

Early embryonic development of the foregut of the Caridean shrimp *Exopalaemon styliferus* (H. Milne-Edwards, 1840)

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Abstract: Introduction: *Exopalaemon styliferus* was reported from the northern part of the Arabian Gulf and the inland waters of Iraq. It is a well-known fact that the recent hatched larvae of crustaceans didn't feed. The aim of the present article is, therefore, to give a descriptive account of the events and changes occurred in the cellular masses of the embryo which leads to the early development of the foregut and to resolve the question, why new hatched zoeas don't feed? **Materials and methods:** specimens of the shrimp *E. styliferus* were collected from Shatt Al-Arab estuary at Al-Fao town, south of Basrah. A conical net, 1 m long and 40 cm mouth aperture was used to collect the oviparous females and brought to the laboratory, placed in 60 L glass tanks filled with water from the same locality. After hatching, the females were removed. Every day, 10 larvae were collected and fixed and this continues until the day 20 after hatching. Embryos were fixed with Bouin's fluid for 24hr, whereas the larvae were fixed with Formalin Acetic Acid Alcohol (FAA) for 24hr. The specimens (embryos and larvae) were processed for light microscopic study. **Results:** the present study investigates the formation of foregut in shrimp *Exopalaemon styliferus* which has five developmental stages. The first four stages are embryonal while the fifth one is after hatching. The foregut is originated in the initial post naupliar stage as embryos at a length of 0.8 mm from a small cellular mass in the second part of the cephalic area. Changes in this stage are divided into two secondary stages according to the arrangement, shape and structure of cells. It is found that esophagus rudiment develops independently of stomach rudiment, and then they are connected together in the second developmental stage. The hole of the mouth is closed in this stage. The length of the embryos in the second stage (mid post naupliar) approximates between 1.5 and 1.7 mm esophagus rudiment appears as a small hollow tube where its wall is constructed of one layer epithelial cells, and at the end of this stage stomach rudiment appears in a pear shape where the beginning of the origination cardiac and pyloric parts is distinguished. In the third stage, final post nauplius stage, where embryos at length of 2 mm, here is clear growth in foregut accompanied with a series of changes and centralized to the emergence of pyloric part of the stomach and cardiac pyloric valve. In the final embryonal stage, pre-hatching embryo stage, the length of the embryo is 2.2-2.3 mm, and the wall of foregut becomes of three layers, two of them are cellular and the third is non cellular which is secreted by epithelial cells of the ling of cavity of gut. In this stage, the limits of the cardiac part but the details of pyloric part are not so clear. Larvae hatch at length of 2.5-3.5 ml in larval phase after hatching (Zoea 1) where many morphological changes take place in the stomach and make it in a marsupial shape curved towards the dorsal part of the larva. In this phase, foregut is connected with midgut after the appearance and clarity of details of the pyloric part and the constitution of its dorsal chamber which is the last events in this stage. **Conclusion:** the study concludes that the reason behind the disability of the first larval phase to nurture after hatching is incompleteness of the foregut as it is without important structures such as folds in the cardiac part and cardiac- pyloric valve and filter of the pyloric part.

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Keyword: Shrimp, *Exopalaemon styliferus*, Development, Foregut.

Introduction

The shrimp *Exopalaemon styliferus* (H. Milne-Edwards, 1840) (Fig. 1) is one of six species of the genus *Exopalaemon* of the family Palaemonidae (Guo *et al.*, 2005; Xuân, 1992). *E. styliferus* is distinguished by its long, slender, upwardly curved rostrum with a hump just above the level of eyes, with 5-7 dorsal and 6-10 ventral teeth, with long, slender 2nd peraeopods, and with the body transparent, whitish in colour, and

with brownish red colour in the region of the rostrum (Fischer & Bianchi, 1984).

E. styliferus inhabits shallow marine and brackish waters from the north coast of Borneo and Indonesia, west ward through Thailand and India to Pakistan (Fischer & Bianchi, 1984). It was also reported from the northern part of the Arabian Gulf and the inland waters of Iraq (Salman & Bishop, 1990).

There are some sporadic and scarce information regarding the distribution and biology of *E. styliferus*

(Fischer & Bianchi, 1984; Salman & Bishop, 1990; Zare *et al.*, 2010), breeding and life cycle (Saud *et al.*, 1991), the effect of the environmental condition on the species (Rajyalakshmi, 1975), its feeding (Al-Khafaji, 2002) and the description of its larval and post larval stages (Al-Abbad *et al.*, 2008).

It is a well known fact that the recent hatched larvae of crustaceans didn't feed. The aim of the present article is, therefore, to give a descriptive account of the events and changes occurred in the cellular masses of the embryo which leads to the early development of the foregut and to resolve the question, why new hatched zoeas don't feed?

Materials & Methods

Specimens of the shrimp *E. styliferus* were collected from Shatt Al-Arab estuary at Al-Fao town, south of Basrah, during July 2010 - September 2011. A conical net, 1 m long and 40 cm mouth aperture was used to collect the specimens.

Oviparous females were brought to the laboratory, placed in 60 L glass tanks filled with water from the same locality, 15-0 females were placed in each tank. Continuous aeration was supplied to each tank and the water of the tank was changed every two days, in addition to daily removal of the water and wastes from the bottom by siphoning. The females were given boiled flesh of fish. Embryos at various developmental stages were daily removed (right from the beginning of the embryogenesis to the time of hatching). The embryos were divided into four stages according to criteria given by Müller *et al.* (2004). Measurements of 25 embryos from each stage were carried out with ocular micrometer.

Females about to release their larvae were having dark eggs, these females were removed from the tank, placed singly in 5 L plastic container filled with the same biotopes water and supplied with food and continuous aeration. After hatching, the females were removed. Every day, 10 larvae were collected and fixed and this continues until the day 20 after hatching.

Embryos were fixed with Bouin's fluid for 24hr (Humason, 1972), whereas the larvae were fixed with Formalin Acetic Acid Alcohol (FAA) for 24hr. The specimens (embryos and larvae) were washed with 50% Ethanol and preserved in 70% Ethanol. Sectioning and tissue preparation were done according to Humason (1972). Staining was done with Eosin-Haematoxyline. Mounting was made with Canada Balsem. Examination was carried out with the aid of Olympus microscope and photos were captured with Sony digital Camera.

Results

The present study focused upon monitoring of events and changes that leads to the formation of the initial foregut. The process of development of the foregut was divided into five stages, four of which are

embryonic and the 5th one is the first larval (zoea) stage.

1. Initial post naupliar stage

This stage follows the gastrulation directly. It includes embryos ranging in length from 0.8-0.9 mm. The embryo was located on one of the lateral sides of the egg that had a length of 1.0 mm. Therefore, the dorsal surface of the embryo was closely connected to the yolk mass.

The embryo at this stage is bent upon itself and segmented along its bend longitudinal axis, in which a distinction of the anterior and posterior regions occurred. The segments in the most anterior part, the future head region, are followed consecutively by the thoracic, abdominal and tail regions. The future cephalic (head) region is composed of three parts. The first part consists of two ovoid segments, the optic lobes. The second part at this stage forms most of the cephalic region of the embryo and made up of large median segment, whereas the third part was formed of a triangular segment located at the base of the second part. This part connects the future cephalic region with the future thoracic region, whereas the histological cross sections show the abdominal area base on the thoracic area (Figs. 2, 3A & 8).

Studies of tissue cross sections of embryos with a length of 0.8 mm, indicated that the second part of the future cephalic region, which appeared as a large single segment (Fig. 2) is in fact of two closely adhered halves (left and right) with a small space between them (Fig. 9). Each half is composed of two different- sized cellular masses, a distinct Small Cellular Mass (SCM), close to the midline of the embryo, and an outer large cellular mass (LCM) (Fig. 9).

The two masses of each half are made up of mesenchymal tissue with cells embedded in interstitial substance stained pale in colour and the cells were spheroidal or ovoid in shape (Fig. 9). The LCM of each half is distinguished by closely packed cells with small or no intercellular spaces, whereas the SCM is consisted of two kinds of cells, small deeply stained cells occupying the half close to yolk mass and large-sized faintly stained cells at the region far from the yolk. Mostly, the two kinds of cells are close to the midline of the embryo as each kind of cells is opposed to its counterpart of the other half (Figs. 3C1 & 9).

The present result shows that the initial foregut originates from the SCM of the second part of the cephalic region.

In an embryo of 0.9 mm length, the two kinds of cells masses undergo large changes including shapes, orientation and colour of cells at the same time and in the two halves of the second segment.

The changes underwent by the SCM occurred in one definite position, close to the middle of the second part of the cephalic region. Two steps can be

distinguished in these changes. The first step begins with the cells acquiring pale colour than that of the 0.8 mm embryo. Just before that, the cells slightly move away from each other and from the LCM. These cells are then distributed in the interstitial second part of the cephalic region (Figs. 3C1, 3C2 & 10).

During this movement, the cells of two masses of the left and right halves overlap with each other and the space that was apparent at the embryo of 0.8 mm long disappeared (Fig. 10). From these two masses of SCM, a loose cellular mass (LOCM) is formed and start to have its distinct boundaries from the LCM (Figs. 3C2 & 10).

During this time, the events of the second stage started, as the cells of LOCM are arranged in irregular rows with a longitudinal axis perpendicular to the longitudinal axis of the embryo. This axis extends from the ventral surface of the embryo to a short distant and ended before reaching the yolk mass, anterior to the mass of cells formed from the opaque- coloured mass of the small cells itself (Figs. 3D1 & 11). This stage ended by all cells and their nuclei acquiring an ovoid shape. These cells appeared parallel to each other and their longitudinal axis is perpendicular to the longitudinal axis of the embryo (Fig. 11).

