PUBLICATIONS


Histological Characterization of Male Morphotypes of *Macrobrachium rosenbergii* (de Man)

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Histological characterization of three male morphotypes of *Macrobrachium rosenbergii*, viz., small male (SM), strong orange clawed male (SOC), strong blue clawed male (SBC) have been carried out to bring out the structural and functional differences in the organ tissues of these morphotypes. The organ tissues studied for morphological characteristics were the reproductive system including androgenic gland, hepatopancreas and the neurosecretory system, viz., eye stalk, brain and thoracic ganglion. The present study indicated clear structural and functional differences in the organ tissues of male morphotypes, lending support to the morphological variations among male morphotypes of *M. rosenbergii*.

Key words: Histological characterization, male morphotypes, *Macrobrachium rosenbergii*.

Among mature males of the freshwater prawn *Macrobrachium rosenbergii*, three distinct morphological types, viz., small males (SM), orange-clawed males (OC) and blue-clawed males (BC) are discernible. These morphotypes represent three developmental stages of male maturation process. SM occupies the initial stage of developmental pathway. They transform to strong orange clawed males (SOC) through an intermediary stage known as weak orange clawed morphotype (WOC). The OC in turn transforms to BC through two transitional stages, viz., pre-transforming strong orange clawed males (t-SOC) and weak blue clawed males (WBC) before culminating in strong blue clawed males (SBC). The terminal stage of this transformation pathway is old blue clawed morphotype (OBC) which is characterised by relatively large second cheliped. (Ra'anan, 1982; Ra’anan & Cohen, 1985; Kutis et al., 1987; Karplus et al., 1992; Harikrishnan & Kurup, 1997a).

Though a lot of work has been carried out to characterize these male morphotypes morphologically, allometrically and biochemically (Cohen et al., 1981; Harikrishnan & Kurup, 1997a; Sureshkumar & Kurup, 1998), no attempt has so far been made to bring out the

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structural and functional differences of different body organs causing morphotype transformation. The present paper aims at histological characterization of three behaviourally distinct male morphotypes of *M. rosenbergii*, viz., small males (SM), strong orange clawed males (SOC) and strong blue clawed males (SBC).

Materials and Methods

Live specimens of male morphotypes of *M. rosenbergii*, viz., SM, SOC and SBC, belonging to a single age group were collected from a growout adjacent to Vembanad Lake (Kerala) during 1998-1999. They were identified into three distinct morphotypes (Kuris *et al.*, 1987; Sagi & Ra'anan, 1988) and brought to the laboratory in live condition. The organ tissues selected for the study consisted of the reproductive system including androgenic gland, hepatopancreas and the neurosecretory system, viz., eyestalk, brain and thoracic ganglion. Samples of these tissues, collected from five specimens, displaying unequivocal morphotypic characteristics were selected for histological study. For microscopical studies, paraffin sections of these organ tissues were taken at 7 μ after fixation in Bouin’s fluid, and then stained with Haematoxylin-eosin, Heidenhain’s Azan and Mallory’s Triple stains for the reproductive system, hepatopancreas and neurosecretory systems, respectively (Humason, 1982). The slides were examined by light microscopy and photographed using a binocular microscope and Nippon camera combination at high power.

Results and Discussion

Testes

The testicular lobes are composed of long cylinders, compactly held together by connective tissue. Light microscopy revealed differences in the content of the cylinders among the different male morphotypes. The testes of small males contained cylinders, most of which were enveloped by a single layer of epithelium. Part of the epithelium is multilayered and included cells of variable size, forming a spermatogenic zone containing germinal cells and sustentacular cells. Mature spermatozoans were seen in the lumen of a few cylinders (Fig. 1).

The testicular cylinder of strong orange clawed male is filled with spermatocytes only, which appeared similar in size, shape and cytological features. It is characterized by a complete absence of spermatozoans. (Fig. 2). The testicular lobules of blue clawed males contained mature spermatozoans almost to the exclusion of other cell types. The spermatogenic zone was barely observable (Fig. 3).

