate nuclei possessing large chromatin granules and mildly eosinophilic cytoplasm. Generally, in Crustacea, non-germinal cells designated as supportive cells, accessory, sustentacular, interstitial, nurse, nutritive or sertoli cells was widely reported which provide nourishment to the germ cells during spermatogenesis (Hinsch,

1980; 1993). Fasten (1918) characterized nutritive cells among the spermatogonial groups in the testicular lobes of *C. magister*. In *B. cunicularis*, sertoli cells were arranged in the periphery of the testicular follicle (Sherkhane et al., 2010). The germinal zone of the seminiferous tubule in *P. pelagicus* contained both spermatogonia and accessory nurse cells (Stewart et al., 2010). Erkan et al. (2009) have attributed the presence of accessory cells in the crayfish *Astacus leptodactylus.* However, supporting cells were not reported in the testis of *Panulirus argus* (Mota & Tome, 1965).

Based on cytological features, six spermatogenic stages were identified in the present study: primary and secondary gonia, primary and secondary spermatocytes, spermatids and spermatozoa (Fig. 2A-F). Primary gonia, the largest of the germ cells (9.1 to 12.0 μm), were found proliferating from the acinar periphery. They have thin, mildly eosinophilic cytoplasm surrounding large, oval nuclei which occupied most of the cell volume and several small chromatin lumps aggregated at the inner nuclear membrane. Secondary gonia were distinguishable from primary gonia by their size (6.1 to 9.0 μm), much basophilic nuclei and indistinct cytoplasm. Their oval nuclei contained chromatin clumps congregated at the inner nuclear membrane. Primary spermatocytes (5.1 to 6.0 μm) have round or oval nuclei with chromatin arranged peripherally as discrete granules. Their cytoplasm appeared thin and mildly eosinophilic. The secondary spermatocytes were relatively small cells, (3.1 to 4.0 μm) with oval nuclei showing fine granular chromatin and thin mildly eosinophilic cytoplasm. The spermatids were smaller in size (2.0 to 3.5 μm) than the spermatozoa; possessed strongly basophilic round nuclei, extremely condensed chromatin and barely visible cytoplasm. During spermiogenesis, the spherical nuclei of spermatids undergo changes to become crescent shaped. The spermatozoa were oval (4.0 to 5.0 μm), basophilic cells with acrosomes in the middle. During the process of spermatogenesis, there was a sharp reduction in the cell diameter, resulting in spermatozoa about 35% of the size of primary gonia.

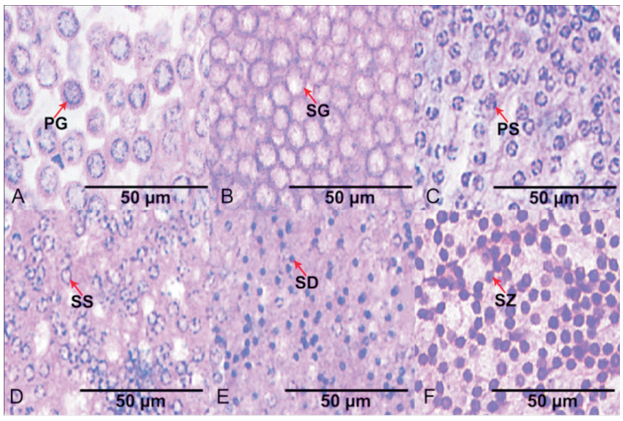

Fig. 2. Developmental stages of germ cells within the testis of adult male *Travancoriana schirnerae.* A, Primary gonia; B, Secondary gonia; C, Primary spermatocytes; D, Secondary spermatocytes; E, Spermatids; F, Mature spermatozoa. Primary gonia (PG), secondary gonia (SG), primary spermatocytes (PS), secondary spermatocytes (SS), spermatids (SD), spermatozoa (SZ)

The six distinct stages of spermatogenesis categorized in the testis of *T. schirnerae* appeared akin to those described in other brachyurans (Gupta et al., 1989; Minagawa et al., 1994; Balasubramanian & Suseelan, 2000). In decapods, during spermatogenesis, a clear reduction in the nuclear volume from spermatogonia to mature sperm was observed (Silva, 1993; Zara et al., 2012; Ravi et al., 2014). In the present study, spermatocytes were found to be relatively smaller than the gonia and spermatozoa appeared larger than the spermatids. Likewise, spermatozoa appeared larger than the spermatids in *C. smithii* (Balasubramanian & Suseelan, 2000). Suganthi & Anilkumar (1999) reported that the size of spermatozoa was comparable to the spermatids in *M. messor.* Conversely, in *M. brachydactyla* and *P. pelagicus*, spermatozoa appeared smaller than the spermatids (Simeó et al., 2009; Stewart et al., 2010) and spermatocytes were the largest germ cells in *M. brachydactyla* (Simeó et al., 2009) . In *C. ornatus*, secondary spermatocytes showed small sized nuclei when compared to spermatids and spermatozoa (Nascimento & Zara, 2013).

