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HISTOLOGICAL DIFFERENTIATION IN THE DIGESTIVE SYSTEM AND BRANCHIAL ARCHES OF THE LIVE- BEARING TELEOST , *POECILIA SPHENOPS*

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Abstract

The development of digestive and branchial systems have been studied in viviparous black molly ,*Poecilia sphenops*, during embryonic stages and at newly born youngs .The pharynx originates as incomplete circle , or tongue likewise structure , with some mucous cells line its mucous , and some taste buds. The liver started as two segment s ,whereas the spleen bud was triangular structure The liver started as two segment s ,whereas the spleen bud was triangular structure in midst of them .The intestine started as closed tube ,where the pancreatic islets beside. Gradually, there were intestine folds and looping , with pancreatic-livery duct flows in small intestine .Then the pharynx connected with intestine by developed esophagus .It is apparent that the morphogenesis of the rostral digestive tract starts before hind gut formation. Three primordial gill arches shown at early stages of developing embryo, before the operculum opening. The gill filaments start to form as short gill rakers .Meanwhile, the kidney tubules being more developed. With birth of youngs, there was completely open lumen for intestine and more differentiated digestive organs. The fourth gill arch still as bud structure, but the gills seem to perform their functions in respiration or water / ion balance, as they continue their development.

Key words: *P.sphenops*, embryo, digestive system, branchial system.

Introduction

Black molly *Poecilia sphenops* is one of live bearer fishes which hold the developing embryo within a body structure until the post larval or really juvenile stage is attained.

In viviparous fish *Gambusia affinis* , where there is mother- to- embryo transfer of nutrients , the individual provisioning of embryos was not related to developmental stage but was related to embryo mass (Marsh-Matthews *et al* . ,2005). It was observed that the reproductive mode in scorpaenid fish *Sebastes mlanops* involved longer retention of embryos until after organogenesis and functional differentiation of the gut , facilitating this rather primitive form of embryonic nutrition (Boehlert and Yoklavish , 1984) .The embryos of blenny *Zoarces viviparus* are able to assimilate analine from ambient micromolar solutions (Korsgaard , 1992) .It was demonstrated that a large endogenous supply of nutrients enables the definite adult phenotype to develop directly (Balon , 1986). The fish –specific apolipoprotein Apo-14 is required for digestive system organogenesis during fish embryogenesis and larval development (Xia *et al*., 2008). On the other hand, Wang *et al.* (2011) observed similar morphology between the liver progenitor cells and the green fluorescent protein(GFP) nuclei on the yolk syncytial layer (YSL) , suggesting that they might originate from the same progenitor cells in early embryos ,and as that in human (Zaret ,2008). The endoderm gives rise to the hepatoblast, and at early gastrula stage, the endoderm cells located in the ventral part tend to differentiate to liver bud while those on the dorsal side are apt to give rise to pancreas (Tao and Peng, 2009). The hepatogenesis in zebrafish started as specification in endoderm, then differentiated before hepatic growth (Chu and Sadler, 2009). In zebrafish itself, the primitive gut tube is formed later from the endoderm between 32 and 40 hours post fertilization (Makkey *et al*., 2007).

It was suggested that the earliest bones to appear are those dermal elements of the branchial skeleton involved with feeding (Morris and Gaudin, 1982). Falk-Petersen (2005) noted that main organs and organ systems become functional by first feeding and differentiate during larval stage and metamorphosis of marine fishes.

The main objective of the present study was to demonstrate the early developmental stages of digestive system, along with branchial system of black molly to detect the complementarity between the two systems and to recognize their role in nutrition of larvae and new-born individuals.

Material and Methods

Twenty individuals of pregnant females of black molly *Poecilia sphenops* were reared in two aquaria containing 40-liters of water, in each one, and fed with artificial commercial diet.

Five to six fishes were sampled randomly, weekly and dissected to take out the ovaries or embryos (at different stages of development) inside. The samples were fixed in Bouin's solution, embedded in paraffin and cut into 5-6 µm thickness, in cross or longitudinal sections that were stained with haematoxylin –eosin stain (Bancroft and Stevens, 1982).