At the time when changes in the first stage of the pale coloured cells begin, the opaque coloured cells of the same mass undergoes some changes as well, extending from the middle of the second part of the future cephalic region to the end of this part. These changes start with the movement of the deeply coloured cells away from the LCM of the cephalic region towards the midline of the embryo slightly towards the yolk mass (Figs. 3C1 & 10). It is apparent that during the movement of these cells, they remain closely packed together. As the movements of the two masses proceeds, the cells of two masses approach each other and push more towards the yolk mass (Fig. 3C2), until they meet at the midline of the embryo. At this time a Closely Packed Longitudinal Cellular Mass (CPLM) is formed along the area that underwent these changes (Figs. 3B & 3D2), in which the most anterior region opposite to the loose cellular mass (LOCM) (Figs. 3A, 3B, 3D1 & 11), formed from the pale-shaped cells, whereas the most posterior region occurred near the third part of the future cephalic region. The CPLM is distinguished into two regions of different size and shape. The first region is in the form of a thickened longitudinal folded lamellated cells, so that its concavity occurred towards the yolk at the dorsal midline of the embryo and this lamella occupied the anterior region of the mass. This lamella appeared in a cross section as a half circle composed of two cellular layers or more (Figs. 3D2a & 12). The second region is larger than the first one and continuous with it, occupies the rest of the middle and posterior parts of

the mass and appeared in the form of solid rod, the C. S. of which is in the form of irregular cellular mass (Figs. 3D2b & 13). The cell mass of the two regions and their nuclei appeared deep colour, depressed dorsally and ventrally, the nucleoli of the nuclei are centrally located, whereas the cytoplasm is pale in colour and some cells appeared in the process of the various cellular divisions (Figs. 11, 12 & 13).

The present results showed that the LOCM, which was formed at about half of the second part of the cephalic region, from which the primordial of the esophagus derived, whereas from the CPLM, which was formed at the dorsal part of the embryo along the middle of the second part to its end, the primordial of the stomach is derived.

2. Mid post naupliar stage

This stage includes embryos 1.5-1.7 mm long. In an embryo 1.5 mm long, the LCOM, which will comprise the primordia of the future oesophagus, undergoes changes in the cells of the outer rows. They become distinguished from the cells at the center of the mass and start to move away from these cells at the center by a small interstitial space and gradually arranged into one row around the central mass. This arrangement starts from the dorsal side of the embryo, near the most anterior of the elongated cell mass, the primordial of the stomach, and continues towards the ventral region of the embryo (Figs. 4C1 & 14).

The start of the arrangement of cells in the outer row is associated with the gradual disappearance of the cells at the center leaving small space near the most anterior part of the elongated cells and is quite apparent at 1.7 mm long embryo. This space represents the primordial of the lumen of the future esophagus. This space is surrounded by a row of cells that completed their arrangement at the end of the events of this stage. The arrangement of the cells of the outer row continues until its completion with the disappearance of the cells at the center (Figs. 4A, 4B, 4D1 & 15).

The lumen of the primordial of the esophagus enlarged more at the following stages towards the ventral region, parallel to the longitudinal axis of the future esophagus. At the end of the process of the rearrangements of cells, a row of cells is apparent around the lumen to form the dorsal lining of the future esophagus. At this time, a short hollow tube is formed with its most anterior end which forms the mouth and pharynx, is closed (Figs. 4D1, 15 & 16).

The cells forming the lining of the primordial of esophagus changed in shape and look pale columnar with large ovoid, pale coloured nuclei and central distinct nucleoli, with a little pale cytoplasm (Fig. 15).

At the end of this stage, the events and changes underwent by the CPLM, leads to the distinction of the cardiac and pyloric future portions of the stomach. The CPLM increases in length and diameter, and the

anterior and posterior halves of this mass undergoes changes in shape and structure as well as changes in the shapes of its cells. The anterior half of the CPLM, the folded cellular lamella undergoes changes which lead to the formation of a small hollow tube representing the primordial of the stomach, as the two lateral edges of the lamella gradually approach each other, in an embryo of 1.5 mm long (Fig. 4C2) and fused in the ventral midline of an embryo of 1.7 mm long (Figs. 4D2 & 17). Before the completion of the formation of the tube, the two ventral edges at the most anterior part of the folded lamella united from each side with the lining of the primordial of the esophagus and forms a continuous single row of cells (Fig. 15). This keeps the lumen of the esophagus continuous with that of this tube, thus, the beginning of the anterior gut is formed from the fusion of the primordial of the esophagus with that of the stomach (Figs. 4D1 & 15).

At the time these events are taken place, the pattern and arrangements of cells changed causing the formation of tube irregular in the C.S., the cells of the dorsal and ventral walls become irregular and appear in certain places, as if they are of more than one layer. Their cells didn't regularly arranged with each other to form a row or rows, and the cells at these walls appear spherical or ovoid, with some remain compressed, whereas the cells of the central walls of the tube are arranged in one row. These cells with their nuclei appear ovoid and slightly elongated and their cytoplasm is clear and pink in colour (Fig. 17). The cells located directly after the lumen differentiation into dorsal lining of the lumen, whereas those of the outer periphery of the tube form the layer of the wall of this region and look flat in shape (Fig. 17).

It is apparent that the increase in length of the CPLM, at this stage is centered at the posterior half of the primordial of the stomach, which was as a solid tube in the previous stage, accompanied by an increase in diameter of this portion.

This occurred due to increase of repetitive mitotic divisions of its cells. Therefore, the posterior half looks wider, solid and polygonal in cross section (Figs. 4D4 & 18), and had a diameter larger than the hollow tube of the anterior half. The cells of this tube look spherical or ovoid with large-sized, pale coloured nuclei, and the cells are separated by distinct intercellular spaces, and some cells are in the process of mitosis. These events and changes lead to change the morphology of the primordial of the stomach to become pear-shaped (Fig. 4A & 4B).

The results show that most of the cardiac portion is originated from the hollow anterior half of the primordia of the stomach, whereas from the solid posterior half, the posterior cardiac part and the pyloric as well as the cardiac- pyloric valve are formed during the later developmental stages. It is also apparent that

some of the cells at the most anterior of the mass of the posterior half (the solid tube), at the ventral side of the primordial of the stomach, become distinct from those of the rest of this portion and look as if they had just separated from the mass by extending further towards the lumen of the anterior half. They, at this stage, also changed into deeper colour than the rest of the tube. These cells remain connected with the mass of the posterior half (Figs. 4D3 & 19).

3. Final post naupliar stage

This stage includes embryos of 2.0 mm long. The changes in the esophagus are associated with slight increase in its length and showed an opening to the exterior at the lower side (the ventral surface) throughout the mouth opening (Figs. 5C1, 20 & 21).

The primordial of the stomach increases further at this stage and the preceding ones with the growth of the embryo, therefore, increase in size and its shape remain as it was in the previous stage (Fig. 5A & 5B). It appeared surrounded by yolk, with a single cellular layer of flat cells around it, which appeared in the previous stage around the first half of the stomach primordial only, separated it from the yolk mass. The major changes at this stage are occurred on the second half more than the first one, which still had a diameter less than that of the posterior half (Figs. 22-25).

During this stage, the cells of the dorsal and ventral walls of the anterior half of the stomach primordial elongate and become more regular than in the previous stage, and the most elongated cells are those of the midline of the stomach primordia in the median sagittal level and the midline of the median frontal level. As a result of this, the shape of the lumen of the anterior half of the stomach primordial changed, and look star-shaped in cross section and narrow than before (Fig. 5C2 & 22).

The most obvious changes occurred at this stage are on the cells mass of the posterior half of the stomach primordial. A small cavity appeared at the center of the mass, enlarged towards the periphery in the form of narrow passages and look like the lumen of the anterior half but narrow and enlarged later to form one continuous passage with that of the anterior half of the stomach primordial. At this time, the shape of the cells of the posterior half changed and become elongated as is the case of the anterior half, and the most elongated cells are those in the median frontal-sagittal level (Figs. 5C4, 5C5 & 24).

The formation of this cavity (lumen) leads to divide the mass of the posterior half into two, dorsal and ventral mass and the lateral walls in between, which are formed of a single row of the elongated cells (Figs. 5A, 5B, 5C5 & 25). At this time, the cells mass at the most anterior of the posterior half, become clearly distinguished and form a deep coloured pyramidal cells mass (Figs. 5C3 & 23). The ventral

wall of the posterior half is therefore, composed of two cellular masses which differ in shape and size. The median sagittal sections reveals that the first mass occurred at the middle of the ventral wall of the stomach primordial, in front of the second mass, which was recently formed, and occurred in the most posterior part of the ventral wall of the stomach primordial. The first mass is smaller in size, pyramidal in shape and formed earlier than the second mass, which is large in size and of irregular shape, whereas the dorsal mass is ovoid in shape (Figs. 5B, 5C3 & 5C5, 23 & 25).

Associated with these events, the lining of the lateral wall of the posterior half is folded and extends along the dorso-lateral wall and starts from the level of the formation of the first mass in the ventral wall to the end of the stomach primordial. It was noticed that the folding becomes more apparent towards the posterior end (Fig. 25).

The cells at the periphery of the lumen are differentiated into the dorsal lining of the stomach, whereas the flat cells formed the rest of layers of the wall (Figs. 24 & 25). However, the cells masses formed by splitting of the mass of the posterior half will form the internal structures of the stomach, the cardiac-pyloric valve originates from the first mass (Figs. 5B & 23), and from the second mass, the pyloric region of the stomach is formed (Figs. 5B, 5C3 & 25), whereas the dorsal mass will form the future posterior end of the cardiac region of the stomach (Figs. 5B, 5C3 & 25).

4. Pre-hatching stage

This stage is the final embryonic stage, comprising 2.2-2.3 mm long embryos. At this stage, the wall of the anterior foregut primordial is enclosing a cavity seems narrow than the previous stage (Figs. 6B, 6C1-4, 26, 27 & 28). The wall is composed of three layers, two cellular, the dorsal lining which is surrounded from the outside by a single cell layer of flat cells. These cells appeared around the stomach primordial in the previous stage and around the esophagus at this stage, whereas the third layer is non-cellular, secreted by the dorsal cells of the cavity lining of the lateral foregut primordial. This is in the form of a thin layer covering the free cells surfaces secreting it and is a pale in pink colour and hard to be seen sometimes (Figs. 6B & 26).

