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# HISTOLOGICAL AND STATISTICAL STUDIES ON THE OVARIAN CYCLE OF THE CARAMOTE PRAWN *MELICERTUS KERATHURUS* (FORSKÅL, 1775) (CRUSTACEA – DECAPODA)

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## ABSTRACT

The two ovaries of the caramote prawn *Melicertus kerathurus* lie above the hepatopancreas and below the pericardial sinus and the heart. Both ovaries were reduced in general size with white compact and sickle-shaped contents during January- March. Their contents attain gradually a pale green colour in April - June. This stage is an early maturing in which the ovary increased in size and the anterior and middle lobes are developing. When the ovary is immature the surface is relatively smooth and homogenous, but as the organ grows the surface acquires an increasingly irregular ppearance. They contain oocytes and small spherical ova with light-stained cytoplasm free from granules and a conspicuous nucleus. During July -September, mature stage in which the ovary is in a maximal size and filled with vitellogeneic and ripe oocytes. They were dark green. During October- December, the gonad reverted gradually to the immature condition i. e., the ovary undergoes a spent-recovering and was with varying degrees of yellow colour. For each season three ovaries were studied carefully through serial histological preparations. The length and width for each histological section was measured using slide micrometer (4 cm & 3 cm) respectively. The different stages of oocyte developmental stages were counted and analyzed in each season through One Way Analysis of Variance (ANOVA) with $p<0.05$ and Tukey's Multiple Comparison Test. This calibrations clarified that the oogonia predominate the ovary during winter season, the previtellogenic oocytes predominate the ovary during spring season, the vitellogenic oocytes predominate the ovary during summer season. The majority of autolyzed oocyte were observed during winter season.

**KEYWORDS:-** ovaries; *Melicertus kerathurus*; spring; summer; autumn; winter; One Way ANOVA; Tukey's Multiple Comparison Test

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## INTRODUCTION

Studies on the breeding habits of some penaeid prawns have been investigated (Cau, et al. 2002; Aktas, et al.. 2003 ; Dumont and D'Incao, 2004 ; Coman, et al.. 2006 and Marsden and Elizabeth,. 2008). Most of these investigations were restricted to determine the time of spawning and conclusions were based entirely on the data on incidence of mature prawns in the catches obtained in different months. No detailed study on the process of maturation, spawning periodicities and the factors influencing successful spawning has been so far attempted. The present investigation pertains to the study of the different aspects of ovary maturation and together with statistical analysis of the developmental stages of the oocyte in the four seasons of the year. The anatomy and histology of the female reproductive system in the Panaeus stylifera has been described (Shaikmahmud and Tembe,1958). The general shape and appearance of the ovaries are similar in many species studied, and also agree with the descriptions of the ovaries of Penaeus setiferus (King, 1948) and P. duorarum (Minagawa, et al. 2000; Conides, et al. 2008 ). The mature ovaries are paired organs, situated dorsally and extend from the base of the rostrum to the last abdominal segment. They are bilaterally symmetrical and partly fused. Each half of the ovary consists of three lobes, of which the slender anterior lobe occupies the anterior region. The middle lobe has 6 or 7 finger-like lateral lobules which entirely fill the area between the anterior region and the posterior border of the carapace. The posterior lobes extend the length of the abdomen. The two halves of the ovary are united by two commissures, one at the base of the anterior lobes and the other at the tip of the posterior lobes in the 6th abdominal segment. Histologically, the ovary is composed of a number of radially arranged rows of oocytes in various stages of development. The immature ova lie towards the centre, while the mature ones towards the periphery (Yano, et al. 1996; Avarre et al. 2003 and Okumura, et al.. (2004). . Each developing ovum is being surrounded by a number of small nutritive cells. A mature oocyte has a rounded appearance with a large nucleus and a cytoplasm full of yolk-granules. Each ovary is enclosed within in a membranous capsule. The difference in morphology suggests differences in chemical compositions. Proteins and glycoproteins have been identified in these layers in S. ingentis (Xue, et al. 1987 and Sobha, et al. (2007). However, each layer may serve a different function. In S. ingentis, the vitelline envelope serves as the primary site for sperm attachment, and the cortical surface of the egg further stimulates the sperm to undergo the second phase of the acrosome reaction. Vitellogenesis is the process of the biosynthesis of proteins and their transport and storage in the ovary (Laubier – Bonichon & Laubier, 1976 ; Charniaux-Cotton, 1985; Tseng, et al. 2001and Coman, et al. 2006). The yolk proteins occur as large molecular weight proteins (300–500 kDa) that also have carotenoid, sugar and lipid moieties attached during biosynthesis. It is these characters which distinguish yolk proteins from the other proteins circulating in the hemolymph. The primary role of the yolk proteins is to provide nutrition to the developing crustacean embryos. In many cases, crustaceans rely on the yolk proteins for several days after release from the females for their nutrition (Castille & Lawrence, 1991; Aktas and Kumlu, 1999 and Longyant et al. 2003).

Concerning the spawning season: M. dobsoni showed all the maturity stages of the ovary in all months, except in September, when immature and early maturing prawns only are encountered. The presence of late maturing and mature stages in almost all months suggests that M. dobsoni breeds throughout the year. However, peaks of spawning activity are evident in June, November to December, and April. Menon (1952; 1955) recorded this species as breeding for at least 6 months of the year, both on the Malabar coast and in the Cochin area, while the same species breeds throughout the year. It appears to be usual for the species to show two main peaks of breeding activity in the year, but the timing of these peaks may vary from place to place and from year to year (George, et al. 1955; Primavera, 1985 ; Dall, et al.. 1990 and Lumare. &Scordella, 2001). The seasonal distribution of M. affinis in different stages of maturity shows that non-spawning prawns dominate the catches from April to September, and that the species breeds from October to March with a peak in December. The species P. indicus has a prolonged breeding period in Cochin waters, with the greatest breeding activity between October and April. Panikkar and Menon (1955) recorded two breeding periods, in October to November and in May to June. Galil (2007) and Ohtomi, et al. (2003) observed that the species breeds throughout the season with spawning peaks in October to November and May to June. Subrahmanyam (1963) reports that in Madras waters, the highest breeding activity of the species is seen during March and May to September. The seasonal distribution of mature P. stylifera shows that this species also breeds throughout the year in the Cochin area, with peaks during December and June to August. The spawning pattern of Penaeus kerathurus, in the Persian Gulf, as measured by the variation in percentage of mature females and by post larval occurrence, shows two peaks per year, one in spring and the other in autumn. These peaks vary in relative importance from year to year, although the spring peak is the more important one. Recruitment in autumn apparently stems from the spring spawning, while the autumn spawning produces recruitment the following spring. Age at spawning is thus 12 months for the majority of the shrimps. Spawning occurs near shore on muddy sand bottoms (Teshima and Kanazawa, 1983 and Lumare, 1998.). The maturity stages of female shrimps were visually determined and recorded. The maturity stages have been classified according to simple macroscopic criteria such as ovary appearance and colour (King, 1948), which have already been validated through histological studies.

## MATERIALS AND METHODS

### Animals

Specimens of the caramote prawn Melicertus kerathurus (Forskål, 1775) were sampled regularly twice every month at depth 1- 2 meters during the four seasons in the year (2012 - 2013) along the northern estruarine harbour of the Arabian Gulf - Saudi Arabia. Identification of this species under investigation was carried out according to Adiyodi & Subramouian (1983) and Kosmas and Maria (2013). Each collection was transported in well-aerated sea water to the laboratory. Few amount of magnesium sulphate was added in the aquaria as a relaxing agent. Individuals of each collection were used to prepare sections of ovaries for histological studies.

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## MACROSCOPIC OBSERVATIONS

All specimens were fixed in 10% neutral formalin, ovaries were isolated and photomacrographed using two different magnifications. The size (length and width) of ovaries were measured and tabulated. Considerable number of shrimps from each collection have been dissected alive in the laboratory, ovaries have been isolated and classified into two groups. The ovary showed different colours according to maturity. These colour differences were investigated along the year.

## MICROSCOPIC OBSERVATIONS

The same specimens of the macroscopic observation were washed in distilled water for 24 hours. These specimens were dehydration through ascending series of ethyl alcohol, followed by another dehydration series of tertiary butyl alcohol. then tertiary butanol and paraffin oil (1:1) , absolute paraffin oil. All preparations then washed (carefully ) in tissuemat or paraplast with melting point 54-58 Ċ and blocked in fresh paraplast. Sections of 5-8 µ were obtained. Ehrlich haematoxylin and eosin were used as a basic routine stain. A number of triple stains were tried to enable differentiation of the different components inside the ovary (Pantin,1948 ; Pearse, 1968 &1980 ) (Table 1). Statistically the number of the oocyte developmental stages, oogonia, previtellogenic and vitellogenic, postvitellogenic oocytes and autolysed ones were counted in three ovaries for each serial histological preparation in all the seasons of the year and commented.

For examination of the different preparations investigated, normal macroscope with 10 , 40 and 100 magnification capacities was used. Ortholux Leintz Wetzler Stereoscope microscope with light house 250 with external light source of Schott KL 1500 was used. Olympus IMT-2 phace Contrast microscope following the method of Nomarski Differential Interferrence contrast attachment was used. Lenses and light source were normal.The Camera used was full automatic microscope camera for research purposes.