Spermatogenesis in decapods can be synchronous or asynchronous depending on the species (Krol et al., 1992). In *T. schirnerae,* cells within individual acinus seemed to be in a single stage of development and acini from a single lobe presented germ cells in one or two stages of development, indicating a synchronous progress of spermatogenesis. Synchrony in spermatogenic activity has also been observed in *Geryon maritae*, *C. sapidus*, *Telmessus cheiragonus* and *A. leptodactylus* (Melville-Smith, 1987; Johnson, 1980; Nagao & Munehara, 2003; Erkan et al., 2009). Conversely, asynchronous development has been observed in *M. mercenaria* (Binford, 1913), in the

crayfish *Cherax quadricarinatus* (Lopez Greco et al., 2007) and the shrimp *Sicyonia ingentis* (Shigekawa & Clark, 1986). In *C. quadricarinatus*, each testicular lobe contained cells undergoing a single stage of spermatogenesis regardless of different stages occurring in the adjacent lobes (Lopez Greco et al., 2007).

In the present study, seasonal and moult related changes were perceptible in the annual spermatogenic activity. The annual spermatogenic activity got revived during March/April. Acini appeared large with well developed basal lamina and active accessory cells at the periphery. In March, the testis was dominated by primary (42%) and secondary gonia (22%) with a large number of primary gonia undergoing mitotic prophase and metaphase. Primary spermatocytes exhibited meiotic prophase and metaphase stages in 16% of the acini examined (Fig. 3A, B). Secondary spermatocytes were densely packed in some acini (20%). By April, percentage of gonia was apparently reduced (21%); there occurred a perceptible increase in the percentage of secondary spermatocytes (31%) and spermatids

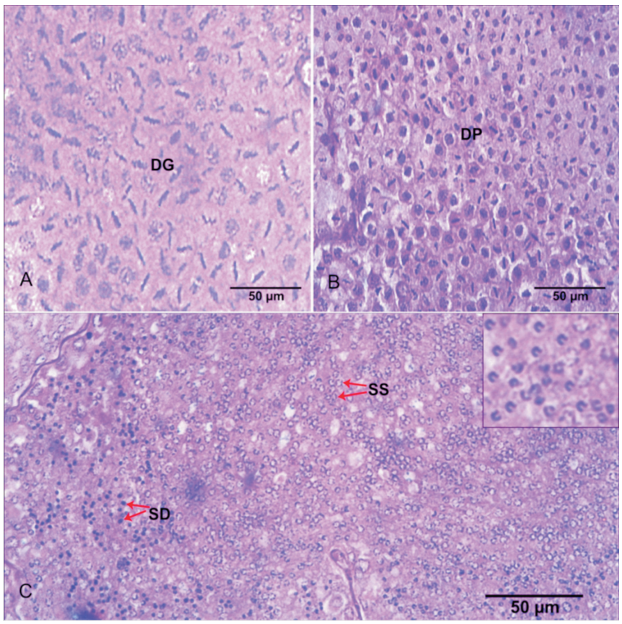

Fig. 3. Revival of spermatogenic activity during March/April in *Travancoriana schirnerae.* A, Primary gonia undergoing intensive mitotic division; B, Primary spermatocytes under meiotic division; C, Acini packed with secondary spermatocytes and spermatids (inset showing spermiogenesis). Dividing gonia (DG), dividing primary spermatocytes (DP), secondary spermatocytes (SS), spermatids (SD)

(40%) (Fig. 3C). Large numbers of accessory cells were observed in the periphery of acini containing spermatids. Residual sperms (8%) were evident in some acini which indicated that the sperm resorption process was not complete.