The sections were viewed under x 25 -1000 magnification and photographed with aid of a digital camera (Sony DSC -77). Cell measurements were made under resolving power of 0.2 µm.

Results and Discussion

In gross morphology, the growing embryos lie in a groove in the surface of the yolk and enclosed by ovarian follicle, as they live in a fluid medium, inside some eggs in different stages.

The abundant yolk supply in the egg maybe sufficient to provide the nutrient material required by the embryo. Scove *et al*. (2010) observed that during the last months of gestation in *Zoarces viviparous* , yolk reserves depleted and the embryos depend on an external source of nutrient . Korsgaard (1992), in turn, referred to an evidence for uptake and metabolism of amino acids by the embryo of the same species *in vitro* and *in vivo*.

Digestive System:

The Pharynx originates as incomplete circle, or tongue likewise structure in the first place, composed of cuboidal stratified epithelium rests on collagenous connective tissue, with some chromatophores and mucous cells among or above the epithelium. The pharynx wall attains 25-37 µm in height, whereas the epithelium height 12-20 µm. There were some capillary blood vessels among the tongue wall, as there was large artery filled by RBC at a boundary to its wall. There were mucous cells line the mucosa of pharynx, in addition to other cells over the epithelium that attains 12-13 µm in height, and so, there is mucous gland. Some taste buds were shown on epithelial surface or among it, as there were chromatophores among submucosa, along with vascular ducts filled with blood corpuscles (Fig.1). However, no clear folds yet could be observed.

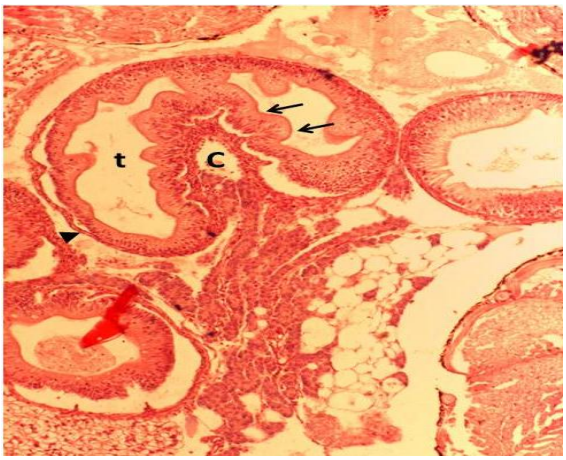

Fig.1: Start of pharynx, with mucus cells (→), connective epithelium (→), spleen tissue (►), blood vessel(c) and taste bud (t)

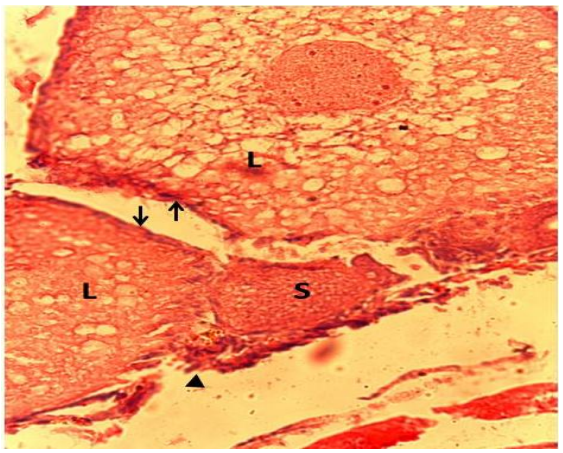

Fig.2: Liver bud (L) covered by bud (s) and pancreas (►)