**(Table 1): Morphological nature of the cytoplasm and the nucleus of the oocyte of Marsopenaeus japonicus at its different stages of development.**

| Stages of oocyte develop-ment / Stain Referenes | Oogonia | | Previtelloge (young) ooc | | Vitellogenic oocyte | | | | Post-vitellogenic oocyte | |
|---|---|---|---|---|---|---|---|---|---|---|
| | Cyto-plasm | Nucleus | Cyto-plasm | Nucleus | Step 1 Small granule | Step 2 Large granule | Step 3 patche | Nucleus | Yolk materia (homog enous) | Nucleus |
| Ehrlich haematoxylin & eosin Pearse 1968 | red | blue | blue | deep blue | Deep da blue | opaque blue | light blu | deep bl | blue | deep blue |
| Heidenhain's iron haematoxylin Pearse 1968 | red | blue | red | deep blue | deep da blue | opaque blue | light blu | deep blue | blue | deep blue |
| Mallory triple stain Pearse 1968 | red | deep blue | dark r | deep blue | deep da blue | opaque blue | light blu | deep blue | red | deep blue |
| Masson trichrome stain Pearse 1968 | red | deep blue | dark r | deep blue | deep da blue | opaque blue | light blu | deep blue | red | deep blue |
| Weigert's haematoxylin & Van Gieson stain Pearse 1968 | red | deep blue | dark r | deep blue | deep da blue | opaque blue | light blu | deep blue | red | deep blue |

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## RESULTS

Examination of fresh specimens of caramote prawn Melicertus kerathurus (Forskål, 1775) along the year round revealed that the ovarian morphology had affected by the seasonal variations not only in size but also in their histological structure. Consequently, special emphasis will be given in the following description to the changes that took place inside the the ovary.

## MACROSCOPIC OBSERVATION:

The two ovaries are situated above the hepatopancreas and below the pericardial sinus and heart. Both the anterior and posterior ends of the ovaries tought each other and leaving a gap in the middle for the passage of the cardio-pyloric strand. The ovaries extend anteriorly up to the renal sac and posteriorly up to the anterior margin of the first abdominal segment. Both ovaries were with white compact and sickle-shaped contents seen during January- March. They were with varying degrees of yellow colour during the period of October till December. Ovaries contents attain gradually a pale green colour with the beginning of April till June. They were dark green in colour and extend about the whole length of the animal during July till September. (Figs. 1-5). The mature ovaries are paired organs, situated dorsally, extending from the base of the rostrum to the last abdominal segment. They are bilaterally symmetrical and partly fused. Each half of the ovary consists of three lobes, of which the slender anterior lobe occupies the anterior region. The middle lobe has 6 or 7 finger-like lateral lobules which entirely fill the area between the anterior region and the posterior border of the carapace. The posterior lobes extend the length of the abdomen. The two halves of the ovary are united by two commissures, one at the base of the anterior lobes and the other at the tip of the posterior lobes in the 6th abdominal segment.

### (Table 2): Size of the ovary in the four seasons of the year.

| Time of the year | Specimen number | Length in µm (25 X) | Width in µm (25 X) |
|---|---|---|---|
| Winter season | 1 | 13000 | 2000 |
| 2012 | 2 | 12100 | 2000 |
| | 3 | 11000 | 3100 |
| Spring season | 1 | 15000 | 4550 |
| 2013 | 2 | 16000 | 5000 |
| | 3 | 16300 | 4000 |
| Summer season | 1 | 17000 | 6000 |
| 2013 | 2 | 18500 | 5700 |
| | 3 | 19000 | 5700 |
| Autumn season | 1 | 1400 | 4500 |
| 2013 | 2 | 13500 | 4000 |
| | 3 | 14500 | 4800 |

This calibration concluded that the ovary attains its maximal size during Summer season. During Autumn season the ovary gets smaller in size gradually and the smallest size was observed during Winter season. In spring season, the ovary gets larger gradually in its length and width.

## MICROSCOPIC OBSERVATION:

The external lining of the ovary appears verrucose and entirely covered by branched and overlapping fibres. When the ovary is immature the surface is relatively smooth and homogenous, but as the organ grows the surface acquires an increasingly irregular and nubbly appearance (Figs. 6-7). Longitudinal and cross-sections of the ovary show its lining which composed of several layers of filamentous or fibrous material disposed in lamellae. Some of the inner layers invaginate toward the centre of the ovary forming groups of spherical bodies resembling cells of different sizes. The grainy material observed among the fibres possibly corresponds to the hemolymph circulating the ovary internally (Fig. 8). A number of larger spherical bodies between the lamellae were observed. The bodies formed by the lamellae and their internal components, termed nodes or cysts, were identified during dissection of premature ovaries. The nodes tend to be organized internally with the smaller cells, which may be either follicles or early stage germ cells, near the centre (Fig. 9). However, because of their similar morphology and size, these two cell types could not be clearly distinguished. The large previtellogenic oocytes and vitellogenic oocytes of the subsequent stages are easier to distinguish due to differences in

diameter, cytoplasmic aspect and nucleus/cytoplasm ratio. Previtellogenic oocytes are smaller than mature oocytes but larger than oogonia near which they are located. The nucleus and nuclear envelope are visible and the cytoplasm appears slightly granular (Figs. 9-12). In mature oocytes, the nucleus/cytoplasm ratio is reduced; the nucleus is at this point quite evident and is filled with spherical vesicles containing yolk granules (Figs. 13-15). These cells, which come closest to the external lining of the ovary, are separated by increasingly conspicuous envelopes. The external lining of intact cells appears membranous and branched, and may originate from extensions of the fibrous layer or follicular cells (the technique employed does not allow to make the distinction). The periphery of cross-sectioned mature oocytes displays a rather homogeneous and continuous band, possibly corresponding to the plasma membrane. In addition, extensions resembling microvilli may be seen projecting outward from the band (Fig. 15) and occupying an empty space corresponding to the chorion. Mature oocytes are covered by another protective envelope formed probably by surrounding follicular cells. Premature and mature gonads are mostly filled with previtellogenic and vitellogenic oocytes and are bound by a slender external lining. Histologically, the ovary consists of a number of radially arranged rows of ova in various stages of development (Fig. 8). The immature ova lie towards the centre, while the mature ones towards the periphery. Each developing ovum is being surrounded by a number of small nutritive cells. A mature ovum has a rounded appearance with a large nucleus and a cytoplasm filled of yolk granules. Each ovary is enclosed within in a membranous capsule.

Five maturation stages of the oocyte were recognized: during January till March, immature stage in which the ovaries are thin, translucent, unpigmented and confined to the abdomen. They contain oocytes and small spherical ova with light-stained cytoplasm free from granules and a conspicuous nucleus (Fig. 15). During April to June, early maturing stage in which the ovary increases in size and the anterior and middle lobes are developing. The dorsal surface is light yellow to yellowish green. Opaque yolk granules are formed in the cytoplasm and partly obscure the nuclei. The developing ova are clearly larger than the immature stock. Late maturing stage in which the ovary is light green and is visible through opacity of its ova, due to the accumulation of yolk (Fig. 16). During July to September, mature stage in which the ovary is dark green and clearly visible through the exoskeleton. The ova are larger than in the preceding stage and the peripheral region becomes transparent. This stage is believed to be the last stage of maturity before actual spawning as the largest ova are encountered only in this stage (Fig. 17-18). During October to December, It is probable that after the extrusion of eggs, the gonad revert gradually to the immature condition in other words the ovary undergoes a spent-recovering.

The histological examination of the ovary around the year indicates that the female caramote prawn has only one reproductive cycle in the year which begin with January and continues till December. The ovaries produce oocytes which undergo an important vital activity named deutoplasmogenesis (vitellogenesis) to change these oocytes into comparatively large ones condensed with yolk. Oocyte maturation undergoes definite stages which are:

## Stage 1: During January till March

The epithelial wall of the saccular ovary divides the interior into lobules. The ovarian lobules are crowded with oogonia which appear as if they are budded from the germinal epithelium. They have a linear arrangement on the epithelial layer (Fig. 8). Few number of young oocytes (previtellogenic and endogenous vitellogenic) are also present. The cytoplasm of these stages, oogonia and young oocytes, is somewhat homogeneous and with comparatively large nucleus with a prominent nucleolus. The number of young oocytes increases gradually at the expense of oogonia. These oogonia could be differentiated from young oocytes by their variable sizes and the ratio nucleus/ cytoplasm. Both stages have the same histological appearance and the same affinity to the different histological stains applied. Great number of autolyzed large oocytes appeared in the preparations of ovaries of March (Fig. 9). This indicates that this period of time can be interpreted as the interbreeding period. However, the number of oogonia increases gradually during this period (February-March). The previtellogenic oocytes are present in the centre and the endogenous vitellogenic oocytes are at the periphery. The previtellogenic oocytes are 30–70 µm in diameter. The endogenous vitellogenic oocytes are 70–290 µm in diameter, and larger oocytes (130–290 µm in diameter) are enveloped by follicle cells. There are unstained vesicles in the cytoplasm of the endogenous vitellogenic oocytes, which are considered to be lipid droplets. The ratio of the nucleus to the cytoplasm in case of oogonia is about 2:1. Affinity to stain: with Ehrlich haematoxylin & eosin and the different stains available as Heidenhain's iron haematoxylin, the cytoplasm accepts a red colour while the nucleus accepts blue colour ( see table 1). previtellogenic oocytes are typically rounded or oval in outline. The cytoplasm is eosinoplelic and appears homogeneous in nature. Its outline is not differentiated from the internal bulk of the cytoplasm by any of the stains used, i.e., no membrane can be defined. The nucleus is large and central in position. It is acidophilic and the nucleoli are of various sizes. The nucleolus in the previtellogenic oocyte is a solid rounded body. It is highly basophilic. With the growth of the oocyte, this nucleolus increases in size, develops vacuoles inside it, and becomes acidophilic. Later on during this period, these previtellogeni oocytes become no longer lineary arranged along the germinal epithelium. Its Size range from 550 to 630 µm. The ratio of the nucleus to the cytoplasm 1:2. The cytoplasm increases in size gradually, therefore it is possible to differentiate the oogonia from the young oocyte through the ratio of the nucleus to the cytoplasm.

