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Study of ovarian maturation of freshwater female prawn, *Macrobrachium rosenbergii* with Reference to histological and visual changes

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ABSTRACT

According to the general appearance and macroscopic observations of ovary, oocyte diameter ad histology of ovarian tissues four different stages of ovarian development were found viz., Immature, Maturing, Mature and Spent. The distinction of different stages of the oocyte depends upon their cytoplasmic content, changes in the nucleus and size of the oocytes which can be classified as, Oogonia which are small spherical cell with round nuclei which lack stainable yolk material. The average cell diameter was (24.02 µ), nuclear diameter (17.4 µ) and cell volume (7416.955 µ). Pre-vitellogenic oocytes **-** These oocytes increase in volume and acquire a large amount of basophilic cytoplasm with devoid of yolk material. Oocytes measures (38.16 µ), (24.48) and (29080.61 µ) in cell diameter, nuclear diameter and cell volume respectively. Vitellogenic oocytes **-** The oocytes enter a synthetic phase resulting in the formation of yolk. The appearance of follicle cells is noticed in these oocytes. The nucleus, nucleolus, ooplasm and follicular cells undergo marked changes in their cytology *viz.,* Vitellogenic-I oocytes which measured (60.24 µ), (11.28 µ) and (114401.9 µ) in cell diameter, nuclear diameter and cell volume respectively. Vitellogenic-II oocytes measures about (94.51 µ), (34.32 µ), (216482.13 µ) in cell diameter, nuclear diameter and cell volume respectively. Vitellogenic-III oocytes measures about (156.96 µ), (27.84 µ), (2023696.3 µ) in cell diameter, nuclear diameter and cell volume respectively. Whereas, degenerating oocytes are almost of the same size as tertiary vitellogenic oocytes and can be located by disintegration of the nucleus and appearance of vacuoles.

Key words: *Ovarian maturation, Histology, Ovarian colour, Macrobrachium rosenbergii.*

Introduction

In marine and freshwater invertebrates, the annual reproductive cycle may be assessed by various methods like observations of spawning, percentage of ovigerous female against time and presence of ripe gametes in gonad, occurrence of larvae in the plankton etc. Three standard methods for determination of the reproductive cycle in crustacean decapods were used; gonadosomatic index (GSI), oocyte diameter and proportion of ovigerous females. Analysis of the histological sections of each ovary determined the morphological characteristics, size and frequency of different oocyte types and the observation of visual features coupled with histological characteristic was found to represent a reliable procedure to evaluate the ovarian maturation (Peixoto *et al*., 2003; Lin Wang *et al*., 2020). The morphological aspects of the gonads and germ cells used by most workers to determine the stage of sexual maturity, especially in the more economically important crustacean species. Visual observation of

females, like shape and color of their ovaries, has been routinely used to assess the phase of maturation of penaeids. In captivity, exact identification of ovarian development is essential in order to avoid excessive handling stress and waste of fertile spawns into the tanks (Browdy, 1992). Histological analysis has been widely used to describe ovarian maturation stages of penaeids but there is lack of information showing its relationship with visual changes during ovarian development (Tan Fermin and Pudadera, 1989; Quinitio *et al*., 1993). The classification of gonadal stages vary from author to author. In penaied shrimp, Tan Fermin and Pudadera (1989) have recognized three to eight stages. First and the last stages are generally termed "immature and spent" (out spawn). The intermediate phases are variously named development, inactive, prematuration, near mature, maturing, active and mature. Peixoto *et al*., (2003) reported the description of the ovarian development of *F. paulensis* connecting histological sections and photographic features using a chromatic scale. The knowledge of reproductive biology, reproductive cycle, ovarian maturity stages and the timing of breeding, are required for both marine and freshwater commercially important crustaceans is used in the stock assessment, developing, and evaluating strategies for fisheries management. The present study describes the ovarian development stages of freshwater female prawn, *M. rosenbergii* through the combined observations of histological and visual characteristics.

