Journal of Environmental Biology **April 2006,** 27(2) 225-233 (2006)

Changes in neurosecretory cells of eyestalk, brain and thoracic ganglia of female giant freshwater prawn, *Macrobrachium rosenbergii,* in relation to gonadal maturation

A.K. Pandey and Anjani Kumar
Central Institute of Freshwater Aquaculture, Kausalyaganga, Bhubaneswar-751 002, India

(Received: 30 March, 2004 ; Accepted: 25 October, 2004)

Abstract: *Histomorphological changes in neurosecretory cells of eyestalk, brain and thoracic ganglia of Macrobrachium rosenbergii during different stages of ovarian maturation were recorded. There were five types of neurosecretory cells (NSCs) in eyestalk having size in range of 5-35 μm with or without axons. They were distributed along medulla externa, medulla interna and medulla terminalis. Axonal terminals of these neurosecretory cells were found to terminate in sinus gland. Brain and thoracic ganglia possessed five types of neurosecretory cells such as giant neuron (>80 μm), A (60-80 μm), B (40-60 μm), C (20-40 μm) and D (<20 μm). They were seen arranged in several groups in different parts of brain - in anterior region B, C and D cells were observed, in posterior region giant neurons and A cells dominated while in lateral regions A, B, C and D cells were recorded. Thoracic ganglionic mass was divided into anterior, middle and posterior regions. NSCs were distributed in anterior and posterior portions but were lacking in middle portion. A and B cells were present in anterior-most region followed by C and D cells. In posterior-most region, giant neurons and A cells were present. The present study suggests that ovarian maturation in the giant freshwater is controlled by the secretion of B and C cells of eyestalk, giant neuron (GN), A and B cells of brain and thoracic ganglia.The neurosecretory cells of eyestalk were active in immature stage whereas cells of brain and thoracic ganglia were active in mature stage. The active stage of secretion was characterized by hypertrophy, increase in number of nucleolus, staining intensity, granulation and migration of secretory material towards axons. Histochemical tests demonstrated that the neurosecretory cells were strongly positive to acid fuchsin, paraldehyde fuchsin but exhibited feeble reaction to Sudan black B and periodic acid-Schiff's reagent (PAS).*

Key words: *Eyestalk, Brain, Thoracic ganglia, Ovarian maturation, Macrobrachium rosenbergii.*

Introduction

Macrobrachium rosenbergii is an important species for diversifying freshwater aquaculture in India due to its attributes like reproduction in captivity, established technique for larval rearing, excellent growth rate and survival, absence of major diseases, wide consumer acceptance and high market value (New, 1995). It is widely cultured on commercial scale in many Asian countries including Thailand, China, Taiwan, Philippines, Vietnam, Malaysia, Indonesia, Bangladesh and India (Kutty, 2001). As the natural seed resources have been declined drastically due to several anthropogenic interventions, it is imperative to understand the reproductive physiology of the candidate species for successful broodstock management and captive breeding. The neuroendocrine systems play pivotal role in reproductive endocrinology of crustaceans by transducing the environmental stimuli into physiological processes (Adiyodi and Adiyodi, 1970; Anilkumar and Adiyodi, 1985; Quackenbush, 1986; Nagabhushanam *et al*., 1992; Yano, 1992; Yano and Wyban, 1992; Subramoniam, 1999; Huberman, 2000). Since we lack information regarding the neuroendocrine mediation of reproduction in giant freshwater prawn, an attempt was made to classify the different reproductive stages of the female *M. rosenbergii* and record correlative changes in neurosecretory cells of the eyestalk, brain and thoracic ganglia during ovarian maturation. Histochemical tests on the neurosecretory cells were also conducted to furnish more information on chemical nature of the neurosecretion.

Materials and Methods

Live female *Macrobrachium rosenbergii* (age 3-8 months; body weight range 15-59 g; intermoult stage), were collected from the Freshwater Prawn Farm, Central Institute of Freshwater Aquaculture, Bhubaneswar. They were segregated according to a five point maturity scale ranging from stage 1 (immature) to stage 5 (mature) following the classification of Damrongphol *et al.* (1991), Rao (1991) and Ra'anan *et al.* (1991). The eyestalk, supra-oesophageal ganglion (brain), thoracic ganglia and ovary were dissected out, fixed immediately in freshly prepared Bouin's solution and calciun formol. After fixation, all the tissues were washed thoroughly in running tap water, dehydrated in ascending series of alcohol, cleared in xylene and embedded in paraffin wax at 60 ºC. Serial sections were cut at 6 μm. Tissues were stained with hematoxylin-eosin (H and E), Mallory's triple, aldehyde fuchsin (AF), paraldehyde fuchsin, Sudan black B and periodic acid-Schiff's reagent (PAS) for histological as well as histochemical studies (Pearse, 1968; Bancroft and Stevans, 1977).

Neurosecretory cells (NSCs) of the eyestalk, brain and thoracic ganglia were classified according to their size, shape, position and staining characteristics. During differernt stages of maturity, NSCs were grouped into three phases - quiescent