## Stage 2: During April till June

Oocytes with moderate size are present in the ovary. Autolyzed oocytes and their debris disappear. Very few number of oogonia can be seen or may be absent at all. The majority of the oocytes differ from the previtellogenic oocytes described in January till March. They gradually undergo two synchronously vital activities. They become follicular and grow gradually in size (Figs. 12-13). Opaque granules are deposited in the form of a ring around its concentric nucleus. The cytoplasm is almost completely opaque so that the nucleus becomes inconspicuous under a stereomicroscope. Hence, the translucent stage oocyte can be easily differentiated from the dark opaque stage oocyte . Histologically, the presence of a few rows of peripheral vacuoles seems to be the most predominant characteristics of vitellogenic oocyte. In addition, more than one

nucleolus, can be observed in each cell. The changes undergone by these oocytes can be differentiated into three stages: The oocyte in the first stage becomes follicular. The opacity of cytoplasm increases in the step I oocyte. Histologically, the number of vacuoles gradually increases, and they become dispersed towards the central area. Small granules of the same nature of the ring are scattered randomly in the cytoplasm. Cortical rods are formed on the periphery of the cytoplasm (Figs. 18-20). The oocyte of second stage is larger in size. Large granules appear in the cytoplasm and increase in number. These granules gain dark colour with the different stains. With Best's Carmine reaction they gain a red colour due to the presence of glycogen (Fig. 21). Vitellogenesis proceeds in step II. The vitelline envelope also becomes conspicuous under the follicle cells. Pigmented granules first appear in this stage and are located at the periphery of the oocyte. The size range of these oocytes is from 650 to 750 µm. The ratio of the nucleus to the cytoplasm 1:2. In the early developing stage of the ovary, the exogenous vitellogenic oocytes are present at the periphery, and the endogenous vitellogenic oocytes are present in the centre. At the beginning of this stage, small eosin-positive yolk globules are observed in the cytoplasm of the oocytes (300-340 µm in diameter, and at the end of this stage, yolk globules fill the cytoplasm (Fig. 22). The exogenous vitellogenic oocytes were positively stained by Mallory`s Trichrome but the previtellogenic oocytes, endogenous vitellogenic oocytes, oogonia, and follicle cells were not stained (Fig. 23). Oogonia, the previtellogenic oocytes, and the endogenous vitellogenic oocytes are present in the centre of the ovaries. The diameter of the oocytes reaches 875–950 µm at the end of this stage. In electron microscopy observations, yolk globules become larger (2–15 µm), and fill the cytoplasm. Among the accumulated yolk globules, rough endoplasmic reticulum is present.

## During July till September

The oocyte in this third stage had maximal size 750 – 800 µm. The peripheral vacuoles are decreased in number while the yolk accumulation increases. The thickness of the vitelline envelope gradually increases in stage 3 oocytes and then reaches its maximal thickness. This indicates that the chorion is formed around the oocyte during the process of vacuolization of the inner follicle epithelium (Figs. 24-25). In addition, yolk bodies also stained positive with this dye and appeared first in the stage 3 oocyte as bright brown clusters on the cell periphery (Figs. 25-26). The Post-vitellogenic oocyte is characterized by a homogenous cytoplasm and the yolk material accepted a clear blue colour with the different triple stains applied (Fig. 27).

Therefore three histological views can be identified: Individuality of cytoplasmic granules is well-differentiated. The follicle cells are well-developed. Individuality of granules disappear. The follicle cells loosen from the outer surface of the oocyte and this surface begins to accept definite colour with different stains. With Mallory triple stain, it gains a dark blue colour. i.e., deposition of chorion surrounding the oocyte. Individuality of granules disappear completely and the components of the cytoplasm become homogenous in appearance. All the test cells or most of them fade in the cytoplasm and intermingle with the components of the oocyte. Finally they degenerate completely. The inner follicle cells degenerate gradually and debris of these follicle cells appear around the oocyte i.e., the fully formed oocyte in the ovary is free from follicle cells. In the ripe stage of the ovary, the maturing oocytes are present at the periphery. The maturing oocytes (875–975 µm) are still enveloped by the follicle cells, and vitellin membrane develops on the oocyte surface. The vitellin membrane is 4 µm thick, and the oocytes project microvilli toward the follicle cells. In the spawned stage of the ovary, the ovulation traces are present at the periphery of the ovary, and oogonia, the previtellogenic oocytes, and the endogenous vitellogenic oocytes are present in the centre. The ovary often contains regressing mature oocytes. (Table 4) shows the variable affinities of the oocyte in its different stages of development: Figure 28 shows the process of autolysis of the postvitellogenic oocyte at any time.

## STATISTICAL ANALYSIS

For each season three ovaries were studied carefully through serial histological preparations. The length and width for each histological section was measured using slide micrometer (4 cm & 3 cm) respectively.The different stages of oocyte development were counted and tabulated.

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**(Table 3 ) Total number of oocyte developmental stages taken from three ovaries per season.**

| Time of the year / stage | oogonia | | | Previllogenic oocyte | | | Vitellogenic. oocyte | | | Post-vitellogenic. oocyte | | | Autolyzed oocyte | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 1* | 2* | 3* | 1* | 2* | 3* | 1* | 2* | 3* | 1* | 2* | 3* | 1* | 2* | 3* |
| Winter season 2012 | 182 | 174 | 193 | 41 | 67 | 58 | 6 | 4 | 2 | 64 | 78 | 45 | 75 | 36 | 29 |
| Spring season 2013 | 96 | 84 | 113 | 175 | 183 | 97 | 79 | 93 | 68 | 16 | 23 | 36 | 2 | 15 | 24 |
| Summer season 2013 | 53 | 37 | 46 | 84 | 105 | 91 | 173 | 159 | 147 | 4 | 15 | 9 | 1 | 0 | 4 |
| Autumn season 2013 | 21 | 17 | 32 | 47 | 39 | 26 | 92 | 87 | 76 | 84 | 79 | 93 | 12 | 32 | 28 |

1* first ovary

2* second ovary

3* third ovary

**(Table 4) Mean of the total number of oocyte developmental stages taken from three ovaries per season.**

| Time of the year / stage | oogonia | Previllogenic oocyte | Vitellogenic. oocyte | Post-vitellogenic. oocyte | Autolyzed oocyte |
|---|---|---|---|---|---|
| Winter season 2012 | 549 | 166 | 12 | 187 | 140 |
| Spring season 2013 | 293 | 455 | 240 | 75 | 41 |
| Summer season 2013 | 136 | 280 | 479 | 28 | 5 |
| Autumn season 2013 | 70 | 112 | 255 | 256 | 72 |

From these calibrations, it can be concluded that the oogonia predominate the ovary during winter season, the previtellogenic oocytes predominate the ovary during spring season, the vitellogenic oocytes predominate the ovary during summer season. The majority of autolyzed oocyte were observed during winter season (Tables 3-10 & Histograms 1-5 & 7).

Histogram (6) clarifies that the ovary is vital and active all the year round but it showed a definite histological appearace in each season.

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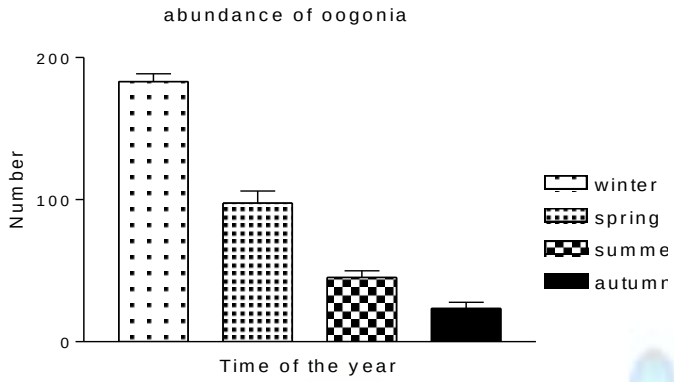
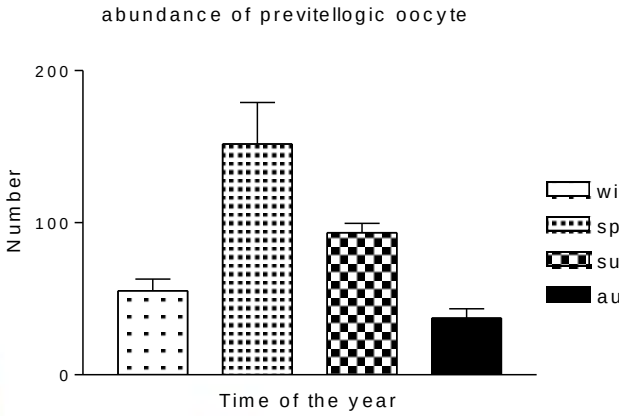
## Histograms showing the abundance of oocyte developmental stages in the year