The annual spermatogenic activity reached its peak in May/June. Acini were decidedly large, fully packed with germ cells. The percentage of mature spermatozoa was greatly increased (70%) while the proportion of gonia (4%) and spermatocytes (16%) decreased (Fig. 4A). Acini extended with sperms was noted to be the prodigious feature of the testis of this period (Fig. 4B). Testis contained relatively less spermatids (8%) and spermiogenesis was progressing normally in a few acini (2%).

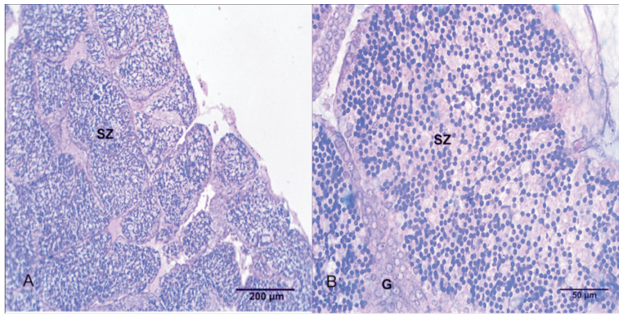

Fig. 4. Spermatogenic activity in peak during May/June in *Travancoriana schirnerae.* A, Testicular lobe with acini compactly packed with mature spermatozoa; B, Acinus filled with mature spermatozoa. Gonia (G), spermatozoa (SZ).

A perceptible decline in spermatogenic activity was evident through July/August. Most of the acini contained inactive and resting secondary spermatocytes (30%). Primary gonia (20%) appeared loosely arranged and a few of them initiated signs of chromatin condensation. Some secondary gonia (16%) were located at the periphery of acini containing spermatozoa (Fig. 5).

The number of acini enclosing spermatids (6%) was considerably reduced. Residual sperms (28%) were seen aggregated either towards the periphery or in the lumen of many acini. The testis remained inactive and showed signs of degeneration during September/October. Both inter and intra-acinar cell free spaces were found prominent. Accessory cells nuclei appeared pycnotic and clumped in nature. Phagocytes were detectable among degenerating germ cells. Acini contained primarily of pycnotic secondary spermatocytes (43%), residual sperms (27%), gonia (27%) and a few pycnotic spermatids

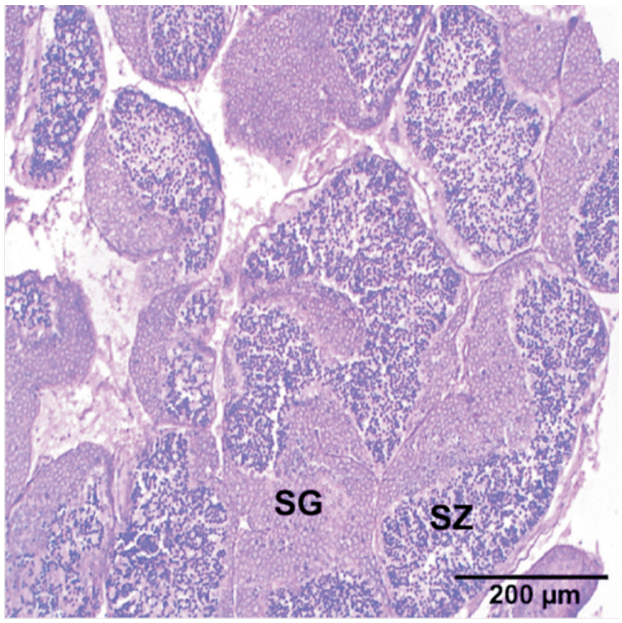

Fig. 5. Testis showing decline in activity in July/August. Secondary gonia (SG), spermatozoa (SZ)

