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Ovarian biology of *Melicertus kerathurus* (Forskäl, 1775) - (Crustacea-Penaeidae) in the Arabian Gulf

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Abstract

The economic potential in the shrimp industry in captivity represents a significant role in world food production. Improvement of gamete production, increasing density of fertilization, providing a suitable medium for the larval stages and maintaining eugenic generations are the most important purposes for aquaculture farms. In this study adults of *Melicertus kerathurus* were sampled from the Ad-dammam estuarine beach of the Arabian Gulf from April 2017 to March 2018. Supraesophogeal ganglia, eyestalks, mandibular organs, hepatopancreas and ovaries of *Melicertus kerathurus* were prepared for histological and ultrastructure studies. Neurosecretory cells of the supraesophogeal ganglia and eye stalks varied significantly in size, shape and secretory activity in different seasons of the year. In an active phase they had large nuclei and conspicuous granules in their neuroplasm. The eyestalk bear three neuronal structures and sinus gland. The neurosecretory neurons of the eyestalk were in aggregates. They had the same morphology seen in the supraesophageal ganglion. The secretory activity of the sinus gland varied in the different months of the year. The maximal activity was observed during October – June and the minimal activity was observed during July - September. The secretory cells of the mandibular organ contained extensive SER, Golgi apparatus, ribosomes and abundant mitochondria. These organelles showed an active phase of secretion during spring and summer and an inactive phase of its cells were observed during autumn and winter when few organelles were observed. During spring and summer, the hepatopancreas showed folding of the tubules with elongated lumen, B -, M - and R cells were seen with rich cisterns of RER and Golgi apparatus, many secretory and

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endocytotic vesicles. During autumn and winter the hepatopancreas showed minimal activity of B -, M - and R cells. Ovary morphology varied according to the season of the year. It was dark green and massive during July – September; with yellow color during October - December and white during January- March. Our study found that ovary maturation is dependent on the activity of secretory cells through the seasons.

Keywords: Neurosecretory cells - supraesophogeal ganglia - eyestalks – mandibular organs -hepatopancreas - ovaries

INTRODUCTION

The economic potential in the shrimp industry in captivity represents a significant role in world food production [1]. Improvement of gamete production, increasing density of fertilization, providing a suitable medium for the larval stages and maintaining eugenic generations are the most important parameters for aquaculture farms [2]. Elimination of undesirable waste products from aquaculture tanks is necessary [3]. Malnutrition is a major problem in shrimp aquaculture farms where the shrimp's diet lacks sufficient protein and calories to maintain growth and reproduction. Previous studies thought to improve the crustacean aquaculture [4]. To increase productivity in crustacean aquaculture, it was found that eyestalk ablation unilaterally or bilaterally induces growth and reproduction [4]. On the other hand eyestalk ablation leads to hemolymph loss, many ablated shrimps die and suffer from hormonal imbalance [5]. Shrimps of *Melicertus kerathurus* (Forskäl, 1775) are highly abundant in estuarine ecosystems which are characterized to sustain environmental conditions [6]. This allows their easy use as research crustaceans for laboratory purposes. They are easily collected from the field and cultured in the laboratory in their breeding season so that their larval stages can be obtained naturally or artificially for investigation. Aquaculture is the fastest growing sector in food production [7].

Research has been focused to demonstrate the hormonal control on reproduction of marine invertebrates. Crustaceans have definite neuronal organs controlling most physiological activities including reproduction. The different types of neurosecretory cells found in the brain and eyestalk in the matured male and female sand lobster *Thenus orientalis* have been investigated [8]. Shrimps are broadcast spawning, releasing fertilized eggs into the sea beds. Oocyte maturation in penaeids depends on vitellogenesis that increases the oocyte diameter gradually from ≈ 50 µm - 350 µm depending on the macruran species. In most crustaceans, oogonia and developing oocytes continue throughout adult life. In definite months of the year, relying upon habitat temperature and other factors, these immature ova undergo cytological modifications during their transformation to secondary oogenesis [1]. Males tend to be physically bigger and this allows competitive behavior between males for access to females. Fertilization by the males quickly follows ovulation. A ripe female (≈ 40–55 g weight) spawns about 60,000 - 200,000 mature eggs every time. At oviposition, the mature eggs measure ≈ 300 µm in diameter, and are bounded with a granular protein [9]. The developing larvae depend on their yolk protein stores as a nutritive

supplement for ≈ 100 hours post fertilization. Decapod larvae are hatched without feeding appendages. So, inadequate amount of yolk materials in the developing larvae leads to mortality [10]. Larvae undergo four molts and their feeding appendages develop, so they are able to scavenge microorganisms like small worms and other larvae. This proves the significance of yolk protein synthesis for the survival of nauplii [11].

The objectives of this study were to analyze the control of the ovarian cycle in adult *Melicertus kerathurus* (Forskäl, 1775) in the four seasons of the year beginning from March 2017 in the Arabian Gulf, Ad-dammam estuarine waters. Increasing productivity in aquaculture relies principally on how to establish the optimal hatchery conditions, improving the outcome of cultured shrimps and the successes in the artificial propagation of cultured species in the estuarine beaches. The kingdom of Saudi Arabia has many shrimp aquaculture farms like Red Sea shrimp farm, Saudi Fisheries Company, Arabian Shrimp Company (ASCO), Saudi Red Sea Tiger and Prawn Farm National Aquaculture Group (NAQUA) which is one of the largest aquaculture operations in Saudi Arabia with 16 shrimp farms. Our findings can be applied to improve gamete production, increase fertilization density, providing a suitable medium for the developing larval stages and improve eugenic manner of the new generations.

MATERIAL AND METHODS

Shrimp collection and macroscopic observation

Adults of *Melicertus kerathurus* (Forskäl, 1775) were collected from the inshore waters of the Arabian Gulf of the Ad-dammam estuarine beach from April 2017 to March 2018. From each collection of *Melicertus kerathurus*, three females were incised dorsally to expose the genital system and were photomacrographed. From each season another three females were chosen, wet weighed (≈ 55 g), then incised dorsally, ovaries were isolated and weighed. The gonadosomatic index (GSI) was calculated according to GSI = 100 X wO / (wB- wO) where wO is ovary weight and wB is wet weight of female [12]. Ovary diameters were measured. A normal stereomacroscope was used to investigate the different whole mount preparations of ovaries, eye stalks and supraesophogeal ganglia.

Histological analysis of the gonads of *M. kerathurus*

Preparation of histological sections of ovaries, eye stalks, hepatopancreas, mandibular organ and supraesophogeal ganglia were done to observe the different maturation phases of gonads and to observe the different histological appearances of the tissues inside the other organs. These were fixed in 10% neutral formalin, washed in distilled water for 24 hours, dehydrated in ethyl alcohol, followed by tertiary butyl alcohol and paraffin oil (1:1), embedded in paraffin wax (melting point 54-58 ºC). For histological studies sections of 5-8 µm were prepared. A number of stains were applied; Eherlich haematoxylin and eosin [13], Weighert's haematoxylin & Van-Gieson stain [14],

Mallory triple stain [13], Heidenhain's iron haematoxylin (Pantin,1948), Masson's trichrome stain [13]. Other sections (10-12 µm) were obtained for histochemical observations. Several histochemical stains were applied to observe vitellogenesis and neurosecretions inside the cells of the eye stalk, the supraesophogeal ganglion, the mandibular organ, the hepatopancreas and the ovary [15]. Ortholux Leintz Wetzler stereoscope with external light source was used for imaging. Olympus IMT-2 phase Contrast microscope following the method of Nomarski differential interference contrast attachment was used.

Transmission electron microscopy (TEM)

Parts of supraesophogeal ganglia, eyestalks, mandibular organs, hepatopancreas and ovaries of *Melicertus kerathurus* were isolated and prepared for TEM. The fixative used was 2.5% Glutaraldehyde in PBS and 0.33 M NaCl at 4 °C. Preparations were washed in PBS and post-fixation in 2% Osmium tetroxide with PBS was carried out for 30-60 min. The samples were washed again with PBS, dehydrated in ethyl alcohol and propylene oxide, and embedded in araldite resin. A Leica UC6 microtome with diamond knives was used to prepare semithin and ultrathin sections. Sections were picked by formvar-coated singleslot copper grids, stained with uranyl acetate and lead citrate in a Nanofilm TEM stainer. Ultrathin sections were examined on a Phillips CM 120 transmission electron microscope at 60 kV. Semithin sections were stained with toluidine blue (1% toluidine, 1% $Na_2B_4O_7$, 20% sucrose) for 1 min at 60°C.

Statistical analysis of oocyte development

Three typical ovaries of *Melicertus kerathurus* were chosen from the histological preparation of three females from each season and the number of oocyte developmental stages were counted. Each trial was in triplicate ; the mean of the of oocyte developmental stages was calculated and subjected to One-way analysis of variance [16].

RESULTS

Activity of the supraesophageal (cephalic) ganglion

The supraesophageal ganglion of *Melicertus kerathurus* was bilobulated and located at the base of rostrum anterior to the esophegus. It was embedded in a thick mass of fatty tissue and formed by the fusion of three ganglia since three nerves arise to innervate the eyes, antennules, and antennae. A pair of stout optic nerves arose from the dorsal surface of the supraesophageal ganglion, one on each side, to supply the compound eye of its side. Different neurosecretory cells were scattered in the supraesophageal ganglion and varied significantly in diameter (9 – 78 µm), shape (polygonal to pyriform) and secretory activity (Fig. 1A). The majority of axons were thin and small (100-300 nm) in diameter. Most small mylinated nerve fibers ended in a dense glial neuropil. The neurons and the associated glial cells were imaged using

TEM. Glial cells were of several types; astrocytes, macroglia cells, perineural glia, periaxonal cells and neuropil. These cells formed cytoplasmic extensions which were always observed surrounding many small nerve fibers (Fig. 1B).

