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β-Carotene Analysis and Histological Investigation on Different Egg Stages of Anomuran Crab, *Emerita Asiatica* (H. Milne Edwards, 1837)

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Abstract - **Histological investigation and β-carotene analysis of different egg stages of anomuran crab, *Emerita asiatica* was studied. This investigation characterized the morphological and histological features of the ovaries; eggs and also resulted in the identification of the following structures such as Previtellogenic Oocyte, Vitellogenic Oocytes, Atretic Oocyte, matured Oocyte and its associated structures which are quiet common structures that are found in almost every Sand Crab. This study can be further used in studying the reproductive biology of this Sand Crab (*Emerita asiatica*). It can also be used in determining the structural and morphological changes that can happen in gonads due to the action of various microorganisms such as bacteria, viruses and fungi by artificial infections. The observation described in this study will serve as a reference for future research aimed at studies based on environmental factors, temperature, climatic conditions, and feeding that might have an effect on this species. The conversion of β-carotene into vitamin A (retinol), for clear eyesight and healthy eyes, a robust immune system, and healthy skin and mucous membranes, we require vitamin A. Large amounts of vitamin A can be hazardous, but our bodies only make as much of the vitamin from β-carotene as is required. The sand crab serves as a different source of β-carotene than vitamin A, even though *E. asiatica* is not widely used. Though *E. asiatica* is not a commercially viable crab, it plays a vital role in the environment to maintain a stable marine ecosystem. Several steps and preventive measures should be taken to conserve these members of the marine food chain to have a stable ecosystem and also to protect this species from extinction. Further, this study can potentially benefit as baseline information for the future research on the species.**
***Keywords**: **Emerita asiatica*, β-carotene, Cleavage, Gastrulation, Previtellogenic Oocyte**

I. INTRODUCTION

Fish, crustaceans, and mollusks made up 43% of the aquatic animal food consumed by humans in 2007, but mammals, reptiles, and aquatic plants were not included. To accommodate future demand, more growth is anticipated. Using either all or part of the natural production, the dominance of shellfish, herbivorous, and omnivorous pond fish is quite different and incompatible in many ways. The globalization of the commerce and the advantageous economics of large-scale intensive farming have helped to dramatically increase the production of species like salmon, shrimp, and catfish. Globally, aquaculture is expanding, and the idea of sustainable aquaculture is becoming more widely understood to include both spatial and temporal aspects of environmental, economic, and social characteristics [1].

Aquaculture has roots in ancient China and may be at least 4,000 years old. Asia currently produces the majority of the world's aquaculture. The yearly increase in aquaculture output is being hampered by increased competition for adequate land and water, issues with effluent from aquaculture facilities, disease outbreaks, and potential shortages of animal protein for aquatic animal diets. A comeback of industry growth might be sparked by new technologies, such as the use of genetic engineering techniques to enhance aquatic species' performance and disease resistance, the development of water reuse systems, and the construction of offshore facilities [2].

An overarching blue economy/blue growth plan looks at measures to lessen the overall impact these economic sectors have on biodiversity, live aquatic resources, and ecosystem services. Blue Economy/Blue Growth policies have been undertaken in a number of coastal developing nations, especially Small Island Developing States (SIDS), to support the early promotion of food security and decent livelihoods. According to the situation, they initially focus on fisheries, aquaculture, ecosystem services, marine tourism, and coastal tourism with the intention of eventually incorporating other significant sectors. The present state of fisheries and aquaculture is examined in this research in relation to the Blue Economy/Blue Growth and its applicability to African coastal nations.

With appropriate specifics on the FAO's strategy and initiatives, it outlines the current status of ideas and action plans for the blue economy and blue growth, in particular for sustainable fisheries and aquaculture. It will soon be enhanced by insights from other organizations focused on the development of African fisheries and aquaculture [3]. Aquaculture is the area of food production that is expanding the quickest, and marine aquaculture supplies 38% of the world's needs. Many nations, including the United States, are seeking sectoral expansion because to projections of

rising demand for seafood and the role that aquaculture will play in meeting it (US).The Trump administration's Promoting American Seafood Competitiveness and Economic Growth Executive Order (EO) identifies the growth of aquaculture as a means of reducing the country's seafood trade imbalance, enhancing seafood security, creating employment, and boosting rural prosperity. The EO's terminology matches a broader narrative that characterizes marine aquaculture as an "immense opportunity" for economic growth with minimal physical or biological limitations and opportunities for market expansion [4].

To enable and encourage capital investment, the Trump EO offers the possibility of expediting permits and lowering regulatory hurdles to entry. A broad interest in the Blue Economy, a concept that was introduced at the 2012 UN Conference on Sustainable Development (Rio+20), is reflected in the enthusiasm for the growth of marine aquaculture. Blue Economy, which was first defined differently by different ocean stakeholders, places a greater emphasis on economic growth by portraying the oceans as "development zones" and a "commodity frontier" [5]. Even potential conflict with commercial fishing is reframed as an opportunity because fishermen in communities historically dependent on declining wild capture fisheries may find new employment in aquaculture while also supplying the skilled labour needed for a developing and diversified seafood industry.

The rhetoric on opportunity, however, is silent on the precise processes through which global economic expansion will convert into tangible local economic gains. Regarding associated decision-making procedures that might direct aquaculture growth in order to offer such advantages, particularly in methods that take justice and fairness in benefit quality and distribution into consideration, it is mostly quiet. Although MSP is suggested as a way to settle disputes, how it will do so (and by whom) is not mentioned. Overall, "trade, ecological, and technological incentives are taking precedence over local socio-economic ramifications of aquaculture expansion" [5].

With over 91% of the world's aquaculture production currently produced in Asia (102.9 million tonnes in 2017), aquaculture continues to dominate the production of aquatic food both in Asia and around the world. Total global aquaculture production has now surpassed total global capture fisheries production by over 18.32 million tonnes. Developing nations presently account for more than 95% of the world's aquaculture production, which is growing there at an average annual rate of 6.13%.