Histological examination of the testes of these three morphotypes revealed that the testes show difference in structure and function. The available reports suggest that the male morphotypes of *M. rosenbergii* such as SM, SOC and SBC, show perceptible difference in reproductive activity and somatic growth among them (Sagi & Ra’anan, 1988; Sagi *et al.*, 1988; Sureshkumar & Kurup, 1998; Joseph & Kurup, 2000). SM and SBC are sexually active participating in mating
Fig 1: CS of testes of SM (x100) S2 - Spermatogenic zone, S Sperrn

Fig 2: CS of testes of SOC (x100) showing spermatocytes

Fig 3: CS of testes of SBC (x100) showing sperm

Fig 4: CS of vas deferens of SM (x40) showing sperm

Fig 5: CS of vas deferens of SOC (x40) showing sperm

Fig 6: CS of vas deferens of SBC (x40) showing sperm
Fig 7: CS of androgenic gland of SM (x100) showing type I androgenic gland tissue

Fig 8: CS of androgenic gland of SOC (x100) showing type II androgenic gland tissue

Fig 9: CS of androgenic gland of SBC (x100) showing type III androgenic gland tissue

Fig 10: CS of hepatopancreas of SM with fibrillar cells (x100)

Fig 11: CS of hepatopancreas of SOC with secretory cells (x100)

Fig 12: CS of hepatopancreas of SBC with absorptive cells (x100)
**Fig 13:** LS of eyestalk of SM showing groups of neurosecretory cell types of C, D and E (x100).

**Fig 14:** LS of eyestalk of SOC showing groups of neurosecretory cell types of C, D and E (x100).

**Fig 15:** LS of eyestalk of SBC showing groups of neurosecretory cell types of C, D and E (x100).

**Fig 16:** TS of brain of SM showing the localisation of neurosecretory cell types of B, C, D and E (x100).

**Fig 17:** TS of brain of SOC showing the localisation of neurosecretory cell types of B, C, D and E (x100).

**Fig 18:** TS of brain of SBC showing the localisation of neurosecretory cell types of B, C, D and E (x100).
and fertilization and thereby utilizing little energy in somatic growth. On the contrary, orange-clawed males are characterized by a fast growth rate and reduced reproductive activity. (Sagi & Raman, 1988; Sagi et al., 1988; Sureshkumar & Kurup, 1998).

The results of the present study revealed that there exists a very strong correlation between the structural properties of the testes with the reproductive activity of these morphotypes.

**Vas deferens**

The vas deferens of male morphotypes of *M. rosenbergii* contains four distinct regions, viz., proximal vas deferens, medial vas deferens, distal vas deferens and seminal vesicles and this is comparable with that of *P. setiferus* (King, 1948; Ro et al., 1990). The present study revealed that mature spermatozoans are observed in the vas deferens of all these male morphotypes (Fig. 4, 5 & 6). However, histological study of the testes of SOC morphotype revealed that they are devoid of spermatozoans in their testicular lobules. It was also reported that SOC male morphotype has a low reproductive activity during breeding processes (Sagi et al., 1988; Joseph & Kurup, 2000; Telecky, 1984; Sureshkumar & Kurup, 1998). Therefore, it can be inferred that SOC male morphotypes which are characterized by the total absence of mature spermatozoans in their testes are capable of performing low rate of reproductive activity during breeding processes, like other sexually active male morphotypes and this may be due to the
presence of spermatozoans retained in the seminiferous tubules (i.e., vas deferens and seminal vesicle) by the SM morphotypes.