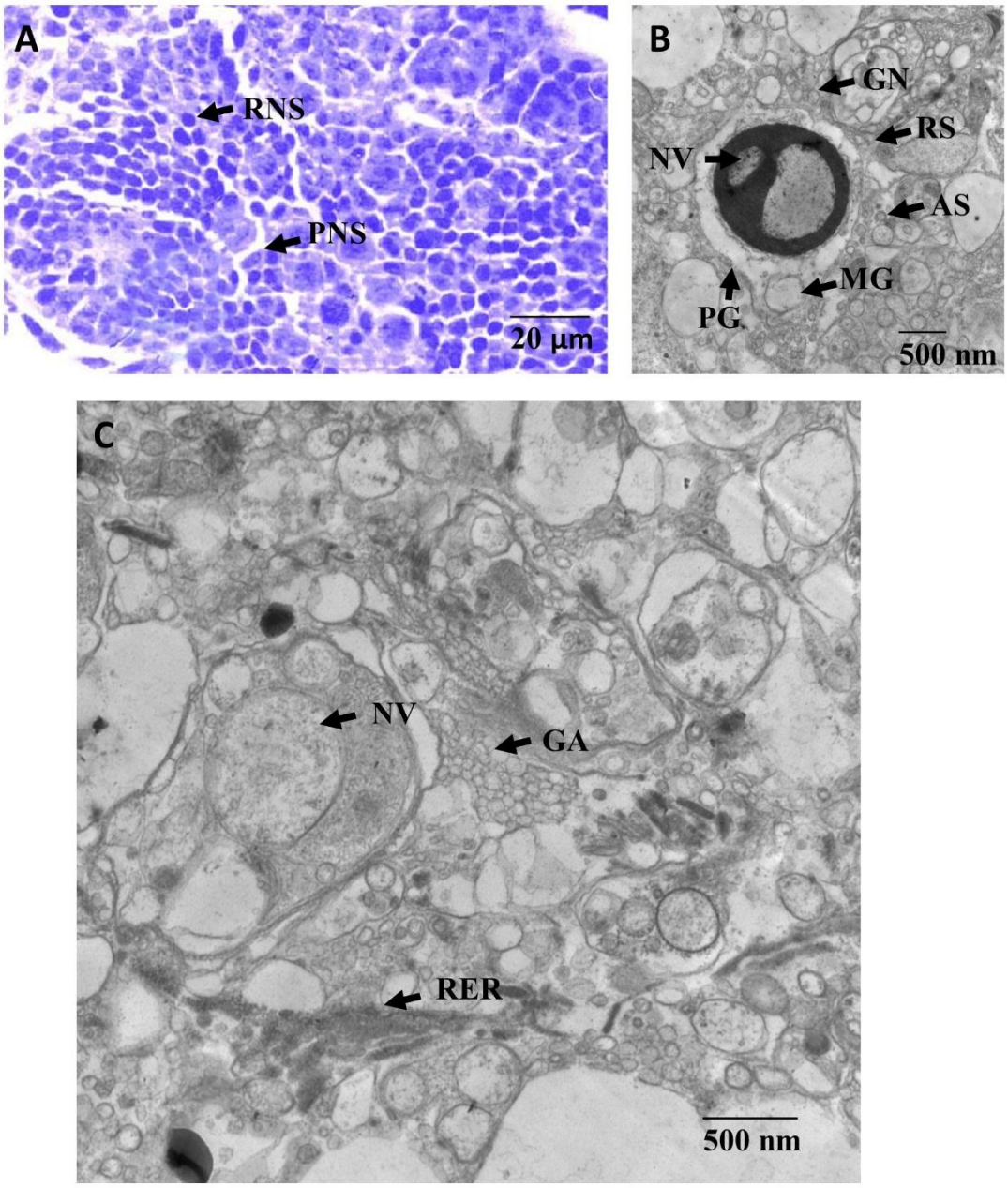

Fig. 1 Transmission electron microscopy of the supraesophageal ganglion. A. Semithin section showing active polygonal to pyriform neurosecretory neurons. PNS= Polygonal Neurosecretory Cell; RNS= Pyriform Neurosecretory Cell. **B.** TEM showing thin and small nerve fibers (100-300 nm ϕ) ending in a dense glial neuropil. Astrocytes, macroglia cells, perineural glia, periaxonal cells and neuropil. These cells formed cytoplasmic extensions which were always observed surrounding many small

nerve fibers. NF= Nerve Fiber; AS= Astrocytes; MC= Macroglia Cell; PG= Perineural Glia; PS= Periaxonal Cell; GN= Glial Neuropil. **C.** TEM showing the secretory phase during July – September neurosecretory neurons were rich RER, Golgi Apparatus and neuroplasmic vesicles. RER= Rough Endoplasmic Reticulum; GA= Golgi Apparatus; NV= Neuroplasmic Vesicle.

During July – September / October - June

Large and small neurosecretory neurons were polygonal in shape, with a centrally placed vesicular nucleus and varied number of nucleoli in the karyoplasm**.** These neurons were rich with neuroplasm, as evidenced by a low nucleus- neuroplasm ratio. Perinuclear vacuoles, pericellular capillary plexus and glial cells were observed in the pericellular region. These neurons had conspicuous granules in their neuoplasm. A secretory phase of these neurons, during July – September, was characterized by rich rough endoplasmic reticulum, Golgi Apparatus and neuroplasmic vesicles (Fig. 1C). During October – June the neuroplasm of these neurosecretory neurons was clear, pale stained, non-vacuolated and agranulated (Figs. 2A-C). Other small neurosecretory neurons with relatively less neuroplasm were observed in aggregates. They were polygonal to pyriform in shape (Fig. 3A). During July – September, two successive events occurred: firstly, large numbers of vacuoles were noticed with capillary plexuses and glial cells in their pericellular regions; secondly, secretory activity was found as shown by active RER and Golgi Apparatus (Figs. 3B). The histochemical test showed glycogen, proteins, amino acids and lipids in the neuroplasm (Figs. 2D-E).

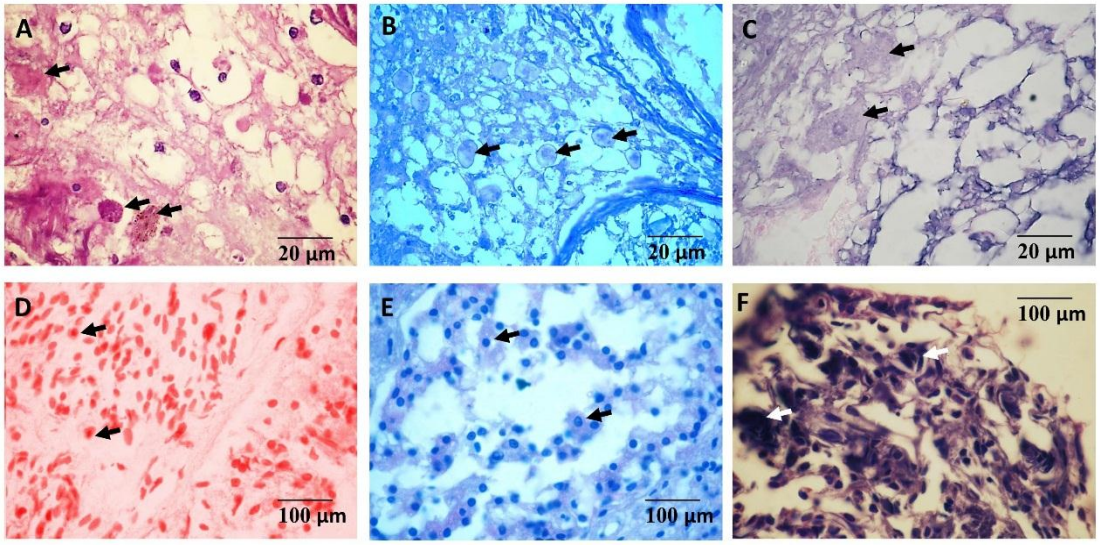

Fig. 2 Histochemical analysis of supraesophageal ganglion. A-B. CS showing neurons in the inactive phase with little glycogen, MPS, proteins and lipids during October - June. **D-F.** CS showing neurons were rich in glycogen, MPS, proteins and lipids from July - September. Indicated by arrows.