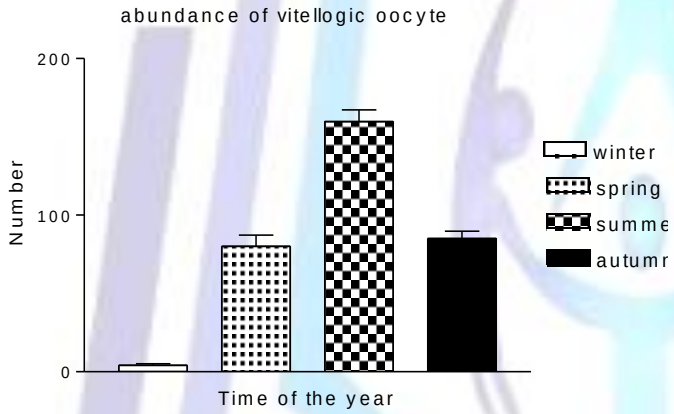
Histogram 1



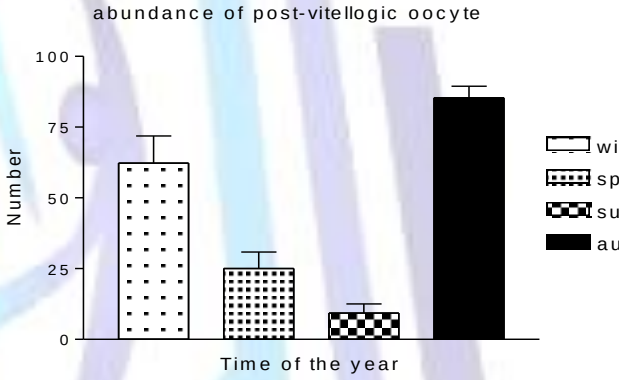

Histogram 2



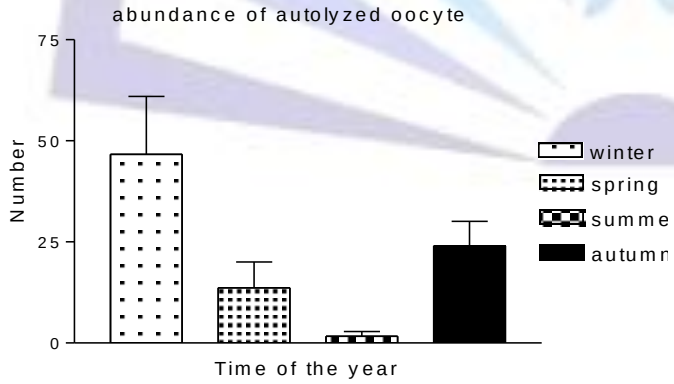

Histogram 3



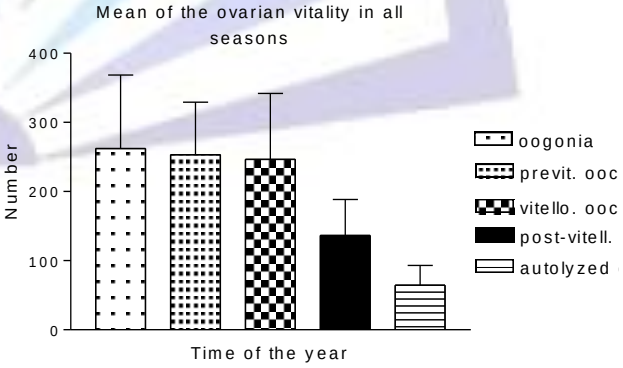

Histogram 4




Histogram 5




Histogram 6



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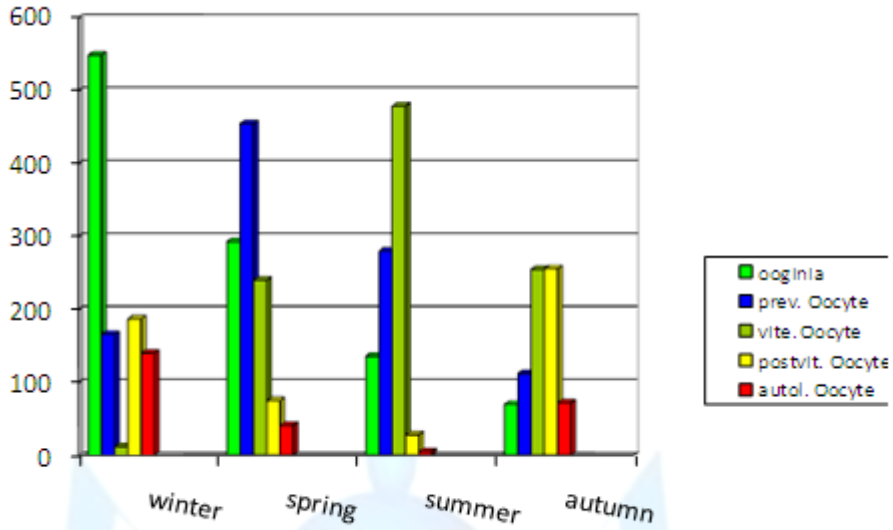
**Histogram 7 Mean of the different stages of oocyte development along the four seasons**




**(Tables 5-10) Turkey`s Multiple Comparison Test and One Way ANOVA of the oocyte developmental stages along the year.**

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed (Table 5) | | | | | |
| Data Table-oogonia | | | | | |
| One-way analysis of variance | | | | | |
| P value | P<0.0001 | | | | |
| P value summary | *** | | | | |
| Are means signif. different? (P <0.05) | Yes | | | | |
| Number of groups | 4 | | | | |
| F | 141,3 | | | | |
| R squared | 0,9815 | | | | |
| ANOVA Table | SS | df | MS | | |
| Treatment (between columns) | 45360 | 3 | 15120 | | |
| Residual (within columns) | 856 | 8 | 107 | | |
| Total | 46210 | 11 | | | |
| Tukey's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff | |
| winter vs spring | 85,33 | 14,29 | P < 0.001 | 58.29 to 112.4 | |
| winter vs summer | 137,7 | 23,05 | P < 0.001 | 110.6 to 164.7 | |
| winter vs autumn | 159,7 | 26,74 | P < 0.001 | 132.6 to 186.7 | |
| spring vs summer | 52,33 | 8,763 | P < 0.01 | 25.29 to 79.38 | |

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| | | | | | |
|---|---|---|---|---|---|
| spring vs autumn | 74,33 | 12,45 | P < 0.001 | 47.29 to 101.4 | |
| summer vs autumn | 22 | 3,684 | P > 0.05 | -5.048 to 49.05 | |

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed (Table 6) | | | | | |
| Data Table-previtellogenic oocyte | | | | | |
| One-way analysis of variance | | | | | |
| P value | 0,0028 | | | | |
| P value summary | ** | | | | |
| Are means signif. different? (P < .05) | Yes | | | | |
| Number of groups | 4 | | | | |
| F | 11,53 | | | | |
| R squared | 0,8122 | | | | |
| ANOVA Table | SS | df | MS | | |
| Treatment (between columns) | 22990 | 3 | 7665 | | |
| Residual (within columns) | 5317 | 8 | 664,6 | | |
| Total | 28310 | 11 | | | |
| Tukey's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff | |
| winter vs spring | -96,33 | 6,472 | P < 0.01 | -163.7 to -28.92 | |
| winter vs summer | -38 | 2,553 | P > 0.05 | -105.4 to 29.41 | |
| winter vs autumn | 18 | 1,209 | P > 0.05 | -49.41 to 85.41 | |
| spring vs summer | 58,33 | 3,919 | P > 0.05 | -9.075 to 125.7 | |
| spring vs autumn | 114,3 | 7,682 | P < 0.01 | 46.92 to 181.7 | |
| summer vs autumn | 56 | 3,762 | P > 0.05 | -11.41 to 123.4 | |
| Table Analyzed (Table 7) | | | | | |
| Data Table-vitellogic oocyte | | | | | |
| One-way analysis of variance | | | | | |
| P value | P<0.0001 | | | | |
| P value summary | *** | | | | |
| Are means signif. different? (P < 0.05) | Yes | | | | |
| | | | | | |
| Number of groups | 4 | | | | |
| F | 122,1 | | | | |
| R squared | 0,9786 | | | | |
| ANOVA Table | SS | df | MS | | |
| Treatment (between columns) | 36390 | 3 | 12130 | | |
| Residual (within columns) | 794,7 | 8 | 99,33 | | |

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| | | | | | |
|---|---|---|---|---|---|
| Total | 37180 | 11 | | | |
| Tukey's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff | |
| winter vs spring | -76 | 13,21 | P < 0.001 | -102.1 to -49.94 | |
| winter vs summer | -155,7 | 27,05 | P < 0.001 | -181.7 to -129.6 | |
| winter vs autumn | -81 | 14,08 | P < 0.001 | -107.1 to -54.94 | |
| spring vs summer | -79,67 | 13,84 | P < 0.001 | -105.7 to -53.61 | |
| spring vs autumn | -5 | 0,8689 | P > 0.05 | -31.06 to 21.06 | |
| summer vs autumn | 74,67 | 12,98 | P < 0.001 | 48.61 to 100.7 | |