With 328 species reported in 2017, the aquaculture industry represents a very diverse group of aquatic plant and animal species, with total production valued at over US$250 billion. This includes the production of unicellular Chlorella algae in indoor bioreactors and Atlantic salmon (*Salmo salar*) in marine environments. Fish continue to make up the largest major species group in aquaculture by weight (53.4 million tonnes, or 47.7% of all aquaculture production), followed by aquatic plants (31.8 million tonnes, or 28.4%), molluscs (17.4 million tonnes, or 15.4% of all production), crustaceans (8.4 million tonnes, or 7.5% of all production), amphibians and reptiles (471,784 tonnes), and other invertebrate animals (422,124 tonnes); [6]. About 2.36 million hectares of ponds and tanks are used in India's freshwater aquaculture industry, which produces almost 55% of the nation's fish. To raise and grow aquatic creatures (fish, shrimp, crab, shellfish, etc.) and plants for commercial gain, ponds, reservoirs, lakes, rivers, and other inland waterways are used in freshwater aquaculture.

Fish, breeding shrimp made up of Macrobrachium and Hainan prawn, and crabs are the principal breeding targets [8].Sand crabs, sand bugs, sand fleas, or sand crabs belong to the tiny decapod crustacean genus Emerita. These tiny creatures utilize their antennae for filter feeding and burrow in the sand in the swash zone. The Pacific sand crab is a tiny crustacean that can reach lengths of up to 35 mm (1.4 in) and widths of up to 25 mm (1.0 in). The orange egg mass carried beneath the telson, which is approximately twice as large as the male and frequently used to identify the female [9].The adult lacks claws and spines and is sand-colored and well-camouflaged. It contains three pairs of pleopods and five pairs of legs. Periodically, sand crabs moult, causing their exoskeletons to wash up on the shore. The sand crab has an extended dome form that is intended for quick burrowing, and it is well adapted to live in the sand, which presents an unstable substrate [10]. The antennules are also elongated, and the eyes are on long stalks so as to protrude above the sand's surface. These come together to form a tube that directs water via the gills downward. The retractable antennae are substantially longer. They also extend above the sand's surface when there is water overhead to catch food particles. To aid in digging, as well as for use in gathering food and transferring it to the mouth, the legs and uropods have hairy edges [9].

If the sand crab is moving or digging, it always goes backward. Additionally, it has the ability to tread water and swim backward. It is a feeder in suspension. It faces the ocean and sinks back into the beach. Each wave extends its antennae and captures floaters as it recedes. The creature pulls back its antennae and scrapes the debris into its mouth. This might happen more than once every wave. It coils its antennae and digs rearward deeper into the sand when exposed by water [11]. Plankton makes up the majority of the food, particularly dinoflagellates. In the spring and summer, sand crabs mate.

Each month, the female may lay batches of up to 45,000 eggs, which she carries tucked under her belly. The eggs take roughly four weeks to hatch. Five planktonic zoeal stages and a final megalopal stage make up the larvae. Up to 130 days are spent in the zoeal phases. The megalopae disperse onto sandy beaches where they moult and grow into juveniles before becoming adults in a matter of weeks

[12]. The larvae may move far and colonize new places thanks to the prolonged planktonic stage. The adults often die in the fall of their second year after reproducing in both their first and second summers [13]. [14]. The names beta carotene and carota are derived from Greek and Latin, respectively (carrot). The yellow/orange pigment is what gives fruits and vegetables their bright colors. Processing of the tissues was placed at room temperature. The goal of tissue processing is to eliminate water from biological tissues and replace it with a medium that freezes and sets exceedingly hard, enabling the slicing of incredibly thin portions. In order to make the tissue sufficiently rigid but elastic to be sliced into portions of desired thickness using a microtome, the tissue must be treated to impregnate it into a solid medium.

A. Embedding of Paraffin

The tissues were processed and then put in a wax tissue container. Orienting the tissue in molten paraffin, which when it hardens offers a firm surface for maintaining the integrity of all the tissue's components when portions were sliced. Greek "beta" and Latin "carota" is where the name beta carotene originates (carrot). The vibrant colors of fruits and vegetables are due to the yellow/orange pigment. Carotene was first given the name by H. Wachenroder, who crystallized beta carotene from carrot roots in 1831. Vitamin A, a necessary vitamin, is created from beta-carotene. At excessive doses, vitamin A is poisonous. Onions, carrots, peas, spinach, and squash are among the foods high in vitamin A. According to one study, smokers with high levels of beta carotene may be able to prevent cognitive aging. Supplemental beta carotene can interfere with some medications, such as mineral oil and stains. As people get older, beta carotene may help them maintain lung power.

β-carotene is an antioxidant, much like the other carotenoids. Anything that stops other molecules from oxidizing is an antioxidant, protecting the body from free radicals. Free radicals damage cells as a result of oxidation. Numerous chronic diseases can eventually be brought on by free radical damage. Numerous studies have shown how including antioxidants in the diet boosts immune function, protects against free radicals and lowers the risk of cancer and heart disease.

According to several studies, persons who consume at least four servings daily of fruits and/or vegetables high in beta-carotene had a decreased risk of heart disease or cancer. There are few reports on the developmental stages and b carotenoid level of the sand crab *E. asiatica* from the aforementioned accounts and the literature review, and there haven't been any reports on thesis biology for the past 8 years, so the current investigation was carried out to present an authenticated report. From the above said literature there is a need for the study on the analysis of β-carotene on the different egg stages of *E. asiatica* and embryonic developmental stages to conserve these sandy shore fauna in the marine ecosystem.

II. MATERIALS AND METHODS

A. Collection and Maintenance of (Emerita Asiatica) Sand Crab

The Sand Crab, *E. asiatica* were collected from the Injambakkam and Latitude and Longitude lane to be mentioned (130 061 N, 800 241 E), ECR, Chennai - 600115, Tamil Nadu, India 20 Km away from Chennai (Fig. 1). The specimens were collected by hand picking method during the early morning and transported to the Guru Nanak College, Zoology laboratory in polythene bags containing wet sand with adequate aeration and water. The Sand Crabs (Fig. 2) were maintained in a rearing tank with conventional aeration. The Sand Crab had a mean weight of 15g.The water was added on daily basis.