At this stage the vertical- longitudinal axis of the esophageal primordial shifts and looks slightly oblique towards the anterior region (Figs. 6B & 26). The cells of the lining of the esophageal primordial look more elevated and more crowded, thus, its cavity looks narrow than the previous stage (Figs. 6C1 & 27).

The stomach seems to move towards the yolk mass, separated from the body segments from which it arises and the stomach primordial look opened at the posterior end (Figs. 7A & 26).

The changes in the stomach primordial at this stage are centered on the cells mass in the dorsal side of the posterior half (the hind part of the future cardiac region). This mass grew slightly different in relation to the growth of the stomach primordial. Therefore, its posterior end looks slightly projected and this becomes apparent in the stages following hatching, and it changed in shape to a solid rod- like structure (Figs. 6B & 26). During this time, a small central cavity is formed in the middle of that solid rod, extending rapidly along the length of the rod, thus, leading to form a hollow tube which seems as if it is suspended from the roof of the stomach primordial (Fig. 6C4, 26 & 29). It is a narrow tube and becomes wider than after hatching as the growth proceeds.

The tube is opened through both ends and its cavity is continuous with that of the stomach primordial. Then it closes from the posterior end in the following stage directly. This is timed with the start of formation of the posterior wall of the stomach primordial, whereas its anterior end remained opened; therefore, its cavity is continuous with that of the stomach primordial located in front of it.

The cells around the cavity of this tube differentiate to form its lining which is continuous from the dorsal side with the cavity of the anterior part of the stomach primordial (the future anterior part of the cardiac region) and is the dorsal lining of the roof of the primordial of the future pyloric portion of the posterior half of the stomach primordial. Therefore, the cells of the cardiac- pyloric portions are forming together, the wall of the opening of the stomach primordial and the dorsal half is formed from the dorsal cells of the future cardiac region, these cells are those of tube suspended in the stomach primordial, whereas the ventral half is the cells of the dorsal lining of the future pyloric region (Figs. 7A & 26). At this stage the midgut and the foregut greatly approached each other (Fig. 6A).

5. Zoea stage I

The larva ranges in length from 2.5-3.5 mm. New differentiated cells in the wall of the beginning of the esophagus (or esophageal primordial) is apparent and is representing the skeletal muscles which are the first muscles of the wall of the digestive tract to be differentiated and appeared in the region (Figs. 30 & 31).

The esophageal primordial looks arched towards the anterior of the larvae and connected with the cells of its epithelial lining from the outside, recently differentiated skeletal muscle fibers with narrowing of the esophageal cavity at the attachment of these muscles with the epithelial cells (Figs. 30 & 31). Also, the general morphological and structural formations of the stomach are completed at this stage. The most anterior connected with the esophagus bend slightly

towards the ventral side, and the turning of the posterior end of the stomach ventrally, at this time the posterior opening of the stomach is directed ventrally more than posteriorly (Fig. 30). The most anterior end of the stomach primordial is widened at the junction with the most posterior of the esophagus, which is becoming wider as well (Fig. 30). Longitudinal sections of that end indicate the bending of the epithelial lining of the stomach primordial towards the outside and this becomes quite apparent at the stages of the growth after hatching. A constriction is also obvious in the ventral wall of the stomach primordial at the level of the region of the future cardiac- pyloric valve (Fig. 30). At these changes lead to change in the shape of the stomach primordial which looks like a sac arched towards the dorsal side of the larva (Fig. 30).

During these morphological changes of the stomach, the posterior stomach wall starts to develop and separates the end of the cardiac region from the pyloric region, as the cells of the ventral wall of the tube, which was suspended at the stomach wall in the previous stage (Fig. 26), undergoes mitotic divisions producing new cells arranged with the row of maternal cells in the ventral wall of the tube, which end become closed, then the development of the cardiac region is completed (Fig. 30).

During this stage the stomach opening is still formed of the epithelial cells of the future cardiac and

pyloric regions (Fig. 7B). With the persistence of the mitotic divisions, the row of the maternal cells bend ventrally at the most anterior end and forms another row parallel to the ventral wall of the tube. Therefore, the ventral wall is doubled, with the maternal row becomes the epithelial lining of the ventral side of the posterior part of the cardiac region, whereas, the new row forms the roof of the pyloric region, which forms the future of the posterior wall of the pyloric region. Therefore, the end of the cardiac portion will be separated from the pyloric region (Fig. 7C). When the length of the new row of cells becomes equal to that of the maternal row, the opening of the stomach will be ventral (Fig. 7D). At this time, the wall of this opening is formed of cells of the epithelial lining of the pyloric region only, as the new row of cells occupy that of the cardiac region from which it developed (Fig. 7E), and the ventral mass become ventrally located at this stage. Therefore, the posterior chamber of the pyloric stomach is completely developed then the most posterior end of the opening of the stomach becomes connected with the lining of the mid gut (Figs. 30 & 32). The present results indicate that the cardiac-pyloric valve at this time is covered by a layer of thick cuticle, thicker than the rest parts of the stomach (Figs. 30 & 33).

Abbreviations

AB= Abdominal region, ABM = Abdominal mass, AH=Anterior half, APP = Anterior pyloric part, CPV = Cardiac pyloric valve, C= Cavity, CM = Circular muscles, Cp = Cardiac part, Cplm = Closely packed longitudinal cellular mass, CPV= Cardiac- pyloric valve, CPW = Cardiac part wall, CS = Cross section, CTR = Cephalothoracic region, CU = Cuticle, D = Dorsal, DM = Dorsal mass, EPT = Epithelial tissue, ES = Esophagus, ESC = Esophagus cavity, ESR = Esophagus rudiment, EY= Eye, Fg = Foregut, FC = Flat cell, H = Head ,HR= Head region, L = Left, Lcm = Large cellular mass, Locm = Loose cellular mass, M = Muscles, MF = Muscles fiber, MT = Mesenchymal tissue, OC = Oral cavity, OL = Optical lobe, P = Posterior, PCP = Posterior cardiac part, PH= Posterior half, Pp = Pyloric part, PPP = Posterior pyloric part, PPW = Pyloric part wall, R = Right, SC = Stomach cavity, Scm = Small cellular mass, T= Tail, TA = Thoracic appendages, TM = Thoracic mass, TR = Tail region, V =Ventral, VMS = Ventral masses, Y=Yolk



Figure 1: Female of the shrimp *E. styliferus*.

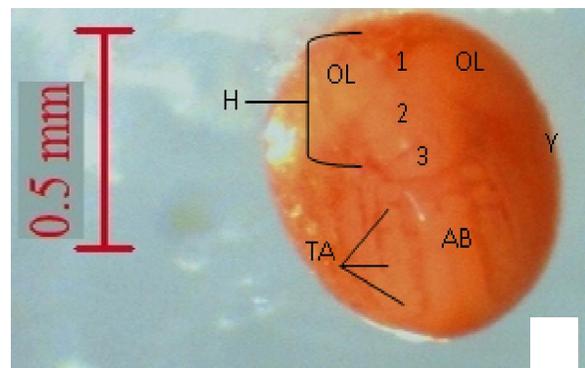


Figure 2: General aspects of the embryo of the shrimp *E. styliferus* at the initial post naupliar stage (1, 2 & 3 shows the parts of the future of the head region).

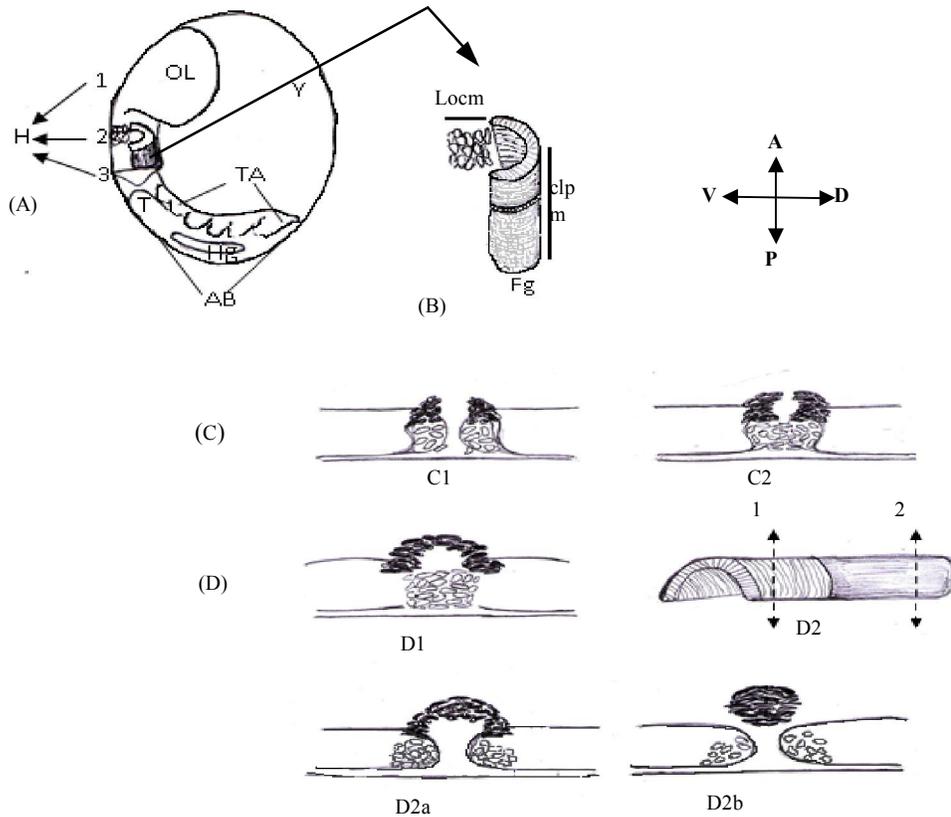


Figure 3: Sketches of lateral cross and superficial levels of 0.9 mm long embryo of *E. styliferus* at the initial post naupliar stage representing the events of early stages of the formation of the foregut.