The liver started as tow segments as elliptical structures measured 310 x 230 µm and 400 x 150 µm, and covered with stratified squamous epithelium. Between the two buds there was triangular, dark stained structure, which represents the spleen bud (Fig.2), and gall bladder with its duct. Besides, undifferentiated closed intestine and undifferentiated islets of pancreas, along with large vascular ducts filled by blood corpuscles. The genesis of such structures seems as current phenomena in digestive system morphogenesis. That is the process observed in another fishes as zebrafish where the liver and pancreas arise from endoderm independent of a primitive gut tube (Wallace and Pack, 2003). However, the morphogenesis of rostral digestive tract (pharynx and esophagus) in *P.sphenops* was before completion of the hind gut formation, and not just as in zebrafish. Tao and Peng (2009) hypothesized that liver progenitors in zebrafish might be differentiated before the initiation of alimentary canal morphogenesis and the liver bud was formed later by migration of these progenitor cells, as shown in pancreas. On the other hand, the strong decrease in the mass and size of liver considered as hatching control (Huynh-Delerme *et al*., 2005). It was noted that different stages of hepatogenesis could be seen during same time. Makkey *et al*. (2007) stated that organ development requires coordination between multiple cellular processes to yield an organ of proper size and tissue architecture.

The structure represents tongue was shown holding 2-5 taste buds, in addition to some ones observed on the outer wrap of the embryo.

Cross sections illustrated the looping of hind gut which showed distinct folds and possessed 37-62 µm height stratified epithelium.

In following, the esophagus connected to the pharynx which lined by stratified cuboidal epithelium that attaining 25-50 µm in height and embraced some sizable chromatophores towards the lumen. The esophagus showed 120 µm in length among its lumen (Fig.3), but it still not open to intestine. However, there is brush boarder over simple columnar basal nucleated epithelium lining the lumen of both latter organs (Fig.4).

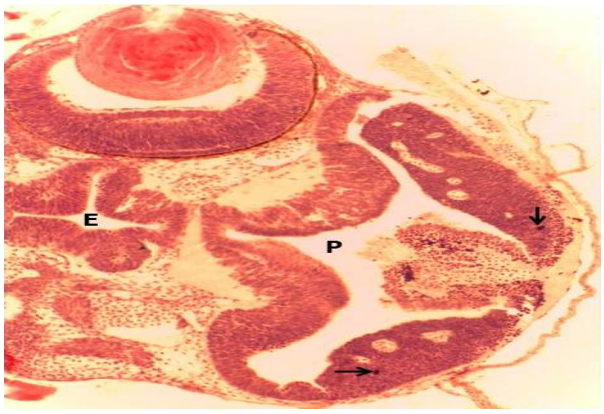

Fig.3: The pharynx (P) approaches to connect the esophagus pharynx (P) (E) and some chromatophores (→) are shown

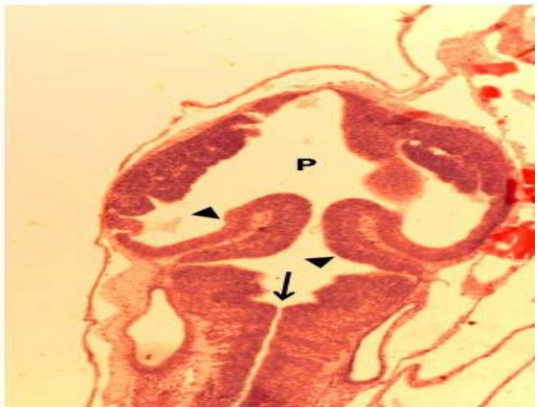

Fig.4: The brush boarder (►) lining the and esophagus(→)

In subsequent stage, the pancreatic islets being more differentiated beside the liver that has incomplete hepatocytes, as there was initiation of pancreatic-livery duct towards the intestine (Fig.5).

The medial intestine still has no differentiated wall, although there is central lumen inside most of its length, meanwhile, the mouth and anus still not open.