Materials and Methods

Freshwater prawns, *M. rosenbergii* were collected monthly from the "Girna Dam", Tq. Malegaon Dist. Nasik, Maharashtra State, for period of two consecutive years. Collected animals were brought in the laboratory in the first week of every month on fixed date and time to avoid fluctuations if any. Other parameters like temperature, pH, salinity, and photoperiod etc. were not considered. From the collection, only healthy female prawns were selected and immediately sacrificed to record the histomorphological observations of ovary. For histological studies of ovaries were dissected out and fixed in bouin's fluid. After 24hrs fixation, the tissues were kept under running tap water with the help of muslin cloth for removal of bouin's fluid. Then tissues were passed through different alcoholic grades for dehydration, followed by cold impregnation, hot impregnation and embedded in the paraffin wax (m.p. 58-60 °C). The tissues embedded in the paraffin wax were trimmed and sectioned at 8µ and 6µ for gonads. Sections of the ovaries were stained with Delafield's haematoxylin-eosin stain. Aspects such as gonad colouration and stages of maturity were registered by macroscopic observation of the ovary. The stained sections of ovary were observed under microscope at different magnifications for histological studies. Measurements of oocytes were recorded with stage occularmicrometer.

Results and Discussion

In crustaceans, the ovarian maturation assessed by various methods like observations of spawning, the percentage of ovigerous female against time and presence of ripe gametes in gonad, occurrence of larvae in the plankton etc. Three standard methods for determination of the reproductive cycle in crustacean decapods were used; (1) gonadosomatic index (GSI), (2) oocyte diameter and (3) proportion of ovigerous females. Based on the visual changes in colour, position, and microscopical examination of ovary four maturity stages were recognized, immature, maturing, mature and spent. Colour change in the ovary during maturation is well known for crustaceans; mainly for shrimps (Dall *et al*., 1990). Sethuramalingum *et al*., (1982) identified on the basis of colour of ovary and suggested three stages of ovarian development (immature, mature and spent) in *Portunus sinipes* and in *Thalamitachaptali*. In present study the ovarian development were also grouped in four stages viz., Immature – in this slight development of the ovaries with slender anterior and posterior lobes were restricted to the body cavity. Ovaries were white, translucent, and difficult to distinguish from surrounding muscles, maturing – in this stage ovaries were grown in volume, showing light yellow in colour and the organ appears distended, firm to touch, indicating increase of germ cells suggesting early stage of vitellogenesis. Mature – in this stage gonad was fully developed occupying all available space in the body cavity. It became more sinuos and extended up to the second abdominal segment. The colour observed was orange or reddish. Spent - after ovulation oocytes and other cells resorbed by the ovaries, the gonads become flaccide with pigmented areas and empty spaces internally. At this stage of ovary, colour and size was more similar like in the immature stage, and the

gonad of spwaned prawn was newer entirely restored to the condition of original maturity. In the present study histological studies showed that the ovarian wall in *M. rosenbergii* is covered with two membranes, an outer most epithelial layer and inner layer of germinative epithelium (Fig. 2). The ovarian wall is continuous with ovarian stroma and is thicker during post spawning period but becomes thin at maturing and mature stages of the ovary. A germinal zone is restricted only to medioventral portions of the ovarian lobes. This portion is distinguished by the presence of compact mass of oogonia and small oocytes. During primary stages of maturation, the germinal zone consists of oogonia and pre-vitellogenic oocytes whereas; the developing oocytes are displaced towards the marginal region of the ovary (Fig. 3). A few primary or residual oogonia, which occur throughout the year in the germinal zone, divide mitotically shortly after ovulation and give rise to additional oogonia for further growth of ovary. Oogonia are a small spherical cell with round nuclei surrounded by a thin rim of poorly basophilic oocortex, which lacked stainable yolk material (Fig. 2). Oogonia develop into previtellogenic oocytes but a few remain undifferentiated (residual oogonia) until the ovulation. The average cell diameter was (24.02) and nuclear diameter (17.4) with (7416.955) cell volume (Table 1, Fig. 2). In pre-vitellogenic oocytes the oocytes increase in volume and acquire a large amount of basophilic cytoplasm with devoid of yolk material. The nucleus appears vesicular containing peripherically arranged chromatin clumps and 2-3 nucleoli, which appear solid and stained black with heamatoxylin. There is no yolk formation and oocytes measures (38.16), (24.48) and (29080.61) in cell diameter, nuclear diameter and cell volume respectively. Cytoplasmic volume (21403.23) increased compared with oogonia (4660.0026) (Table 1, Fig. 3). In