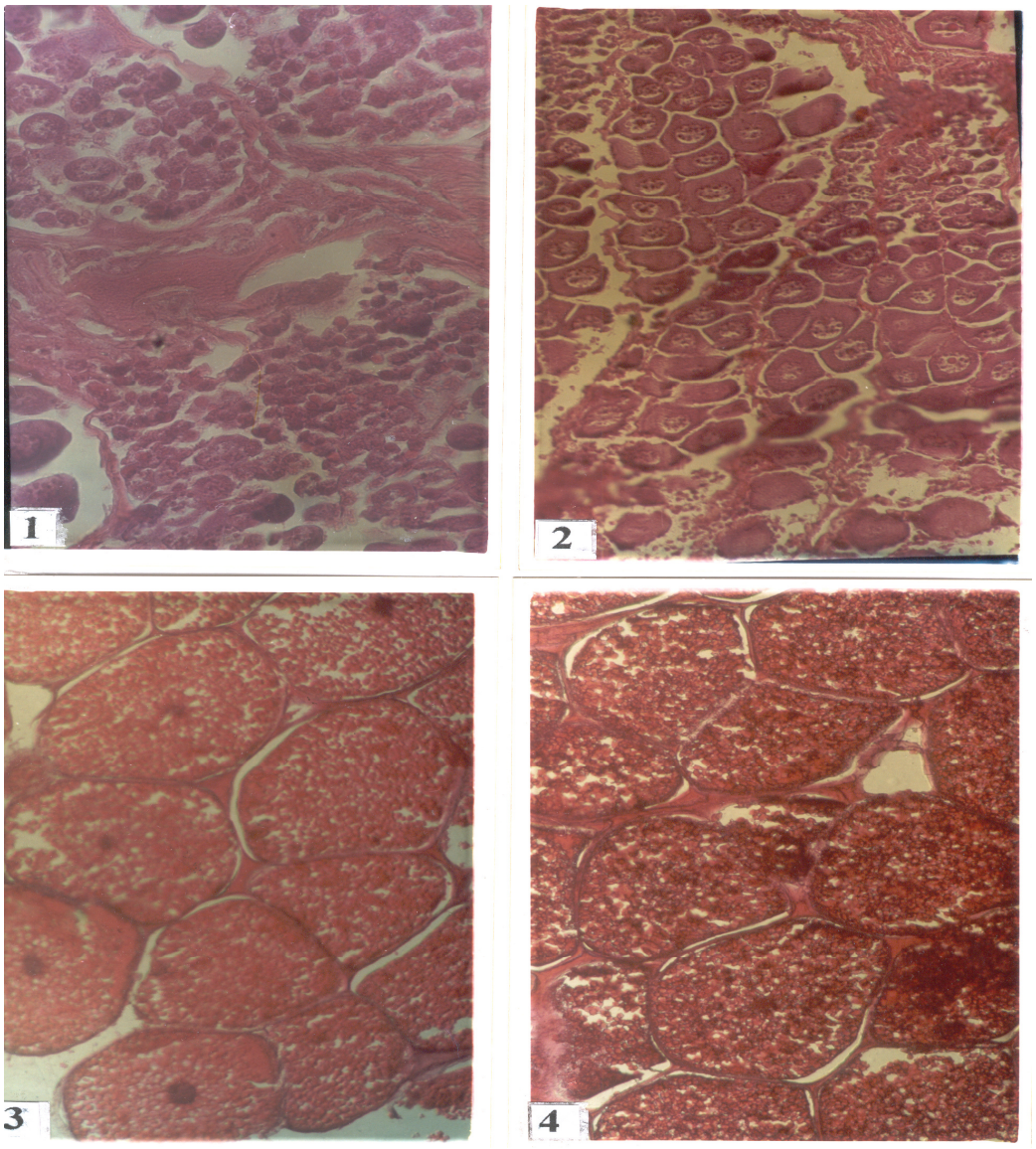

Fig. 1: Ovary of immature *Macrobrachium rosenbergii* (b w 18 g) showing the zone of proliferation in the germinal layer. H and E.x 200.
Fig. 2: Ovary of *M. rosenbergii* (b w 24 g) in previtellogenic stage exhibiting opaque yolk granules in the cytoplasm. H and E. x 200.
Fig. 3: Ovary of *M. rosenbergii* (b w 38 g) showing yolk ladden vitellogenic oocytes. H and E. x 200.
Fig. 4: Ovary of *M. rosenbergii* (b w 58 g) exhibiting matured oocytes. H and E. x 100.

(Q), vacuolar (V) and secretory (S) (Mohamed and Diwan, 1991). The cell and nuclear diameter from 5 aminals (25 from each) at every stage were measured with the help of ocular micrometer in short and long axes and the mean values were calculated. Further, to assess the secretory activity, the degree of cytoplasmic granulation of the NSCs were also recorded.

Results

Morphology of ovary: Female giant freshwater prawn possessed paired ovaries located dorsally to the stomach and hepatopancreas in cephalothorax region. They gave paired oviducts which opened into gonopores on basal segment of the third pleopods. Morphologically, ovaries showed marked variations in relation to maturity. It was commonly observed that ovaries in each stage contained eggs of more than one stage but high proportion of ova were in a single maturity stage. Initially, the prawns were segregated into different maturity stages on external morphology but they were examined histologically too. Based on the size of ova, position as well as size of nucleus, yolk deposition and distribution, maturity of the female *M. rosenbergii* could be classified into the following five stages:

(i) Immature stage: In this group females could be recognized by clear, transparent ovary inside the rostrum. It was small in size just arising at the junction between carapace and first abdominal somite. Gonadosomatic index (GSI) of the animal

Table – 1: Different types of neurosecretory cells in eyestalk of female *M. rosenbergii*.

Cell type	Cell diameter (μm)	Nucleus diameter (μm)	Shape	Stainig intensity	Other features (in Mallory's triple)
A	30-35	10-12	Round/ oval	+++	Largest cells found in the eyestalk in medulla externa region, few in number, stained orange or red.
B	25-30	8-10	Round/ oval	++	Medium sized cells in medulla interna and medulla terminalis, few in number, stained orange or blue.
C	20-25	6-8	Round	++	Small in size, abundant, found in all the regions in bunch, stained orange, blue or red.
D	10-15	<6	Round	++	Small in size, abundant, mostly in medulla interna and terminalis, stained orange, blue or red.
E	<10	_	Round	++	Smallest in size, nucleus not visible, abundant in number, orange or blue.

(n = 50, each cell type) (+ Low; ++ Medium; +++ High)

was 0.63±0.26. Histologically, oocytes were in the first stage of meiotic prophase. They were small, round and irregular cells with large cytoplasm. Maximum size of oocytes was 19 μm and minimum 6 μm with average 11.54±0.25 μm and standard deviation 2.30 μm (Fig. 1). Generally, weight of the animal in this stage was <20 g.