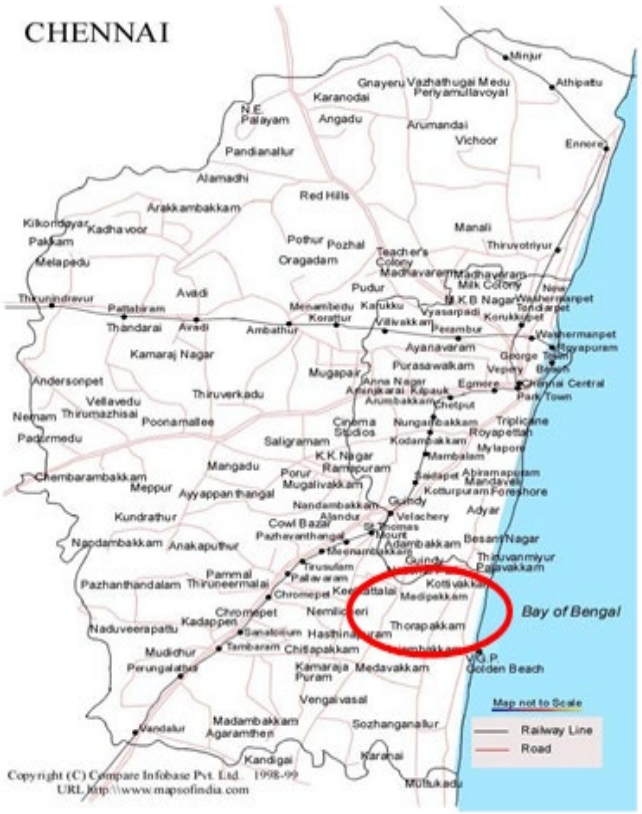

Fig. 1Map showing the Collection Site

Fig. 2 Photograph showing the ventral view of sand crab *E. asiatica* with egg mass in the abdomen

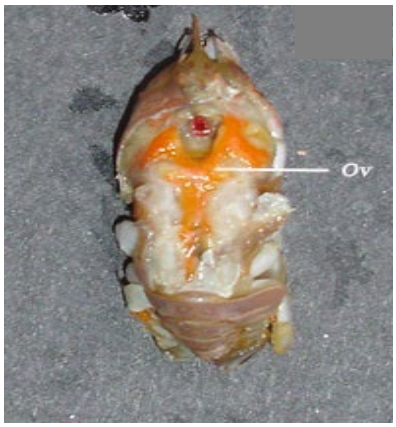

Fig. 3 Dorsal view showing Y shaped ovary for β carotene analysis

B. Histology of (Emerita Asiatica) Sand Crab

For histological investigation, the different stages of gonads of ovaries of Sand crabs were dissected. The organ was fixed in Davidson's alcohol formalin acetic acid fixative. The organ was stored in screw-capped tubes containing the fixative. Initially, the isolated eggs were fixed for 48 hours; thereafter the materials were transferred to 70%alcohol and stored.

C. Preparing Tissue Samples for Histology

Fixing specimens for histology has been performed with the handling of potentially dangerous chemical products. With safety gloves and goggles (or protective glasses), the fixatives were handled throughout the fixation process.

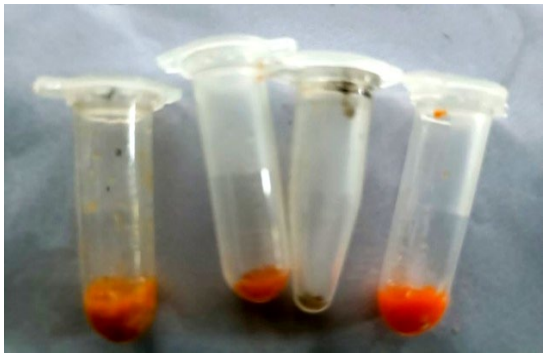

Fig. 4 Eppendorf tubes containing the different egg stages for developmental studies through histology

D. Sample Dissection and Fixing

The Sand Crab was anesthetized or chilled and immediately fixed with appropriate alcohol formalin acetic acid fixative. The Crab was sacrificed, and the target organ (gonads) was dissected out (Fig. 3). It was immediately placed into the fixative and carefully labeled, and it was recommended to take at least ten volumes of fixative for each volume of tissue samples (ratio of 1 part tissue to at least 9 parts formalin). The sample was allowed to fix for at least 24-48 hours before processing. Cassettes were placed on to cold plate and when the wax solidified the samples were removed from the molds and stored at room temperature.

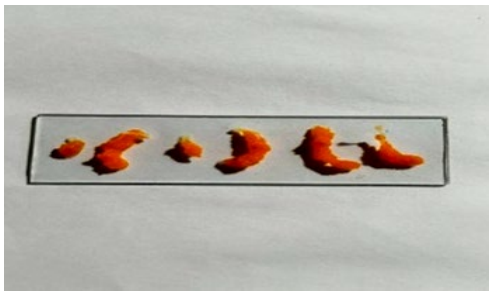

Fig. 5a Separated eggs with pleopods for developmental studies

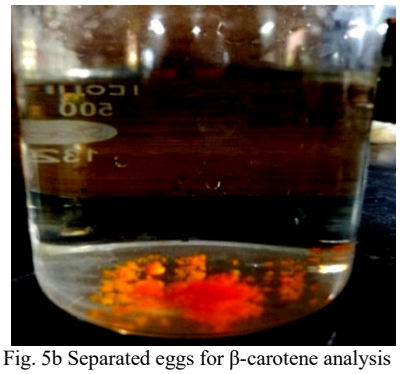

Fig. 5b Separated eggs for β-carotene analysis

E. Microtome Sectioning

Sectioning an embedded tissue sample produced sufficiently thin slices of the sample that the details of the microstructure of cells/tissues were clearly observed using a microscope. A microtome is a mechanical device used for cutting uniform sections of tissue of appropriate thickness by trimming the surface of the paraffin tissue block. The transverse sections were cut at 4-5µm thickness using a manual rotatory microtome. The sliced paraffin block becomes a fragile ribbon made of wax. It was gently placed into warm water bath and picked it up on adhesive coated slides.

F. Hematoxylin and Eosin Staining

Standard stain, the Bennett modification of Mayer's Hematoxylin and Eosin stain, was commonly used in routine histological preparations, and it gave an outstanding routine. With this technique, cell nuclei were stained blue with hematoxylin and eosin, while the cytoplasm and several extracellular components were stained with various hues of pink. The mounting agent was a resinous mounting medium (D.P.X.). The slides were inverted onto the coverslip after being stained with Hematoxylin and Eosin and cleaned off. A drop of the mount was then applied to the cover slip. It was carefully avoided that air bubbles would develop. The coverslip or slide wasn't pushed since doing so may harm the part. Carl Zeiss's binocular compound microscope was used to examine the histology sections. A calibrated ocular micrometer scale with an accuracy of up to 10 m was mounted on a Carl Zeiss microscope for measuring cells. With a Carl Zeiss microscope's projection eyepiece set to 10 X and its objectives set at 10, 20, 40, and 100 X, photomicrographs were obtained.