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed (Table 8) | | | | | |
| Data Table-postvitellogic oocyte | | | | | |
| One-way analysis of variance | | | | | |
| P value | P<0.0001 | | | | |
| P value summary | *** | | | | |
| Are means signif. different? (P < 0.05) | Yes | | | | |
| Number of groups | 4 | | | | |
| F | 31,43 | | | | |
| R squared | 0,9218 | | | | |
| ANOVA Table | SS | df | MS | | |
| Treatment (between columns) | 10800 | 3 | 3598 | | |
| Residual (within columns) | 916 | 8 | 114,5 | | |
| Total | 11710 | 11 | | | |
| Tukey's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff | |
| winter vs spring | 37,33 | 6,043 | P < 0.05 | 9.354 to 65.31 | |
| winter vs summer | 53 | 8,579 | P < 0.01 | 25.02 to 80.98 | |
| winter vs autumn | -23 | 3,723 | P > 0.05 | -50.98 to 4.980 | |
| spring vs summer | 15,67 | 2,536 | P > 0.05 | -12.31 to 43.65 | |
| spring vs autumn | -60,33 | 9,766 | P < 0.001 | -88.31 to -32.35 | |
| summer vs autumn | -76 | 12,3 | P < 0.001 | -104.0 to -48.02 | |

| | | | | | |
|---|---|---|---|---|---|
| Table Analyzed (Table 9) | | | | | |

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| Data Table-autolyzed oocyte | | | | | | |
|---|---|---|---|---|---|---|
| One-way analysis of variance | | | | | | |
| P value | 0,0287 | | | | | |
| P value summary | * | | | | | |
| Are means signif. different? (P < 0.05) | Yes | | | | | |
| Number of groups | 4 | | | | | |
| F | 5,132 | | | | | |
| R squared | 0,658 | | | | | |
| ANOVA Table | SS | df | MS | | | |
| Treatment (between columns) | 3283 | 3 | 1094 | | | |
| Residual (within columns) | 1706 | 8 | 213,3 | | | |
| Total | 4989 | 11 | | | | |
| Tukey's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff | | |
| winter vs spring | 33 | 3,914 | P > 0.05 | -5.184 to 71.18 | | |
| winter vs summer | 45 | 5,337 | P < 0.05 | 6.816 to 83.18 | | |
| winter vs autumn | 22,67 | 2,688 | P > 0.05 | -15.52 to 60.85 | | |
| spring vs summer | 12 | 1,423 | P > 0.05 | -26.18 to 50.18 | | |
| spring vs autumn | -10,33 | 1,226 | P > 0.05 | -48.52 to 27.85 | | |
| summer vs autumn | -22,33 | 2,649 | P > 0.05 | -60.52 to 15.85 | | |

| Table Analyzed (Table 10) | | | | | |
|---|---|---|---|---|---|

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| Data Table-Mean of ovarian vitality | | | | | | |
|---|---|---|---|---|---|---|
| One-way analysis of variance | | | | | | |
| P value | 0,3135 | | | | | |
| P value summary | ns | | | | | |
| Are means signif. different? (P < 0.05) | No | | | | | |
| Number of groups | 5 | | | | | |
| F | 1,303 | | | | | |
| R squared | 0,2578 | | | | | |
| ANOVA Table | SS | df | MS | | | |
| Treatment (between columns) | 123800 | 4 | 30960 | | | |
| Residual (within columns) | 356400 | 15 | 23760 | | | |
| Total | 480300 | 19 | | | | |
| Tukey's Multiple Comparison Test | Mean Diff. | q | P value | 95% CI of diff | | |
| oogonia vs previt. oocyte | 8,75 | 0,1135 | P > 0.05 | -327.8 to 345.3 | | |
| oogonia vs vitello. oocyte | 15,5 | 0,2011 | P > 0.05 | -321.1 to 352.1 | | |
| oogonia vs post-vitell. oocyte | 125,5 | 1,628 | P > 0.05 | -211.1 to 462.1 | | |
| oogonia vs autolyzed oocyte | 197,5 | 2,562 | P > 0.05 | -139.1 to 534.1 | | |
| previt. oocyte vs vitello. oocyte | 6,75 | 0,08758 | P > 0.05 | -329.8 to 343.3 | | |
| previt. oocyte vs post-vitell. oocyte | 116,8 | 1,515 | P > 0.05 | -219.8 to 453.3 | | |
| previt. oocyte vs autolyzed oocyte | 188,8 | 2,449 | P > 0.05 | -147.8 to 525.3 | | |
| vitello. oocyte vs post-vitell. oocyte | 110 | 1,427 | P > 0.05 | -226.6 to 446.6 | | |
| vitello. oocyte vs autolyzed oocyte | 182 | 2,361 | P > 0.05 | -154.6 to 518.6 | | |
| post-vitell. oocyte vs autolyzed oocyte | 72 | 0,9341 | P > 0.05 | -264.6 to 408.6 | | |

## LEGENDS

### Fig. 1

Photomacrograph of a dissected female caramote prawn Melicertus kerathurus showing the ovary of January- March. Note, the ovary is white and compact with sickle-shaped contents.

### Fig. 2

Photomacrograph of a dissected female caramote prawn Melicertus kerathurus showing the ovary of April-beginning of June. Note, the ovary is pale green.

### Fig. 3

Photomacrograph of a dissected female caramote prawn Melicertus kerathurus showing the ovary of end of June. Note, the ovary is green.

### Fig. 4

Photomacrograph of a dissected female caramote prawn Melicertus kerathurus showing the ovary of July – September.Note, the ovary is dark green and extend about the whole length of the animal

### Fig. 5

Photomacrograph of a dissected female caramote prawn Melicertus kerathurus showing the ovary of October- December.. Note, the ovary had varying degrees of yellow to orange colours.

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**Fig. 6**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing the external lining of the ovary. It is covered by branched and overlapping fibres. The specimen represents ovaries of April – June

**Fig. 7**

Photomicrograph of a transverse section of an ovary of female caramote prawn Melicertus kerathurus showing an immature ovary in growth stage. Note, the surface acquires an increasingly irregular appearance. The specimen represents ovaries of April –June.

**Fig. 8**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing some of the inner layers invaginate toward the centre of the ovary forming groups of spherical bodies resembling cells of different sizes. The specimen represents ovaries of April –June

**Fig. 9**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing the nodes or cysts. The nodes tend to be organized internally with the smaller cells, which may be either follicles or early stage germ cells. The specimen represents ovaries of April –June.

**Figs. 10-12**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing previtellogenic and vitellogenic oocytes. Previtellogenic oocytes are smaller than mature ones but larger than oogonia near which they are located. The specimen represents ovaries of April –June,

**Figs. 13-15**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing mature oocytes, the nucleus/cytoplasm ratio is reduced; the nucleus is quite evident and is filled with spherical vesicles containing yolk granules. The specimen represents ovaries of January- March.

**Fig. 16**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing early maturing stage during April to June. Opaque yolk granules are formed in the cytoplasm and partly obscure the nuclei. The developing ova are clearly larger than the immature stock. The specimen represents ovaries of January- March.

**Figs. 17-18**

Photomicrographs of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing mature stage during July to September. Note, This stage is believed to be the last stage of maturity before actual spawning.

**Figs. 19-20**

Photomicrographs of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing mature stage during July to September: The oocyte becomes follicular. The opacity of cytoplasm increases; the number of vacuoles gradually increases, and they become dispersed towards the central area.

**Fig. 21**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing mature stage during July to September: The oocyte of this step is larger in size. Large granules appear in the cytoplasm and increase in number.

**Fig. 22**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing an early developing stage of the ovary, the exogenous vitellogenic oocytes are present at the periphery, and the endogenous vitellogenic oocytes are present in the centre.

**Fig. 23**

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing the exogenous vitellogenic oocytes , previtellogenic oocytes, endogenous vitellogenic oocytes, oogonia, and follicle cells.

**Figs. 24-26**

Photomicrographs of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing mature stage during July to September: The oocyte attains its maximal size 750 – 800 µm. The peripheral vacuoles are decreased in number while the yolk accumulation increases. The thickness of the vitelline envelope gradually increases and reaches its maximal thickness. This indicates that the chorion is formed around the oocyte during the process of vacuolization of the inner follicle epithelium.

## Fig. 27

Photomicrograph of a transverse section of an ovary of a female caramote prawn Melicertus kerathurus showing the Post-vitellogenic oocyte. Note, it is characterized by a homogenous cytoplasm and the yolk material accepted a clear blue colour with the different triple stains applied.