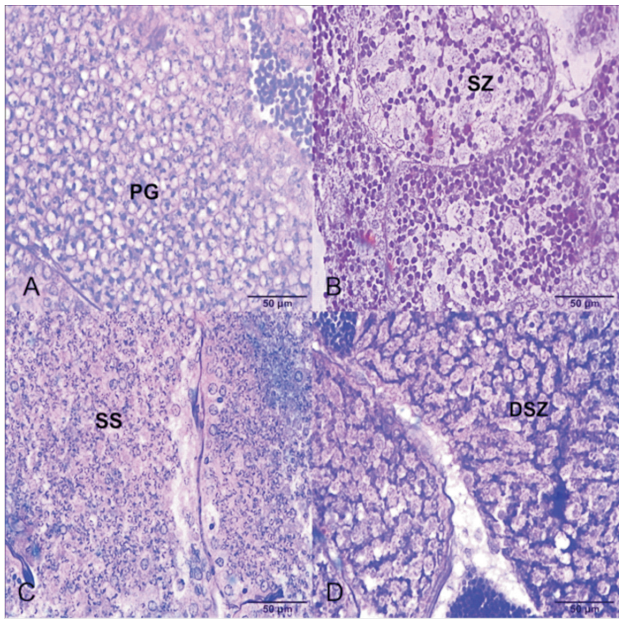

Fig. 6. Histological sections through testis showing inactivity from September to February. A, Acini showing pycnotic primary gonia during September/october; B, Residual sperms clumped inside the acini during November/December; C, Secondary spermatocyte degeneration in January/February; D, Residual sperms undergoing degeneration (January/February). Primary gonia (PG), secondary spermatocytes (SS), spermatozoa (SZ), degenerating spermatozoa (DSZ)

(3%) (Fig. 6A). Residual sperms were congregated as pycnotic masses towards the acinar periphery.

A total pause in spermatogenic activity was observed during November/December. Inter and intra-acinar cell free spaces were more pronounced and the germ cells appeared loosely packed within the acini. Both gonia (34%) and spermatocytes (42%) exhibited chromatin condensation and degeneration. In a few acini, residual sperms (24%) clumped to form pycnotic masses (Fig. 6B). During January-February, the testis presented a degenerated appearance. Acini appeared small and shrunken; inter and intra-acinar spaces were perceivable in the testis. The acini consisted primarily of pycnotic gonia (29%) and residual sperms (25%). Gonial and spermatocytic (40%) degeneration marked a common feature of the testis (Fig. 6C). Residual sperms were fairly loose and noted either in the centre or in the periphery of some acini. Spermatolysis was common in acini enclosing sperm masses (Fig. 6D). A few primary gonia (6%) were aligned at the periphery of the acini containing residual sperms.

From the above observations, it is affirmed that *T. schirnerae* has an annual cyclicity in testicular activity. Seasonal cyclicity was observable in the spermatogenic process of many freshwater crabs. In *Potamon koolooense*, spermatogenesis initiated in January/February, reached its peak by April/May and ceased gradually by December (Joshi & Khanna, 1982). In *Paratelphusa hydrodromous*, November to May was the reproductively inactive season and June/July through October, the active season which also included the mating season (Gupta et al., 1989). Even though species specific differences were observed in the annual spermatogenic process in freshwater crabs, which probably related to differences in environmental conditions, the pattern of cyclicity was quite similar in all the species. Seasonal changes in testicular activity was also reported from certain estuarine and marine brachyurans (Pillay & Nair, 1971; Dhawan et al., 1976; Batoy et al., 1987). However, spermatogenesis was a continuous process without apparent seasonal changes in the estuarine crab *Ucides cordatus* (Castilho et al., 2008) and the deep sea crabs *C. opilio* (Kon & Honma, 1970), *G. maritae* (Melville-Smith, 1987) and *R. ranina* (Minagawa et al., 1994). In *T. schirnerae*, the seasonal changes in testicular activity were attributed to the changes in the secretory activity of the androgenic gland (Sudha Devi & Smija, 2014).

Our observations on moult related fluctuations in spermatogenesis revealed that both the premoult and postmoult testes demonstrated inactivity. The testis remained inactive during the first half of the intermoult (September to February) and active during the second half (March to August). The premoult testis contained large number of pycnotic primary gonia (32%) undergoing degeneration. In some acini, secondary gonia (20%) were observable in the periphery of acini containing degenerating spermatozoa. Acini contained relatively few spermatocytes (28%) which evinced nuclear pycnosis and degeneration. Phagocytes were noticed in acini containing degenerating germ cells. Residual sperms, evident as pycnotic masses, found decreased in number (20%) (Fig. 7A). The postmoult test is showed signs of inactivity with an increased percentage of spermatocytes undergoing chromatin condensation (50%) (Fig. 7B). Phagocytes were observed among degenerating germ cells. Primary gonia (22%) were loosely arranged, exhibited chromatin condensation and degeneration. Residual sperms (21%), loosely arranged either in the centre or periphery of the acini, exhibited degeneration. A few secondary spermatogonia (7%) were conspicuous near the periphery of acini enclosing degenerating sperms.