(ii) Previtellogenic stage: In this stage, animal could be recognized by development of colouration and increase in size of the ovary. Pigmentation (light orange or yellow) in the ovary was noticed inside the rostrum. GSI of the animal during this stage was 2.28±0.45. Development of ovaries towards rostrum was clearly visible. Histological observations showed that maximum and minimim size of oocytes were 70 and 30 μm, respectively with mean value 55.52±1.89 μm and standard deviation 13.42 μm (Fig. 2). Average weight of the animal in this stage ranged between 20-25 g.

(iii) Primary vitellogenic stage: In this stage, ovaries could be distinguished by deep orange colour. There was marked increase in size and it extended anteriorily upto base of the rostrum. GSI of the animal was 4.70±1.52. Maximum and minimum size of the oocytes was 160 and 100 μm, repectively. Mean ova diameter was 128.70±2.69 μm with standard deviation 14.99 μm. The cytoplasm was acidophilic at the periphery but was still basophilic around the nucleus. The animals weighed between 25-30 g.

(iv) Vitellogenic stage: This stage was recognized by considerable increase in the ovarian size as it extended upto first spine of the rostrum. It was clearly visible from outside as deep orange mass. GSI of the prawn during this stage was 6.62±1.08. Maximum and minimum sizes of oocytes were 400 and 250 μm, respectively. Mean value and standard deviation were recorded as 347.0±5.29 and 39.27 μm, respectively. Heavy accumulation of yolk globules was observed in the oocytes (Fig. 3). The cytoplasm of oocytes displayed acidophilic character. Weight of prawn during this stage ranged between 30-40 g.

(v) Ripe (mature) stage: In this stage, ovary was large, deep orange in colour, extended anteriorly upto base of second and third spine of the rostrum. Ovary could be recognized as bulky mass beneath the rostrum. GSI of the prawn was computed as 6.79±1.10. Maximum and minimum sizes of the oocytes ranged between 700 and 180 μm, respectively. Average ova diameter was 544.0±15.29 μm and standard deviation 155.59 μm (Fig. 4). The animal weighed >40 g during this stage.

In spent ovary, partially empty cordons as well as space could be seen due to release of eggs. Though cortical vesicles could not be seen but the eggs were full of lipid globules and protein plaquets. As *M. rosenbergii* has perennial breeding habit, there was a large variation in oocyte size. Though oocyte development showed multimodal peak, the predominent group of ova represented the stage of maturation of the particular individual. It is pertinent to remark that after spawning, ovaries returned either to stage 1 or 2 depending upon the type of successive moult. Intermoult period inducing ovarian growth lasted longer than that causing somatic growth. Some females followed somatic growth just after spawning while reproductively active females followed ovarian growth after spawning. During this study, it was observed that even in egg-bearing stage, the individual could attain maturity stage 3.

Eyestalk X-organ complex: In *M. rosenbergii,* neurosecretory cells were observed in the medulla externa (ME), medulla interna (MI) and medulla terminalis (MT) of eyestalk. No such types of cells were noticed in lamina ganglionaris (LG). Based on the morphological (size and shape) and histochemical (staining) characteristics, these cells were divided into five categories (Table 1). In all the three congregations more than one type of cells were observed. First type of cells (A) were

