Indian J. Fish., 59(1) : 119-124, 2012

Histological and morphological changes associated with ovarian development of speckeled shrimp *Metapenaeus monoceros* (Fabricius, 1798)

JOICE ABRAHAM* AND MARY K. MANISSERI
Central Marine Fisheries Research Institute, Kochi - 682 018, Kerala, India
**Fish Farmers Development Agency (Department of Fisheries), Kochi - 682 018, Kerala, India*
e-mail: joiceabraham@gmail.com

ABSTRACT

Histological techniques and gonadosomatic index (GSI) were applied to validate macroscopic classification of ovaries in *Metapenaeus monoceros*. The five ovarian maturity stages based on gross morphology were correlated with those based on histomorphology *viz.*, pre-vitellogenic, early vitellogenic, late vitellogenic, vitellogenic and spent. Mean values of GSI increased from 0.353 (immature ovary) to 6.98 (fully mature), thus recording a 20-fold increase. Pre-vitellogenic ovary contained primary oogonial cells, secondary oogonial cells and pre-vitellogenic oocytes. Early vitellogenic stage was characterized by the dominance of perinucleolar oocytes. Folliculogenesis commenced during this stage. Late vitellogenic and vitellogenic ovaries were characterized by rough granular cytoplasm which was fully eosinophilic due to accumulation of yolk and the mean ova diameters during these stages were 170 µm and 230 µm respectively. Cortical rods, which are characteristic features of fully mature oocytes of penaeid shrimp were not encountered during the present study in any of the oocyte development stage.

Keywords: Cortical rods, Gonadosomatic index, Histology, *Metapenaeus monoceros*, Oocyte diameter, Vitellogenic

Introduction

Oogenesis in penaeids involves sequential deposition of yolk protein, vitellin in developing oocytes which consists of two distinct processes: proliferative and differentiative (Adiyodi and Subramoniam, 1983). Histological observations on the oogenesis of penaeid shrimp have been made in several species (Dall *et al*., 1990). In most of the studies, developmental changes in size and colouration of the ovary have been related to cytomorphological changes (Rao, 1968; Yano, 1988; Vasudevappa, 1992; Mohamed and Diwan, 1994; Quintero and Garcia, 1998; Sakaji *et al*., 2000; Peixoto *et al*., 2003).

Metapenaeus monoceros (Fabricius, 1798), commonly called as speckled shrimp is one of the commercially important penaeid species found along both the coasts of India. The estimated landing of penaeid shrimps in India in 2010 was 2,17,900 t, forming 51% of the total crustacean landings (CMFRI, 2011). *M. monoceros* is a commercially important species among large sized penaeids of the country. The species forms an important component of the shrimp catch from the Pokkali fields of Kerala in India and the rice-prawn filtration units in Bangladesh. *M. mon*oceros attains a maximum length of about 190 mm and has high export potential. There is very good scope for this species to be taken up for semi-intensive culture practices in India, due to its large size among the *Metapenaeus* spp. Based on the morphology, five ovarian maturity stages have been identified in *M. monoceros* (Nandakumar, 2001). However, unlike in other penaeids, in *M. monoceros* no attempt has been made to correlate gross morphology of the developing ovarian stages with histology.

Cortical crypts are rod shaped bodies encountered in the ripe oocytes of fully mature, ready to spawn ovary of penaeid shrimp (Dall *et al*., 1990). Previously referred to as jelly-like substance, cortical specializations, rod-shaped bodies or peripheral bodies, they exist in all specimens observed in genera *Penaeus* and *Sicyonia* (Anderson *et al*., 1984; Yano, 1988). Vasudevappa (1992) and Sakaji *et al*. (2000) reported that cortical rods were not found in oocytes of *M. monoceros* and *Metapenaeopsis dalei* respectively. Contrary to the assumption that cortical rods are a characteristic feature of genus *Penaeus*, Ayub and Ahmed (2002), reported their presence in mature eggs of *Metapenaeus affinis*.