**Androgenic gland**

The androgenic gland of male morphotypes of *M. rosenbergii* consists of strands of cells forming a pyramidal cluster, loosely associated with the posterior portion of the ejaculatory duct. The strands consist of three principal cell types. Cell of type-I are small with dense cytoplasm, often containing two nuclei. Cells of type-II are slightly larger and vacuolated. Cells of type-III are large cells in which most of the inter cellular space consists of vacuoles. The androgenic gland of these male morphotypes showed variations in their activity (Thampy & John, 1972; 1973; Sagi, 1988; Sreekumar et al., 1982). In small males the androgenic gland cells are constituted mainly by type-I cells and in SBC, type-II cells constitute the gland cells (Fig. 7&8). On the contrary type-III cells forms the major component of SOC androgenic gland (Fig. 9). The presence of active nuclei surrounded by rich homogenous cytoplasm throughout the gland, indicate high activity of the gland in SM and SBC. In SOC, the high incidence of nuclear pycnosis and cytoplasmic vacuolization of the gland cells along with the presence of areas showing late phases of degeneration suggest that the activity of the gland is low in this morphotype. Therefore, there is a positive correlation between the activity of the androgenic gland and the reproductive activity of these morphotypes. As the SM and SBC morphotypes exhibits high reproductive activity while the SOC morphotypes show low reproductive activity, in the presence of BC morphotypes.

Eyestalk neuropeptides such as GSH and GJH apparently act directly on the female ovaries (Cotton & Payen, 1988; Quackenbush, 1991; Fingerman, 1995) whereas, in males, their action on testes appears to be indirect, via a direct action on the androgenic gland as suggested by Adiyodi (1984) and Gupta *et al.* (1989) and Hasagawa *et al.* (1993). According to Thampy & John (1973) and Sreekumar *et al.* (1982), the androgenic gland shows signs of increased secretory activity as evidenced by increase in the size of the gland as well as the type of cells. The changes in the primary and secondary sexual characteristics of the male morphotypes of *M. rosenbergii* along with the changes in the activity of androgenic gland, indicates that there is a hormone produced in the eyestalk, which has got an inhibitory effect on the androgenic gland, in male primary and secondary sex characters as well as on the growth of these morphotypes.

It may be possible that androgenic gland which controls the primary and secondary sex characters of male crustaceans has a role in the development of male morphotypes and growth variation among them, as growth rate of *M. rosenbergii* is closely associated with morphotypic status (Rajaman, 1982; Rajaman & Cohen, 1985).
Hepatopancreas

Hepatopancreas of *M. rosenbergii* is a large compact organ which occupies the greater part of the cephalothoracic cavity, posterior to the cardiac foregut. They are composed of compact arrays of blind ending tubules and are held ventrally with the gut at the junction of the pyloric foregut and anterior end of the midgut. The bulk of the tissue comprises of simple tubules, whose wall consists of a single layer of secretory epithelium. In between the individual tubules are found interstitial cells and small blood spaces. The tubules of the hepatopancreas are lined with an epithelium in which four cells can be recognized. They are the Embryonic (E-) cells, Absorptive (R-) cells, Secretory (B-) cells, and the Fibrillar (F-) cells.

i. Embryonic (E-) cells: These are small undifferentiated columnar cells seen at the distal end of the tubules. The only part where the epithelium is more than one cell thick. These cells are continuous with a short region where they undergo differentiation to become other cells of the tubules. All cells in this region appear to be morphologically similar.

ii. Absorptive (R-) cells: These cells are found lining the lumen of the hepatopancreatic tubules. These cells have a dense granular cytoplasm with a large round nucleus. Their nuclei lie medioproximally within the cells, and have a prominent nucleolus.

iii. Secretory (B-) cells: The secretory cells are mainly limited to the proximal region of the tubules. These cells have a large vacuole occupying 80-90% of the total cell volume. The nucleus of the B- cell is proximal to the large vacuole and appears to be compressed as the latter enlarges.

iv. Fibrillar (F-) cells: These cells are frequently seen among the R- cells and B- cells. Their nuclei are located at the basal region of the cell cell (Travis, 1955; Web, 1955; Davis & Burnett, 1964; Al-Mohanna et al., 1985a).