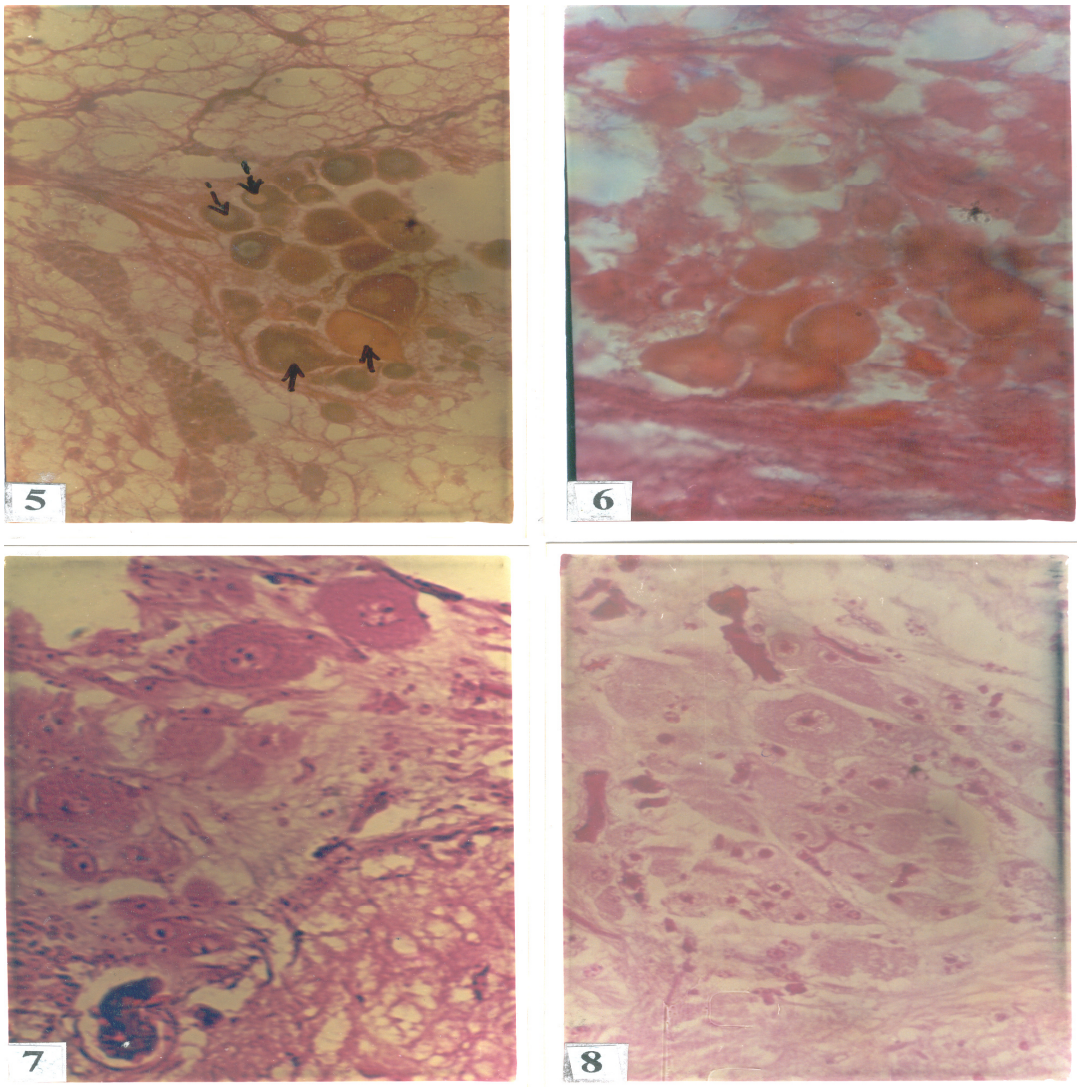

Fig. 5: Eyestalk medulla externa of the immature *M. rosenbergii* showing A (arrow) and B cells (broken arrow). Mallory's triple. x 400.

Fig. 6: Eyestalk medulla interna of the matured *M. rosenbergii* showing neurosecretory B and C cells in quiescent (Q) phase. Mallory's triple. x 1000.

Fig. 7: Brain of the immature *M. rosenbergii* showing different types of neurosecretory cells. Most of them are in Q phase. Mallory's triple x 400.

Fig. 8: Brain of the matured *M. rosenbergii* showing active neurosecretory cells. Most of them are in secretory (S) and vaculation (V) phases. Mallory's triple x 400.

mostly oval in shape having cell diameter between 30-35 μm and nuclear size 10-12 μm (Fig. 5). These cells were recorded at the junction of medulla externa and medulla interna. Most of these cells were with axons but few cells were without axons also. They displayed homogenous staining behaviour in all stages of prawns and no cyclicity was observed. Second type of cells (B) more in number was present along with bigger cells in medulla externa. They were also located in medulla interna and medulla terminalis. Cell and nuclear size were 25-30 and 8-10 μm, respectively. Axonal endings of these cells were terminated in the medulla interna region. Changes in cell diameter, granulation and staining intensity were noticed in these cells at different stages of maturity. Third category of cells (C) was more in number as compared to A and B. They were present in medulla interna and medulla terminalis regions. These cells were round in shape with or without axons. Diameter of cell and nucleus were between 20-25 and 6-8 μm, respectively. Axonal endings of these cells were also terminated in the medulla interna. Alterations in cell diameter, degranulation and staining intensity were observed in them at different maturity stages. Fourth group of cells (D) was located in medulla interna and medulla terminalis regions. Diameter of the cells

Table – 2: Different types of neurosecretory cells in brain and thoracic ganglia of *M. rosenbergii*.

Cell type	Cell diameter (μm)	Nucleus diameter (μm)	Shape	Staining intensity	Other features (Mallory's triple)
Giant neurons	80-120	20-40	Oval or round	++ to ++++	Very few in number, giant cells having bigger nucleus, with or without axons, stain very deep.
A	60-80	15-36	Oval or round	++ to ++++	Large cells with comparatively large nucleus, with or without axons, granulated in secretory phase, more in number, generally stain red.
B	40-60	15-25	Oval or round	+ to +++	High in number, with or without axons less secretory activity and granulation, stain red.
C	20-40	7-15	Oval to round	+ to +++	Very high in number, with or without axons, secretory activity and granulation is less, stain red.
D	5-20	2-10	Oval to round	+ to +++	Very numerous, with or without axons cytoplasm content is very less, nucleus prominent, secretory activity is very less, stain faint red.

(n = 50, each cell type), (+ Low; ++ Medium; +++ High)

ranged between 10-15 μm and nucleus less than 6 μm. These cells were round in shape. No marked changes in morphological as well as staining affinity were recorded in these cells in relation to ovarian maturity. The last category of cells (E) was located in medulla interna and medulla terminalis regions. These cells were round in shape with diameter less than 10 μm and the nucleus was too small to measure in light microscopy. They did not display marked changes in morphological and staining behaviour in relation to ovarian maturation.

The sinus gland (SG) measuring 40-65 μm in size was observed in the medulla interna. As axonal terminals of the neurosecretory cells were found to terminate in this structure, it looked like a depository of the secretory materials. Variations in staining intensity of the contents of the gland were noticed at different maturity stages.

Based on the changes such as cell and nuclear diameter, granulation, staining intensity and migration of secretory granules towards axons, it was found that more than 70% NSCs were active in immature while active cells were below 50% among the mature individuals (Fig. 6).

Brain and thoracic ganglia: Nervous system of *M. rosenbergii* consisted of a large supra-oesophageal ganglionic mass (brain) and a ventral nerve cord with a pair of ganglia corresponding to each embryonic somite. The ganglia were joined longitudinally by connectives and tranversely by commissures. The nerve cord passing through thoracic region is known as thoracic ganglia. In brain and thoracic ganglia of female *M. rosenbergii*, five categories of cells such as giant neuron (GN), A, B, C and D were encountered at different stages of maturity (Fig. 7, 9, 10) (Table 2).The first category of cells were giant neuron (GN) with cell and nuclear diameter >80 and 20-40 μm, respectively. They were oval or round in shape with or without axon. These cells though very few in number were mostly confined to the peripheral region but also seen in the middle area. They displayed marked changes in cell size, number of nucleolus, granulation and vacuolation as well as migration of secretory granules towards axonal portion (Fig. 11). Cell and nuclear diameter of the second group cells (A cells) ranged between 60-80 and 15-36 μm, respectively. They were oval or round in shape and located mostly in middle region of the brain. A type of cells are more in number than giant neurons in brain and thoracic ganglia. They showed marked changes such as increase in size and number of nucleolus as well as staining intensity at different maturity stages (Fig. 8, 10, 12). Migration of secretory granules towards axons has also been noticed in matured specimens. The third group of cells (B cells) was more in number in comparison to giant neurons and A cells. They were oval or round in shape with or without axons. Their size ranged between 40-60 μm with bigger nucleus (15-25 μm). Histological changes were observed in these cells in relation to maturation. The fourth group of neurosecretory cells (C cells) were having size between 20-40 μm and nuclear diameter 7-15 μm with scant cytoplasm. Cells were round with or without axon. They exhibited less secretory activity as compared to GN, A and B cells. Last type of cell (D cells) was more in number with size range between 5-20 μm and nuclear diameter 2-10 μm. They were oval to round in shape and appear to show less secretory activity in relation to ovarian maturation.