In the present work, an attempt has been made to validate macroscopic classification of the ovaries of *M. monoceros,* applying histological techniques and Gonado-somatic index (GSI) for the first time.

Materials and methods

Specimens of *M. monoceros* were collected during November 2001- June 2003 from trawlers operating from Kalamukku and Murikkumpadam fish landing centres of Vypeen Island (lat.10° 08' N, long. 76°21' E). Live adult shrimp of size ranging from 90 to 160 mm were used for the study. Plastic bins of 25 l capacity with aerators were used for live transport of shrimp from the fishing ground to the landing centres. Shrimp were then transported live to CMFRI laboratory where they were segregated sex-wise and kept in 1 t capacity fibre glass tanks with aeration. Total length (TL) and carapace length (CL) of the shrimps were measured to the nearest millimeter (mm) and total weight (TW) to the nearest milligram (mg).

Ovaries of shrimp were dissected out carefully and observed under stereoscopic dissection microscope to study their morphology. All dissections were done in normal saline (0.85% NaCl). The exposed ovary was then carefully excised out and weighed to the nearest mg. The gonadosomatic index (GSI) was calculated as percentage of the ovarian weight relative to the body weight.

For conducting routine histological studies, one of the middle lobes of the ovary was cut and fixed in Bouin's fluid for 24-48 h. The tissue was processed for light microscopy according to Bell and Lightner (1988). The tissues were washed overnight to remove excess picric acid, dehydrated in propanol series (30-100%), cold impregnated in a mixture of wax and chloroform (1:1), tissue blocks were prepared, serial sections of 5-6 μm were taken and affixed to glass slides and subjected to routine haematoxylin-eosin staining. Stained sections were repeatedly washed in an ascending series of propanol grades to remove excess eosin and cleared in xylene. Sections were then mounted with DPX and examined under a Leica MPS 60 binocular microscope and photomicrographs were taken. Micrometric measurements of oocytes in different stages of maturation were taken using an ocular micrometer calibrated with a stage micrometer. As oocytes are not spherical in shape, the largest and smallest axes of the oocyte diameter were measured and the average was taken as the actual oocyte diameter. A minimum of 100 cells were measured from a single slide.

Results

Based on the colour and size of the ovary as observed through the dorsal exoskeleton and upon dissection, the ovary in *M. monoceros* was classified into five stages (Table 1).

Stage I or Immature

Ovary not visible through exoskeleton. Upon dissection, it was thinner than gut, strand like, translucent and smooth in texture. Ovary was formed of developing anterior lobes confined to the posterior half of the cephalothorax, and posterior lobes, which were situated on the dorsal aspect of the abdomen, extending up to the middle of the sixth abdominal segment.

Stage II or Early maturing

Ovary was visible through the dorsal exoskeleton as a thin strand. Upon dissection, it was observed to be light yellow in colour and granular. The anterior lobes had further extended forward into the cephalothorax, the middle lobes and the rudiments of their lobules were developing. The posterior lobes were larger than the gut with minute brown pigments appeared on the dorsal surface of the ovary.

Stage III or Late maturing

Ovaries were clearly visible through the dorsal exoskeleton as a dark thick band. Dissected out ovaries appeared light green with branched brownish

Table 1. Gonadosomatic index and morphological and histological changes in relation to maturity stages in female *M. monoceros*