With advanced development , there were deeper intestine folds that attaining 30-50 µm thickness , with 20-30 µm of which simple columnar epithelium characterized by basal nuclei, and rests on 7-10 µm thick connective tissue . The intestine appeared with more distinct looping, as it contained amorphous diet, along with some RBC (Fig.6). The existence of such materials within intestine before mouth opening indicates maternal nutrition. The liver in being more developed attaining 700x330 µm in dimensions with more differentiated hepatocytes and blood vessels permeates its mass in addition to scarcity of chromatophores (Fig. 7), as there were flourished pancreas scattered among small intestine looping. The spleen shown as oval mass between the intestine and liver, its length 100 µm, stained darkly that indicates an assembly of immature blood cells. It is being mature organ before birth or mouth opening, whereas it was observed as immature organ with mouth opening in oviparous fish common dentex *Dentex dentex* (Stantamaria *et al*., 2004).

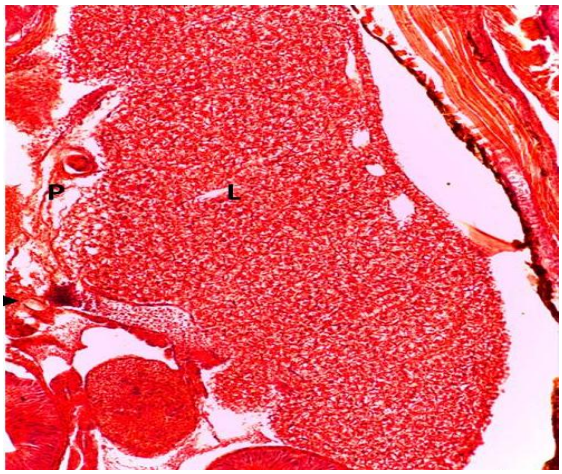

Fig.5: Liver (L), pancreatic islets (P) and livery -pancreatic duct (►)

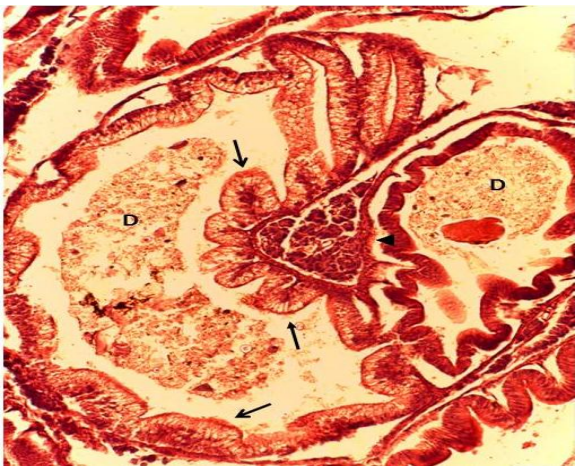

Fig.6: Intestine folds (→), pancreas (►) and amorphous diet (D)

At this stage, there were kidney tubules with blood vessels and blood corpuscles diffuse among them (Fig.8), in the meanwhile the operculum still with partial aperture.

At subsequent stage, the intestine seems with less mucous cells around lumen, where the spleen is larger in mass as flows into the intestine through slender duct.

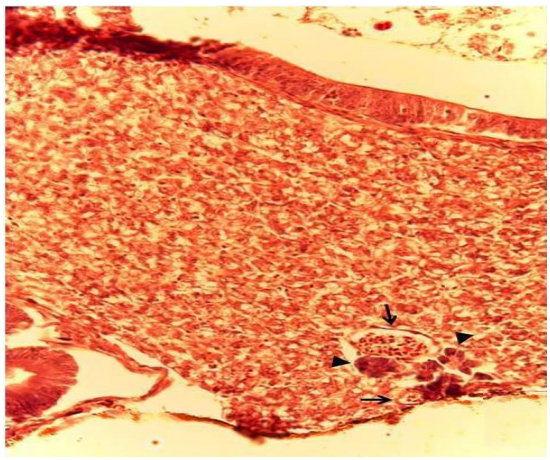

Fig.7: Differentiated hepatocytes with blood blood vessels (→) and chromatophores (►)