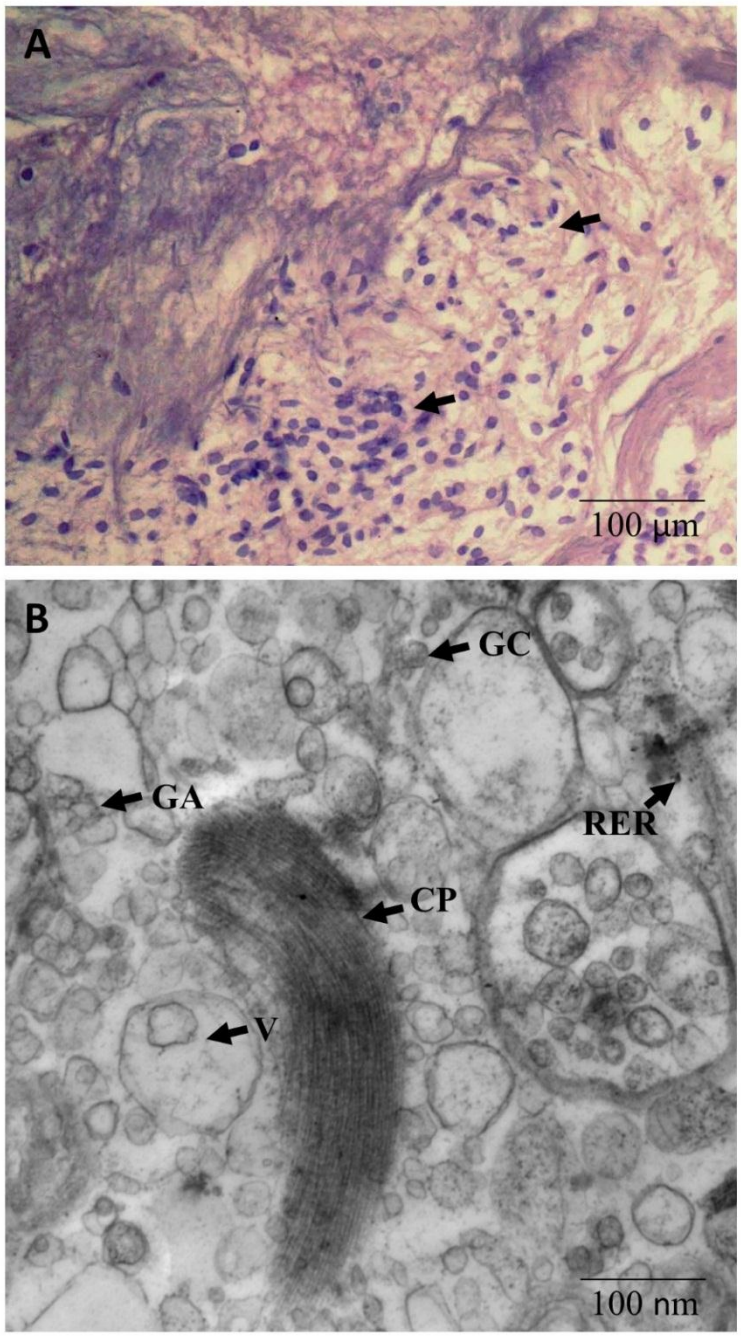

Fig. 3 Neurosecretory neurons of the supraesophageal ganglion. A. CS showing small neurosecretory neurons with relatively less neuroplasm observed in aggregates during October – June indicated by an arrow. **B.** TEM during July - September showing large numbers of vacuoles were noticed with capillary plexuses and glial cells in their pericellular regions; secretory activity was found as shown by active RER and Golgi Apparatus. V= Vacuole; CP= Capillary Plexuses; GC= Glial Cell; RER= Rough Endoplasmic Reticulum; GA= Golgi Apparatus.

Activity of the Eyestalk

The eyestalk of *Melicertus kerathurus* bear neuronal structures and a sinus gland. The neurosecretory neurons were in aggregates. They had the same morphology seen in the supraesophageal ganglion (Figs. 4A, B). The four types of neurons were distributed with different amounts in each aggregate. According to Longyant, et al. (2003), these aggregates were described in the eyestalk as medulla terminals and medulla externa of gangalionic X-organ [17] (Fig. 4B).

Two types of neurosecretory neurons were inactive during July – September and showed vacuolization and secretion phases during October – June. Two other types of neurosecretory neurons underwent secretory phase during July – September and were inactive during October – June (Figs. 4A, C, D).

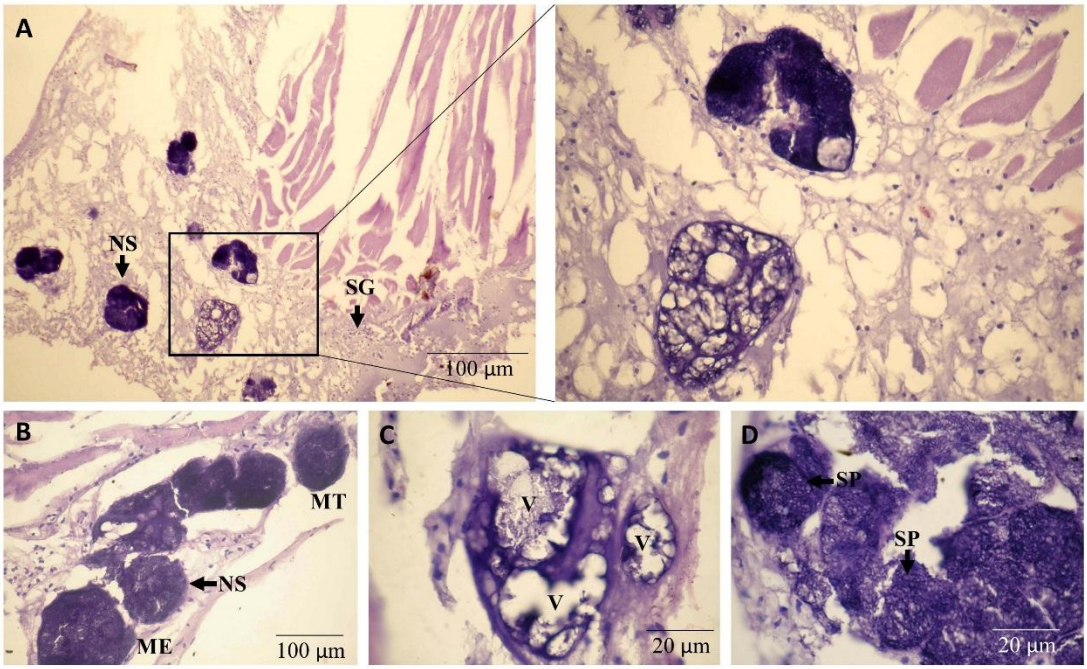

Fig. 4 Histology of the eyestalk. A. CS showing the neuronal structures and a sinus gland. NS= Neurosecretory Cell; SG= Sinus Gland. **B.** CS showing aggregates of neurosecretory neurons as medulla terminals and medulla externa of ganglionic X-organ. NS= Neurosecretory Cell; MT= Medulla Terminals Ganglionic X-organ, ME= Medulla Externa of Ganglionic X-organ. **C.** CS showing the first two types of neurosecretory neurons described in the supraesophogeal ganglion were inactive during July – September and showed vacuolization and secretion phases during June – October. INS= Inactive Neurosecretory Neuron; V= Vacuolization; SP= Secretion Phase. **D.** CS showing the last two types of neurosecretory neurons underwent secretory phase during October – June.

The sinus gland was associated with external blood sinuses around the medulla lobes (Fig. 5A). The gland consisted of many cells which had undergone cytological changes in the cytoplasm. The nuclei of the glandular cells were prominent with spherical to ovoid shape and contained round nucleoli. Chromatin was aggregated around the nucleoli and peripheral to the nuclear membrane. The cytoplasm contained mitochondria, ribosomes and endoplasmic reticulum. Cytoplasmic inclusions of moderate electron dense materials were observed with different sizes and enclosed by limiting membranes. The secretory activity of the sinus gland varied in the different months of the year. Maximal activity was observed during June – October and minimal activity was observed during July – September (Fig. 5B).

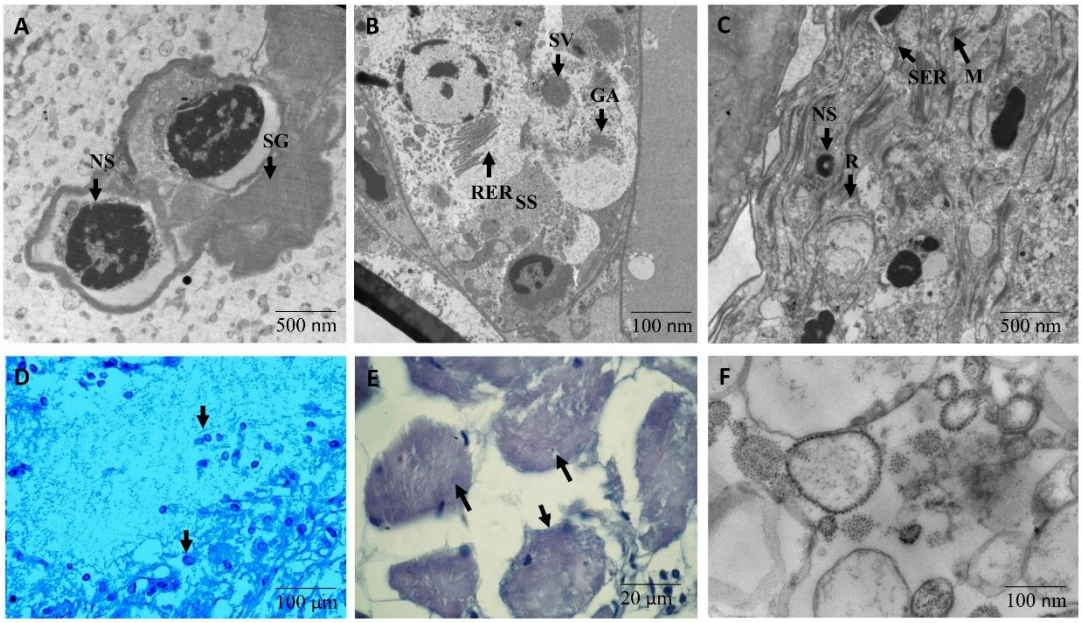

Fig. 5 Activity of the sinus gland and mandibular organ. A, B. TEM showing Maximal activity of the sinus gland was observed during June – October and minimal activity was observed during July – September. SS= Sinus Gland Secretion; RER= Rough Endoplasmic Reticulum; GA= Golgi Apparatus; SV= Secretory Vesicle. **C.** TEM showing the secretory cells of the mandibular organ contained extensive SER, Golgi apparatus, ribosomes and abundant mitochondria. The plasma membrane was highly folded because of bulk transport and the organelles were numerous. SER= Smooth Endoplasmic Reticulum; GA= Golgi Apparatus; R= Ribosome, M= Mitochondrion, NS= Neurosecretory Neuron. **D, E.** Histochemical analysis showing an active phase of secretion during spring and summer when proteins and steroids were secreted; Indicated by arrows. **F.** While the inactive phase of its cells was observed during autumn and winter when few organelles were observed.