Maturity Stages	Colour and appearance	GSI ± S.D (n=25)	Ovary stages	Oocyte diameter (μm ± S.D)	Nucleus diameter (μm ± S.D)
Stage I (Immature)	Translucent Smooth	0.353 ± 0.21	Pre-vitellogenic		
			Primary oogonial cells	6.43±1.92	4.8±1.83
			Secondary oogonial cells	17.64±3.84	14.23±2.84
			Pre-vitellogenic oocytes	35.68±12.36	21.3±5.28
Stage II (Early maturing)	Light yellow Smooth-granular	2.15±0.83	Early vitellogenic	129.46±18.34	42.64±9.56
Stage III (Late maturing)	Light green Granular	3.98±1.20	Late vitellogenic	174.38±16.8	55.68±9.38
Stage IV (Mature)	Dark green/ Dark brown Granular	6.98±1.52	Vitellogenic	230.56±15.89	58.39±6.39
Stage V (Spent)	Pale cream Flaccid-Granular	0.894±0.29	Spent	21.87±3.86	14.32±2.18

chromatophores distributed over the entire dorsal surface and with a firm and granular texture. The anterior, middle and posterior lobes were fully formed, but did not fill the cephalothorax completely.

Stage IV or Mature

Ovaries were clearly visible through the exoskeleton as a thick band on the entire dorsal side of the animal, with the characteristic diamond shaped expansion of the first abdominal segment. Fully mature female specimens had dark green ovaries, while a few had dark brown ovaries. The texture was firm and granular. The ovarian lobes were considerably larger and filled up all the available space in the body cavity, both in the cephalothoracic and abdominal regions.

Stage V or Spent

Ovaries were not visible through the exoskeleton as in the case of the immature stage. Hence spent stages were differentiated from immature stages based on the size of the specimens. Upon dissection, the ovaries appeared whitish or creamy in colour with a flaccid appearance. Incompletely spawned specimens were also encountered with the anterior and middle lobes whitish/creamy and posterior lobes green in colour.

Gonadosomatic index (GSI)

GSI values during the various maturity stages are presented in Table 1. There was an increase in GSI values with the progression of maturity, except from stage IV to stage V when there was a sharp decrease. The GSI values ranged from 0.353 in immature females (Stage I) to 6.98 in mature specimens (Stage IV) showing a 20-fold increase.

Histology

The histological studies led to the classification of the ovary in *M .monoceros* into five stages depending on the prominent cell types present and these stages could be correlated to the maturity stages I to V.

Pre-vitellogenic stage (Stage I ovary)

Ovary during the pre-vitellogenic stage was observed to possess three types of cells – primary oogonial cells and secondary oogonial cells arising from the germarium and moving towards the periphery in a graded manner and deeply basophilic pre-vitellogenic oocytes further away from the germinal zone (Fig. 1). The primary oogonial cells had a mean diameter of 6.43 μm and a nucleus of 4.8 μm (Table 1) with pale eosinophilic cytoplasm. The nucleus was basophilic with the nucleoli arranged along the periphery. Secondary oogonial cells were formed by the mitotic division of the primary oogonial cells and had a mean diameter of 17.64 μm with a nucleus of 14.23 μm.

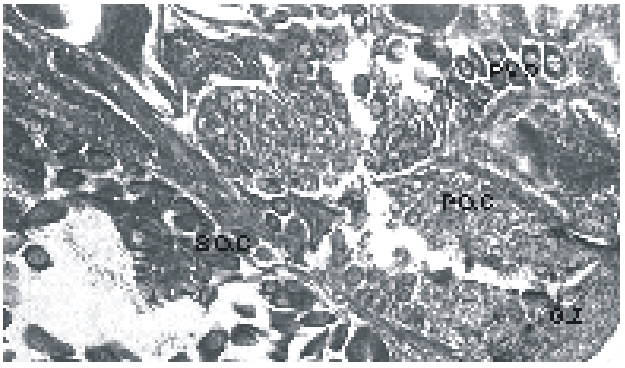

Fig. 1. Stage I ovary showing germinal zone (GZ), primary oogonial cells (POC), secondary oogonial cells (SOC) and pre-vitellogenic oocytes (PVO) x 200

Early vitellogenic stage (Stage II ovary)

Oocytes in the early vitellogenic stage were characterized by the arrangement of 8-10 basophilic nucleoli as a circular ring along the periphery of the nuclear membrane called the perinucleolar oocytes (Fig. 2). Perinucleolar oocytes had a mean diameter of 129.46 μm and nuclear diameter of 42.64 μm (Table 1). Cytoplasm of perinucleolar oocytes was eosinophilic and appeared granular due to the presence of vesicular primary yolk. A germinal zone with oogonial cells was apparent at this stage. Folliculogenesis, the investment of follicle cells around developing oocytes, was also completed during this stage. Basophilic follicle cells were found moving along the periphery of the germinal zone towards the centre of the ovary encircling individual oocytes.