- A.** Sketch of the foregut primordial showing the beginning of the origin of the foregut.
- B.** Sketch of the foregut primordial showing structural details.
- C.** Sketch showing events of the early stage of the formation of foregut primordial.
- C1. Beginning of the movement of the small cell mass (Pale in colour) away from the large cell mass.
- C2. Movement of the large cells (Pale in colour) towards the midline of the embryo and the formation of the loss cell mass, it also shows the beginning of movement of the small cells (Deep in colour) of the small cell mass towards the midline of the embryo maintaining their compaction.
- D.** The second stage of the formation of foregut primordial.
- D1. Arrangement of the pale coloured cells of the loss cell mass, in irregular rows and the formation of deep coloured cells, an elongated compact cellular mass.
- D2. Lateral view of the elongated and compacted cellular mass.
- D2a. C.S. in the anterior region of the mass looking arched in shape.
- D2b. C.S. in the posterior region of the mass looking as spherical cellular mass.

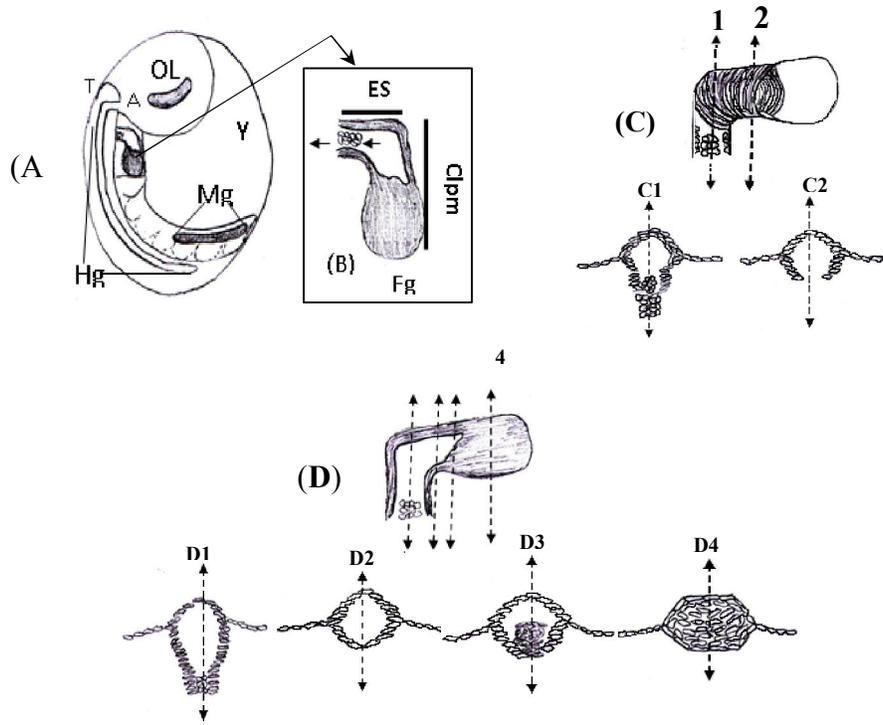
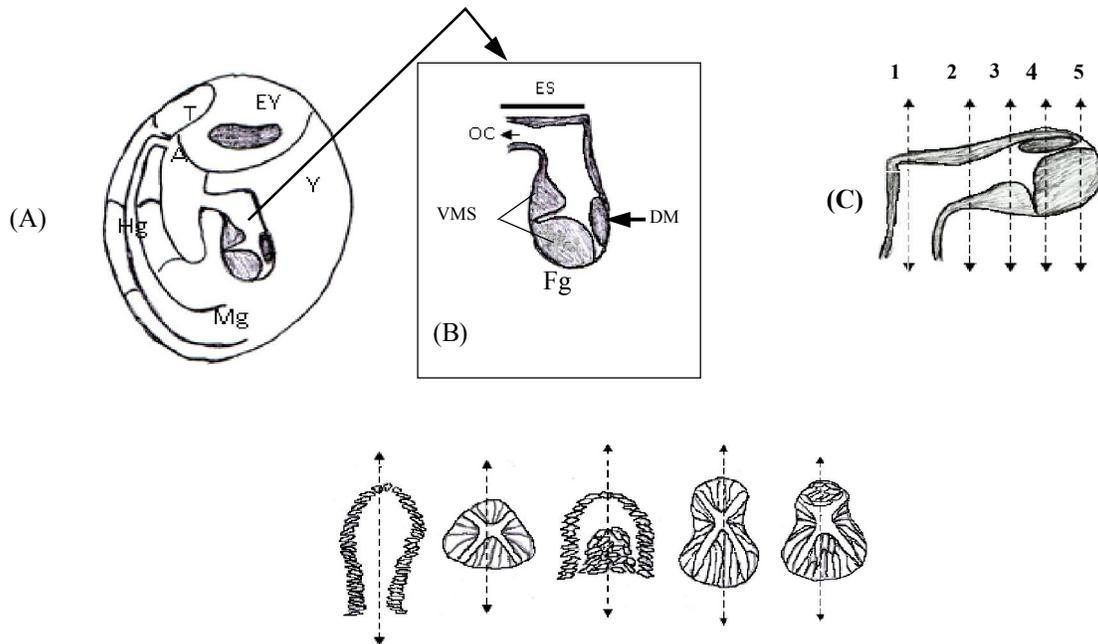


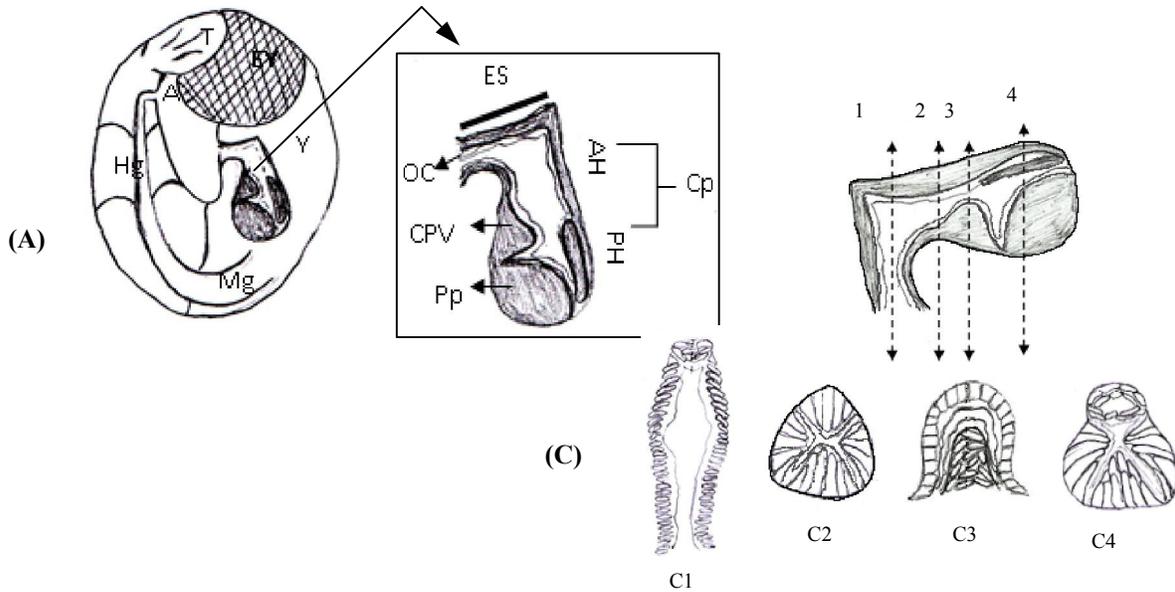
Fig. 4: Sketches in lateral and cross levels of 1.5-1.7 mm long embryo of the *E. styliferus* at the midgut post naupliar stage showing the events of the formation of the digestive tract.

- A. Latero- longitudinal drawing of an embryo at the mid post- naupliar stage showing increase of diameter of the posterior part of the stomach primordial and the withdraw of the central cell mass of the esophageal primordial to the outside and the beginning of the formation of the midgut as a solid tube in the thoracic region as well as the formation of the anal opening at the end of the hindgut primordial.
- B. Enlarged scale of the foregut primordial indicating structural details.
- C. Latero- longitudinal section in the foregut primordial of 1.5 mm long embryo showing movement of the out rows of the cells of esophageal primordial away from the central cells (1) and the movement of the edges of the folded lateral lamella towards each other (2).
- D. Latero- longitudinal section of the foregut primordial of a 1.7 mm long embryo showing arrangement of the cells of the esophageal wall with the most anterior of the stomach wall and their fusion with each other and the withdrawal of the central cells towards the outside to the ventral side of the embryo (1), C. S. of the anterior of the folded lamella showing the fusion of their edges at the midline of the embryo (2), C. S. in the anterior of the posterior half of the elongated cell mass of the compact cells showing the differentiated dark cells from the ventral side of the solid tube (3) and C. S. at the posterior of the posterior half (the solid tube) of the elongated cell mass of the compact cells which looks as a polygon (4).



Figuer. 5: Sketches at lateral and cross levels of 2 mm long embryo of *E. styliferus* at the final post naupliar stage.