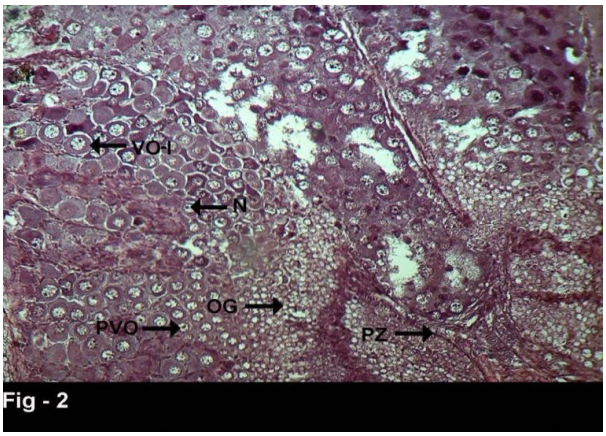

Fig. 2. Photomicrograph of the ovary showing immature stage of *M. rosenbergii*. Haematoxylin- eosin X 100. VO-I : Vitellogenic oocyte-I PVO: Previtellogenic oocyte N : Nucleus OG : Oogonia PZ : Proliferating Zone

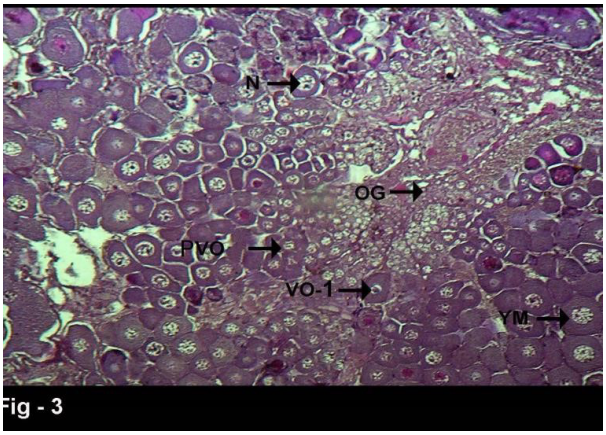

Fig. 3. Photomicrograph of the ovary showing maturing stage of *M. rosenbergii.* Haematoxylin-eosin X 100. N: Nucleus, OG: Oogonia, PVO: Previtellogenic oocyte VO-I: Vitellogenic oocyte-I YM: Yolk material

Table 1. Changes in the size of oocytes during oogenesis in *M. rosenbergii*

Oocyte stage	Cell diameter (μ)	Nuclear diameter (μ)	Cell volume $(\mu)^3$	Nuclear volume $(\mu)^3$	Cytoplasmic volume $(\mu)^3$	Nucleo-cytoplasmic ratio N/C
Oogonia	24.02	17.4	7416.955	2756.93	4660.0026	0.591616
Previtellogenic	38.16	24.48	29080.61	7677.37	21403.23	0.3587014
Vitellogenic-I	60.24	11.28	114401.9	751.11	113650.79	0.0066
Vitellogenic-II	94.51	34.32	216482.13	21155.34	195326.19	0.1083
Vitellogenic- III	156.96	27.84	2023696.3	11292.39	2012404.01	0.00559

Cell Volume = $4/3\pi r^3$, Nuclear Volume = $4/3\pi r^3$, Cytoplasmic Volume = Cell Volume – Nuclear Volume