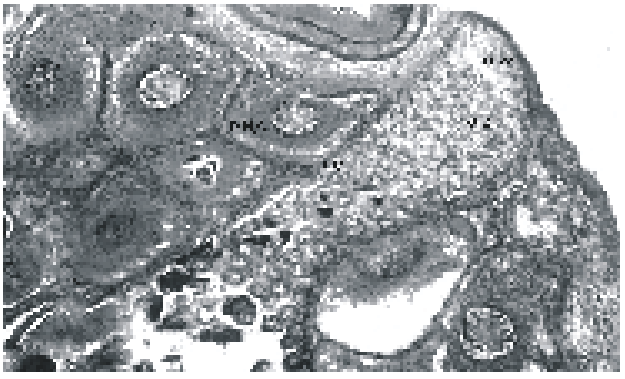

Fig. 2. Stage II ovary with perinucleolar oocytes (PNO), follicle cells (FC) and ovarian wall (OW) x 200

Late vitellogenic stage (Stage III ovary)

Late vitellogenic oocytes were characterized by a rough granular cytoplasm, which was fully eosinophilic (Fig. 3). The mean oocyte diameter was 174.38 μm with a nuclear diameter of 55.68 μm. The nucleus was basophilic and nuclear material was found to diffuse into the cytoplasm (Fig. 4). Owing to the increased diameter of growing oocytes which accumulated densely packed yolk platelets, the follicle cells were stretched and flattened.

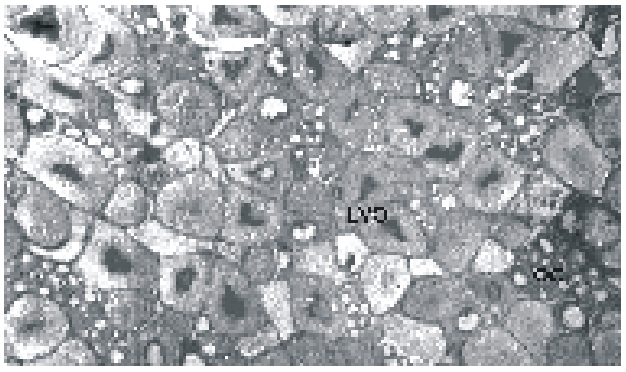

Fig. 3. Stage III ovary showing late vitellogenic oocytes (LVO) and oogonial cells (OC) x 100

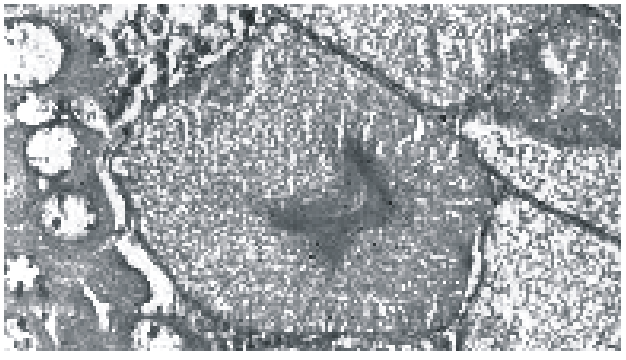

Fig. 4. Late vitellogenic oocyte x 400

Vitellogenic stage (Stage IV ovary)

Ovary in the vitellogenic stage was filled with vitellogenic oocytes with a mean diameter of 230.56 µm and nuclear diameter of 58.39 µm (Table 1). Fully mature oocytes appeared more elongate than circular with a thin rim of basophilic follicle cells around it (Fig. 5 and 6). Follicular detachment from the oolemma, which occurs just prior to ovulation, was observed. Vitellogenic oocytes had a granular and eosinophilic cytoplasm filled with yolk. Nucleus was basophilic and nucleoli were not apparent. Cortical bodies, which is characteristic of fully mature oocytes of most penaeid species, were absent in the vitellogenic oocytes of *M. monoceros*.