- A.** Latero- longitudinal section showing the divisions of the posterior part of the stomach primordial into three cellular masses and the complete withdrawal of the central cells of the esophagus.
- B.** Enlarged drawing of the foregut primordial showing the withdrawal of the central cells from the esophagus to the outside and the formation of the mouth opening and the division of the posterior half of the stomach into three cellular masses as a result of the formation of the cavity chamber in the center.
- C.** Latero- longitudinal section of the foregut primordial of a 2 mm long embryo showing Longitudinal section in the esophagus showing the withdrawal of the central cell mass and the formation of the mouth opening at the ventral side of the esophagus, also shown the union of its chamber with that of the stomach (C1), C. S. in the middle of the anterior half of the stomach primordial showing a star-shaped chamber, lumen or cavity (C2), C. S. at the end of the anterior half of the stomach primordial showing the first ventral mass with pyramidal shape (C3), C. S. at the anterior of the posterior half of the stomach primordial showing its chamber which is similar to that of the anterior half but narrower than it (C4) and C. S. at the end of the posterior half of the stomach primordial showing the dorsal cellular mass and the second ventral cellular mass and also indicates the movements of the lateral walls to the interior (C5).

**Figuer 6**

Figuer 6: Sketches at the lateral and cross levels of an embryo of *E. styliferus*, 2.2-2.3 mm long at the pre-hatching stage.

- A. Sketch of latero- longitudinal section of a pre-hatching stage embryo indicate the occurrence of elongation in the dorsal cell mass of the stomach primordial.
- B. Enlarged sketch of the foregut showing its structural detail.
- C. Sketch of the foregut showing C. S. at various regions.
 - C1. Longitudinal section of the esophageal primordial showing narrowness of its lumen.
 - C2. C. S. in the mid anterior half of stomach primordial showing narrowness of its lumen caused by increase movement (or migration) of the cells towards the lumen.
 - C3. C. S. through the cardiac- pyloric valve region.
 - C4. C. S. at the end of the posterior half of the stomach primordial showing the presence of the lumen (chamber) at the mid dorsal cellular mass.

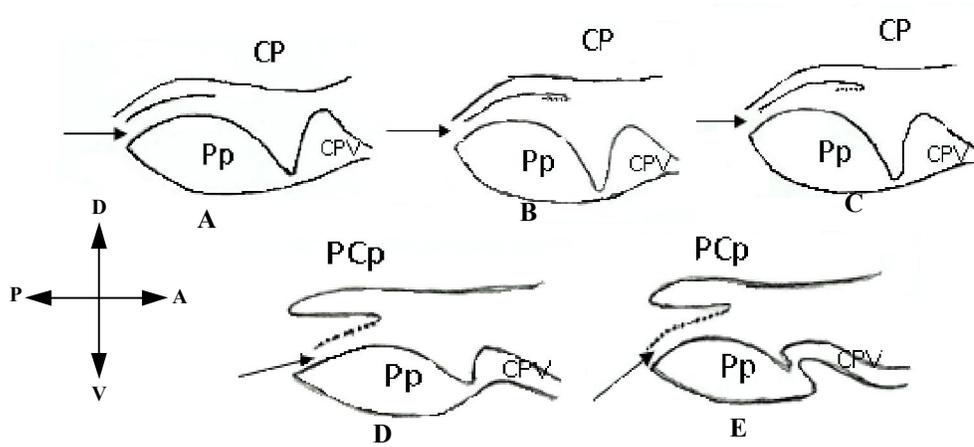
**Figuer 7**

Figure 7: Sketches indicate the stages of splitting of the pyloric portion from the cardiac region and the posterior wall of the pyloric region of the stomach (arrow indicates the opening of the stomach primordial) of the shrimp *E. styliferus*.

A. Latero- longitudinal section in the posterior part of the stomach primordial at the pre- hatching embryo stage showing the wall of the stomach opening which is common between the cardiac and pyloric portions. Its dorsal side is formed from cells belonging to the future cardiac region whereas its ventral side is formed from the dorsal lining of the pyloric region.

B. Latero- longitudinal section in posterior region of the stomach primordial at the zoea I after hatching indicating the beginning of the formation of a new row of cells from the ventral wall of the suspended tube at the stomach roof as a result of mitotic divisions of the maternal cell row.

C. Latero- longitudinal section in the posterior region of the stomach primordial at the zoea I after hatching showing the ventral wall of the tube as a double wall, the upper most represent the ventral wall of the posterior half of the cardiac region and the lower represents the dorsal wall of the pyloric region.

D. Latero- longitudinal section in the posterior part of the stomach primordial at the zoea I after hatching, showing the new cell row equal in length to the maternal row of cells.

E. Latero- longitudinal section at the posterior region of the stomach primordial of zoea I after hatching, showing the opening of the stomach primordial which is now located ventrally as a result of turning of the posterior part of the stomach primordial ventrally, the wall of the stomach opening is formed of dorsal cells of the pyloric portion only

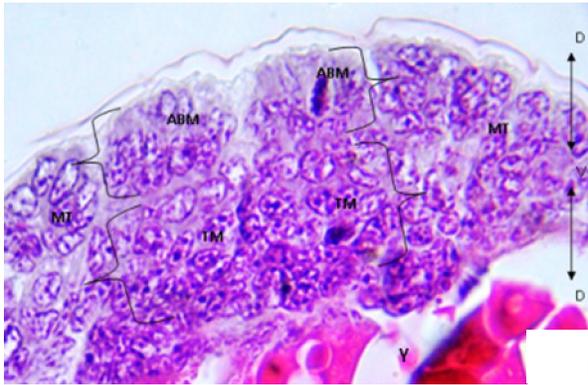


Figure. 8: C. S. in the front area of the thoracic and abdominal region of the embryo at the stage of initial post naupliar stage shows the abdominal area (small bracket) based on the thoracic area (big bracket) (mag. 200x).

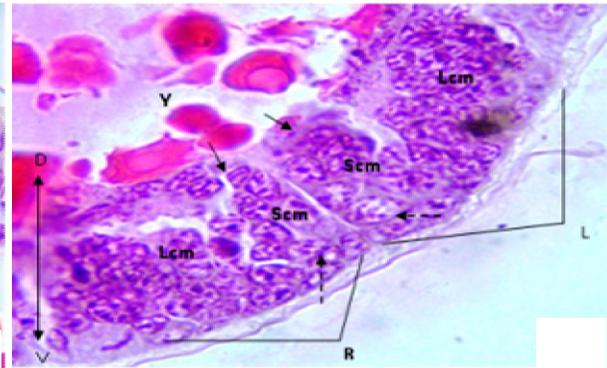


Figure 9: C. S. in the second part of the cephalic region of 0.8 mm long embryo of the shrimp *E. styliferus* at the initial post-naupliar stage showing the two halves of the region, the left and right half, arrows indicate the opaque (deep) coloured cells, broken arrows show the pale coloured cells (mag. 200x).

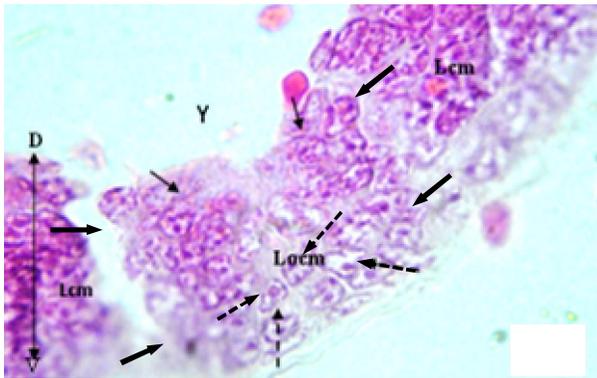


Figure.10: embryo of the shrimp *E. styliferus* at the initial post- naupliar stage, showing the movements of both kinds of SCM, the deep and pale coloured cells (thick arrow), away from the large cellular mass and their dispersion in the intercellular matrix, and the disappearance of the limit between the left and right half of the cephalic region. Brocken arrows indicate the director of the pale coloured SCM towards the midline of the embryo to form LOCM, whereas, the thin arrows indicate the movement away of the deep coloured SCM from the large cells mass (mag. 500x).

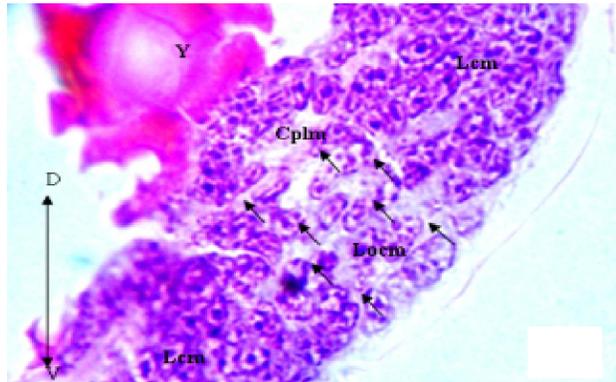


Figure. 11: C. S. in the second part of the cephalic region of 0.9 mm long embryo of the shrimp *E. styliferus* at the initial post-naupliar stage, showing the arrangement of the LOCM in irregular rows. Arrows indicate the direction of arrangement of cells of the mass from the ventral side of the embryo dorsally (mag. 200x).

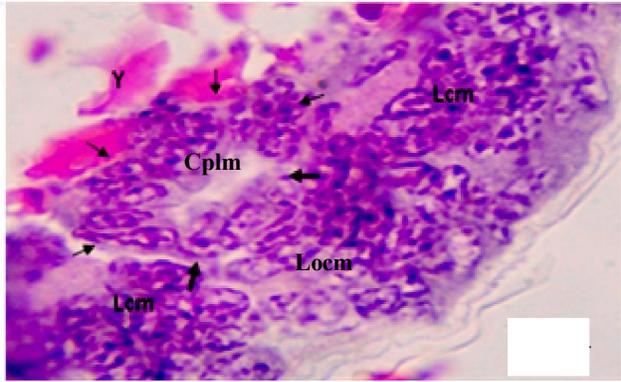


Figure. 12: C. S. of the second part of the cephalic region of an embryo 0.9 mm long of the shrimp *E. styliferus* at the initial post-naupliar stage, passing through the anterior region of the closely packed longitudinal cellular mass CPLM, indicating the presence of this mass as an open ring (arrow). Thick arrows show the two lateral ends of the folded cellular lamella (mag. 200x).