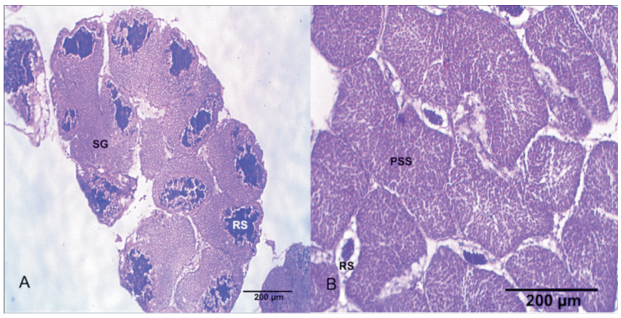

Fig. 7. Moult related fluctuations in the histology of the testis of *Travancoriana schirnerae.* A, Acini showing secondary gonia and degenerating residual sperms in premoult testis; B, Intense chromatin condensation and degeneration of secondary spermatocytes in postmoult testis. Secondary gonia (SG), pycnotic secondary spermatocytes (PSS), residual sperms (RS).

In brachyurans, reproduction and moulting are programmed as antagonistic events (Adiyodi & Adiyodi, 1970); the intermoult period chiefly devoted to reproduction, whereas the premoult and postmoult for somatic growth (Adiyodi, 1988). The present study revealed that the first half of the intermoult (September to February) constituted the reproductively inactive phase while the second half (March to August) represented active phase which accommodated the mating season. By contrast, in the Calicut population of *P. hydrodromous*, the first half of the intermoult corresponded to the reproductively active phase and the second half represented the somatic phase (Kurup & Adiyodi, 1981). Our observations also revealed that both premoult and postmoult testes exhibited inactivity with pycnotic gonia, spermatocytes and residual sperms. Comparable findings were described in other brachyurans. In *M. messor*, premoult and postmoult testes exhibited inactivity with a reduction in the maturation of germ cells and intense chromatin condensation of spermatocytes whereas proliferation and maturation phases of germ cells were noticed in intermoult testis (Suganthi & Anilkumar, 1999) thus exhibiting an antagonism between growth and reproduction. Similarly, Gupta et al. (1989) defined extreme chromatin condensation of spermatocytes in the postmoult testis of *P. hydrodromous*. On the contrary, increased gonial proliferation was observed in the premoult testis of the freshwater prawn *Macrobrachium idella* (Sreekumar & Adiyodi, 1983). Anilkumar & Adiyodi (1985) and Adiyodi (1988) reported that the oocyte maturation and somatic growth are mutually exclusive events in female brachyurans.

In conclusion, seasonal and moult related fluctuations were detectable in the spermatogenic process of *T. schirnerae*. The annual spermatogenic process revived during March/April; reached its peak in May/June and the testis was reproductively inactive from September to February. The premoult and postmoult testes showed inactivity while normal spermatogenic process was carried out in the intermoult period. In view of the above, an antagonism is suggested to exist between growth and reproduction in male *T. schirnerae*.

Acknowledgements

The authors wish to thank the Kerala State Council for Science, Technology & Environment for the financial support in carrying out this work.

References

Adiyodi, R. G. (1988) Reproduction and development. In: The Biology of the Land Crabs. (Burggren, W.W. and Mc Mahon, B.R., Eds), pp 139-185, Cambridge University Press, Cambridge

Adiyodi, K. G. and Adiyodi, R. G. (1970) Endocrine control of reproduction in decapod Crustacea. Biol. Rev. 45: 121-165

Anilkumar, G. and Adiyodi, K. G. (1985) The role of eyestalk hormones in vitellogenesis during the breeding season in the crab, *Paratelphusa hydrodromous* (Herbst). Biol. Bull. 169: 689-695

Balasubramanian, C. P. and Suseelan, C. (2000) Male reproductive system and spermatogenesis in the deep water crab *Charybdis smithii* McLeay (Brachyura: Portunidae). Indian J. Fish. 47: 275-282

Batoy, C. B., Sarmago, J. F. and Pilapil, B. C. (1987) Breeding season, sexual maturity, and fecundity of the blue crab, *Portunus pelagicus* (L.) in selected coastal waters in Leyte and vicinity, Philippines. Ann. Trop. Res. 9: 157-177

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

Castilho, G. G., Ostrensky, A., Pie, M. R. and Boeger, W. A. (2008) Morphology and histology of the male reproductive system of the mangrove land crab *Ucides cordatus* (L.) (Crustacea, Brachyura, Ocypodidae). Acta. Zool. 89: 157-161