The absorptive (R-) cells functioning as a storage site for lipid and glycogen was reported by Travis (1955) and Davis & Burnett (1964). The secretory (B-) cells are suggested to function in the synthesis and release of digestive enzymes. The Fibrillar (F-) cells can be considered to be immature (B-) cells. The hepatopancreas play an important role in food assimilation, storage and secretion of digestive enzymes. The present study revealed that there exists a distinct difference in the cell types of hepatopancreas of SM, SOC and SBC. In SM, the hepatopancreas mainly constituted by embryonic cells and fibrillar cells, indicated low growth rate (Fig. 10). In SOC stage during which active feeding takes place, secretory cells are dominated in the hepatopancreas, as evidenced by the high somatic growth (Fig. 11). While in SBC, characterized by reduced growth rate, majority of cells are constituted by the absorptive cell type (Fig. 12). From the
high content of lipid droplets and glycogen present in these cells. It may be concluded that they function also as a storage site. In the intermoult storage, the main food reserve is fat and glycogen, which form the energy store in prawns.

Neurosecretory system

The neurosecretory system located in the eyestalk, brain and the thoracic ganglion produce neurohormones apparently responsible for growth, moulting, metabolic rate, water balance, dispersion of retinal pigments and sexual activity (Lockwood, 1968). Neurosecretory cells can be defined as neurons with axonal terminals that show specialization and localization for release of substances to the haemolymph (Cook & Sullivan, 1982). With the view to investigate the structure of neurosecretory cells of these systems and the role of the neurohormones produced by them, the same have been studied in SM, SOC and SBC male morphotypes of *M. rosenbergii*.

Detailed examination of the serial sections of the eyestalk, brain and the thoracic ganglion revealed that they contain certain specialized cells. These cells differ from the ordinary nerve cells in having neurosecretory material in them and hence are taken to be the neurosecretory cells. Depending on the cytological characteristics, such as the shape, size, with or without axons, condition of cytoplasm, the shape and size of their nuclei and the staining properties, the neurosecretory cells of the eyestalk, brain and thoracic ganglion of these morphotypes can be classified and designated as type A, B, C, D & E. The eyestalk neurosecretory system possesses only three types (C, D and E) of the neurosecretory cells, the brain four types (B, C, D & E) and the thoracic ganglion possesses all the five types (A, B, C, D & E) of neurosecretory cells. The neurosecretory cells are found either in scattered condition or in the form of specific groups. It has been observed that, the type C cells in the eyestalk exhibit decrease in the nuclear activity in SM and SOC than SBC, while the type D and E cells do not show any variation in these morphotypes (Fig. 13, 14, 15).

The neurosecretory cells of the brain also exhibit variations in their cytological characteristics in these morphotypes. Type B, C, and D cells become more active in SBC when compared to SM and SOC. The E cells do not show any response in these morphotypes (Fig. 16, 17 & 18). Type A, B and C cells of thoracic ganglion exhibit marked changes in its cytological characteristics, in these morphotypes. It shows an increase in activity in SBC than SM and SOC. Type D and E cells do not appear to play any apparent role in these morphotypes (Fig. 19, 20 & 21).

In short, it can be concluded that the type C cells of the eyestalk, type B, C and D cells of the brain and type A, B, C and D cells of the thoracic ganglion play active roles in these morphotypes. The present work establishes that type A, B and C cells of the thoracic ganglion and the B, C and D cells of the brain releases the gonad stimulating hormones in SM and SBC, accelerating the reproductive activity. The C cells of the eyestalk
produce the neurohormones, which inhibit the testicular development and sexual inactivity in SOC morphotypes.

Gomez (1965). Estman-Reks & Fingerman (1984) and Yano (1993) showed that the brain and thoracic ganglion are the source of a gonad-stimulating hormone. The two antagonistic factors control gonadal maturation. One factor (inhibitory) is produced by the X-organ neurosecretory cells, the other factor(s) (stimulatory) is produced by the thoracic ganglia and brain. However, the interaction mechanism of those two antagonistic factors is not completely understood.

Hence, it can be postulated that small male possesses relatively slow growth rate, having the potential to develop, when environmental and social situations permit, through the intermediate phase of the orange claw morphotype into a dominant blue claw male morphotype.

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