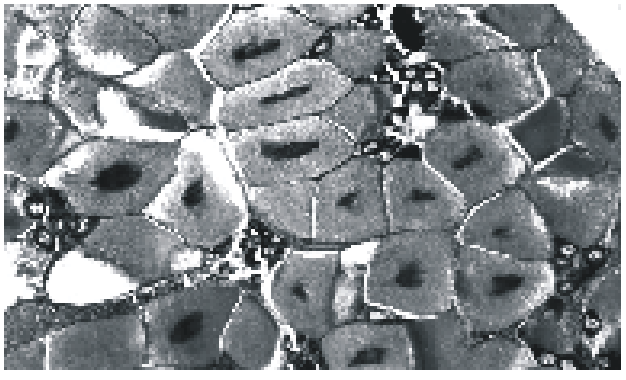

Fig. 5. Stage IV ovary showing vitellogenic oocytes x 100

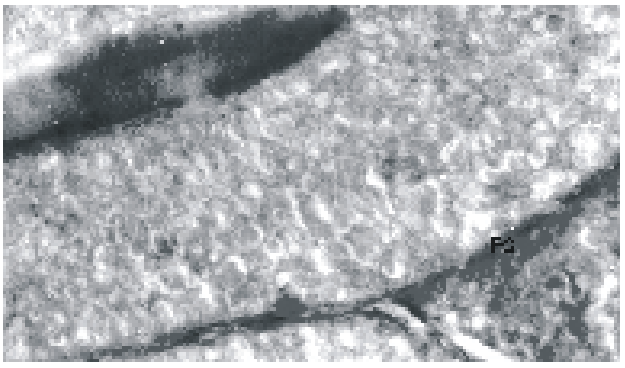

Fig. 6. Vitellogenic oocyte surrounded by flattened follicle cells (FC) x 1000

Spent stage (Stage V ovary)

Oocytes in spent ovary were similar to those present in pre-vitellogenic stage except for the larger number of resorbing oocytes. Strongly basophilic oogonial cells and pre-vitellogenic oocytes were present (Fig. 7 and 8). Vacuolated follicle cells were found to encircle the resorbing oocytes.

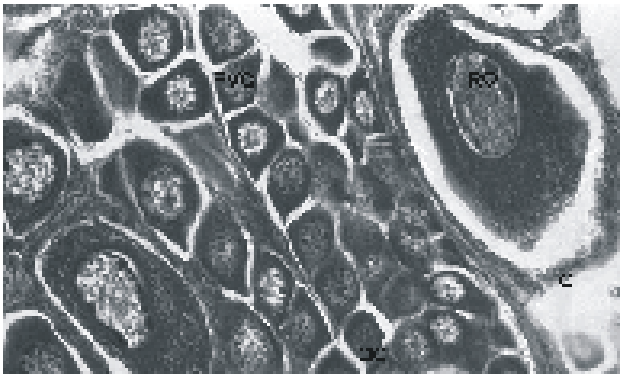

Fig. 7. Stage V ovary showing resorbing oocytes (RO) x 400

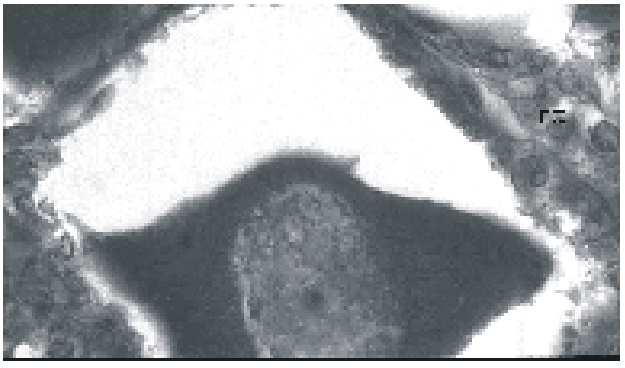

Fig. 8. Resorbing oocyte (RO) surrounded by vacuolated follicle cells (FC) x 1000