All the above types of neurosecretory cells (NSCs) were seen in different parts of brain as several groups. In anterior region of the brain B, C and D cells were more in number whereas in posterior region giant neurons (GN) and A cells dominated. In lateral groups, A, B, C and D cell were uniformly distributed. The neurosecretory cells in thoracic ganglia were divided into anterior, lateral (anterolateral, midlateral and posterolateral), posterior and two median groups. B and C cells were noticed in the anterior group. C and D cells were located in lateral groups of thoracic ganglia. In posterior and median groups only A and B cells were noticed.

Secretory activity of the NSCs: On the basis of histological as well as histochemical characteristics, secretory activity of the NSCs has been grouped as quiescent or resting (Q), secretory (S) and vacuolar (V) phases. When the NSCs in both brain and

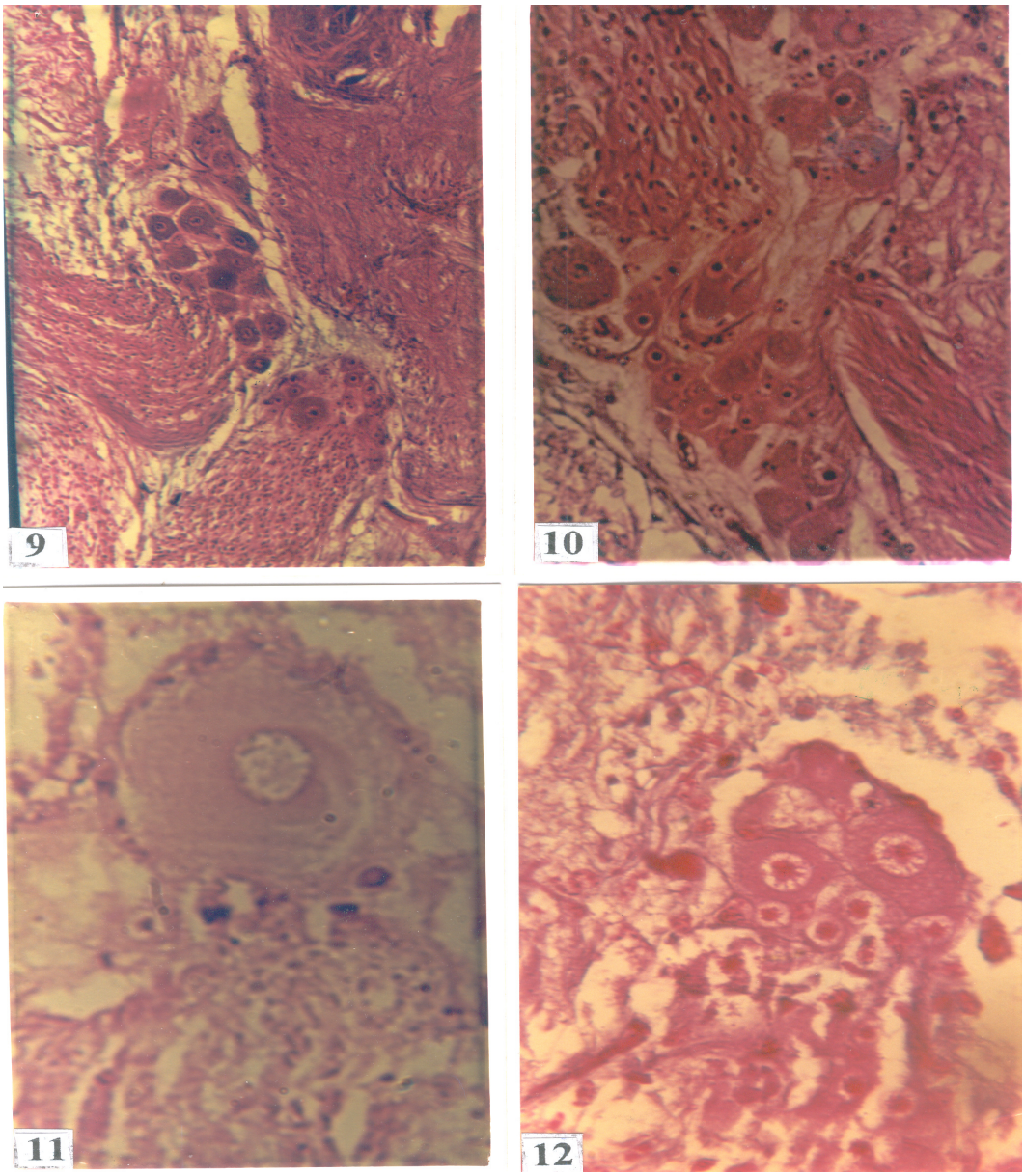

Fig. 9: Thoracic ganglion of the immatured *M. rosenbergii* showing inactive (Q phase) neurosecretory cells. Mallory's triple x 200.
Fig. 10: Thoracic ganglion of the previtellogenic *M. rosenbergii* showing a few cells in S phase. Mallory's triple x 400.
Fig. 11: Thoracic ganglion of the vitellogenic *M. rosenbergii* showing active vacuolated (V) giant neuron. Mallory's triple x 400.
Fig. 12: Thoracic ganglion of the matured *M. rosenbergii* showing highly active vacuolated (V) A and B cells. Mallory's triple x 400.

Table – 3: Percentage of neurosecretory cells during different maturity stages of female *M. rosenbergii* (n = 50, each cell type).