Activity of the mandibular organ

The mandibular organ [18] of females was isolated from the ventral base of each mandible. The secretory cells of the mandibular organ contained extensive SER, Golgi apparatus, ribosomes and abundant mitochondria (Fig. 5C). These organelles showed an active phase of secretion during spring and summer when proteins and steroids were secreted (Figs. 5D, E). During this active phase, the plasma membrane was highly folded because of bulk transport and the organelles were numerous. While the inactive phase of its cells was observed during autumn and winter when few organelles were observed (Fig. 5F).

Activity of the hepatopancreas

The hepatopancreas was a conspicuous lobular brown colored gland surrounded with a connective tissue capsule. It occupied the posterior region of the stomach. These lobules were branched into blind-end tubules. Its lining consisted of simple columnar secretory epithelium separated by interstitial connective tissue (Fig. 6A). These cells were of many types; E-cell, short cells with a round large nucleus and basophilic cytoplasm; F-cell, elongated cells with microvilli and a round nucleus located at base of the cell with a basophilic cytoplasm; B-cell, large cells with few basophilic vacuolated cytoplasm and a large peripheral nucleus; R-cell, the most common cell with microvilli scattered in the hepatopancreas with basophilic faint cytoplasm and centrally located nucleus; M-cell, a few small cells with basophilic cytoplasm and central nucleus. This study showed that the hepatopancreas B -, M - and R cells underwent histological alternations with respect to months. During spring and summer the hepatopancreas showed folding of the tubules with an elongated lumen. B -, M - and R cells were with rich cisterns of RER, Golgi apparatus, mitochondria and many secretory and endocytotic vesicles (Fig. 6B). Histochemical preparations showed that the glandular epithelium of M - and R cells stained dark blue due to the xanthoproteic reaction, Millon reaction, Ninhydrin Schriff reaction, and the oxidized tannin azo method with respect to the presence of phenyl groups, Nh2 groups, and amino groups. Other granules stained red with PAS due to the presence of glycogen. Sudan black or Nile blue sulphate gave a black color due to the lipid droplets (Figs. 7A-C). During autumn and winter the hepatopancreas showed minimal activity of B -, M - and R cells. Their cytoplasm was weakly reactive to the same histochemical stains applied (Figs. 7D-F).

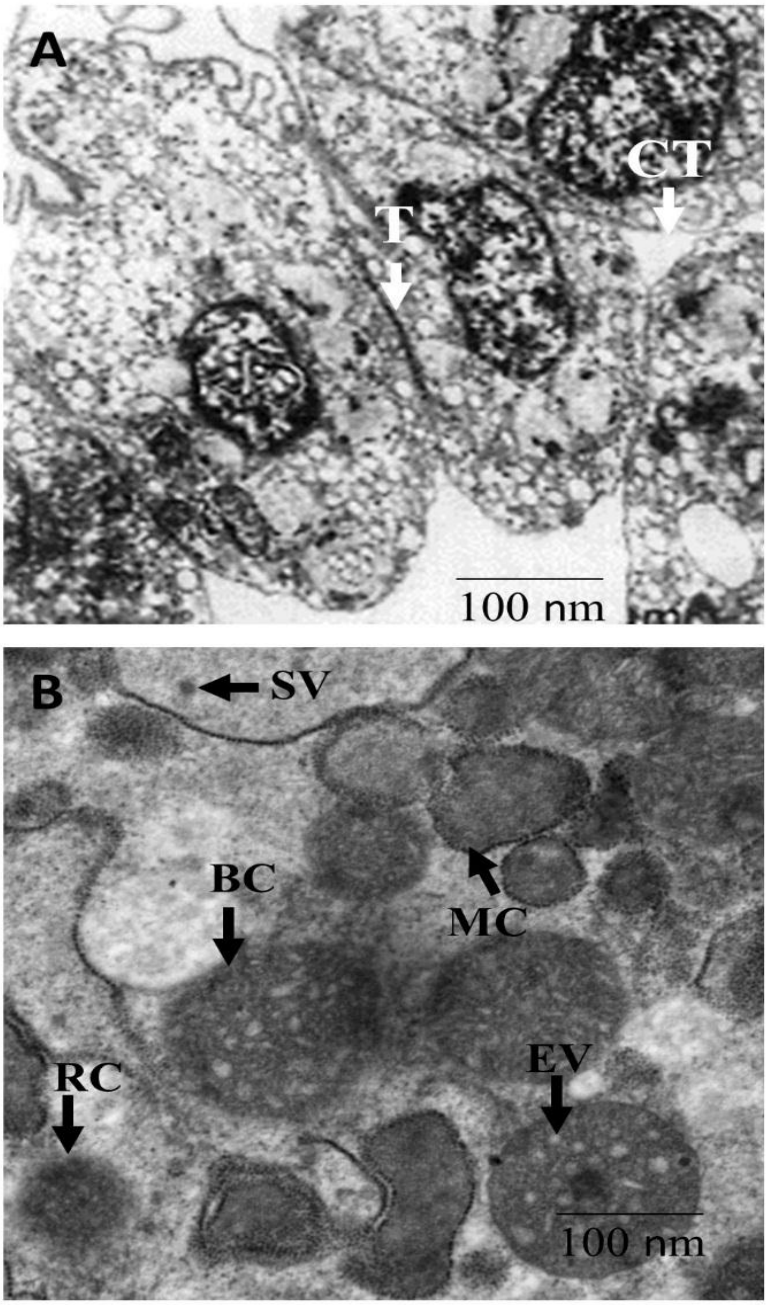

Fig. 6 Activity of the hepatopancreas. A. TEM showing lobules were branched into blind-end tubules. Its lining consisted of simple columnar secretory epithelium separated by interstitial connective tissue. T= blind-end Tubule, CT= Interstitial Connective Tissue. **B.** TEM during spring and summer showing B -, M - and R cells were with rich cisterns of RER, Golgi apparatus, mitochondria and many secretory and endocytotic vesicles. BC=B cell; MC=M cell; RC=R cell; SV= Secretory Vesicle; EV= Endocytotic Vesicle.

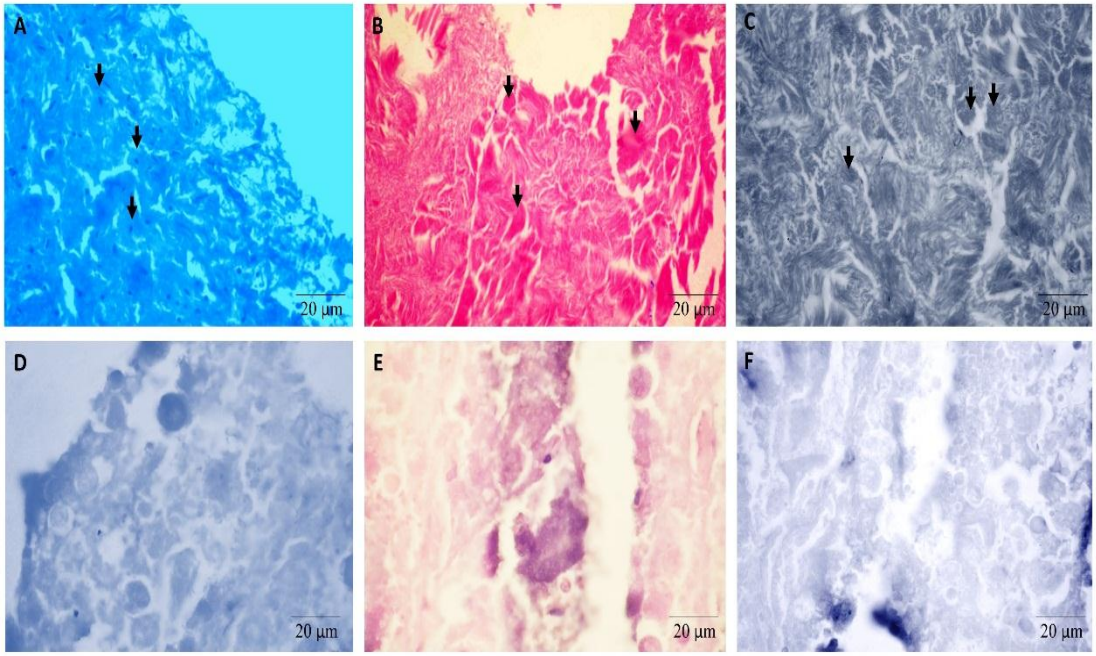

Fig. 7 Histochemical analysis of the hepatopancreas. A. CS showing the glandular epithelium of M - and R cells stained dark blue due phenyl groups, Nh2 groups, and amino groups. **B.** Other granules stained red with PAS due to the presence of glycogen. **C.** Sudan black or Nile blue sulphate gave a black color due to the lipid droplets. **D, E, F.** CS During autumn and winter showing the minimal activity of B -, M - and R cells. Their cytoplasm was weakly reactive to the same histochemical stains applied.