Discussion

Ovarian maturation in *M. monoceros* is accompanied by distinct changes in colour, size as well as appearance and the process is almost similar to that described by Rao

(1968) for four species of penaeids. Colour change in the ovary during maturation is well known for decapod crustaceans, particularly for penaeids (Dall *et al*., 1990). In the present study, for fully mature ovaries of *M. monoceros,* two distinct colours were noticed *viz*., dark green and dark brown. Other workers have reported the ripe ovaries of this species to be dark green or brownish green (Nalini, 1976; Rao, 1989; Nandakumar, 2001).

The mean ova diameter of mature oocytes of *M. monoceros* in our study was 230 µm. The ova diameter values for fully mature oocytes of *M. monoceros* reported hitherto are 260 µm (Mohamed *et al*., 1978), 110 – 270 µm (Rao, 1989) and 174 – 232 µm (Nandakumar, 2001). According to Gurney (1942), the size of eggs of the same penaeid species occurring in different habitats and localities of India varied considerably. Rao (1974) observed that the spawned eggs of *M. dobsoni*, *Fenneropenaeus indicus* and *Parapenaeopsis stylifera* collected from the Cochin waters were larger than the eggs of penaeid prawns in other parts of India.

The process of oogenesis in *M. monoceros* is completed in two phases like that in other crustaceans (Adiyodi and Subramoniam, 1983). First is the proliferative phase, in which the primary oogonial cells undergo mitotic division forming secondary oogonial cells which after meiotic division give rise to primary oocytes as seen in stage I (pre-vitellogenic) ovary in this study. The second phase is the differentiative phase, wherein the immature ova accumulate yolk and develop into mature oocytes corresponding to stage II (early vitellogenic), stage III (late vitellogenic) and stage IV (vitellogenic) ovaries in *M. monoceros*. More or less similar observations have been reported in many other decapod crustaceans (Yano, 1988). Oogonial cells had pale eosinophilic cytoplasm whereas in pre-vitellogenic oocytes, the cytoplasm was deeply basophilic. Mohamed and Diwan (1994) reported that the oogonial cells of *Penaeus inidcus* possessed a large, conspicuous nucleus and weakly eosinophilic cytoplasm. The pre-vitellogenic oocytes in *M. dobsoni* had deeply basophilic cytoplasm, which was homogeneous and granular (Vasudevappa, 1992).

In *M. monoceros,* germinal zone was observed as a thin band along the innermost layer of the ventral ovarian wall. Adiyodi and Subramoniam (1983) reported a wide variation in the placement of germinal zone in the ovary of crustaceans. In the present study, it was observed that from the germinal zone, a continuous crop of oogonial cells are produced, from an area called the zone of proliferation. Similar observations were made by Shaikhmahmud and Tembe (1958) in *P. stylifera* and Vasudevappa (1992) in *M. dobsoni*. The gradual movement of oogonia towards the centre of the lumen of ovary during their transformation into primary and secondary ooctyes is clearly demonstrated in the present study. In *M. monoceros* and in the above mentioned studies, the immature ova present in the centre of the lumen have been observed to move towards the periphery as they grow and become mature.