Maturity stage	Brain			Thoracic ganglia		
	Quiescent phase	Vacuolar phase	Secretory phase	Quiescent phase	Vacuolar phase	Secretory phase
Immature	62.1±0.40	30.1±0.44	7.6±0.45	77.8±0.29	12.7±0.15	9.6±0.26
Previtellogenic	60.2±0.35	23.5±0.34	16.4±0.33	42.3±0.39	24.5±0.52	33±0.61
Primary vitellogenic	50.1±0.45	25±0.42	24.9±0.45	39.9±0.27	28.2±0.72	31.6±0.49
Vitellogenic	29±0.57	34.9±0.50	35.3±0.7	27.5±0.68	34.2±0.46	38.3±0.55
Mature	20.2±0.35	30.4±0.26	49.4±0.52	12.8±0.44	31.6±0.61	55.6±0.68

Table – 4: Staining affinity of the neurosecretory cells of female *M. rosenbergii.*

Test	Eyestalk		Brain			Thoracic ganglion		
	B	C	GN	A	B	GN	A	B
Mallory's triple	++	++	+++	++	++	+++	++	++
Paraldehyde fuchsin	++	+	++	++	+	++	++	+
Sudan black B	+	--	+	+	--	+	+	--
Periodic acid Schiff (PAS)	++	--	++	++	+	++	++	+

(+ = Mild positive; ++ = Positive; +++ = Strong positive; -- = Negative)

thoracic ganglia had comparatively less cell diameter, granulation and staining intensity, they were grouped under quiescent (Q) phase. When the cells were full of secretory granules, large number of nucleolus, migration of secretory granules towards periphery, high staining intensity and hypertrophy, they were grouped as active or secretory (S) phase. In vacuolar (V) phase, vacuoles were seen towards periphery due to release of the secretory materials. Interestingly, numbers of nucleolus in the NSCs were less during quiescent phase in brain and thoracic ganglia as compared to secretory as well as vacuolar phases.

Changes in activity of different types of neurosecretory cells in brain and thoracic ganglia of the prawn in relation to ovarian maturation have been summarized in Table 3. As maturity advanced, number of S as well as V cells increased rapidly in brain and thoracic ganglia. In brain of immature female *M. rosenbergii,* 62.1±0.40% cells (GN+A+B) were in Q, 30.1±0.44% in S and 7.6±0.45% in V phase of the secretory cycle while in matured specimens 20.2±0.35% cells were in Q, 30.4±0.26% in S and 49.4±0.52% in V phase. In thoracic ganglia of immature prawns, 77.8±0.29% were in Q, 12.7±0.15% in S and 9.6±0.26% in V phase as against 12.8±0.44% in Q, 31.6±0.61% in S and 55.6±0.68% in V phase of the matured individuals.

Histochemistry of neurosecretory cells: Different histochemical tests were performed to find out the chemical nature of secretory material released by NSCs (Table 4). Among different cells, giant neuron (GN), A and B cells exhibited strong affinity in almost all tests as compared to C and D cells. Poor reactions of the latter may probably be due to less secretory granules and scanty cytoplasm. Though all these cells were positive for Mallory's triple but staining affinity varied. Neurosecretory cells of eyestalk were positive for Mallory's triple and aldehyde fuchsin (AF). Similarly, the giant neurons (GN), A and B cells of brain and thoracic ganglia also stained intensely with Mallory's triple and aldehyde fuchsin (AF). B cells of eyestalk and giant neuron as well as A cells of brain and thoracic ganglia were positive (with low intensity) to Sudan black B too. B and C cells of eyestalk, giant neuron (GN), A as well as B cells of brain and thoracic ganglia were also positive to periodic acid-Schiff's reagent (PAS).]

Discussion

Rajyalakshmi (1961) and Rao (1991) have described four maturity stages in the prawn but when the virgin female were classified separately, five scale classification pattern was followed by O'Donovan and Cohen (1984), Mohamed and Diwan (1994) and Nandkumar (2001). In *M. rosenbergii,* five scale classifications were observed as females were divided into immature, previtellogenic, primary vitellogenic, vitellogenic and mature. Morphology of the ovary of the giant freshwater prawn changes during maturation. Colouration of the ovary varied from colourless to deep orange as maturity advanced. In immature stage size of the ovary was tiny and confined to the posterior-most region of carapace while in mature ones it occupied the entire carapace cavity. Similar observations have also been recorded by other workers in this species (Rajyalakshmi, 1961; Ling, 1969; Rao, 1991; Damrongphol *et al*., 1991). Detailed histological observations of ovary of the giant freshwater prawn revealed that the ova diameter varied from 11.54±0.25 to 544±22 μm in immature and mature specimens, respectively. O'Donovan and Cohen (1984) have recorded ova diameter in immature *M. rosenbergii* as 8.5 μm and in mature individuals 530 μm. In the present study GSI value was found to be 0.63±0.26 in immature and 6.79±1.10 in mature prawns. Our observations corroborate the findings of O'Donovan and Cohen (1984) and Damrongphol *et al*. (1991) in the same species. *M. rosenbergii* breeds throughout the year but peak may occur in particular month (Rao, 1991). Though oocyte development has shown multimodal peak in the present study, there is a clear indication that a particular batch of ova predominates represnting stage of maturation of the particular individual.