G. Assay for β-carotene Sample Preservation

Samples were swiftly taken from the animals, put in tiny glass vials with Teflon stoppers on the tops or screw caps, sealed, and frozen at 200°C before being kept for examination (Fig.4, 5a and 5b). After being stored for one week, samples were examined.

H. Sample Extraction

A 10ml screw-capped clean glass vial was filled with 1 gm of tissues that had been quickly weighed to the nearest 10 mg on a piece of aluminum foil. Anhydrous sodium sulfate 2.5 grams were added. The sample was carefully crushed against the side of the vial with a glass rod until the sodium sulfate was fairly evenly distributed throughout. 5 cc of chloroform was used to cover it. The glass vial was sealed and left overnight at 0°C (8-24 hours). One to two centimeters the caked residue was free of chloroform. Similar steps were taken to prepare a blank.

I. Spectrophotometric Analysis

The chloroform extract was diluted with ethanol to a level of 3 ml using an aliquot of 0.3 ml. similar procedures were used to prepare the blank, which was then transferred to a 1 cm cuvette (4 ml capacity) and measured for absorption in a spectrophotometer at 290, 350, 380, 450, 475, and 500 nm. For the carotene analysis, the readings were displayed on a graph.

III. RESULTS OF THE STUDY

A gravid female *E. asiatica* was separated, the ventral anatomy was studied, and a stereoscopic microscope was used to take pictures of the developing embryo. Thin slices of a batch of eggs were treated in a manner comparable to histological analysis. Fertilized eggs of *E. asiatica* were studied and captured with a Leica light microscope to describe embryonic development (Figs. 6, 7, 8 and 9). Pleopods and the genital aperture on the coxae of the third pair of legs were used to distinguish between men and females. At 12.0 mm CL, the first two pairs of pleopods were visible, and at 14 mm CL, all three pairs of pleopods were visible. However, the size of the three pairs of pleopods varied, with the last pair being smaller. Although the ovary already had vitellogenic oocytes at 19 mm CL, it wasn't until 20 mm CL when ovarian females were first shown to be fertile.

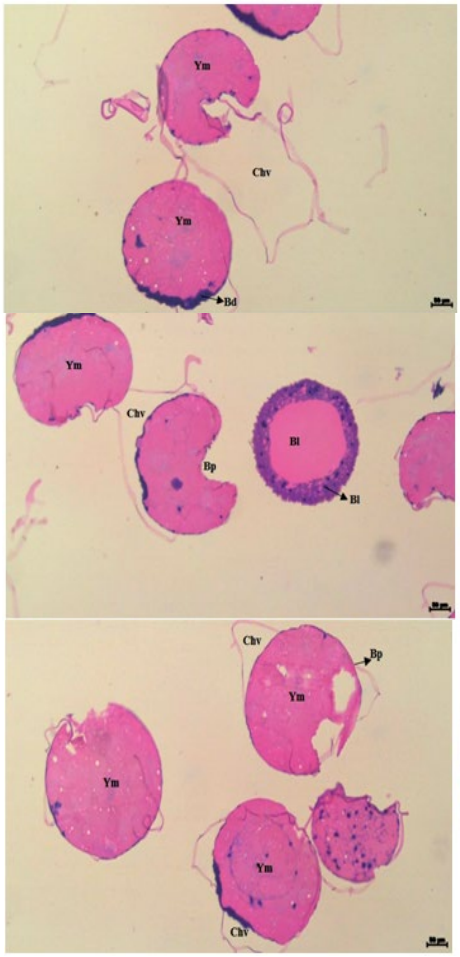

Fig. 6 Photomicrograph showing the histological observation of early embryonic developmental stages of *Emerita asiatica*

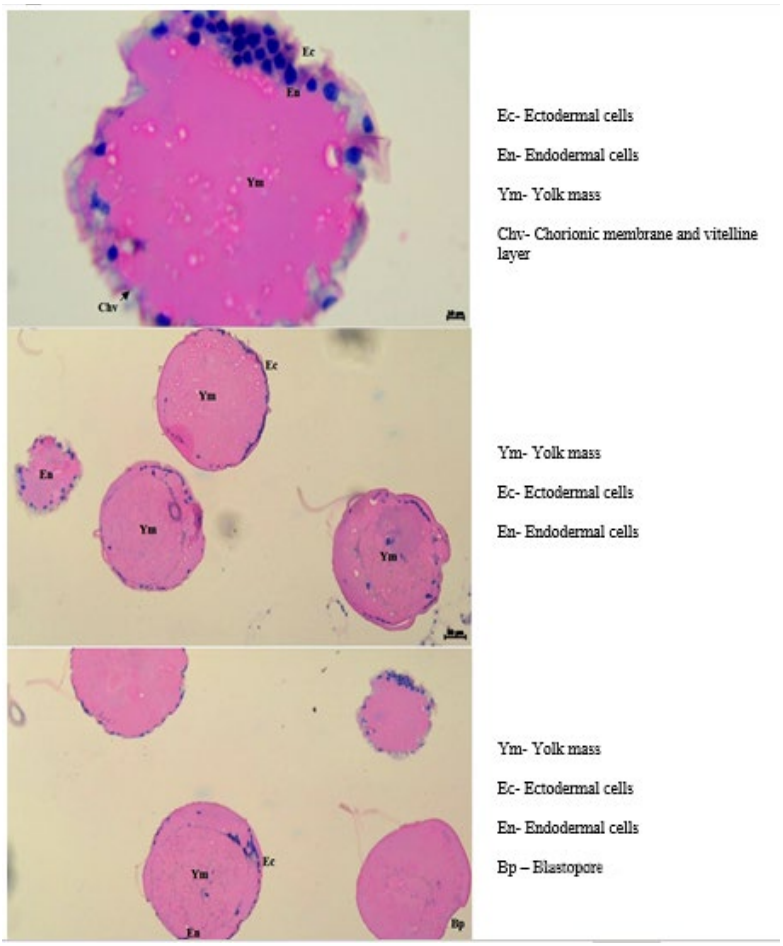

Fig. 7 Photomicrograph showing the histological observation of embryonic development of *Emerita asiatica*; fertilized egg before cleavage; gastrula embryo showing forming blastopore (circle) and cytoplasmic bridges between blastomeres