In *M. monoceros,* early vitellogenic oocytes were characterized by the perinucleolar arrangement of 12–15 nucleoli. Vasudevappa (1992) made similar observations in the early vitellogenic ovaries of *M. dobsoni*. He also noticed abundance of atretic cells in the early vitellogenic oocytes. In *Metapenaeopsis dalei*, Sakaji *et al*. (2000) noticed perinucleolar oocytes in stage III ovaries. The late vitellogenic ovary of *M. monoceros* was characterized by the presence of rough granular cytoplasm which was fully eosinophilic. In *M. dobsoni* also Vasudevappa (1992) reported a clear shift from the basophilic to eosinophilic nature of cytoplasm at the late vitellogenic stage. Mohamed and Diwan (1994) reported that the granular nature of the oocyte cytoplasm of *F. indicus* at this stage was mainly due to the formation of dense yolk platelets and accumulation of lipid globules.

The vitellogenic ovary in *M. monoceros* was filled with mature oocytes which were more elongate than circular with patches of oogonial cells in between. The eosinophilic cytoplasm was filled with yolk granules and the absence of cortical bodies was conspicuous. It is of a general consensus that full ovarian maturation of penaeids is indicated by the presence of large acidophilic oocytes with cortical bodies (King, 1948). Previously referred to as jelly-like substance, cortical specializations exist in all specimens observed in genera *Penaeus* and *Sicyonia* (Hudinaga, 1942; King, 1948; Anderson *et al*., 1984; Yano, 1988). According to Tan–Fermin and Pudadera (1989), in *P. monodon,* the cortical bodies are present along the periphery but as maturation progresses, these bodies elongate and extend towards the nucleus. However, the absence of cortical bodies in fully ripe ova has been reported by Shaikhmahmud and Tembe (1958) in *P. stylifera*; Rao (1968) in *M. affinis*, *M. dobsoni* and *P. stylifera*; Vasudevappa (1992) in *M. dobsoni* and Sakaji *et al*. (2000) in *Metapenaeopsis dalei*. Ayub and Ahmed (2002) detected the presence of cortical rods in mature eggs of *M. affinis* and *P. stylifera.* But they reported that these peripheral bodies remain spherical throughout and never take the appearance of rods. Cortical crypts release materials forming a jelly coat when the eggs are exposed to seawater (Clark and Lynn, 1977). As a result of such a cortical reaction, the egg reduces its volume (Clark *et al*., 1980). Because of the absence of cortical rods in the oocytes of *M. monoceros*, it is expected that there is little or no jelly coat around the eggs exposed to seawater and reduction in volume after the cortical reaction is lesser than that in

species which have them.

The present study assumes importance as a detailed analysis of ovarian development of any species is fundamental to understanding the reproductive dynamics of the species, which is an essential pre-requisite to apply adequate management measures for sustainable resource exploitation.

Acknowledgements

Authors sincerely thank Prof. (Dr.) Mohan Joseph Modayil, Former Director, CMFRI, Cochin for extending all the facilities required to carry out this work and for his constant encouragement. The help and guidance rendered by Dr. R. Paul Raj, Former Head, Post Graduate Programme in Mariculture, CMFRI, Cochin is gratefully put on record. The CIFE-ICAR fellowship granted for the first author's Ph. D. programme is greatly acknowledged.

References

Adiyodi, R. G. and Subramoniam, T. 1983. Oogenesis, oviposition and oosorption in Crustacea, Decapoda. In: Adiyodi, K. G. (Ed.) *Reproductive biology of crustacea*, Vol. I. John Wiley and sons, Chichester, London, p. 443-495.

Anderson, S. L., Chang, E. S. and Clark, W. H. 1984. Timing of post-vitellogenic ovarian changes in the Ridge back prawn *Sicyonia ingentis* (Penaeidae) determined by ovarian biopsy. *Aquaculture,* 42: 257-271.

Ayub, Z. and Ahmed, M. 2002. A description of the ovarian development stages of penaeid shrimps from the coast of Pakistan. *Aquacult. Res*., 33: 767-776.

Bell, T. and Lightner, D. V. 1988. Male reproductive system. In: Bell, T. and Lightner, D.V. (Eds.), *A handbook of normal penaeid shrimp histology*. World Aquaculture Society, Baton Rogue, Lousiana, p. 74-83.