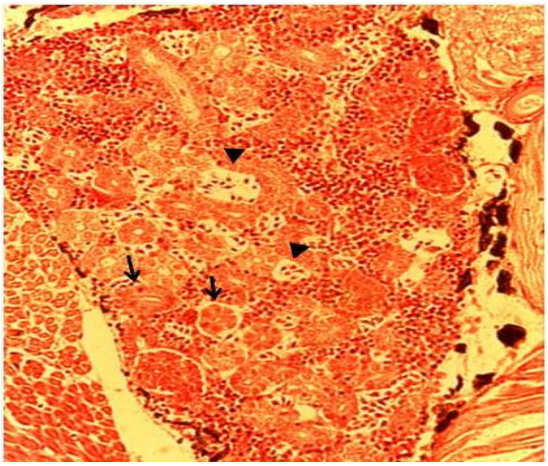

Fig.8: Primordial kidney with tubules (→) and vessels (►)

No folds could be observed in some portions of intestine where measured 180 µm in diameter and lined with stratified columnar epithelium that included scattered mucous cells and some chromatophores. The strata of intestine, in general, are surrounded by fibrous connective tissue and incomplete circle of pigment cells above squamous stratified epithelium (Fig.9). The height of intestinal fold varied from 30-62 µm, where the epithelium varied from 12-25 µm.

The newly born molly have open mouth that lined internally by epithelium communicates with head epidermis and contained some taste buds, as other buds ,which seem larger in size, outside the mouth . Laurila and Holopainen (1990) found that the intestine folds development and mouth opening occurred in *Carassius carassius* after hatching. Zaret (2008) suggested that analogous process could be observed in mammals, with important differences. The digestive tract morphogenesis occurs at a comparatively late stage of development in zebrafish (Wallace and Pack, 2003).

The pharynx open to esophagus as it open towards the gills. The pharynx folds being more developed towards the liver and decreased in wall height down to 15-32 µm, as they included taste buds and mucous cells among or above their thickness. In any case, the mucous cells seem less in density comparing with that at previous stage. The sections showed amount of plankton in pharynx lumen taken as food and being with less digestibility comparing with that shown in lower portions of alimentary tract. The esophagus is currently open to intestine. Simultaneously, the serial sections showed integration of intestines lumens, along with spots of apoptosis, as well as, aggregation of blastocytes. Although the digestive system now is not completely developed, it seems sufficiently efficient to support the growth of the new born fishes by digesting the planktonic preys.

There is olfactory tissue , as well as , some taste buds inside olfactory chamber .The taste buds noticed at embryos about to birth , inside or outside body , as they are more at birth , that facilitate external feed , along with olfactory and vision senses . Kasumyan and DØving (2003) suggested that is a good correspondence between development of the gustatory system in fish ontogeny and its ability to discriminate taste properties of food items. The younger specimens usually less capable to undergo a degree of starvation as declared by Love (1980).

The elongated esophagus characterized by plenty of mucous cells among its mucosa and less on edges, while its folds decreased in height down to 15-23 µm, as it contained more food particles.

The proximal intestine appeared broad in its cross section and marked by stratified cuboidal epithelium in mucosa that attained up to 50 µm in some parts. More mucous cells are seen on brush boarder or among epithelium in comparison with that on esophagus, but no chromatophores. The food items appeared more digested inside the intestine lumen, particularly at rectum.

The liver grew in size up to 950 x 460 µm, as it contained confined blood vessels in addition to marked larger one, beside empty hole. The pancreatic islets, in turn, possessed larger common pancreatic- livery duct. According to the preparatory structures, it seems that the born youngs are ready for external feeding, employing this digestive system and open mouth, in association with developed sense organs.

Branchial System:

There are three gill placods with less apparent fourth one in branchial cavity at early stages of developing embryo , as there was no distinct cartilage among them , although it being more obvious at cranium (Fig.10) . In the meantime, the intestine still not open in most portions. Subsequently, the arches cartilage noted to be more differentiated, where it seems more developed on the oral side, meanwhile, the operculum appeared open partially, accompaniment with heart impulse.