A. Ovary Structure and Oogenic Differentiations

All specimens in the current study had the same patterns of ovarian characteristics (Fig. 3). The ovarian tissue of *E. asiatica* is an extended organ, filling cephalothorax along the dorsal-ventral axis and extending parallel to the digestive gland, according to histological observation. A single layer of thin ovarian capsule, an outermost layer of thin pavement epithelium, a middle layer of connective tissue, and an interior layer of germinal epithelium encircled the ovarian maturation. The germinal epithelium and a fibrocyte layer made up this capsule. Oocyte differentiation and follicular cell structure were seen in the ovarian parenchyma. In this work, we found that the ovary of E. asiatica included oocytes that were developing asynchronously or at different phases of oogenesis.

Oogenesis may be divided into five oogenic phases based on histological analysis. Under primary growth conditions, a previtellogenic egg had its first meiosis. The cell grew to a diameter of 50–60 m. Similar to *Penaeus semisulcatus*, P. monodon, and *Marsupenaeus japonicus*, the conspicuous nucleus was clearly characterized. The ooplasm at this point had a basophilic homogenous stain. The presence of ribosomes, mRNAs, and stored energy reserves may be connected to basophilic cytoplasm. A layer of elongated follicle cells covered the oocyte. Early vitellogenic oocytes at this stage of development underwent secondary expansion, resulting in oocytes that ranged in size from 100 to 120 m in diameter. With a nucleolus, the nucleus was still discernible.

Due to the tiny acidophilic yolk granules present at this stage, histological analysis showed that the oocyte displayed the acidophilic ooplasm. It is well acknowledged that the yolk granule, which is produced from lipoprotein and serves as a precursor to vitellogenin substrate, is a primary source of energy for the development of crustacean embryos. The ovary and/or hepatopancreas are where vitellogenin is produced. In addition, lipid droplets were seen in the oocytes at this stage. Numerous decapods seem to have this inclusion's structural layout in common. This lipid buildup most likely serves as an important nutritional reserve for its development. At this point, layers of spindle-shaped follicular cells encircled the egg.

It understood that the accumulation of follicular cells may be connected to the ingestion of yolk granules during the embryonic development of *Emerita asiatica* through histology. An aerial shot of the early stages of the embryo has been observed.

Emerita asiatica embryonic development as seen through histology before cleavage, the fertilized egg was in position. A gastrula embryo with a developing blastopore (circle) and cytoplasmic bridges between blastomeres (inset). Early organogenesis, segment across the embryo's cephalothorax and belly along the anterior-posterior axis has been noticed clearly. Slice through the cephalothorax-abdominal plates along the dorsal-ventral axis was observed; a section plane demonstrating early appendages of the developing embryo. During late organogenesis 30% of the yolk mass is visible on the dorsal side at an early stage of late organogenesis. Yolk depletion has been observed during the late organogenesis stage of the developing embryo. Compound eye close-up with lens (inset) has been observed. Pigmented cell and retina has shown in the compound eye. Lateral view has shown with eyes and digestive system; the digestive system and compound eyes are seen in the dorsoventral portion. The cell size at this stage gradually rose and ranged from 200 to 250 m in diameter.

Oocytes began to acquire yolk globules at the same time, and they began to display the reddish-stain of acidophilic cytoplasm (H&E method). The yolk granules were about 10-15 m in size. In fact, it was shown that the yolk granules at this time were more responsive to PAS and MT techniques.

Although the nucleus was still visible, it had shrunk and moved towards the ooplasm's cortical zone, which is known as "eccentric nucleus or germinal vesicle migration." At this time, the follicular cell layers remained unaltered. At this stage of maturity, the cell was at its biggest size; as a result, it was at its most developed state, measuring between 320 and 370 m in diameter.

The buildup of proteinaceous yolk platelet caused the cytoplasm to become heavily acidophilic colored. The absence of a nucleus during this stage was distinctive. It was still possible to see the follicular cell layer as a straightforward squamous epithelium. At this point, the atretic oocyte had an uneven form and degenerating yolk granules throughout the reabsorption process.

B. Morphology and Stages of Embryonic Development

The eggs of *E. asiatica*, which appeared bright yellow, light orange and light brown morphologically and histologically, were affixed to each seta of the pleopods. As a result, it was thought that the abdominal chamber served as an incubator for the developing eggs. Although it is widely known that crustaceans display enormous heterogeneity in the patterns and time of their development, we first observed the embryogenesis of *E. asiatica*, which exhibited pattern comparable to other decapods. According to earlier studies, many crabs' embryonic development has been categorized according to several criteria, including six phases that include the newly produced egg, multi-cell stage, eye stage, pigment stage, heartbeat stage, and pre-hatching stage.

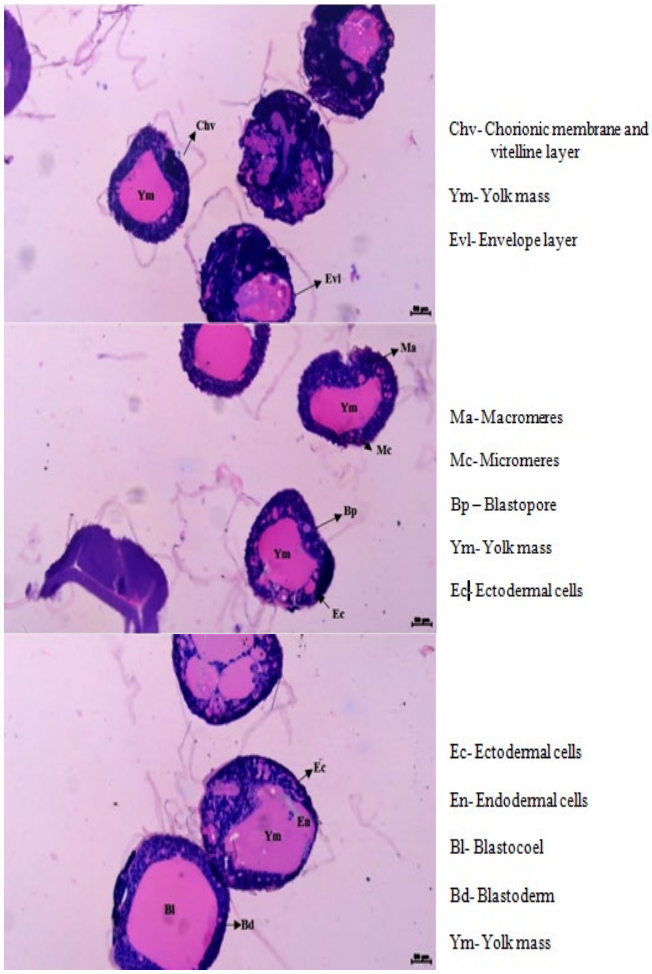

Fig.8 Photomicrograph showing the histological observation of embryonic development of *Emerita asiatica*; early organogenesis; section along an anterior-posterior axis showing cephalothorax and abdomen of embryo;