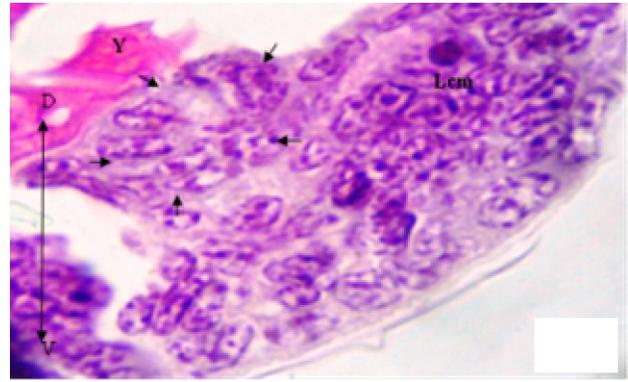


Figure. 13: C. S. of the second part of the cephalic region of an embryo 0.9 mm long of *E. styliferus* at the initial post-naupliar stage, passing through the posterior region of the CPLM, which is represented by cellular mass of compact cells (arrows) (mag. 200x).

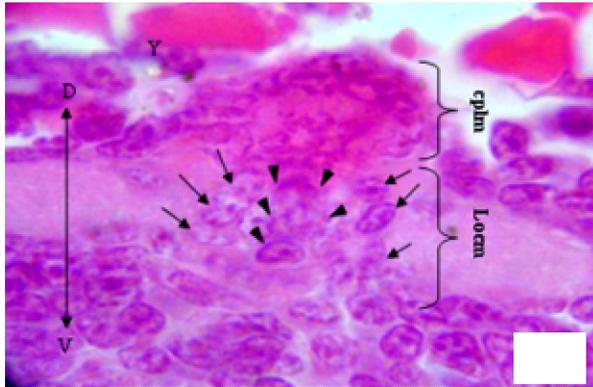


Figure 14: C. S. in the cephalic region of an embryo 1.5 mm long of *E. styliferus* at the mid post-naupliar stage passing through the future esophageal primordial and the anterior end of the stomach primordial. Arrows indicate the movement of the peripheral cells away from the central cells (head of arrows) of the LOCM and their arrangement at the dorsal side of the embryo ventrally (mag. 500x).

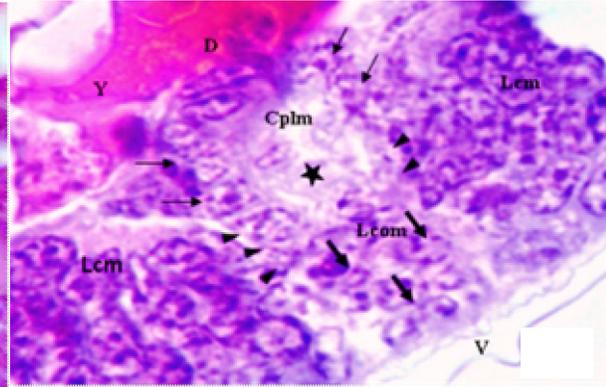


Figure. 15: C. S. in the cephalic region of an embryo 1.7 mm long of *E. styliferus* at the mid post-naupliar stage passing through the future esophageal primordial, indicating the arrangement the peripheral cells of the LOCM towards the ventral and dorsal side (head of arrows). It also shows the arrangements of the cells of the most anterior of the stomach primordial (arrows) and their union with the outer row of the wall of the esophageal primordial. The asterisk shows the start of the chamber at the dorsal side of the esophageal primordial close to the stomach primordial after the withdrawal of the cells at the center of the region (thick arrows) (mag. 200x).

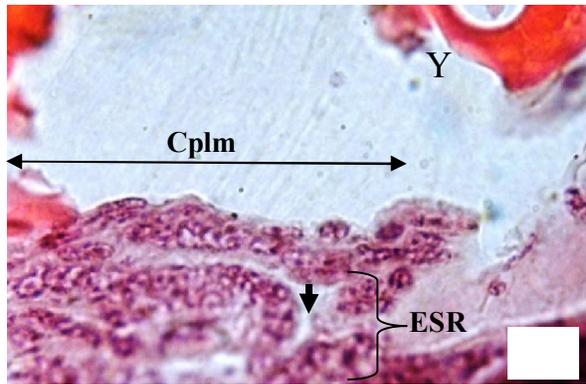


Figure 16: Latero- longitudinal section in the foregut primordial of an embryo 1.7 mm of *E. styliferus* at the mid post-naupliar stage, indicating the withdrawal of the central cells of the future esophageal primordial (small arrow) and the origin of the chamber at its upper part (mag. 200x).

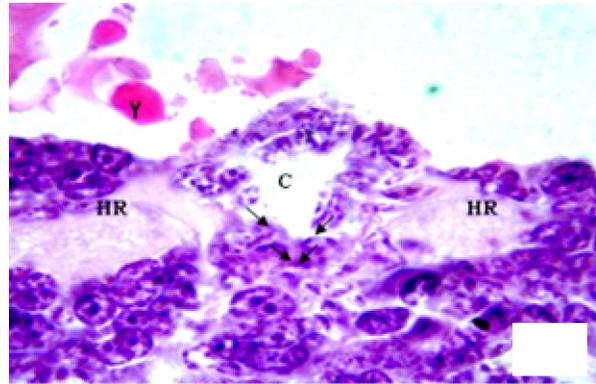


Figure 17: C. S. in the cephalic region of an embryo 1.7 mm long of *E. styliferus* at the mid post- naupliar stage passing through the anterior half of the future stomach primordial. Arrows indicate the union of the lateral edges of the lamella at the midline of the embryo (mag. 500x).

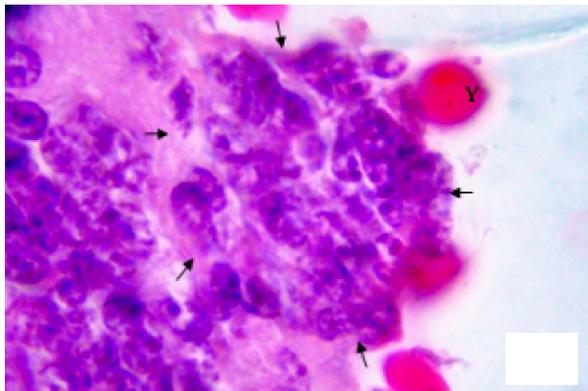


Figure 18: C. S. in the cephalic region of an embryo 1.5 mm long of *E. styliferus* at the mid post- naupliar stage passing through the posterior half of the future stomach primordia with polygonal shape (arrows) (mag. 500x).

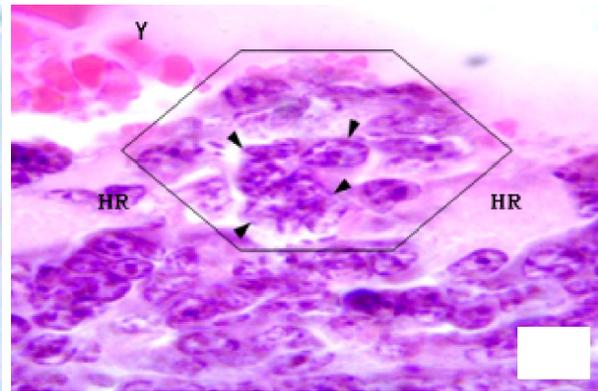


Figure 19: C. S. in the cephalic region of an embryo of *E. styliferus* 1.7 mm long at the mid post-naupliar stage passing through the anterior half of the future stomach primordial. Arrow heads show the deep coloured cells located in front of the posterior half of the stomach primordial. The polygon encloses the posterior half of the stomach primordial (mag. 500x).

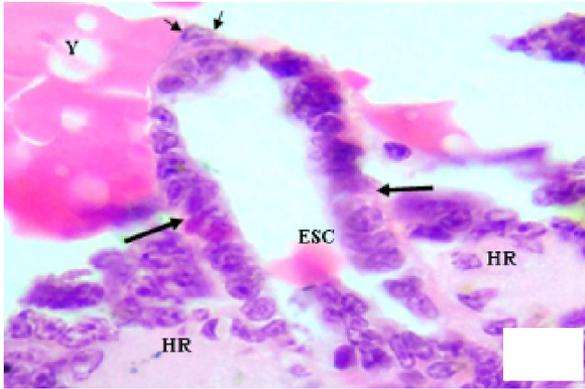


Figure. 20: L. S. of the esophagus and the anterior of the stomach primordial of an embryo 2.0 mm long of *E. styliferus* at the final post-naupliar stage indicating an increase in the length of the esophagus with its chamber (lumen) clearly shown and small arrows indicate to a flat-shaped cell. Thick arrows represent the limit between the esophageal and the stomach primordial (mag. 200x).

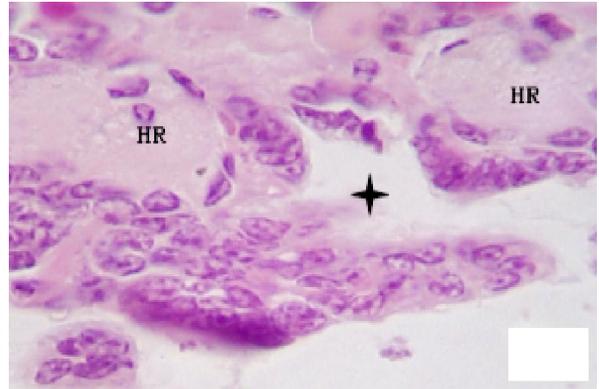


Figure. 21: L. S. through the end of the esophagus of an embryo 2.0 mm long of *E. styliferus* at the final post-naupliar stage showing the buccal cavity originated at this stage (asterisk) (mag. 200x).