Dhawan, R.M., Dwivedi, S.N. and Rajamanickam, G.V. (1976) Ecology of the blue crab *Portunus pelagicus* (L.) and its potential fishery in Zuari Estuary. Indian J. Fish. 23: 57-64

Erkan, M., Yasemin, T. and Sancar-Bas, S. (2009) Male reproductive system morphology and spermatophore formation in *Astacus leptodactylus* (Eschscholtz, 1823) (Decapoda: Astacidae). J. Crust. Biol. 29: 42-50

Fasten, N. (1914) Spermatogenesis of the American crayfish, *Cambarus virilis* and *Cambarus immunis* with special reference to synapsis and the chromatoid bodies. J. Morphol. 25: 587-649

Fasten, N. (1918) Spermatogenesis of the Pacific coast edible crab, *Cancer magister* (Dana). Biol. Bull. 34: 277-307

Garcia, T.M. and Silva, J.RF. (2006) Testes and vas deference morphology of the red-clawed mangrove tree crab (*Goniopsis cruentata*) (Latreille, 1803). Braz. Arch. Biol. Technol. 49: 339-345

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

Gupta, S.N.V., Kurup, K.N.P., Adiyodi, R.G. and Adiyodi, K.G. (1989) The antagonism between the somatic growth and testicular activity during different phases in intermoult (Stage 4) in sexually mature freshwater crab, *Paratelphusa hydrodromous*. Int. J. Invert. Reprod. Dev. 16: 195-204

Hinsch, G.W. (1980) Spermiogenesis in a hermit crab, *Coenobita clypeatus*. II. Sertoli cells. Tissue Cell. 12: 255-262

Hinsch, G.W. (1993) The role of sertoli cells in spermatid maturation in the testis of the crayfish *Procambarus paeninsulanus*. Tissue Cell. 25: 743-749

Hinsch, G.W. and McKnight, C.E. (1988) The vas deferens of the Spanish lobster, *Scyllarus chacei*. Int. J. Invert. Reprod. Dev. 13: 267-280

Johnson, P.T. (1980) Histology of the Blue Crab, *Callinectes sapidus*, A Model for Decapoda. Preger Publishers, CBS Educational and Professonal Publishing, New York

Joshi, P.S. and Khanna, S.S. (1982) Structure and seasonal changes in the testes in freshwater crab, *Potamon koolooense* (Rathbun). Proc. Indian Acad. Sci. 91: 439-450

Kon, T. and Honma, Y. (1970) Studies on the maturity of the gonad in some marine invertebrates–IV: Seasonal changes in the testes of the tanner crab. Nipp. Sui. Gakka. 36: 1028-1033

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

Kurup, K.N.P. and Adiyodi, R.G. (1981) The programming of somatic growth and reproduction in the crab, *Paratelphusa hydrodromous* (Herbst). Int. J. Invert. Reprod. Dev. 3: 27-39

Lopez-Greco, L.S., Vazquez, F.J. and Rodriguez, E.M. (2007) Morphology of the male reproductive system and spermatophore formation in the freshwater red claw crayfish *Cherax quadricarinatus* (Von Martens, 1898) (Decapoda, Parastacidae). Acta. Zool. (Stockholm). 88: 223-229

Manjón-Cabeza, M.E. and Garcia Raso, J.E. (2000) Morphological reproductive aspects of males of *Diogenes pugilator* (Roux, 1829) (Crustacea, Decapoda, Anomura) from southern Spain. Sarsia, 85: 195-202

McLaughlin, P.A. (1983) Internal anatomy. In: The Biology of Crustacea (Venberg, F.J. and Venberg, W.B., Eds), pp 1-52, Academic Press, New York

Medina, A. and Rodríguez, A. (1992) Spermiogenesis and sperm structure in the crab *Uca*

tangeri (Crustacea, Brachyura), with special reference to the acrosome differentiation. Zoomorphology, 111: 161-165

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

Minagawa, M., Chiu, J.R., Kudo, M. and Takashima, F. (1994) Male reproductive biology of the frog crab, *Ranina ranina*, off Hachijojima, Izu Islands, Japan. Mar. Biol. 118: 393-401