Others divided embryonic development into the following stages: cleavage, blastulation, gastrulation, segmentation, and appendage, heart, and chromatophores creation surrounding the body. We identified three different decapods embryonic phases of early *E. asiatica* development by histological observation (cleavage, blastula, and gastrula) 2) Post-gastrula early organogenesis; 3) bellows late organogenesis; The centrolecithal eggs of other decapods are similar in appearance to the eggs of *E. asiatica*, which are light yellow in color, spherical, and filled almost entirely with the yolk. The fertilized egg displayed a distinct vitelline layer and outer membrane (chorionic membrane). After cleavage, the yolk was covered by a single layer of cells known as the blastomere. These blastomeres had polygonal shapes and tiny cytoplasmic bridges linked them to one another. This blastomere layer also had a number of thin grooves and cavities, demonstrating the development from blastulation to gastrulation. We saw distinct clusters of thin, little cells at the unilaminar layer's center in certain embryos, which we believe to be the blastopore area, where germ line cells enter the body. There was no evidence of the establishment of any organs or primordial structures during these early development substages.

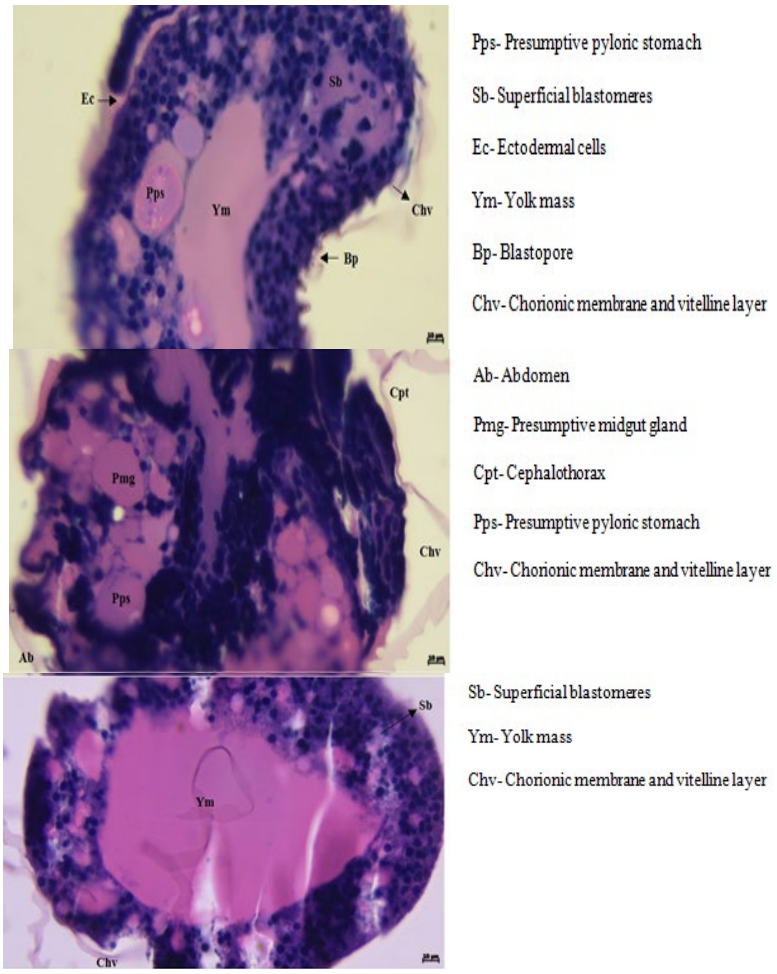

Fig. 9 Photomicrograph showing the histological observation of embryonic development of Emerita asiatica; section along the dorsal-ventral axis through only cephalothorax; late organogenesis; early stage of late organogenesis showing 30% of yolk mass on the dorsal side; late stage of late organogenesis showing yolk depletion

C. β-Carotenoid Analysis

The images below show an embryo carotenoid extract. The presence of astaxanthin in the developing eggs of *E. asiatica* allowed researchers to identify the carotenoid in the highest concentration (Fig. 10). By stage I of embryonic development, astaxanthin concentration was 1.25 g/mg; at stage I, it was 0.61 g/mg. The high level of β-carotene (1.39 g/mg) seen in stage I was significantly reduced in the following stages (0.035 0.003 g/mg). The maximum amount of canthaxanthin, which was found in stage I (0.60 g/mg), decreased to 0.63 g/mg in stage IX (p 0.05); Stage III of the embryonic development process, g/mg (Figs. 11, 12, 13 and 14).