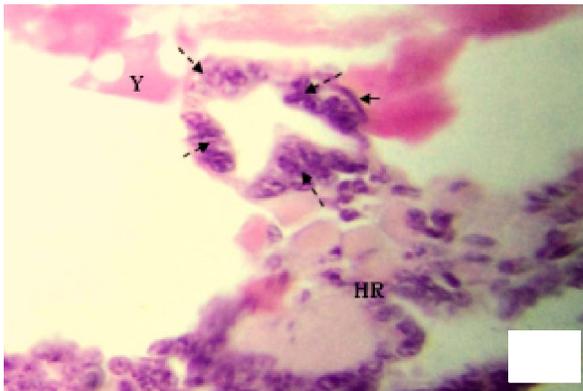


Figure. 22: C. S. through the mid anterior region of the stomach primordial of *E. styliferus* showing the star-shaped lumen. Broken arrows indicate the tallest wall cells at the midline and the walls are surrounded by flat cells (small arrows) (mag. 200x).

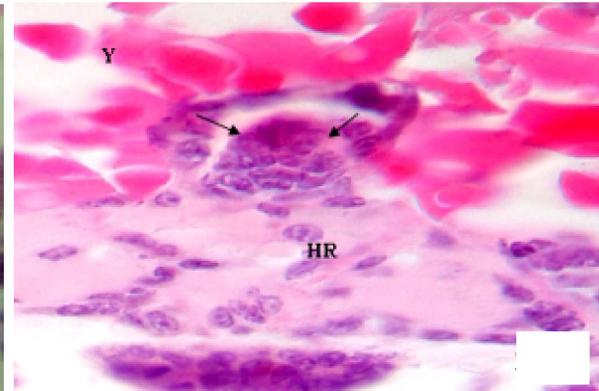


Figure. 23: C. S. through the end of the anterior half of the stomach primordial of 2.0 mm long embryo of *E. styliferus* at the final post-naupliar stage showing the pyramidal mass forming the future cardiac-pyloric valve (arrows). See the lumen (cavity) of the stomach primordial (mag. 200x).

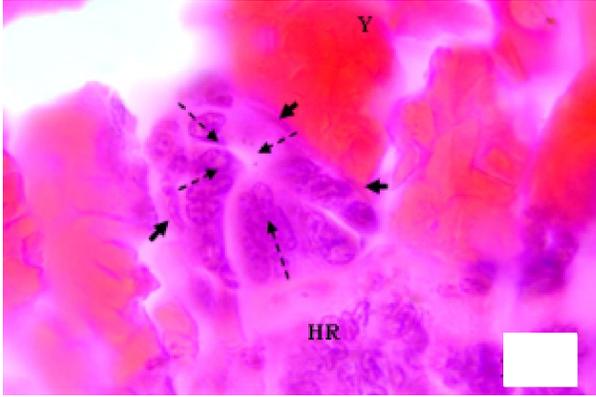


Figure 24: C. S through the anterior of the posterior half of the stomach primordial of an embryo of *E. styliferus*, 2.0 mm long at the final post-naupliar stage, showing the development of small central cavity. This half is closely similar to the anterior half and the cells at the midline are the most fast moving (broken arrows). Small arrows indicate the flat cells surrounding this half (mag. 200x).

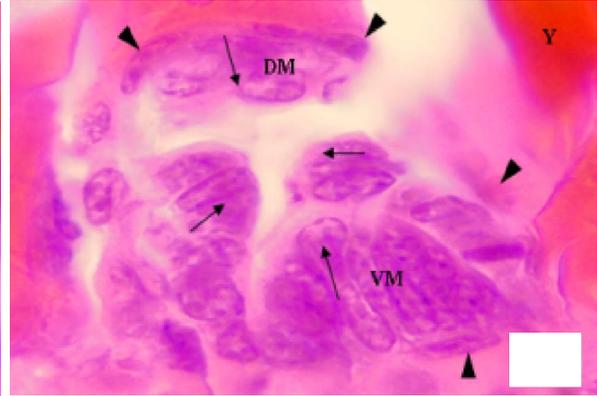


Figure 25: C. S through the anterior of the posterior half of 2.0 mm long embryo of *E. styliferus* at the final post-naupliar stage showing the dorsal mass (DM) and the ventral mass (VS). Arrows show the folding of the lateral walls towards the lumen and the surrounding of the cells of the stomach primordial with a layer of flat cells (small arrows) (mag. 200x)

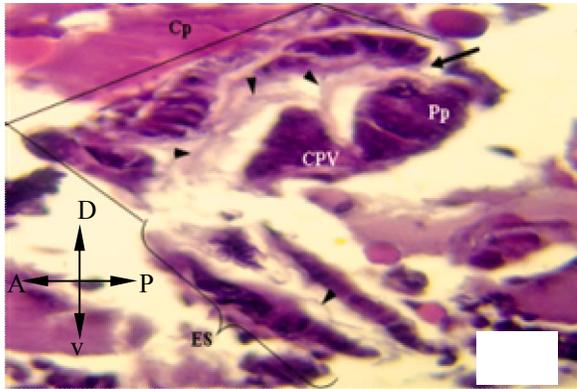


Figure 26: Latero- longitudinal section in the cephalothorax of 2.3 mm long embryo of *E. styliferus* at the pre- hatching embryo stage showing the foregut primordial. Notice the deviation of the sagittal axis of the esophageal primordial. The arrow shows the stomach opening and the cuticular layer (arrow heads) (mag. 200x).

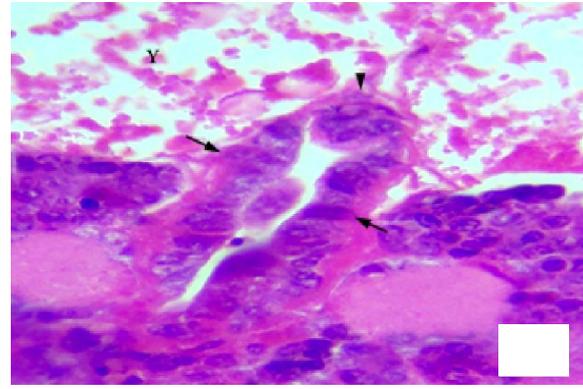


Figure 27: Longitudinal section through the esophageal primordial of 2.3 mm long embryo of *E. styliferus* at the pre- hatching embryo stage. Thick arrows indicate the limit between the esophagus and the stomach. Arrows indicate the flat cells surrounding the stomach primordial. Notice the narrowness of its lumen (mag. 200x).

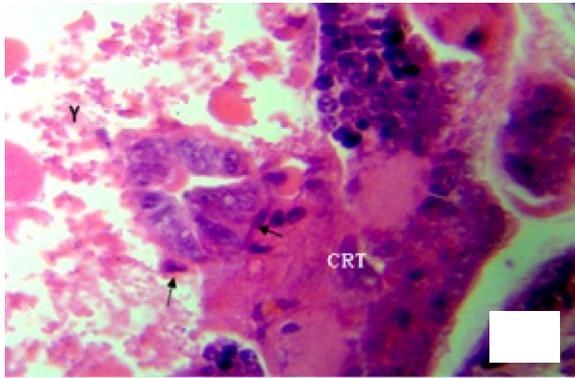


Figure 28: C. S. through the mid anterior cardiac region of the stomach primordia of a 2.3 mm long embryo of *E. styliferus* at the pre-hatching embryo stage showing the narrowing of the star-shaped lumen. Arrows indicate the flat cells (mag. 200x).

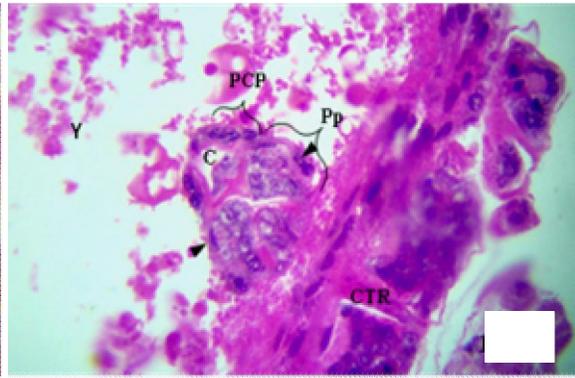


Figure 29: C. S. through the posterior end of the posterior half of the stomach primordia of a 2.3 mm long embryo of *E. styliferus* at the pre-hatching embryo stage showing the presence of a cavity (C) in the tube formed by the dorsal mass and the surrounding of this part by a layer of flat cells (arrow heads) (mag. 200x).

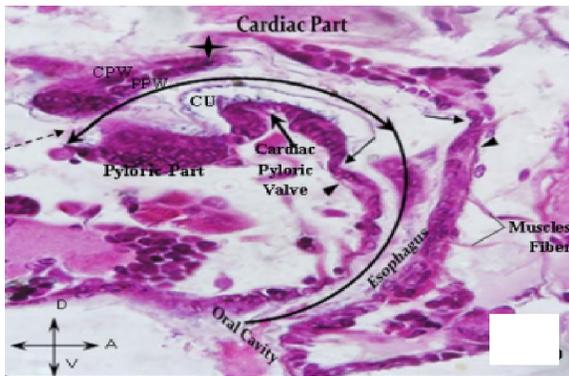


Figure 30: Sagittal section in the zoea I after hatching of *E. styliferus* 3.0 mm long passing through the cephalothorax showing details of the foregut with the arched esophagus and turning of the pyloric portion ventrally (arched arrow). Arrow heads indicate the most posterior of the esophagus and the most anterior part of the cardiac portion of the stomach (arrows), whereas the asterisk indicates posterior part of the cardiac portion of the stomach. Notice the constriction at the position of the cardiac-pyloric valve (large arrow) and the stomach opening (broken arrow). (mag. 200x).