The basic cytoarchitecture of eyestalk of *M. rosenbergii* is quite similar to those reported for *M. kistnensis* (Mirajkar *et al*., 1984). The adult prawn possessed well-developed lamina ganglionaris, medulla externa, medulla interna and medulla terminalis. Giant neurons (GN) were not observed in eyestalk of the giant freshwater prawn. *Metapenaeus affinis* also lacks such structure in eyestalk (Rao *et al.,* 1988). There were five types of neurosecretory cells (A, B, C, D and E) in eyestalk of *M. resenbergii* (size ranging between 10-35 μm). Secretory activity of B and C cells of the giant freshwater prawn exhibited changes with the ovarian maturation. In immature prawns more than 70% of these cells were in active phase whereas activity declined below 50% in mature individuals. Mirajkar *et al.* (1983-*M. kistnensis*), Deecaraman and Subramoniam (1983a-*Squilla holoschista*) and Mohamed and Diwan (1991-*Penaeus indicus*) have also recorded similar changes in the eyestalk of prawns in relation to

gonadal maturity. Sinus gland measured 40-65 μm, took bright red/pink stain and appeared swollen among immature female *M. rosenbergii* while it was shrunken, devoid of granules and staining intensity declined among mature females. In *S. holoschista,* Deecaraman and Subramoniam (1983b) have also recorded higher granulation in immature crabs whereas the gland lacked granules in mature individuals. Involution and decreased staining affinity were also noticed in the sinus gland of matured female *P. indicus* (Mohamed and Diwan, 1991).

Attempts have been made classify different types of NSCs in brain and thoracic ganglia of crustasceans. Nagabhushanam *et al.* (1986) identified eight types of NSCs in *P. stylifera,* Rao *et al.* (1988) reported five types of cells in *M. affinis* while Mirajkar *et al.* (1984) found four types in *M. kistnensis.* Deecaraman and Subramoniam (1983a) observed only three types of cells in stomatopod crustacean (*S. holoschista*). However, in *M. resenbergii* five types of neurosecretory cells such as giant neuron (GN), A, B, C and D were identified (Table 2). In brain of giant freshwater prawn, NSCs were distributed in all portions while the cells were observed only in upper and lower portion of thoracic ganglion similar to those reported by Nagabhushanam *et al.* (1986) and Rao *et al.* (1988). Based on size, shape and stainig affinity, A cells of *M. affinis* could be compared with giant neurons (GN) of *M. resenbergii,* B with A, C with B, D with C and E with D (Rao *et al*., 1988). Joshi and Khanna (1985) and Joshi (1989) also observed four types of cells almost of same size in the crab, *Potamon koolooense*. In *P. indicus*, Mohamed and Diwan (1991) observed giant neurons, A and B cell types in different neuroendocrine centres of the brain and thoracic ganglia. Recently, Jadhav *et al*. (2001) reported three types of cells in brain and four types in thoracic ganglion of the male crab (*Uca latea annulipes*).

Several reports exist on the presence of gonad stimulating hormones (GSH) in brain and thoracic ganglia of the crustaceans (Rao *et al*., 1981; Mirajkar *et al.,* 1983; Deecaraman and Subramoniam, 1983a, b; Rao *et al*.,1988; Jayalakshmi *et al*.,1989; Nagabhushanam *et al*., 1989, 1992; Mohamed and Diwan, 1991; Yano, 1992; Yano and Wyban, 1992; Jadhav *et al*., 2001). Marked changes such as increase in cell diameter (hypertrophy), enhanced granulation of cytoplasm, increase in number of nucleolus, enhanced staining intensity and migration of secretory granules towards axons were observed in giant neuron (GN), A and B cells of the brain and thoracic ganglia of *M. rosenbergii* suggesting their role in ovarian maturation of the species. Mirajkar *et al.* (1983), Deecaraman and Subramoniam (1983b), Mohamed and Diwan (1991) and Jadhav *et al*. (2001) have also recorded similar histological changes in the NSCs of brain and thoracic ganglia of crustaceans in relation to ovarian maturation.

Histochemical tests indicated that giant neuron, A and B cells of brain and thoracic ganglia of *M. rosenbergii* were aldehyde fuchsin (AF) and acid fuchsin positive indicating the secretion to be rich in cysteine/cystine. Mirajkar *et al.* (1984), Victor and Sarojini (1985), Rao *et al*. (1988) and Mohamed and Diwan (1991) have also observed similar staining responses of these cells in decapod crustaceans. Positive response of giant neuron, A and B cells to periodic acid-Schiff's reagent (PAS) indicates the presence of carbohydrate-like compounds/ mucopolysaccharides in the NSCs. Besides, GN and A cells also exhibited mild positive response to Sudan black B suggesting lipoidal nature of the secretion. The present observations corroborate the findings of Mirajkar *et al.* (1984-*M. kistnensis*), Nagabhushanam *et al. (*1986-*P. stylifera*) Rao *et al*. (1988-*M. affinis*) and Mohamed and Diwan (1991-*P. indicus*).

Acknowledgments

We are grateful to Dr. K. Janaki Ram, the then Director, Central Institute of Freshwater Aquaculture, Bhubaneswar for providing the necessary facilities to carry out this work. Financial assistance provided to AK (as JRF) by the Indian Council of Agricultural Research, New Delhi is thankfully acknowledged.

References

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

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

Bancroft, J.D. and A. Stevans: Theory and practice of histochemical techniques. Churchill Livingston, New York (1977).

Damrongphol, P., N. Eangchuan and F. Poolsanguan: Spawning cycle and oocyte maturation in laboratory-maintained giant freshwater prawns, *Macrobrachium rosenbergii. Aquaculture,* **95,** 347-357 (1991).

Deecaraman, M. and T. Subramoniam: Endocrine regulation of ovarian maturation and cement glands activity in a stomatopod crustacean, *Squilla holoschista*. *Proc. Indian Acad. Sci. (Anim. Sci.),* **92,** 399-408 (1983a).

Deecaraman, M. and T. Subramoniam: Histochemistry of the neurosecretory systems in a stomatopod crustacean, *Squilla holoschista. Proc. Indian Acad. Sci. (Anim. Sci.)*, **92,** 387-398 (1983b).

Huberman, A.: Shrimp endocrinology: a review. *Aquaculture*, **191,** 191-208 (2000).

Jadhav, S., M.G. Ragunathan and M. Deecaraman: Changes in the neurosecretory cells of the brain and thoracic ganglion of male crab, *Uca (Celuca) lactea annulipes,* with respect to season. *J. Envrion. Biol*., **22,** 311-314 (2001).

Jayalakshmi, K., R. Sarojini, S.S. Rao and R. Nagabhushanam: An ovary stimulating factor in the freshwater prawn, *Macrobrachium lamerri*. *U. P. J. Zool.,* **9,** 56-60 (1989).