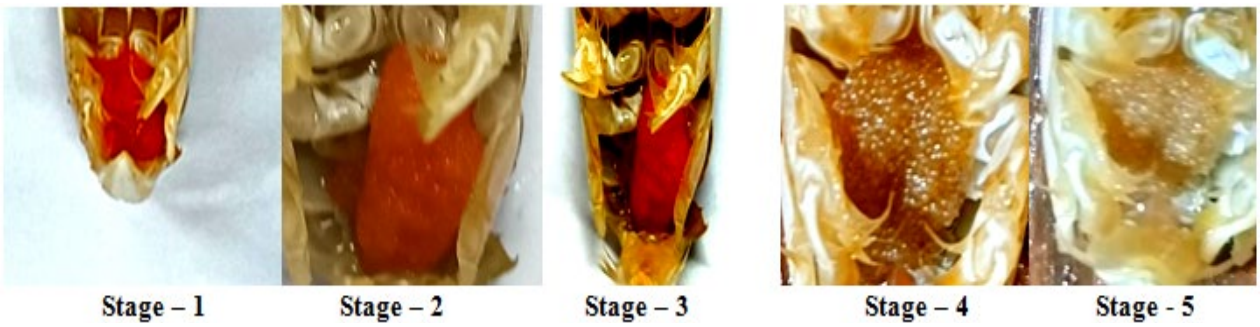

Fig. 10 Photograph showing the morphology of different egg stages of sand crab (Berried females)

IV. DISCUSSION OF THE STUDY

The foregoing observations showed that sexually mature male *Emerita asiatica* range from 3.75 to 11.0 mm CL, while mature females range above 20 mm. This sexual dimorphism in size at sexual maturity has been reported also for other species of Emerita, namely *E. analoga*, *E. talpoida* and *E. rathbunae* [15]. As far as is known, gonadal

development and differentiation have not previously been investigated in *Clarius fuscus*, but this study described structural histological characteristics gonad of *Emerita asiatica*. This revealed that the gonad is composed of previtellogenic oocyte, Vitellogenic oocyte, matured ovum, yolk, yolk granules, and nucleus.

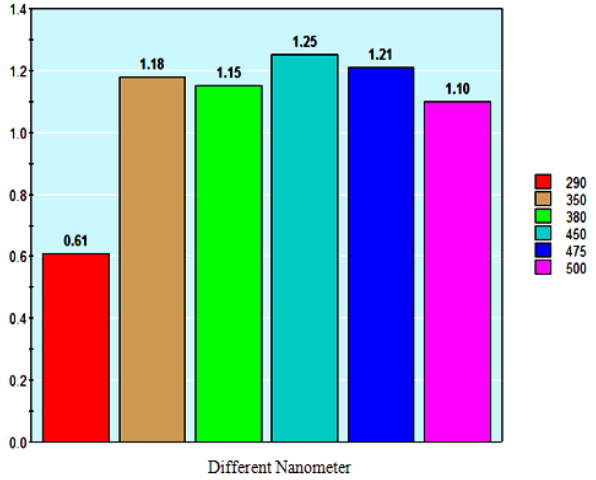

Fig. 11β-carotene Analysis of Stage-I eggs of *Emerita asiatica* under UV Spectrometer

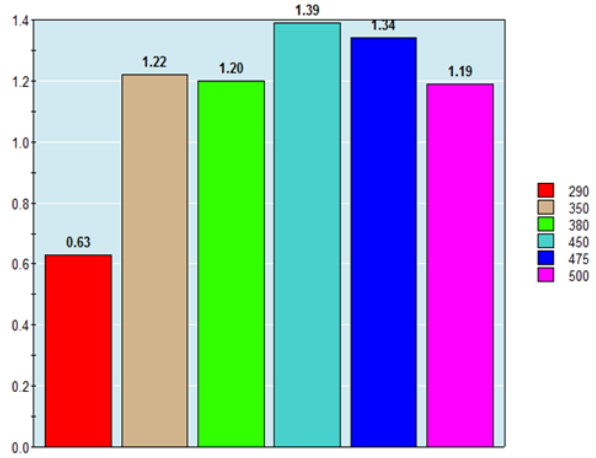

Fig. 12β-carotene Analysis of Stage-II eggs of *Emerita asiatica* under UV Spectrometer

According to the aforementioned studies, mature female *Emerita asiatica* range over 20 mm, whereas sexually mature male *Emerita asiatica* vary between 3.75- and 11.0-mm CL. Other species of Emerita, including *E. analoga*, *E. talpoida*, and *E. rathbunae*, have also been shown to exhibit this sexual dimorphism in size at sexual maturity [15].

As far as is known, *Clarius fuscus* has not yet had its gonadal development and differentiation studied; nonetheless, this study documented the structural histological features of *Emerita asiatica's* gonad. This showed that the gonad is made up of the nucleus, yolk, yolk granules, mature ovum, vitellogenic oocyte, and previtellogenic oocyte.

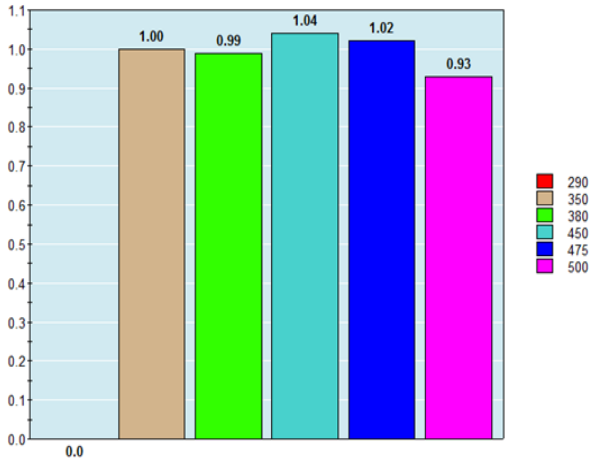

Fig. 13 β-carotene Analysis of Stage-III eggs of *Emerita asiatica* under UV Spectrometer

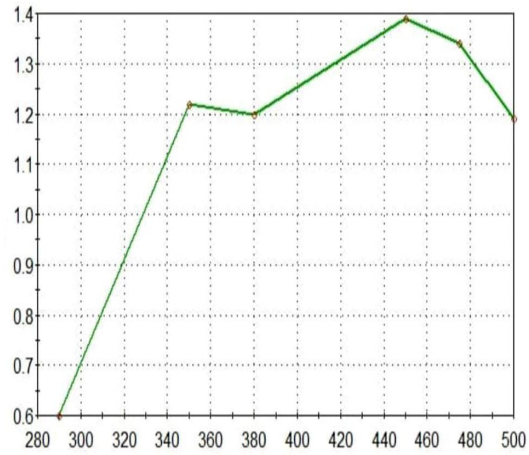

Fig.14 Graph showing the total β-carotene level of egg stages of 1, 2, and 3 at different nanometers

Analysis of the *Clarius fuscus* cyst ovarian morphology revealed in the germinal epithelium, oogonia develop into oocytes and proceeds through the stages of primary and secondary growth until the gametes are mature [16]. In comparison to the diameter of the oogonia, the diameter of *Clarius fuscus*' vitellogenic oocytes rose by roughly 500 times. Different teleost species' vitellogenic oocytes have different diameters, which leads to better circumstances for the development of the embryo and larva [17]. Species-specific adaptations to various ecological situations may be seen in the morphology of the zona pellucida of teleosts, which can also include supplementary structures that have been observed on the surface of oocytes of numerous Neotropical teleosts.