The ovarian cycle

The morphology of the ovary varied according to the seasonality. The two ovaries were massive dark green during July - September; with varying yellow color during October - December and white during January- March. They attained a pale green color during June (Fig. 8A). The ovary was surrounded externally with dense fibrous connective tissue. During January- March the immature ovary had a smooth surface and similar regular cellular make up, but as cells grew, the surface became irregular and massive (Fig. 8B). The lining of the ovary composed of several fibrous materials disposed in lamellae. Hemolymph cells were observed among these fibres. The germinal epithelium budded off spherical oogonia 50-70 µm towards the centre of the ovary (Fig. 8B). During April – June the premature ovary showed large spherical cells among its fibrous lamellae. These spherical cells were observed around the developing oocytes or around oogonia in the ovary centre (Figs. 8B). During July – September the previtellogenic and vitellogenic oocytes of the mature ovary were differentiated with respect to their size, ooplasmic morphometric and nucleus/ooplasm ratio. Previtellogenic oocytes (80-150 µm) had a visible nucleus with a nuclear envelope and the ooplasm appeared slightly granular (Figs. 8C, D). Vitellogenic

oocytes 170 – 250 µm had a reduced nucleus/ooplasm ratio and granules were observed inside spherical vesicles in the nucleus (Figs. 8E). The vitellogenic oocyte was surrounded with the plasma membrane and the chorion was formed progressively. In addition, the spherical cells surrounding the vitellogenic oocyte formed a third outermost follicular epithelium (Figs. 8E). The ratio of previtellogenic and vitellogenic oocytes in premature and mature ovaries differed significantly (Figs. 8C). Histologically, the ovary consisted of radially aligned oocytes at different stages of development. The oogonia and previtellogenic oocytes were centric in position and as development proceeded the mature ones became eccentric. In the ovary stroma, small nutritive cells were observed around developing oocytes (Fig. 8B).

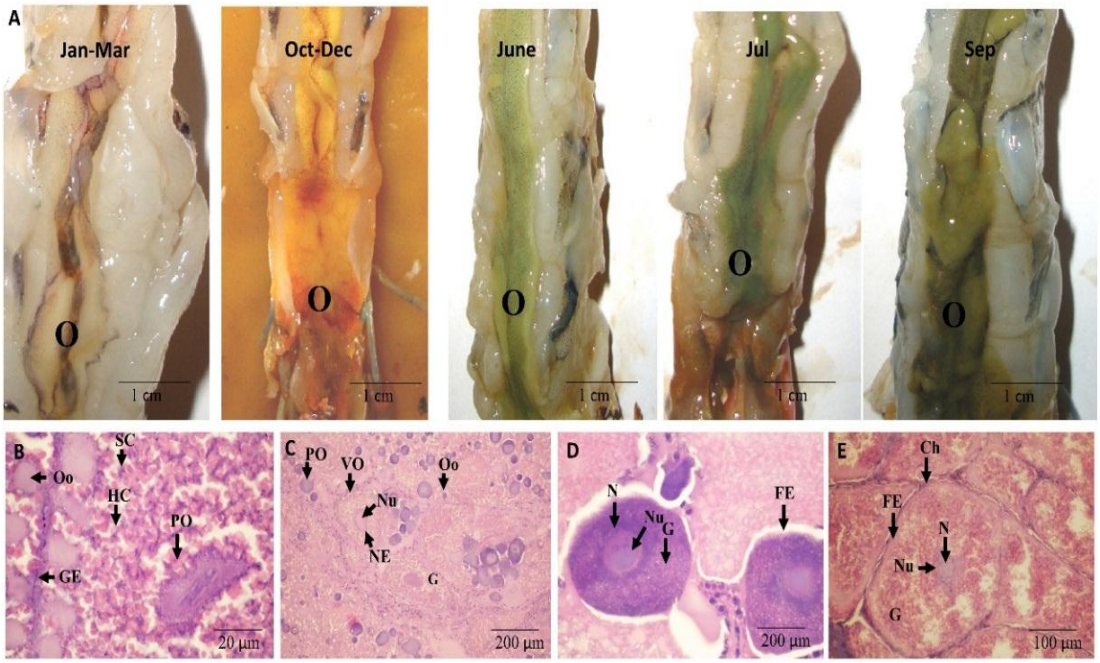

Fig. 8 Histology of the ovary. A. Photomacrograph showing the ovary; white during January- March; with varying yellow color during October - December; dark green during July - September; and. They attained a pale green color during June. O= Ovary. **B.** CS showing the lining of the ovary composed of several fibrous materials disposed in lamellae. Hemolymph cells were observed among these fibres. The germinal epithelium budded off spherical oogonia 50-70 µm towards the centre of the ovary. During April – June the premature ovary showed large spherical cells among its fibrous lamellae. These spherical cells were observed around the developing oocytes or around oogonia in the ovary centre. HC= Hemolymph Cell; FM= Fibrous Material; GE= Germinal Epithelium; Oo= Oogonia; SC= Spherical Cell. **C.** CS showing the previtellogenic and vitellogenic oocytes were differentiated with respect to their size, ooplasmic morphometric and nucleus/ooplasm ratio. Previtellogenic oocytes (80-150 µm) had a visible nucleus with a nuclear envelope and the ooplasm appeared slightly granular. Vitellogenic oocytes 170 – 250 µm had a reduced nucleus/ooplasm ratio and granules were observed inside spherical vesicles in the

nucleus. PO= Previtellogenic Oocyte; VO= Vitellogenic Oocyte; N= Nucleus; NE= Nuclear Envelope; G= Granules. **D, E.** CS showing the vitellogenic oocyte was surrounded with the plasma membrane and the chorion was formed progressively. In addition, the spherical cells surrounding the vitellogenic oocyte formed a third outermost follicular epithelium. N= Nucleus; Nu= Nucleolus; G= Granules; FE= Follicular Epithelium; Ch= Chorion.

Four oocyte maturation stages were identified: In the **immature stage** during January-March, the ovary was 260 µm in width which was thin, transparent, and restricted to the abdomen. Oogonia and developing oocytes with pale-stained agranulated ooplasm and a conspicuous nucleus were observed (Fig. 8B). In the **earlymaturing stage** during April - June, the ovary enlarged in width to 4800 µm and its lobules were undergoing development. Dark yolk granules were observed in the ooplasm and masked the nuclei. The developing oocytes were easily differentiated from the oogonia. The majority of oocytes differed from the oogonia and developing oocytes described in January - March. Follicular epithelium developed and oocytes grew in size. Darkly stained granules were formed around the concentric nucleus. Peripheral vacuoles were observed in the ooplasm which are considered as the most obvious characteristic of vitellogenic oocyte. Follicular epithelium was formed around the developing oocyte. Deposition of yolk granules proceeded intensely in the ooplasm and many vacuoles were developed inside the ooplasm and dispersed gradually to the centre. Protein (peptide linkage), glycogen, basic proteins and acid MPS granules were deposited. Glycoproteins were formed gradually and replaced the centrally located vacuoles (Figs. 9A-D). The vitelline envelope became clearly visible under the follicular epithelia. Pigmented granules were observed at the oocyte periphery. MPS were intense at the oocyte periphery while proteinous granules were perinuclear. Lipid droplets were deposited during July-August (Fig. 9E). In electron microscopy observations, the ooplasm contained electron-dense yolk globules, mitochondria, lipid droplets and RER. The follicle epithelium had mitochondria but no developed RER in its ooplasm. Active endocytosis was observed at the oocyte surface (Fig. 9F). In the **mature stage** during July - September, the ovary 5600 µm in diameter was occupied mostly with vitellogenic oocytes. This stage was considered as the last stage of maturity before actual spawning which took place in this period. The lipid droplets were more concentrated at the peripheral ooplasm compared to the inner perinuclear region. As the oocyte proceeded in development, some protein granules were assembled gradually and formed the cortical rods (Fig. 9G). The vitelline envelope developed, and the yolk granules appeared homogenous. The follicle cells detached from the oocyte. In the **spent-recovering stage** during October - December, the ovary 4000 µm in diameter returned to the morphometry of January-March (Figs. 9h).