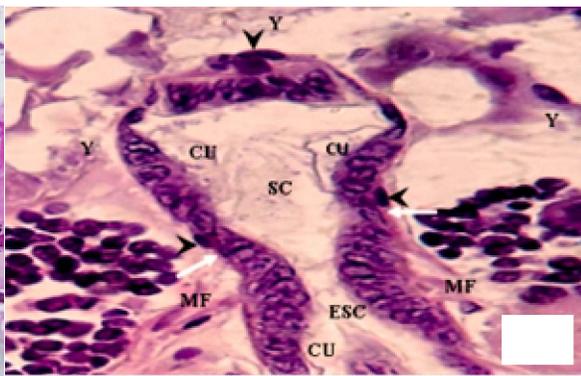


Figure 31: C. S. of the cephalothorax and a longitudinal section in the anterior of the foregut of zoea I of *E. styliferus* 3.0 mm long shows the connection of the esophagus (ESC) lumen with that of the stomach (SC). Notice the narrowness of the esophageal lumen at the region of the attachment of the muscle fibers of the epithelial lining (region of connection) of the esophagus with the stomach (arrows). See also the flat cells (arrow heads) (mag. 30x)

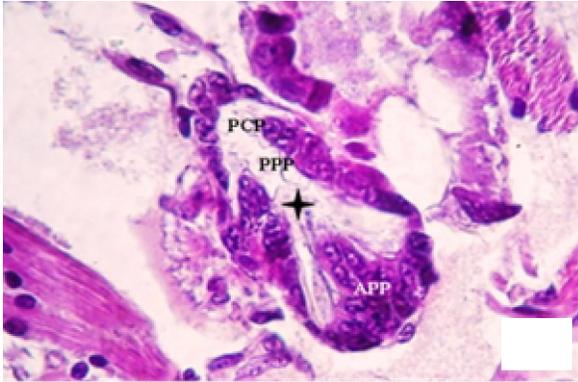


Figure 32: C. S. through the posterior part of the anterior cardiac half of the stomach of zoea I of *E. styliferus* showing the cardiac- pyloric valve with the mesenchymal tissue of the valve. Arrows show the thickness of the Cuticular layer surrounding the valve (mag. 50x).

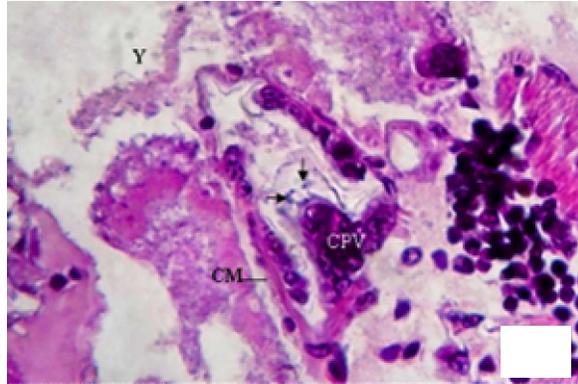


Figure 33: C. S. through the posterior part of the posterior cardiac half of the stomach at the zoea I stage of *E. styliferus* after hatching with a length of 3.0 mm showing the formation of the posterior portion of the pyloric stomach. Asterisk indicates the position of the future central canal (mag. 50x).

Discussion

The digestion tract of the decapods consists of three parts: the foregut which is composed of the oesophagus and the stomach, the mid-gut and the hind-gut which makes the posterior part of the intestine (Mykels, 1979; Factor, 1995). The general morphological features of the early developmental stages of the arthropod embryos are quite similar, with long germ analog with their posterior end represented by folded abdominal region. This was further confirmed by the present results on the shrimp *E. styliferus* in which the posterior end is ventrally folded, as is the case of other species of shrimps like *Macrobrachium potiuna*, *M. olfersi*, *Palaemon pandaliformis* and *Palaemonates argentinus* (Müller *et al.*, 2004), in contrary to the insects in which the ventral region is folded dorsally (Wolpert *et al.*, 1998).

Although, the previous studies were concerned with the development of the digestive tract in some decapods especially the shrimps, but these studies were especially concerned with some aspects of the embryonic development for instance, the formation of important structures of organs in general, but they overlooked many details of the micro changes that lead to the formation of these structure, particularly in the shrimps, and this was of major concern in the present investigation i.e. the region of the three parts of the digestive tract, the foregut and hindgut which are originated from the ectoderm, and the midgut which is originated from the endoderm (Vonk, 1960; Shiino, 1968; Johnston, 1980; Ceccalidi, 1989; Icely & Nott, 1992).

The present results indicate that the foregut and hindgut are differentiated quite early in the

embryonic development than the rest parts of the digestive system and this agrees with the results of Ceccalidi (1989), Li *et al.* (2001) and Zengi *et al.* (2001), but these studies disagree with each other as to the stage at which these parts are originated.

In the present study, the foregut of *E. styliferus* is discriminated at the initial post-naupliar stage, whereas in the crayfish *Cherax quadricarinatus* it was differentiated at the egg-nauplius stage and in the crab *Portunus trituerkulatus* it appeared in the egg-zoea I stage (Li *et al.*, 2001; Zengi *et al.*, 2001). This difference in the stage of the formation of the foregut among these species is likely due to species differences and the type of eggs of each species, for instance in those decapods that have centrolecithal eggs like *E. styliferus*, the amount of yolk allows the embryo to pass through various developmental stages inside the egg which hatch as zoeas (Anderson, 1982; Williamson & Rice, 1996; Scholtz, 2000; Müller *et al.*, 2004), whereas, those decapods with oligolecithal egg is represented by a single developmental stage only (Zilch, 1978; Williamson 1982; Hertzler & Clark, 1992; Hertzler, 2002).

Previous studies showed that the foregut is ectodermal in origin (Vonk, 1960; Shiino, 1968; Johnston, 1980; Ceccalidi, 1989; Icely & Nott, 1992), while in *E. styliferus* it was found that it originates from the area of the midline of the embryo from a mass of mesenchymal cells in the cephalic region of the embryo, as indicated by the presence in the cephalic region of cells of ectodermal origin.

The present results signify the origin of the oesophagus and the stomach from the small cellular mass of the cephalic region in *E. styliferus*. This is perhaps a clear indication of the foregut being include

the oesophagus and the stomach, as well as their position in the digestive system and the embryo as a whole.

The present result showed that the primordial of the stomach in *E. styliferus* is differentiated faster than the oesophagus. This is likely due to the great complexity of the structure of the stomach at the maturity stage in contrast to that of the oesophagus.

The formation of the stomach primordial indicates that it has a special pattern, and during this time the boundaries between the cardiac and pyloric portions were defined early in the embryonic development, as the folding of the dorso-lateral wall of the posterior half of the stomach primordial, represent the boundary separating the cardiac from the pyloric portion, because this folding is not only pushing the cell mass of the ventral wall (the second mass) of the posterior half interiorly, but it would lead to make the posterior half of chamber cavity (lumen) similar to that of the pyloric part of the adult and thus forms an Arabic figure 8. Furthermore, it represents a local specialization from the early stages of development, as it looks like two different halves varied in shape and development. The first is anterior in the form of cellular lamella formed the cardiac portion, whereas the posterior half is represented by a solid mass formed the cardiac- pyloric valve and the pyloric portion together with the posterior end of the cardiac portion.

The present results emphasize that the mesenchymal cellular mass forming the cardiac-pyloric valve had differentiated before that forming the pyloric part. Therefore, when the early formation of the stomach primordial was followed up, it is apparent that the differentiation had occurred in a special pattern from the anterior to the posterior region of the stomach, as the cardiac portion of the stomach primordial at the anterior half was formed before the posterior half. Associated with the formation of the posterior half of the cardiac portion, the differentiation of the cells forming the valve, which occurred before the differentiation of the cell mass forming the pyloric portion, whereas, the posterior wall of the stomach is the last part of the stomach to be formed, which was clearly differentiated at zoea stage 1.

The events in which the stomach primordial past through like the folding of its lateral wall and the constriction at its floor were the process causing the stomach to take an arched shaped. These processes are part of the morphogenetic movements which eventually lead the stomach to take its final shape. These movements initiate the formation of the adult stomach structure of which the formation of the filter at the chamber of the anterior pyloric portion

resembling the Arabic figure 8, which was appeared at the third developmental stage.

It is important to note that these movements are irreversible i.e. remain at their shape formed by the previous movements until new movements had occurred which bring them to their final adult shape. These movements cannot be explained as a result of muscular contraction or amoeboid movements (Verma & Agarwal, 2009).

At the pre- hatching stage of *E. styliferus*, the size of the cardiac region is equal to that of the pyloric region, then the cardiac region becomes larger than the pyloric region at the zoea stage 1, whereas, in the prawn *Macrobrachium amazonicum* both the cardiac and pyloric regions are equal to each other at the zoeal stage 2, then the cardiac becomes doubled the size of the pyloric region at zoea stage 4 (Queiroz *et al.*, 2011). This is apparently due to the rapid growth and development in *E. styliferus* than in *M. amazonicum*.

The present results show that the cardiac-pyloric valve at zoea stage 1 is covered by a thick layer cuticle devoid of setae as is the case in the zoeal stage 1 of *M. amazonicum* (Queiroz *et al.*, 2011).

The present results indicate that the development of the digestive tract is completed after hatching by the fusion of the foregut with the midgut at stage 1 zoea, although the mouth and anal opening in *E. styliferus* were opened at this stage, but the important structures of the digestive tract which make it function like the filter and the folds of the cardiac-pyloric valve were not completed yet. These may answer the question that the zoea 1 is a non-feeding stage (Al-Abbad *et al.*, 2008).

The development of the stomach primordial continued after hatching, as the pyloric portion did not show any specialized structure like the filter. This means that developments of these structures continued after hatching and this is happened also in many organisms which pass through several stages in their life cycle (Schrode *et al.*, 2013). Since the first zoeal stage is a non-feeding stage, therefore, the structure of the type stomach after hatching greatly influenced the type of food consumed by the following zoeas, for the absence of the filter and most other structures of the shrimp *E. styliferus* shown by Ibrahim (2012), may likely indicate a difference in the type of food between the larval (zoea) stages and these stages with the adults are depending upon the degree of development of the digestive tract.

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