Clark, W. H. and Lynn, J. W. 1977. A Mg2++ dependent cortical reaction in the eggs of penaeid shrimp. *J. Expt. Zool*., 200: 177-181.

Clark, W. H., Lynn, J. W. Yudin, A. I. and Persyn, H. P. 1980. Morphology of the cortical reaction in the eggs of *Penaeus aztecus*. *Biol. Bull*., 158: 175-186.

CMFRI 2011. *Annual Report*, Central Marine Fisheries Research Institute, Cochin, 163 pp.

Dall, W., Hill, B. J. Rothlisberg, P. C. and Staples, D. J. 1990. Reproduction. In: Blaxter, J. H. S. and Southward, A. J. (Eds.), *Advances in Marine Biology*, *Vol. 27. The biology of Penaeidae*. Academic Press, London, p. 251-281.

Gurney, R. 1942. *Larvae of decapod*. The Ray Society, London, 129: 1-306.

Hudinaga, M. 1942. Reproduction, development and rearing of *Penaeus japonicus* Bate. *Jap. J. .Zool*., 10(2): 305-393.

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

Mohamed, K. H., Muthu, M. S. Pillai, N. N. and George, K. V. 1978. Larval development of *Metapenaeus monoceros* (Fabricius). *Bull. Cent. Mar. Fish. Res. Inst*., 28: 50-59.

Mohamed, K. S. and Diwan, A. D. 1994. Vitellogenesis in the Indian white prawn *Penaeus indicus*. *J. Aquacult. Trop*., 9: 157-172.

Nalini, C. 1976. Observations on the maturity and spawning of *Metapenaeus monoceros* Fabricius at Cochin. *Indian J. Fish*., 23(1&2): 23-30.

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

Peixoto, S., Cavalli, R. O. D'Incao, F., Milach, A. M. and Wasielesky, W. 2003. Ovarian maturation of wild *Farfantepenaeus paulensis* in relation to histological and visual changes. *Aquacult. Res*., 34: 1255-1260.

Quintero, M. E. S. and Garcia, A. 1998. Stages of gonadal development in the spotted pink shrimp *Penaeus brasiliensis*. *J. Crust. Biol*., 18: 680-685.

Rao, G. S. 1989. Studies on the reproductive biology of the brown prawn *Metapenaeus monoceros* (Fabricius : 1798) along the Kakinada coast. *Indian J. Fish*., 36(2): 107-123.

Rao, P. V. 1968. Maturation and spawning of the penaeid prawns of the south-west coast of India. *FAO Fish. Rep*., 57: 285-302.

Rao, P. V. 1974. Studies on the larval development of the commercially important penaeid prawns of India. *J. Mar. Biol. Ass. India*, 15(1): 95 – 124.

Sakaji, H., Tsuchiya, K. and Segawa, S. 2000. Oocyte development of *Metapenaeopsis dalei* (Penaeidae, Decapoda, Crustacea). *Invertebr. Repr. Dev*., 38(1): 7-12.

Shaikhmahmud, F. S. and Tembe, V. B. 1958. Study of prawns: the reproductive organs of *Parapenaeopsis stylifera* (M. Edw.). *J. Univ. Bom.*, 23: 99-110.

Tan-Fermin, J. D. and Pudadera, R. A. 1989. Ovarian maturation stages of the wild giant tiger prawn, *Penaeus monodon* Fabricius. *Aquaculture*, 77: 229-242.

Vasudevappa, C. 1992. *Growth and reproduction of the penaeid* prawn *Metapenaeus dobsoni* (Miers) *in brackishwater environment.* Ph. D Thesis, Cochin University of Science and Technology

Yano, I. 1988. Oocyte development in the kuruma prawn *Penaeus japonicus*. *Mar. Biol*., 99: 547-553.

Date of Receipt : 06.08.2011
Date of Acceptance : 03.02.2012