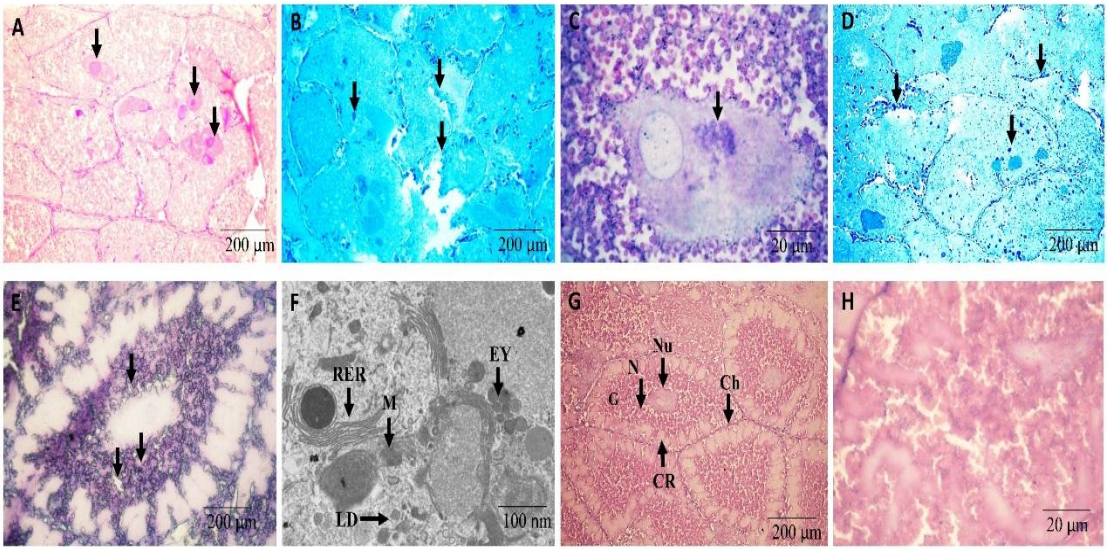

Fig. 9 Histochemical and structure analysis of the ovary. A-C. CS showing oocyte development during July - September. Deposition of yolk granules proceeded intensely in the ooplasm and many vacuoles were developed inside the ooplasm and dispersed gradually to the centre. Protein (peptide linkage), glycogen, basic proteins and acid MPS granules were deposited. Glycoproteins were formed gradually and replaced the centrally located vacuoles. **D.** CS showing the vitelline envelope became clearly visible under the follicular epithelia. Pigmented granules were observed at the oocyte periphery. MPS were intense at the oocyte periphery while proteinous granules were perinuclear. **E.** Lipid droplets were deposited during July-September. **F.** TEM showing the ooplasm contained electron-dense yolk globules, mitochondria, lipid droplets and RER. The follicle epithelium had mitochondria but no developed RER in its ooplasm. Active endocytosis was observed at the oocyte surface. **G**. In the **mature stage** during July - September, as the oocyte proceeded in development, some protein granules were assembled gradually and formed the cortical rods. N= Nucleus; Nu= Nucleolus; G= Granules; FE= Follicular Epithelium; Ch= Chorion. **H.** In the spent-recovering stage during October - December, the ovary 4000 µm in diameter returned to the morphometry of January-March.

The gonadosomatic index (GSI) was used to determine the maturity of female *M. kerathurus*. Our data showed that the GSI was highest during summer and then followed by autumn. Furthermore, it declined to the lowest level during winter and increased in spring (Table 1). From this histological appearance of the ovary of the female *Melicertus kerathurus*, it can be concluded that there was one ovarian cycle annually that began in January and ended in December.

Table 1. Gonadosomatic Index (GSI)

Time of the year	No. of tested females	Female wet weight in g	Ovary wet weight in g	GSI
Spring	1st female	52.7	8.3	18.7
	2nd female	50.2	7.9	18.7
	3rd female	54.7	9.5	21.0
Summer	1st female	55.6	12.1	27.8
	2nd female	53.1	14.2	36.5
	3rd female	52.0	13.7	35.8
Autumn	1st female	49.3	6.6	15.5
	2nd female	48.4	7.1	17.2
	3rd female	42.7	8.0	23.1
Winter	1st female	43.4	3.1	7.7
	2nd female	47.6	4.4	10.2
	3rd female	41.1	4.6	12.6

DISCUSSION

Decapods include lobsters, shrimps and crabs which represent commercially edible and delicacies protein-rich food worldwide. Marine species dwell predominantly in benthic intertidal and sub-littoral zones. There are many environmental factors controlling the fecundity of shrimps in a given ecosystem including temperature [19], photoperiod [20], dissolved oxygen [21], quality of nutrients [22], nitrite concentration in water [23], pollution stress [24], and parasites [25]. In some decapods fluctuations in the environmental factors do not affect their fertility. *Panopeus africanus, Uca tangeri* and *Metopograpsus messor* spawn all the year round while *Aristaeomorpha foliacea* [26] and *Macrobrachium rosenbergii* [27] have a definite spawning period. *Penaeus duorarum* [28] and *Penaeus setiferus* [29] spawn at intervals in each season.

Previous physiological studies focused on the hormones influencing ovarian development [4, 30]. Crustacean reproduction is under the control of stimulatory and inhibitory hormones. X organ sinus gland complex secretes the gonad inhibitory

hormone (GIH) and the brain , thoracic ganglia and mandibular organ secrete the gonad stimulatory hormone (GSH) [30]. High concentration of MIH (molting inhibiting hormone) and GSH and low concentration of GIH and MH (molting hormone) always resulted in reproduction [5]. While GSH acted as a direct acceleratory hormone, MIH acted indirectly as gonad acceleratory hormone by inhibiting the production of MH by Y organ. Reproduction is under endogenous regulation by the nervous and endocrine systems. Reproduction in crustacea is regulated by a complex system of hormones in which hormones of different nature and origin may signal for gonad maturation and spawning. Crustacean mandibular organs regulate reproduction in crustacean females [31]. The mandibular organ which controls growth and reproduction is known to be controlled by mandibular organ inhibiting hormone (MOIH) originating from the eyestalk [32]. Recent developments in crustacean endocrinology have given an insight into the importance of ecdysteroids in female reproduction. Eyestalk neuropeptides such as MIH is found to have an inhibitory control on ecdysteroid synthesis by the Y-organ [33]. Mandibular organ is suggested to have a stimulatory role for Y organ [32]. Different functions of the hormones secreted by the sinus gland were proposed in the available literature. They have profound effects on reproductive processes [34], assimilation , oxygen consumption and molting [35, 36]. Abiotic factors such as temperature & photoperiod and the proteinous richness of nutrients influenced directly the neurosecretroy center (X organ sinus gland complex) to secrete inhibiting hormones [1]. The neurosecretory centre secretes a group of neuropeptide hormones referred to as MIH, MOIH, CHH and GIH [37].

Unilateral eyestalk ablation in *P. monodon* aquaculture is intensively used to induce spawning in many crustaceans. The purpose of this ablation is to diminish the concentration of inhibitory hormones in the hemolymph secreted by the X- organ and sinus gland [17]. This unilateral ablation negatively affects all shrimp physiology [11]. Moreover, ablated females suffered physiological impairment in glucose metabolism and reproductive performance. Unilateral ablation led to an increase in larval mortality [38]; diminished fecundity [4]; impairment in ovary development and spawning [26]. The present study showed that the neurosecretory cells of *Melicertus kerathurus* were grouped into four types based on size, shape and secretory activity and these cells pass into three phases, inactive, vacuolar and secretory. Neurosecretory granules in type I and II neurons of the supraesophageal ganglion were maximal during July - September, diminished gradually during October - June. While types III and IV neurons were active during October till June and inactive during July - September. Many types of neurons were identified in the brain of the freshwater crab *Spirotelphusa hydrodroma* and spiny lobster *Panulirus homarus* [39]. These authors found out that the neurosecretory cells contained precursor materials which became elaborated and mature. Moreover, they suggested that the neurosecretory cells have two phases comprising a formation phase and secretory phase and the less staining reaction is exhibited by the neurosecretory cells of early stages.

Following dissection, GSI was calculated for each female shrimp to assess the degree of ovary maturation [12]. In this study the oocyte development of *Melicertus kerathurus* was demonstrated using a combination of various methods (histological description of ovaries, analyses of oocyte size-frequency distributions, and calculations of the GSI). These methods have been successfully used to describe gonad cycles of numerous temperate and tropical decapod crustaceans [40] and represent standard methods for the study of the reproductive biology [41]. Vitellogenesis of *Melicertus kerathurus*, as described by our results, is in agreement with general patterns of reproductive cycles previously observed in crustaceans [42]. A significant correlation related to inactivity of protein production of the hepatopancreas during non-mature stages, a drop in GSI was observed. During the mature ovarian stage protein was synthesized in the hepatopancreas in secondary vitellogenesis and was accompanied with a high level of GSI: this finding was previously observed in the penaeid shrimp *Marsupenaeus japonicus* [43].

Ovary development and spawning varies in different shrimp species and the abiotic factors exert a great infleunce. This research concluded that vitellogenic and postvitellogenic oocytes were predominated during summer and early fall whereas oogonia and previtellogenic oocytes dominated during late fall to early summer. Egg quality was affected by maternal hygiene [44]. The protein moiety of the yolk is used for larval tissue development while the fat moiety is used for energy production and represent the main constituent of plasma membrane. The present study showed Yolk production and extracellular cortical rod formation in *Melicertus kerathurus* which are considered important factors in determining the quality of the oocyte and the larval hygiene. The extracellular cortical rods were formed and extended into the peripheral ooplasm at the final stage of vitellogenesis. These cortical rods were formed of proteins or / and carbohydrates with respect to oocyte maturation stages. The proteins present in the cortical rods migrated from the ooplasm during oocyte development. This cortical rod protein forms a continuous jelly-like layer around the fertilized egg. Most crustaceans produce the yolk material in a biphasic manner; an Autosynthesis by the oocyte organelles followed by a heterosynthesis phase where the vitellogenin (apolipocrustacein) is formed from the outside [45]. As the oocyte attains vitellogenesis, vitellogenin molecules are carried by hemolymph and enter the ooplasm through pinocytosis as in *E. sinensis* [6]. Accumulation of the extra-ovarian vitellogenin, yolk protein precursor, by vitellogenic oocytes is the role of most of the oviparous animals [11]. Some crustaceans like *Penaeus japonicas* rely entirely on the ovary to complete vitellogenesis [46]. By contrast, in *Penaeus semisulcatus* vitellin is synthesized in the ovary as well as vitellogenin in the hepatopancreas [37].