Joshi, P.C.: Neurosecretion of brain and thoracic ganglion and its relation to reproduction in the female crab, *Potamon koolooense* (Rathbun). *Proc. Indian Natl. Sci. Acad*., **98,** 41-49 (1989).

Joshi, P.C. and S.S. Khanna: Studies on the relationship between the neurosecretion of the brain and testicular cycle in a crab, *Potamon koolooense* (Rathbun) (Crustacea, Decapoda). *Zool. Anz*., **214,** 320-332 (1985).

Kutty, M.N.: Diversification of aquaculture. *In*: Sustainable Indian fisheries (*Ed*: T.J. Pandian). National Academy of Agricultural Sciences, New Delhi, pp. 189-212 (2001).

Ling, S.W.: The general biology and development of *Macrobrachium rosenbergii.* FAO Fish. Rep. No. **57,** 589-606 (1969).

Mirajkar, M.S., R. Sarojini and R. Nagabhushanam: The neurosecretory control of the annual reproductive cycle in the freshwater prawn, *Macrobrachium kistnensis. Curr. Sci.,* **52,** 967-970 (1983).

Mirajkar, M.S., R. Sarojini and R. Nagabhushanam: Histochemistry of the neurosecretory cells in the freshwater prawn, *Macrobrachium kistnensis* (Palaemonidae). *Proc. Nat. Acad. Sci. India*, **54B,** 189-193 (1984).

Mohamed, K.S. and A.D. Diwan: Neuroendocrine regulation of ovarian maturation in the Indian white prawn, *Penaeus indicus* H. Milne Edwards. *Aquaculture,* **98,** 381-393 (1991).

Mohamed, K.S. and A.D. Diwan: Vitellogenesis in the Indian white prawn, *Penaeus indicus* (Crustacea : Decapoda). *J. Aqua. Trop.,* **9,** 157-171 (1994).

Nagabhushanam, R., R. Sarojini and P.K. Joshi: Observation on the neurosecretory cells on the marine penaeid prawn, *Parapenaeosis stylifera. J. Adv. Zool*., **7,** 63-70 (1986).

Nagabhushanam, R., P.R. Machale and R. Sarojini: Pheromonal and hormonal control of reproduction in the freshwater prawn, *Macrobrachium kistnensis*. *Proc. Indian Natl. Sci. Acad*., **98,** 95-98 (1989).

Nagabhushanam, R., R Sarojini and S.S. Rao: Reproductve endocrinology of the Indian marine commercial shrimp, *Metapenaeus affinis*. *In*: Aquaculture research needs for 2000 A.D. (*Eds*: J.K. Wang and P.V. Dehadrai). Oxford and IBH, New Delhi, pp. 163-180 (1992).

Nandkumar, G.: Reproductive biology of the speckled shrimp, *Metapenaeus monoceros* (Fabricius). *Indian J. Fish*., **48,** 1-8 (2001).

New, M.B.: Status of freshwater prawn farming: a review. *Aquacult. Res.,* **26,** 1-54 (1995).

O'Donovan, P. and D. Cohen: The ovarian cycle during the intermoult in ovigerous *Macrobrachium rosenbergii*. *Aquaculture,* **36,** 347-358 (1984).

Pearse, A.G.E.: Histochemistry: Theoretical and applied. Vol. 1. Churchill Livingston, Edenburgh (1968).

Quackenbush, L.S.: Crustacean endocrinology. a review. *Can. J. Fish. Aquat. Sci*., **43,** 2271-2282 (1986).

Ra'anan, Z., A. Sagi, Y. Wax, I. Karplus, G. Hulata and A. Kuris: Growth, size rank and maturation of the freshwater prawn, *Macrobrachium rosenbergii* : anylysis of marked prawns in an experimental population. *Biol . Bull*., **181,** 379-386 (1991).

Rajyalakshmi, T.: Studies on maturation and breeding in some estuarine palaemonid prawns. *Proc. Natl. Inst. Sci*., **27B,** 179-188 (1961).

Rao, K.J.: Reproductive biology of the giant freshwater prawn, *Macrobrachium rosenbergii* (de Man) from Lake Kolleru (A.P.). *Indian J. Anim. Sci*., **61,** 780-787 (1991).

Rao, N.CH., K. Shakuntala and S.R. Reddy: Studies on the neurosecretion of the thoracic ganglion in relation to reproduction of female *Macrobrachium lanchesteri. Proc. Indian Acad. Sci. (Anim. Sci.)*, **90,** 503-511(1981).

Rao, S.S., R. Sarojini and R. Nagabhushanam: Histomorphology and histochemistry of the neurosecretory system in a penaeid prawn, *Metapenaeus affinis*. *J. Adv. Zool*., **9,** 113-122 (1988).

Subramoniam, T.: Endocrine regulation of egg production in economically important crustaceans. *Curr. Sci*., **76,** 350-360 (1999).

Victor, B. and R. Sarojini: Histomorphology of cerebral neurosecretory system in the caridean prawn, *Caridina rajadhari*. *Curr. Sci*., **54**, 817-818 (1985).

Yano, I.: Effect of thoracic ganglion on vitellogenin secretion in kuruma prawn, *Penaeus japonicus*. *Bull. Natl. Res. Inst. Aquaculture,* **21,** 9-14 (1992).

Yano, I. and J.A. Wyban: Induced ovarian maturation of *Penaeus vannamei* by injection of lobster brain extract. *Bull. Natl. Res. Inst. Aquaculture*, **21,** 1-7 (1992).

Correspondence to:
Dr. A. K. Pandey
Central Institute of Freshwater Aquaculture (ICAR)
Kausalyaganga
Bhubaneswar-751002 (Orissa), India
E-mail: akpandey_cifa@yahoo.co.in;
cifa@ori.nic.in; cifa@hun1.nic.in
Tel.: +91-674-2465421, 2465430, 2465446
Fax: +91-674-2465407