The species and stage of oocyte development can affect the morphologies of the follicular cells [18].Additionally, findings from research done with other Neotropical fish by the show that follicular cell-containing oocytes, like those reported in the present study, develop a coating of mucus that covers the oocyte and makes it sticky. Due to the permeability of ions and sand glues, the basal membrane also supports follicular cells, acts as a selective filter, and creates an external barrier for the theca, which is made of

ovarian [19]. At all phases of oocyte development, the basement membrane's ultrastructure was visible, comparable to that for other Siluriformes species [17].

Similar to other teleosts, *Clarius fuscus'* oocyte theca develops from undifferentiated ovarian stromal cells and can have a single layer (as in the present research) or two to three layers [19]. As seen in this work and previously described for the siluriforms Marcelo and Santos follicular atresia is prevalent in vertebrate ovaries and may occur at any stage of oogenesis [20], observed similar findings for additional migratory Siluriformes species [22].

According to the current study's findings, female *Clarius fuscus* have a lengthy reproductive cycle that lasts from August to January. Since post-ovulatory follicles are found near to vitellogenic oocytes, *Clarius fuscus* spawning is likely of the parceled kind, as has been shown in research with this species in lentic habitats.

The ovarian features of each specimen in the current investigation showed the same trends. According to histological observation, the ovarian tissue of E. *asiatica* is an extensive organ that occupies the cephalothorax along the dorsal-ventral axis and extends parallel to the digestive gland. The ovarian maturation was encased in a single layer of the thin ovarian capsule.

A thin pavement epithelium on the outside, a germinal epithelium inside, and a core layer of connective tissue. This capsule was composed of a fibrocyte layer and the germinal epithelium. The ovarian parenchyma displayed follicular cell organization and oocyte differentiation. In this research, we discovered that the ovary of *E. asiatica* had oocytes that were growing at different stages of oogenesis or asynchronously. Based on histological research, oogenesis may be classified into five oogenic phases. A pre-vitellogenic egg performed its initial meiosis under primary growth circumstances.

The cell expanded to a 50-60 m diameter. The noticeable nucleus was clearly described, and it resembled *Marsupenaeus japonicus*, *Penaeus semisulcatus*, and *P. monodon*. At this stage, the ooplasm had a basophilic homogeneous stain. Ribosomes, mRNAs, and stored energy stores could all be associated with basophilic cytoplasm. The Oocyte was encased in a layer of the protruding follicle cells. At this stage of development, early vitellogenic oocytes experienced secondary enlargement, resulting in oocytes with diameters between 100 and 120 m. The nucleus was still visible even with a nucleolus. Histological examination of the Oocyte at this stage revealed the presence of the acidophilic ooplasm due to the small acidophilic yolk granules. The yolk granule, which is created from lipoprotein and acts as a precursor to vitellogenin substrate, is widely regarded as the main energy source for the development of crab embryos. Vitellogenin is made in the hepatopancreas and/or the ovary.

At this point, lipid droplets were also seen in the oocytes. The structural pattern of this inclusion seems to be shared by several decapods. This lipid accumulation most likely acts as a crucial nutritional reserve for its growth. The oocyte was now surrounded by layers of spindle-shaped follicular cells. We postulated that the buildup of follicular cells may be associated with the absorption of yolk granules. In this late vitellogenic oocyte stage, the cell size steadily increased and reached 200 to 250 m in diameter. At the same time as oocytes started to develop yolk globules, they also started to show the reddish-stain of acidophilic cytoplasm (H&E method).

The size of the yolk granules ranged from 10 to 15 m.In fact, it was discovered that the yolk granules were more receptive to PAS and MT methods at this time. It is characterized as "eccentric nucleus or germinal vesicle migration," and even while the nucleus was still discernible, it had contracted and shifted toward the ooplasm's cortical zone. The layers of follicular cells were still intact at this point. The cell was between 320 and 370 m in diameter at this point in its development since it was at its biggest size and most mature form. The cytoplasm's very acidophilic colorings are induced by the accumulation of proteinaceous yolk platelets. This stage was distinguished by the lack of a nucleus. The follicular cell layer might still be seen as a plain squamous epithelium.

The atretic oocyte exhibited an irregular shape and degenerating yolk granules at this stage of the reabsorption procedure. Finally, it should be noted that the current study is the first, most recent and only report on the histological investigation of the egg stages and carotene content of the anomuran crab *E. asiatica*. Despite not being an economically successful species of crab, *E. asiatica* is crucial to the environment in order to maintain a robust marine ecosystem. A variety of processes and preventative measures should be put in place to conserve these components of the marine food chain in order to preserve a healthy environment and protect this species from extinction.

V. CONCLUSION

In conclusion, the morphological and histological characteristics of the ovary of the Sand Crab *Emerita asiatica* are being described in this current work. These structures, which are very typical and are present in practically every Sand Crab, were discovered as a result of this investigation: Previtellogenic oocytes, Vitellogenic oocytes, Atretic oocytes, matured oocytes, and their related structures. This research can be utilized to learn more about this sand crab's reproductive biology (*Emerita asiatica*). The structural and morphological changes that the gonad may undergo as a result of the action of various microorganisms, including bacteria, viruses, and fungi, can also be identified using this method. The findings from this study will be used as a guide for future research focused on studies based on environmental variables, temperature,

climatic conditions, and nutrition that may have an influence on this species. the process through which -carotene becomes vitamin A (retinol). We need vitamin A for strong immune systems, good vision, and healthy eyes, as well as for healthy skin and mucous membranes. Even though excessive amounts of vitamin A can be hazardous, our bodies only make as much of the nutrient from beta-carotene as is required. The sand crab serves as a different source of beta-carotene than vitamin A. even E. Although Asiatica is not a crab that is commercially viable, it is essential to the environment in order to preserve a healthy maritime ecology. In order to maintain a healthy ecosystem and safeguard this species from extinction, a number of procedures and preventive measures should be implemented to conserve these marine food chain constituents. Additionally, this data may be useful as a baseline for future studies on the species.

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