In decapods the hepatopancreas is a storage organ of food reserves, it accumulates lipids and glycogen which differ with seasons and with the stages of matuirity or molt cycle [47]. It secretes most of the digestive enzymes and involved in carbohydrate metabolism [48]. Cyclic fluctuations in the carbohydrate content of the hepatopancreas of the crab *P. hydrodromous* with respect to ovarian growth has been reported [6]. The consumption of the reserves of hepatopancreatic has been reported in some decapods such as *M. messor* [49], *Portunus pelagicus* (Linnaeus,1758), *Uca*

pugilator [50] and *Aegla platensi* [51]. In female crabs, *Sesarma intermedia* the physiology of the hepatopancreas is correlated to the ovarian growth [52]. Crustacean hepatopancreas is considered to be the site of vitellogenin synthesis [53]. Hepatopancreas functions in digestion , absorption, accumulation and metabolism of stored nutrients of which some molecules are transported to the ovary during vitellogenesis. [54].

The present study concluded that vitellogenesis in *Melicertus kerathurus* was done inside the ovary and hepatopancreas. RER, and Golgi apparatus of the vitellogenic oocyte synthesized proteins, carbohydrates and lipid of yolk. Mitochondria were numerous and the nucleus was prominent. This result agreed with the finding of *Penaeus monodon* [55]; *Macrobrachium resenbergii* [27]; *Cherax quadricarinatus* [56] ; *Pandalus hypsinotus* [45]; *Penaeus monodon* [57]. [58] concluded that the fatty acids are carried in the haemolymph from the GI tract on carrier proteins to the hepatopancreas where metabolism takes place. There are controversial opinions in the available literature concerning vitellin and its precursor vitelloginin [57]. Vitellin was found in the ovaries of *Penaeus monodon* [59] and the oocytes [57] ; vitellin precursor was found and quantified in the body fluid of *Macrobrachium resenbergii* [60] ; in the hepatopancreas of *Penaeus monodon* [61]. Other authors concluded that both the ovary and the hepatopancreas synthesize the yolk precursors as in *Penaeus mondon* [55]. Minagawa & Sano (1997) [62] examined the gene expression of vitellogenin during the gonadal cycle and concluded that both the ovary and hepatopancreas synthesize the yolk precursors for *P. monodon.* Longyant et al. (2008) [17] found that the vitellogenin concentration in the in hemolymph of *P. monodon* showed a drop as the ovary matured while Vincent et al. (2001) [63] did not prove this drop during maturity. Although these two separate studies used the same quantifification technique of ELISA, they showed significant differences of vitelloginin concentrations during the different stages of ovary development of *P. monodon*. During the immature stage of the ovary vitellogenin was found in the haemolymph [63] by Vincent et al. (2001) while Longyant et al. (2008) [17] concluded that vitellogenin was found in the hemolymph during the late stage of oocyte development. Avarre *et al*. (2001) [64] concluded that extracts of the sinus gland contain neuropeptides which inhibited vitellinin synthesis and retarded ovary development. Subramoniam (2017) [32] concluded that production of vitellin in the ovary in *P. semisulcatus* decreased as the cortical rods form and proposed that the oocyte began to build cortical rod proteins rather than vitellin.

Our study concluded that the maturation of the ovary is dependent on other organs during the year. Most importantly, the supraesophogeal ganglion activates ovarian maturation whereas the eyestalk suppresses this activity.

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REFERENCES

1. Coman, G.J., et al., *Reproductive performance of reciprocally crossed wild-caught and tank-reared Penaeus monodon broodstock.* Aquaculture, 2006. **252**(2-4): p. 372-384.
2. Ibarra, A.M., T.R. Famula, and F.G. Arcos, *Heritability of vitellogenin in hemolymph, a pre-spawning selectable trait in Penaeus (Litopenaeus) vannamei, has a large genetic correlation with ovary maturity measured as oocytes mean diameter.* Aquaculture, 2009. **297**(1-4): p. 64-69.
3. Gutierrez-Wing, M.T. and R.F. Malone, *Biological filters in aquaculture: Trends and research directions for freshwater and marine applications.* Aquacultural Engineering, 2006. **34**(3): p. 163-171.
4. Pamuru, R.R. and N. Hosani, *Natural and Induced (Eyestalk Ablation) Molt Cycle in Freshwater Rice Field Crab Oziothelphusa Senex Senex.* Journal of Aquaculture Research & Development, 2016. **07**(04).
5. Hesni, M.A., et al. *Influence of Eyestalk Ablation and Temperature on Molting and Mortality of Narrow-clawed Crayfish (Astacus leptodactylus)*. 2008.
6. Alfaro-Montoya, J. and L.A. Vega, *The effect of environmental cues and neurotransmitters on male sexuality of the eastern Pacific Penaeus (Litopenaeus) species.* Aquaculture, 2011. **316**(1-4): p. 60-67.
7. Alfaro-Montoya, J., *The reproductive conditions of male shrimps, genus Penaeus, sub-genus Litopenaeus (open thelyca penaeoid shrimps): a review.* Aquaculture, 2010. **300**(1-4): p. 1-9.
8. Padmaja, M., M. Deecaraman, and M. Jaganathbose, *Study of neurosecretory cells in sand lobster Thenus orientalis of Royapuram Coast-Chennai.* J. Fish Mar. Sci, 2010. **2**: p. 82-85.
9. Figueiredo, J., et al., *Fecundity, brood loss and egg development through embryogenesis of Armases cinereum (Decapoda: Grapsidae).* Marine Biology, 2008. **154**(2): p. 287-294.
10. Huang, P., et al., *Identification of the small heat shock protein, HSP21, of shrimp Penaeus monodon and the gene expression of HSP21 is inactivated after white spot syndrome virus (WSSV) infection.* Fish & Shellfish

Immunology, 2008. **25**(3): p. 250-257.

11. Quackenbush, L.S., *Yolk Synthesis in the Marine Shrimp,Penaeus vannamei.* American Zoologist, 2001. **41**(3): p. 458-464.
12. Pawson, M., G. Pickett, and P. Witthames, *The influence of temperature on the onset of first maturity in sea bass.* Journal of Fish Biology, 2000. **56**(2): p. 319-327.
13. Glass, B., *Notes on Microscopical Technique for Zoologists. C. F. A. Pantin.* The Quarterly Review of Biology, 1947. **22**(2): p. 162-162.
14. Mahoney, R., *Laboratory Techniques in Zoology. Roy Mahoney.* The Quarterly Review of Biology, 1976. **51**(1): p. 139-140.
15. Pearse, A.G.E., *Histochemistry.* Theoretical and applied, 1968. **1**: p. 1-759.
16. Grd, D., et al., *Analysis of Heavy Metals Concentration in Wastewater along Highways in Croatia.* Journal of Computing and Information Technology, 2012. **20**(3).
17. Longyant, S., et al., *Identification of Vibrio spp. in vibriosis Penaeus vannamei using developed monoclonal antibodies.* Journal of Invertebrate Pathology, 2008. **98**(1): p. 63-68.
18. Le Roux, A., *Description d'organes mandibulaires nouveaux chez les crustaces decapodes.* Comptes Rendus de'lAcademie des Sciences, 1968. **266**: p. 1414-1417.
19. Pervaiz, P.A., M. Sudan, and S. Manohar, *Studies on the effect of photoperiodism and temperature on moulting of a freshwater prawn Macrobrachium dayanum.* International Journal of Fisheries and Aquatic Studies, 2015. **3**(1): p. 325-328.
20. Shpigel, M., et al., *The effect of photoperiod and temperature on the reproduction of European sea urchin Paracentrotus lividus.* Aquaculture, 2004. **232**(1-4): p. 343-355.
21. Cheng, W., C.-H. Li, and J.-C. Chen, *Effect of dissolved oxygen on the immune response of Haliotis diversicolor supertexta and its susceptibility to Vibrio parahaemolyticus.* Aquaculture, 2004. **232**(1-4): p. 103-115.
22. Little, D.C. and P. Edwards, *Impact of nutrition and season on pond culture performance of mono-sex and mixed-sex Nile tilapia (Oreochromis niloticus).* Aquaculture, 2004. **232**(1-4): p. 279-292.
23. Wang, W.-N., et al., *Effects of nitrite on lethal and immune response of Macrobrachium nipponense.* Aquaculture, 2004. **232**(1-4): p. 679-686.
24. Pan, C.-H., Y.-H. Chien, and B. Hunter, *The resistance to ammonia stress of Penaeus monodon Fabricius juvenile fed diets supplemented with astaxanthin.* Journal of Experimental Marine Biology and Ecology, 2003. **297**(1): p. 107-118.

25. Reed, L.A., T.C. Siewicki, and J.C. Shah, *Pharmacokinetics of oxytetracycline in the white shrimp, Litopenaeus setiferus.* Aquaculture, 2004. **232**(1-4): p. 11-28.