## ABBREVIATIONS

| | |
|---|---|
| AB | Abdomen |
| AO | Autolyzed Oocyte |
| BF | Branched Fibres. |
| CX | Cephalothorax |
| C | Chorion |
| CR | Cortical Rods |
| DO | Dark Green Ovary |
| EO | Endogenous Vitellogenic Oocyte |
| EG | EXogenous Vitellogenic Oocyte |
| FC | Follicles Cell |
| GC | Germ Cell |
| GE | Germinal Epithelium |
| GO | Green Ovary. |
| H | Hemolymph |
| HS | Homogenous Surface |
| II | Inner Layers Invagination |
| IS | Irregular Surface |
| MO | Mature Oocyte |
| ML | Middle Lobe |
| N | Nodes |
| NS | Nucleus |
| NU | Nucleolus |
| O | Oogonium |
| OY | Opaque Yolk Granules |
| PG | Pale Green Ovary |
| PO | Previtellogenic oocyte |
| TO | Postvitellogenic oocyte |
| SB | Spherical Bodies |
| V | Vacuoles |
| VO | Vitellogenic oocyte |
| WO | White ovary |
| YO | Yellow Ovary |
| YG | Yolk Granules |

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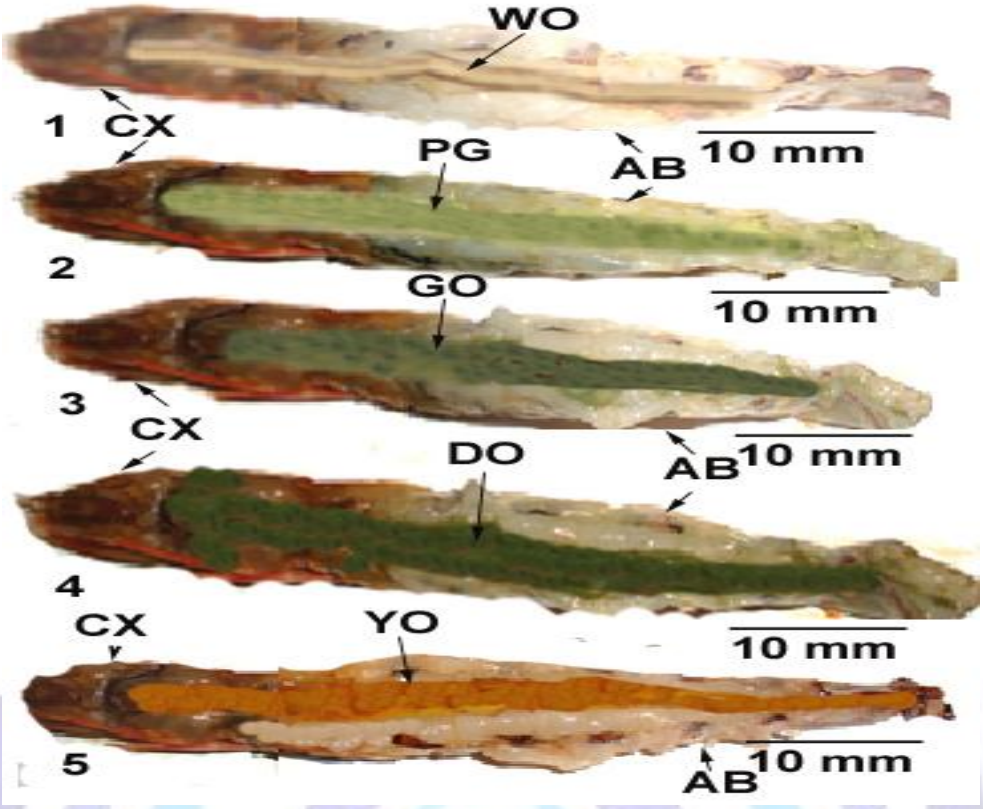


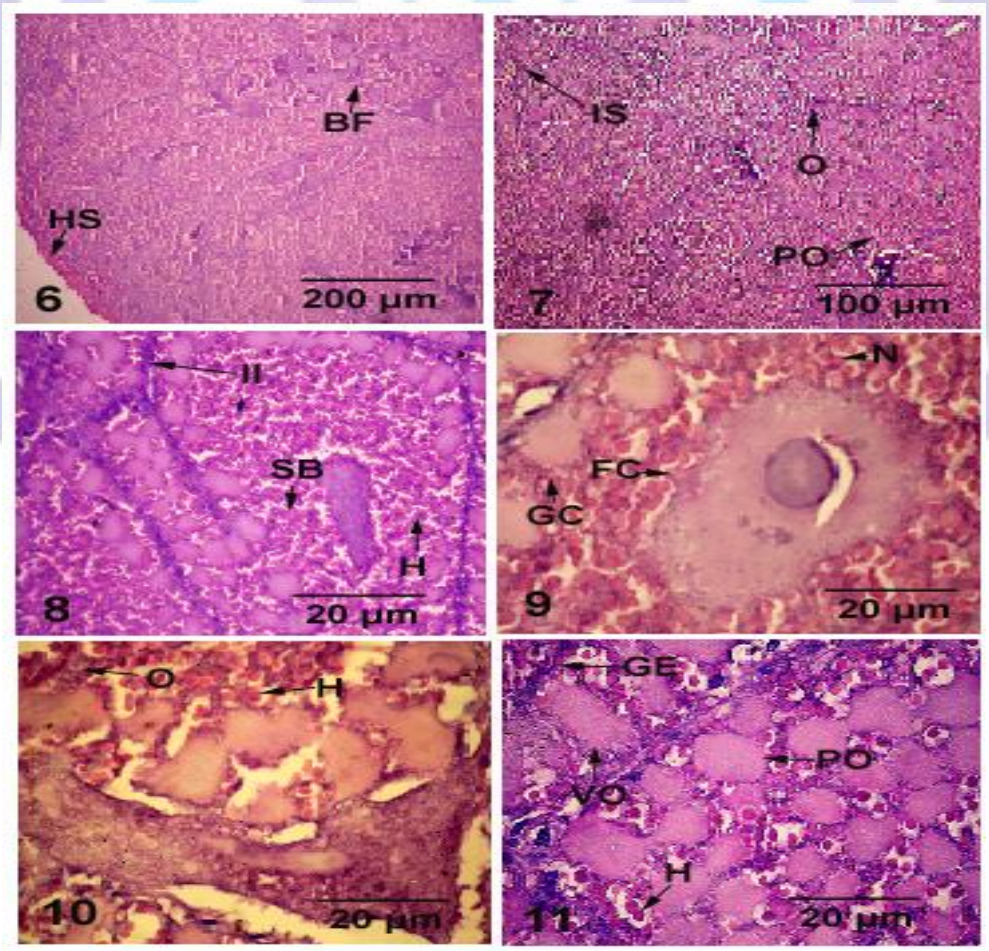

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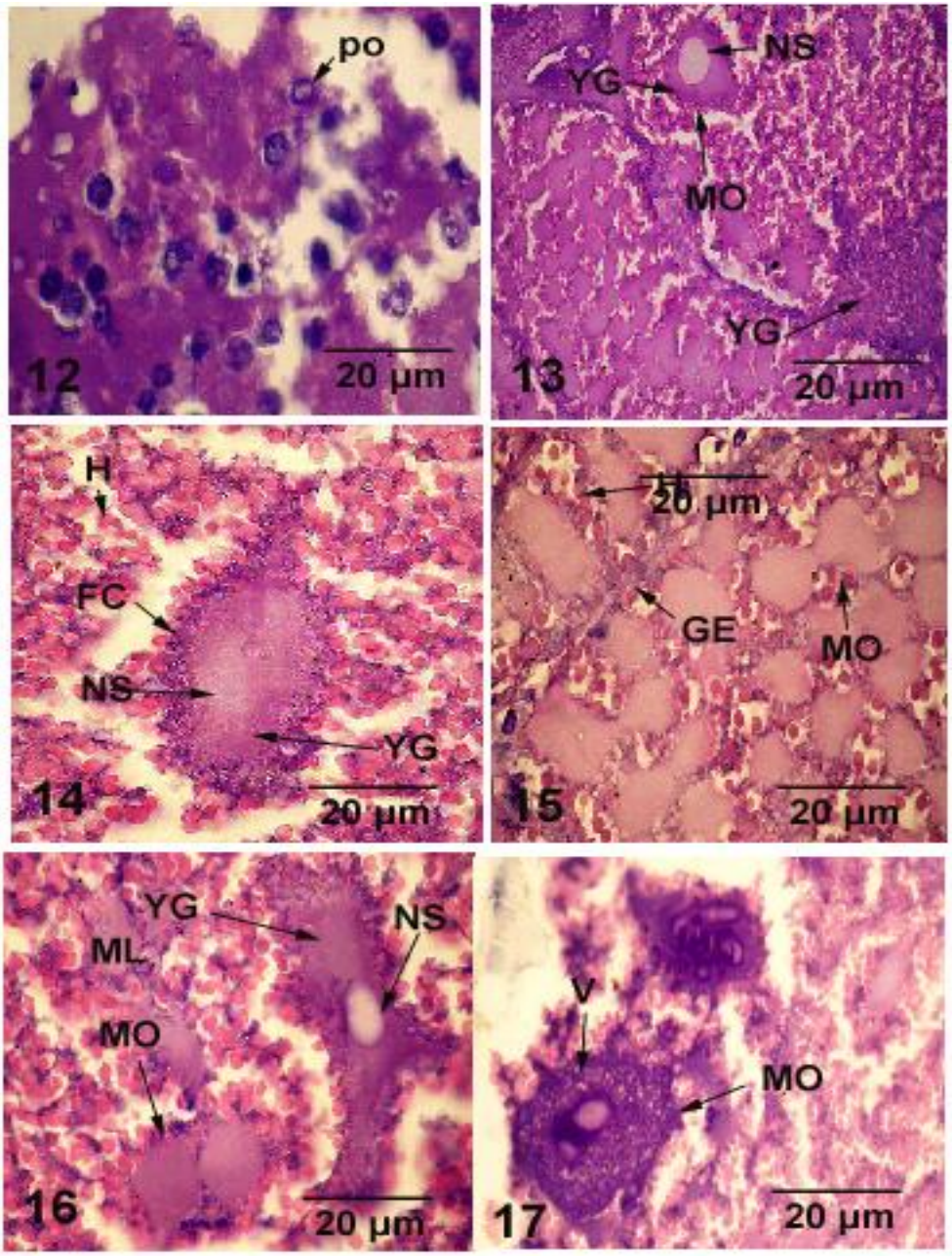