vitellogenic oocytes the oocytes enter a synthetic phase resulting in the formation of yolk. The appearance of follicle cells was noticed in these oocytes. The nucleus, nucleolus, ooplasm and follicular cells undergo marked changes in their cytology as described as; Vitellogenic-I oocytes - Increase in amount of ooplasm and the appearance of small yolk droplets in peripheral ooplasm takes place. Yolk droplets stain purple to black with haematoxylin. Nucleus is solid central in position. Thin layer follicular cells, which are small, found around the oocytes. Increase in oocyte diameter is accompanied by increase in amount of yolk droplets, which progressively extends towards the perinuclear region (Table 1, Fig. 3). These oocytes measured (60.24), (11.28), (114401.9), in cell diameter, nuclear diameter and cell volume respectively. Nuclear volume and cytoplasmic volume increased compared with oogonia and pre-vitellogenic oocytes. Vitellogenic-II oocytes - These oocytes are characterized by the synchronous growth to large size and the unstainable vacuoles increase in number. Vacuoles merged with each other and finally form large unstainable yolk vesicles, initially in marginal ooplasm. This is followed by the arrival of small eosinophilic yolk granules in the extra vesicular ooplasm. The follicular cells are same as in the primary vitellogenic oocytes. These oocytes measure about (94.51), (34.32), (216482.13) in cell diameter, nuclear diameter and cell volume respectively (Table 1, Fig. 3). Vitellogenic-III oocytes - With further growth of oocytes, the amount of yolk globules increases. Yolk droplets are absent because of their alteration into yolk vesicles. Ooplasm thus becomes entirely acidophilic. Yolk globules occupy most of ooplasm including the perinuclear region. Follicle cells appears in round to oval with spindle shapes nuclei. These oocytes measure about (156.96), (27.84), (2023696.3) in cell diameter, nuclear diameter and cell volume respectively. Highest nuclear volume and cytoplasmic volume observed for vitellogenic-III oocytes with rest of stages of oocytes (Table 1, Fig. 4). Degenerating oocytes - In *M. rosenbergii,* the process of degeneration (oosorption or resorbtion) may be simultaneous with oocyte growth. The gathering and competition among the oocytes rendered some of them in a degenerating stage. During favorable conditions, the mature oocytes undergo ovulation. Degenerating oocytes are almost of the same size as tertiary vitellogenic oocytes and can be located by disintegration of the nucleus and appearance of vacuoles. The degenerating ova are surrounded by nutritive phagocytes and some of them enter in to the ova (Fig. 5). Ovarian maturation of several species has been described according to the external appearance of the ovary (King, 1948; Crocos and Kerr, 1983; Pexioto *et al.*, 2003). Ovarian size and colours were related to histological changes in *Callinectes sapidus* and *Geryon quinquedens* (Sigana, 2002). Pillay and Ono (1978) grouped the developing ovaries of grapsid crabs based on colour and size of the ovaries. The results

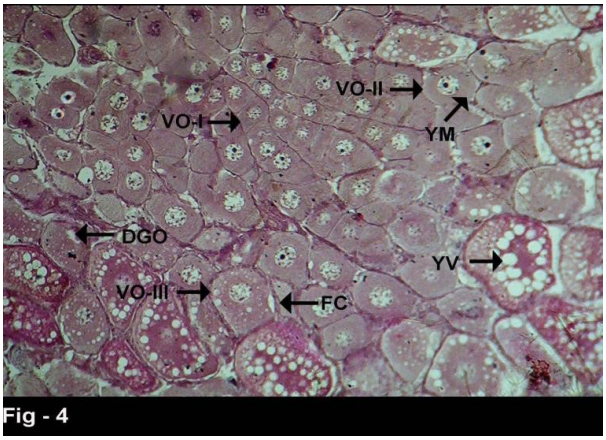

Fig. 4. Photomicrograph of the ovary showing mature stage of *M. rosenbergii*. Haematoxylin-eosin × 100.
DGO: Degenerating oocyte VO-I:Vitellogenic oocyte-I, VO-I I:Vitellogenic oocyte-II VO-III: Vitellogenic oocyte-III
FC: Follicular cell YM: Yolk material, YV: Yolk vesicle

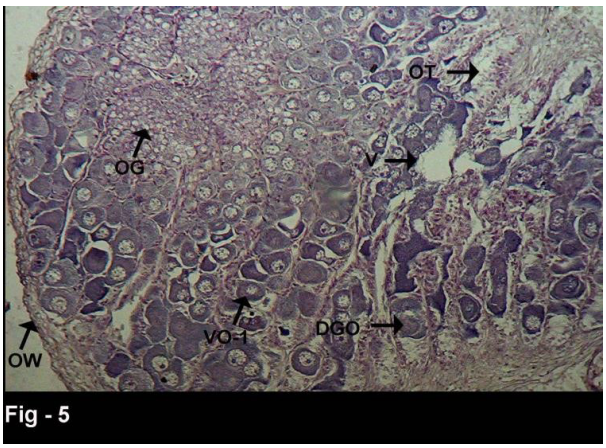

Fig. 5. Photomicrograph showing spent stage of the ovary. Haematoxylin-eosin × 100.
DGO : Degenerating oocyte VO-I : Vitellogenic oocyte-I OT : Ovarian tissue V : Vesicle OG: Oogonia ,OW : Ovarian wall

from histological study of ovarian tissue showed that changes in colour and shape of the ovaries match very well with development of oocytes. The classification of the ovarian maturation stages using the colour scale and histological analysis described here may be used practically for the management of *M. rosenbergii* female maturation in captivity.

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