26. Kao, H.-C., T.-Y. Chan, and H.-P. Yu, *Ovary development of the deep-water shrimp Aristaeomorpha foliacea (Risso, 1826)(Crustacea: Decapoda: Aristeidae) from Taiwan.* ZOOLOGICAL STUDIES-TAIPEI-, 1999. **38**(4): p. 373-378.

27. Revathi, P., et al. *Vitellogenesis during the ovarian development in freshwater female prawn Macrobrachium rosenbergii (De Man)*. 2012.

28. Caillouet Jr, C.W. *OVARIAN MATURATION INDUCED BY EYESTALK ABLATION IN PINK SHRIMP, Penaeus duorarum BURKENROAD 1*. in *Proceedings of the annual workshop-World Mariculture Society*. 1972. Wiley Online Library.

29. Lindner, M.J. and W.W. Anderson, *Growth, migrations, spawning and size distribution of shrimp Penaeus setiferus*. 1956: US Government Printing Office.

30. S B Sainath, C.H.S., *Reproductive Endocrinology of Female Crustaceans: Perspective and Prospective.* Journal of Marine Science: Research & Development, 2011. **s3**.

31. Qi, Z., et al., *Probiotics in aquaculture of China — Current state, problems and prospect.* Aquaculture, 2009. **290**(1-2): p. 15-21.

32. Subramoniam, T., *Crustacean ecdysteriods in reproduction and embryogenesis.* Comparative Biochemistry and Physiology Part C: Pharmacology, Toxicology and Endocrinology, 2000. **125**(2): p. 135-156.

33. Lachaise, F., et al., *Ecdysteroid synthesis by crab Y-organs.* Journal of Experimental Zoology, 1989. **252**(3): p. 283-292.

34. Padmaja, R., et al., *Brine shrimp lethality bioassay of selected Indian medicinal plants.* Fitoterapia, 2002. **73**(6): p. 508-510.

35. Jeon, J.-M., et al., *Two type I crustacean hyperglycemic hormone (CHH) genes in Morotoge shrimp (Pandalopsis japonica): cloning and expression of eyestalk and pericardial organ isoforms produced by alternative splicing and a novel type I CHH with predicted structure shared with type II CHH peptides.* Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2012. **162**(4): p. 88-99.

36. Webster, S.G., R. Keller, and H. Dircksen, *The CHH-superfamily of multifunctional peptide hormones controlling crustacean metabolism, osmoregulation, moulting, and reproduction.* General and comparative endocrinology, 2012. **175**(2): p. 217-233.

37. Kang, B.J., et al., *Dynamics of Vitellogenin and Vitellogenesis-Inhibiting Hormone Levels in Adult and Subadult Whiteleg Shrimp, Litopenaeus*

vannamei: Relation to Molting and Eyestalk Ablation1. Biology of Reproduction, 2014. **90**(1).

38. Palacios, E., C. RodrÍGuez-Jaramillo, and I.S. Racotta, *Comparison of ovary histology between different-sized wild and pond-reared shrimpLitopenaeus vannamei(=Penaeus vannamei).* Invertebrate Reproduction & Development, 1999. **35**(3): p. 251-259.

39. Fernandez, R. and E.V. Radhakrishnan, *Classification and mapping of neurosecretory cells in the optic,supraoesophageal and thoracic ganglia of the female spiny lobster Panulirushomarus (Linnaeus, 1758) and their secretory activity during vitellogenesis.* 2010.

40. Turkmen, G. and H. Yilmazyerli, *Some biological aspects of Melicertus kerathurus (Forskål, 1775) (Decapoda, Penaeidae) inhabiting İzmir Bay (Aegean Sea), Turkey.* Crustaceana, 2006. **79**(5): p. 583-591.

41. Peixoto, S., et al., *Comparison of reproductive output, offspring quality, ovarian histology and fatty acid composition between similarly-sized wild and domesticated Farfantepenaeus paulensis.* Aquaculture, 2008. **285**(1-4): p. 201-206.

42. Ansell, A., et al., *Life—cycle and life—history diversity in marine invertebrates and the implications in community dynamics.* Oceanography and Marine Biology: An Annual Review, 1994. **32**: p. 305-333.

43. Qiu, G.-F., K. Yamano, and T. Unuma, *Cathepsin C transcripts are differentially expressed in the final stages of oocyte maturation in kuruma prawn Marsupenaeus japonicus.* Comparative Biochemistry and Physiology Part B: Biochemistry and Molecular Biology, 2005. **140**(2): p. 171-181.

44. Whitmore, K., et al., *ASSESSMENT REPORT.* 2012.

45. Tsutsui, N., et al., *Molecular characterization of a cDNA encoding vitellogenin in the coonstriped shrimp,Pandalus hypsinotus and site of vitellogenin mRNA expression.* Journal of Experimental Zoology, 2004. **301A**(10): p. 802-814.

46. Raikhel, A.S., *Reproductive Biology of Invertebrates, Vol. 12, Part B*. 2005, CRC Press.

47. Gutiérrez, R. and R. Sánchez, *Diagnostico de la actividad pesquera artesanal en el Estero Real.* Centro de Investigaciones Pesqueras y Acuicolas (CIPA), Instituto Nicaraguense de la Pesca y Acuicultura (INPESCA), Managua, 2007.

48. Bliss, D.E., *The Biology of Crustacea*, in *Internal Anatomy and Physiological Regulation*. 1983, Elsevier. p. ii.

49. Sudha, K. and G. Anilkumar, *Elevated Ecdysteroid Titer and Precocious Molt and Vitellogenesis Induced by Eyestalk Ablation in the Estuarine Crab, Metopograpsus Messor (Brachyura: Decapoda).* Journal of Crustacean Biology, 2007. **27**(2): p. 304-308.

50. Pillay, K.K. and N.B. Nair, *Observations on the biochemical changes in gonads and other organs of Uca annulipes, Portunus pelagicus and Metapenaeus affinis (Decapoda: Crustacea) during the reproductive cycle.* Marine Biology, 1973. **18**(3): p. 167-198.

51. Sokolowicz, C.C., G. Bond-Buckup, and L. Buckup, *Dynamics of gonadal development of Aegla platensis Schmitt (Decapoda, Anomura, Aeglidae).* Revista Brasileira de Zoologia, 2006. **23**(4): p. 1153-1158.

52. Kyomo, J., *Analysis of the relationship between gonads and hepatopancreas in males and females of the crab Sesarma intermedia, with reference to resource use and reproduction.* Marine Biology, 1988. **97**(1): p. 87-93.

53. Charniaux-Cotton, H., *Vitellogenesis and Its Control in Malacostracan Crustacea.* American Zoologist, 1985. **25**(1): p. 197-206.

54. Minh Sang, H. and R. Fotedar, *Growth, survival, haemolymph osmolality and organosomatic indices of the western king prawn (Penaeus latisulcatus Kishinouye, 1896) reared at different salinities.* Aquaculture, 2004. **234**(1-4): p. 601-614.

55. Tseng, D.-Y., et al., *Hepatopancreas is the extraovarian site of vitellogenin synthesis in black tiger shrimp, Penaeus monodon.* Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology, 2001. **129**(4): p. 909-917.

56. Abdu, U., et al., *The vitellogenin cDNA of Cherax quadricarinatus encodes a lipoprotein with calcium binding ability, and its expression is induced following the removal of the androgenic gland in a sexually plastic system.* General and Comparative Endocrinology, 2002. **127**(3): p. 263-272.

57. Chen, C.-C. and S.-N. Chen, *Isolation and partial characterization of vitellin from the egg of the giant tiger prawn, penaeus monodon.* Comparative Biochemistry and Physiology Part B: Comparative Biochemistry, 1993. **106**(1): p. 141-146.

58. Chang, E.S., S.A. Chang, and E.P. Mulder, *Hormones in the Lives of Crustaceans: An Overview.* American Zoologist, 2001. **41**(5): p. 1090-1097.

59. Tom, M., M. Goren, and M. Ovadia, *Localization of the Vitellin and Its Possible Precursors in Various Organs ofParapenaeus longirostris(Crustacea, Decapoda, Penaeidae).* International Journal of Invertebrate Reproduction and Development, 1987. **12**(1): p. 1-12.

60. Jasmani, S., et al., *Hemolymph vitellogenin levels during ovarian development in the kuruma prawn Penaeus japonicus.* Fisheries Science, 2000. **66**(3): p. 535-539.

61. Quinitio, E.T., et al., *Isolation and characterization of vitellin from the ovary ofPenaeus monodon.* Invertebrate Reproduction & Development, 1990. **17**(3): p. 221-227.

62. Minagawa, M. and M. Sano, *Oogenesis and ovarian development cycle of the spiny lobster Panulirus japonicus (Decapoda:Palinuridae).* Marine and Freshwater Research, 1997. **48**(8): p. 875.

63. Prakash Vincent, S.G., R. Keller, and T. Subramoniam, *Development of Vitellogenin-ELISA, an In Vivo Bioassay, and Identification of Two Vitellogenesis-Inhibiting Hormones of the Tiger Shrimp Penaeus monodon.* Marine Biotechnology, 2001. **3**(6): p. 0561-0571.

64. Avarre, J.C., et al., *Inhibition of de Novo Synthesis of a Jelly Layer Precursor Protein by Crustacean Hyperglycemic Hormone Family Peptides and Posttranscriptional Regulation by Sinus Gland Extracts in Penaeus semisulcatus Ovaries.* General and Comparative Endocrinology, 2001. **124**(3): p. 257-268.