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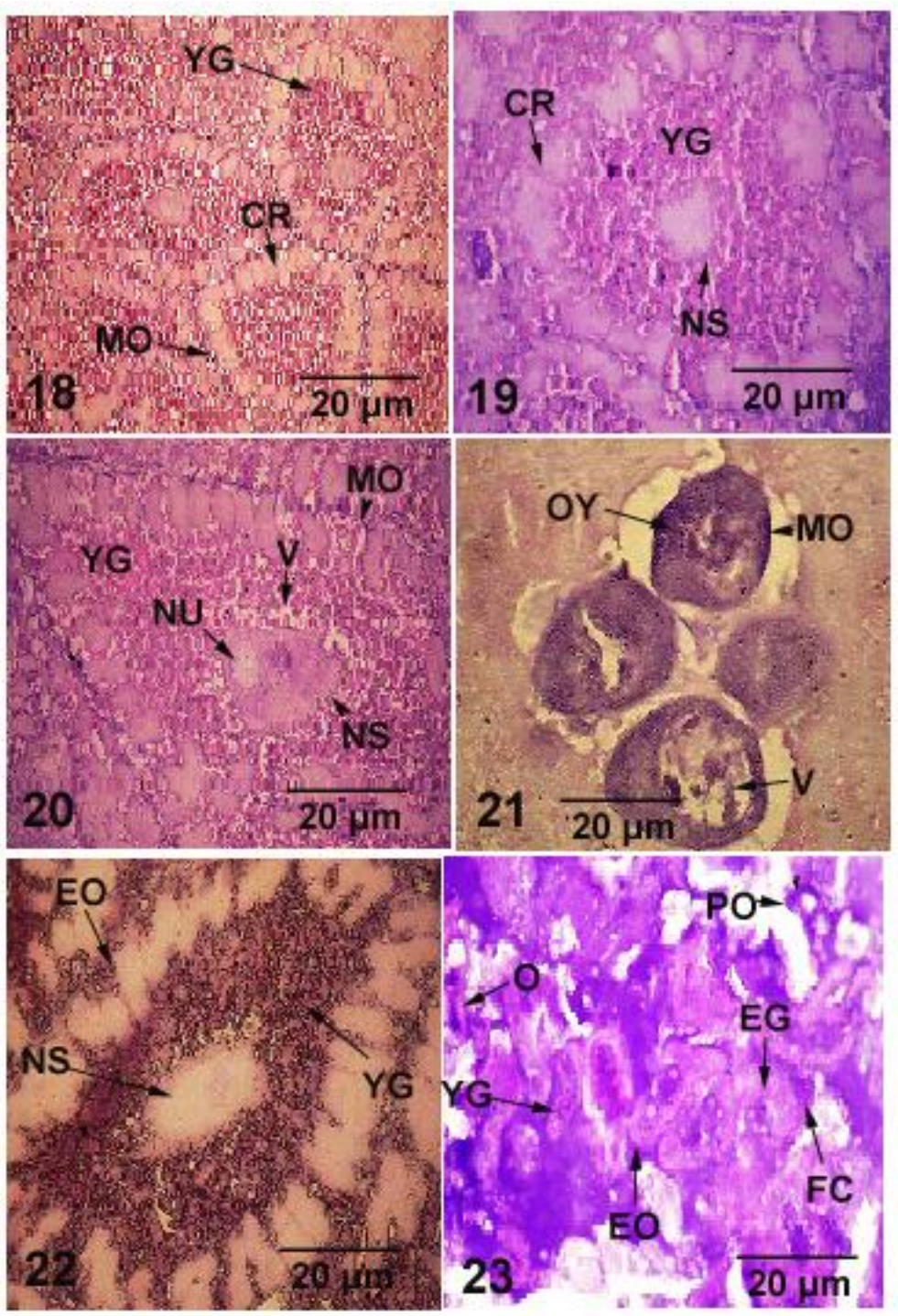

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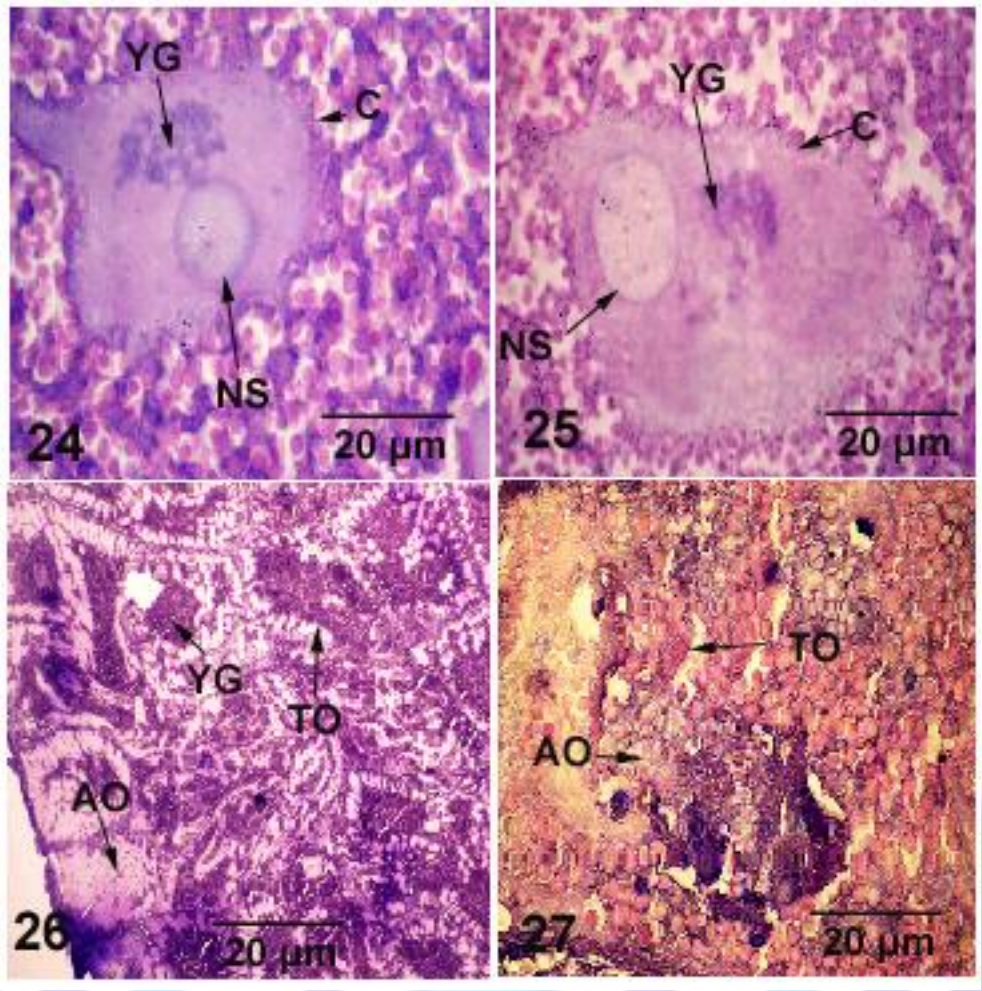


## DISCUSSION

The present study revealed that the two ovaries are situated above the hepatopancreas and below the pericardial sinus and heart. Both the anterior and posterior ends of the ovaries touching each other and leaving a gap in the middle for the passage of the cardio-pyloric strand. The shape and size of the ovaries vary considerably according to the season of the year. The ovaries extend anteriorly up to the renal sac and posteriorly up to the anterior margin of the first abdominal segment. The two ovaries were dark green in colour and extend about the whole length of the animal during the period from July - September. They were with varying degrees of yellow colour during the period of October - December. They were with white compact and sickle-shaped and dardly seen during -January- March and gradually attain a pale green colour with the beginning of April - June. The mature ovaries are paired organs, situated dorsally, extending from the base of the rostrum to the last abdominal segment. They are bilaterally symmetrical and partly fused. Each half of the ovary consists of three lobes, of which the slender anterior lobe occupies the anterior region. The middle lobe has 6 or 7 finger-like lateral lobules which entirely fill the area between the anterior region and the posterior border of the carapace. The posterior lobes extend the length of the abdomen. Macroscopic investigation of the ovary along the year revealed that the ovary attains its maximal size during July - September. During - October - December the ovary gets smaller in size gradually and the smallest size was observed during- January- March.

When the ovary is immature the surface is relatively smooth and homogenous, but as the organ grows the surface acquires an increasingly irregular and nubbly appearance. Cross-sections of the ovary show the lining to be composed of several layers of filamentous or fibrous material disposed in lamellae. Some of the inner layers invaginate toward the centre of the ovary forming groups of spherical bodies resembling cells of different sizes. The grainy material observed among the fibres possibly corresponds to the hemolymph circulating the ovary internally. Results from two studies carried out on P. monodon failed to agree on the pattern of changes in vitellognic witch ovary development. The study by Longyant et al (2003) showed a drop in haemolymph levels when the ovary reached maturity . This drop was not shown in the study of Vazquez-Boucard, (1985) . In addition. these two studies showed significant difference in the quantities detected at the various ovary development stages for P. monodon despite both studies using the same techniques. Vincent et al (2001) found vitellogenin in the haemolymph of what they termend ´0´´ stage of ovary development, while Longyant et al. ( 2003) showed it to be undetectable in their first stage of ovary development. Some of the differences between these two studies may be attributed to the criteria used for staging the ovary develogment, which was poorly defined in both publications .