Mota-Alves, M.I. and Tome, G.S. (1965) On the histological structure of the gonads of *Panulirus argus* (Latreille). Arq. Est. Biol. Mar. Univ. Ceará. 5: 15-26

Nagao, J. and Munehara, H. (2003) Annual cycle of testicular maturation in the helmet crab *Telmessus cheiragonus*. Fish. Sci. 69: 1200-1208

Nascimento, F.A. and Zara, F.J. (2013). Development of the male reproductive system *Callinectes ornatus*, 1863 (Brachyura: Portunidae). Nauplius 21

Pillay, K.K. and Nair, N.B. (1971) The annual reproductive cycles of *Uca annulipes*, *Portunus pelagicus* and *Metapenaeus affinis* (Decapoda: Crustacea) from the southwest coast of India. Mar. Biol. 11: 152-166

Pochon-Masson, J. (1962) Le chondriofusome des gamètes males du Crustacé Décapode *Carcinus maenas*. Compt. Rend. Acad. Sci. Paris. 254: 4076-4078

Ravi, R., Manisseri, M.K. and Sanil, N.K. (2012). Structure of the male reproductive system of the blue swimmer crab *Portunus pelagicus* (Decapoda: Portunidae). Acta. Zool. (Stockholm). 95(2): 175-185

Santos, C.M., Lima, G.V., Nascimento, A.A., Sales, A. and Oshiro, L.M.Y. (2009) Histological and histochemical analysis of the gonadal development of males and females of *Armases rubripes* (Rathbun 1897) (Crustacea, Brachyura, Sesarmidae). Braz. J. Biol. 69: 161-169

Sherkhane, U.D., Patil, M.U. and Pande, G.S. (2010) Gross anatomy of male reproductive system and histology of testis and vas deferens in freshwater crab *Barytelphusa cunicularis* (Westwood 1836) (Decapoda: Crustacea). Bioscan. 5: 599-603

Shigekawa, K. and Clark, W.H. (1986) Spermiogenesis in the marine shrimp, *Sicyonia ingentis*. Dev. Growth Differ. 28: 95-112

Silva, J.R.F. (1993) Histomorfometria da reprodução da lagosta verde *Panulirus laevicauda* (Latreille, 1817) (Crustacea: Decapoda: Palinuridae) do litoral do Estado do Ceará. Dissertação (Mestrado em Ciências Biológicas) - Universidade Federal da Paraíba, João Pessoa

Simeó, C.G., Ribes, E. and Rotllant, G. (2009) Internal anatomy and ultrastructure of the male reproductive system of the spider crab *Maja brachydactyla* (Decapoda: Brachyura). Tissue Cell. 41: 345-361

Sreekumar, S. and Adiyodi, R.G. (1983) Role of eyestalk principle(s) in spermatogenesis of the freshwater shrimp, *Macrobrachium idella*, Proceedings of 1st International Biennial Conference on Warm Water Aquaculture (9-11 February), Brigham Young University, Hawaii, 406-411

Stewart, M.J., Stewart, P., Sooklang, N., Linthong, V., Hanna, P.J., Duan, W. and Sobhon, P. (2010) Spermatogenesis in the blue swimming crab, *Portunus pelagicus*, and evidence for histones in mature sperm nuclei. Tissue Cell. 42: 137-150

Sudha Devi, A.R. and Smija, M.K. (2014) Seasonal changes in the structure and secretory activity of the androgenic gland of *Travancoriana schirnerae*. Cent. Eur. J. Biol. 9: 70-79

Suganthi, A.S. and Anilkumar, G. (1999) Moult-related fluctuation in ecdysteroid titre and spermatogenesis in the crab, *Metopograpsus messor* (Brachyura: Decapoda). Zool. Stud. 38: 314-321

Wang, L., Du, N-S. and Lai, W. (1999) Studies on spermatogenesis of freshwater crab *Sinopotamon yangtsekiense* (Crustacea, Decapoda). Act. Hydrobiol. Sin. 23: 29-33

Yasuzumi, G. (1960) Spermatogenesis in animals as revealed by electron microscopy: VII. Spermatid differentiation in the crab, *Eriocheir japonicus*. J. Biophys. Biochem. Cytol. 7: 73-78

Zara, F.J., Toyama, M.H., Caetano, F.H. and López-Greco, L.S. (2012). Spermatogenesis, and seminal fluid production in the adult blu crab Callinectes danae (Portunidae). J. Crust. Biol. 32: 249-262