A number of larger spherical bodies were observed between the lamellae. The bodies formed by the lamellae and their internal components, termed nodes or cysts, were identified during dissection of premature ovaries. The nodes tend to be organized internally with the smaller cells, which may be either follicles or early stage germ cells, near the centre. However, because of their similar morphology and size, these two cell types could not be clearly distinguished. The large previtellogenic oocytes and vitellogenic (mature) oocytes of the subsequent stages are easier to distinguish due to differences in diameter, cytoplasmic aspect and nucleus/cytoplasm ratio. Previtellogenic oocytes are smaller than mature oocytes but larger than oogonia near which they are located. The nucleus and nuclear envelope are visible and the cytoplasm appears slightly granular. In mature oocytes, the nucleus/cytoplasm ratio is reduced; the nucleus is at this point quite evident and is filled with spherical vesicles containing yolk granules. These cells, which come closest to the external lining of the ovary, are separated by increasingly conspicuous envelopes. The external lining of intact cells appears membranous and branched , and may originate from extensions of the fibrous layer or follicular cells (the technique employed does not allow to make the distinction). The periphery of cross-sectioned mature oocytes displays a rather homogeneous and continuous band, possibly corresponding to the plasma membrane. In addition, extensions resembling microvilli may be seen projecting outward from the band occupying an empty space corresponding to the chorion. Mature oocytes are covered by another protective envelope formed probably by surrounding follicular cells. Premature and mature gonads are mostly filled with previtellogenic and vitellogenic oocytes and are bound by a slender external lining (Yano & Chinzei, 1987 ; Medina et al.. 1996 ; Vaca and Alfaro, 2000 and Peixoto, et al.. 2003).

. Histologically, the ovary consists of a number of radially arranged rows of ova in various stages of development. The immature ova lie towards the centre, while the mature ones towards the periphery. Each developing ovum is being surrounded by a number of small nutritive cells. A mature ovum has a rounded appearance with a large nucleus and a cytoplasm filled of yolk granules. Each ovary is enclosed within in a membranous capsule. The primary role of the yolk proteins is to provide nutrition to the developing crustacean embryos. In many cases, crustaceans rely on the yolk proteins for several days after release from the females for their nutrition. Crustaceans may have as much as 60 to 90% of the total egg proteins as yolk protein. The sites of yolk biosynthesis vary according to the species examined. Some crustaceans rely solely on the ovary to complete vitellogenesis as in case of Penaeus japonicas (King, 1948; Rodriguez, 1985; Cripe, 1994; Yano and Chinzei, 1987; Yang et al., 2000 ). Others may use the fat body as a primary source of yolk (Amphipods and Isopods) as well as in the case with most insects (Gohar et al., 1985 ; Charniaux-Cotton, 1985). The hepatopancreas and ovary are common sites for yolk biosynthesis in several penaeid shrimp (Penaeus vannamei, Penaeus semisulcatus, Penaeus monodon, Parapenaeus longirostris (Shlagman, et al. 1986; Quackenbush, 1989a, b; Quintio et al. 1990; Browdy et al., 1996 ; Chen and Chen, 1993, 1994; Browdy, 1998). Coordination of reproduction and yolk biosynthesis is achieved via the endocrine system. The eyestalk neuroendocrine system is the critical component (Fingerman, 1987; Quackenbush, 1986).

Five maturation stages of the oocyte were recognized: during January till March, immature stage in which the ovaries are thin, translucent, unpigmented and confined to the abdomen. They contain oocytes and small spherical ova with light-stained cytoplasm free from granules and a conspicuous nucleus. During April to June, early maturing stage in which the ovary increases in size and the anterior and middle lobes are developing. The dorsal surface is light yellow to yellowish green. Opaque yolk granules are formed in the cytoplasm and partly obscure the nuclei. The developing ova are clearly larger than the immature stock. Late maturing stage in which the ovary is light green and is visible through ova are opaque, due to the accumulation of yolk. During July to September, mature stage in which the ovary is dark green and clearly visible through the exoskeleton. The ova are larger than in the preceding stage and the peripheral region becomes transparent. This stage is believed to be the last stage of maturity before actual spawning as the largest ova are encountered only in this stage. Crustacean reproduction is characterized by the production of eggs laden with yolk protein. Vitellogenesis is the process of the biosynthesis of these proteins, and their transport and storage in the ovary (Charniaux-Cotton, 1985 ; Conides, et al.. 1988 ; Burighel & Cloney, 1997 and Dall, et al.. 1990). Histological changes associated with oocyte maturation in wild caught P. monodon have been described in detail by Tan - Fermin and Pudadera (1989) . In this study , ovary sections were examined microscopically and classified into three ovarian development stages ( prevetitellogenic, vitellogenic or cortical rod) using criteria reported by Tan -Fermin and Pudadrea (1989).

Reproduction must be timed to allow for maternal yolk investment and optimal larval survival. For many years, it has been known that eyestalk removal and the consequent loss of the eyestalk neuroendocrine system results in rapid loss of inhibitory control of both molting and yolk biosynthesis (Lumare, 1979 and Quackenbush, 1989b). As a result of the common use of Penaeus vannamei in aquaculture in both North American and South American shrimp farms, a considerable knowledge regarding their basic biology and reproductive patterns is considered (Quackenbush, 1989b). This widespread genus has many species under cultivation worldwide. There are some very significant differences among the species with regards to the details of reproduction, but the overall and basic pattern remains quite similar (Browdy et al., 1990; Chen and Chen, 1993; Quintio et al., 1990; Shlagman, et al., 1986). Penaeid shrimp are broadcast spawnners; they release fertilized eggs directly into the sea or ocean, with little or no parental care. Fertilization by the males quickly follows ovulation, and generally occurs at night. A mature female (30–60 g weight) will produce about 60,000 to 200,000 eggs per spawning event. At release, the eggs are about 300 µm in diameter, and they are covered with a cortical granule protein (Rankin, 1989; Aragón-Noriega & Alcántara-Razo, 2005). During a normal reproductive season, females are capable of producing ripe collections of eggs (4–6% total body weight) in about four weeks. As the female grows, she is capable of producing larger broods. After egg release, the developing shrimp larvae are entirely dependent on their yolk reserves for nutrition. In addition to studies on yolk reserves a significant body of research is now directed toward defining and understanding the reserves that comprise the cortical rods within mature oocytes. The yolk reserves and cortical rod reserves have physiologically distinct roles and therefore impact on egg quality in different way . The vitellogenic stages (s) in the development of prawns ovaries include the appearance of rod like bodies during

the final stages of development (Ben Meriem,1993) . After the completion of yolk accumulation , prawn oocytes are surrounded by an ´´acellular cytoplam´´ (Avarre et al. 2001 ). Cortical rods can comprice 10 % of the oocyte volume (Aragón-Noriega and Alcántara-Razo, 2005) ; or more in prawns where the rods are larger compared to other crustaceans such as crabs (Sastry, 1983 ). The biochemical composition of prawn cortical rods is not fully know, however, precursors in the ovary of P . aztecus are 70- 75 % protein and 25 -30 % carbohydrates (Montgomery, et al. 2007). In P . vannamei it was found that Cr protein constitute approximately 11 % of the total ovarian proteins (Rankin and Davis, 1990; Aragón-Noriega & Alcántara-Razo, 2005 ). Cortical rod therefore represent a significant amount of accumulated oocyte protein. Histological section of a Cortical oocyte showing cortical rods around the periphery of the late vitellogenic oocytes (Peixoto, et al. 2005). It was shown that cortical crypts and cortical rods (Pillay and Ono,1978). Cortical rods proteins have been located in the ovary (Avarre et al. 2001 , Yamada, et al. 2007 and Lumare, et al. 2011), also found the cortical rods protein - carbohydrate complex was only present in vitellogenic ovaries and that it was synthesized within the oocyte . Gene expression has however shown that for the prawn M japonicas transcription of the cortical rod proteins occurs in the previtellogenic oocytes (Yamano et al 2004). During vitellogenesis, and in the cortical rods during late vitellogenesis, Yamada, et al. (2007) concluded than most , if not all the cortical rods protein are produced from early stages of oocyte development, accumulated as yolk substances during oocyte development and finally assembled to create the cortical rods. Yamano et al ( 2004). Further concluded that , transcription, translation and formation of the cortical rod structure occurred at different stages of ovarian development .The cortical rods proteins are used the construct a jelly layer that surrounds the fertilized eggs after spawning . It is of critical importance during the earliest stages of embryonic development (Yamano et al 2004), as it offers the only protection until the hatching envelop forms (Avarre et al. 2001). The jelly layer formation is believed to help maintain a suitable microenvironment for the embryonic development and prevent polyspermy (Ben Meriem,1993) . Interestingly, studies on P .monodon egg activation have shown egg- sperm interaction occurs within 1 minute of spawning (Pillay and Ono, 1978) . This is very fast compared to P . aztecus were sperm –egg interaction took place between 20 and 40 minutes post spawning (Ben Meriem,1993; Yang, et al. 2000 and Peixoto, et al. 2005). Penaeid larvae are not hatched with functional mouthparts. They require four post hatch molts before they are capable of independent prey capture and feeding. This emphasizes the importance of yolk biosynthesis for the success of the developing nauplii. This pattern of early independent youngs also favored this species for systematic aquaculture operations (Laufer & Landau, 1991; Browdy et al., 1996 and D'Incao, et al.. 2002). Ovarian maturation in penaeid shrimp is divided into primary and secondary vitellogenesis. Primary vitellogenesis is described by little change in overall size or diameter. Secondary vitellogenesis is where the eggs actually grow in size from around 50 to 300 µm. In most crustaceans, the production of primary oocytes derived from oogonia continues throughout adult life.

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