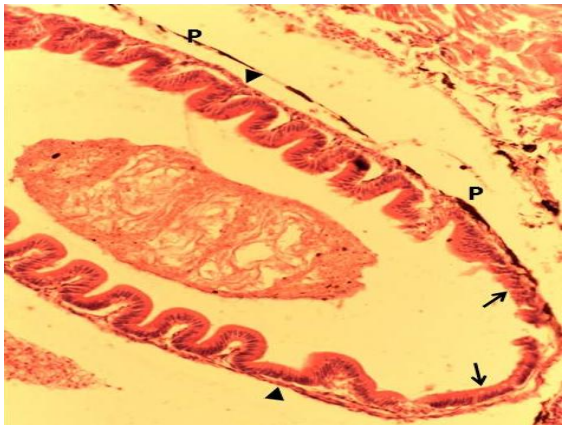

Fig.9: Folds lack in some portions (→), connective tissue (►) and pigments (P)

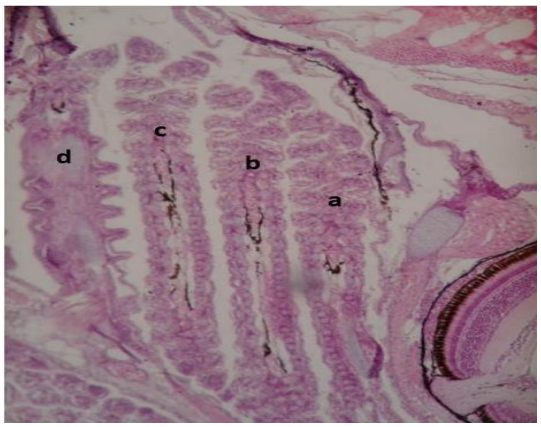

Fig.10: Three gill placods (a, b, c) with less apparent one (d)

In subsequent stage, the arch cartilage showed more differentiation in two arches on oral side, with more illustrated pigments, where the gill rakers started to establish as short protuberances, composed of stratified cuboidal epithelium (Fig.11).

It is clear that the gills began their functions during embryological stages, before operculum opening, where there were three gill buds bearing less developed rakers and no apparent cartilage to regulate water and ions balance . Conte (2012) observed that the gills begin to control gaseous movement and water / ion transport shortly after fertilization of the teleost egg. Some taste buds were seen on the base of arch buds opposite to others rested on the membrane that lined gill pouch.

With opening of operculum, the gill arches being more differentiated, the oral arch extends 260 µm in length, including 38 µm height gill rakers. These arches contain a number of mucous cells and scarcity of chloride cells (Fig.12). Rainbow trout *Oncorhynchus mykiss* gills appear initially to be more important in terms of ion balance than gas exchange before secondary lamellae begin to form (Rombough, 1999). Simultaneously, the kidney tubules of molly being more developed, with plenty of filled blood vessels and cut of chromatophores circle. The kidneys regulate the relationship between ions, as well as, excrete less diffusible nitrogenous nutritional end-products.

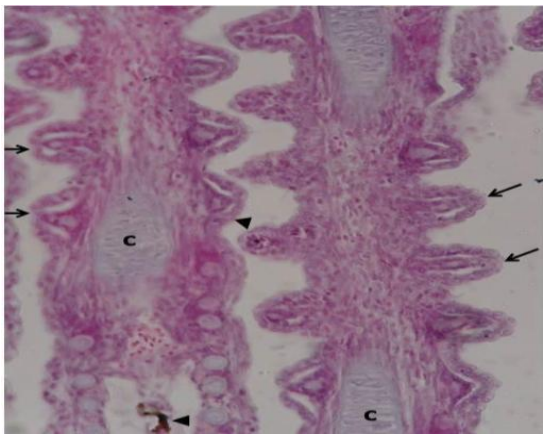

Fig.11: Short gill rakers(→), pigments(►) and mucous differentiated cartillage(C)

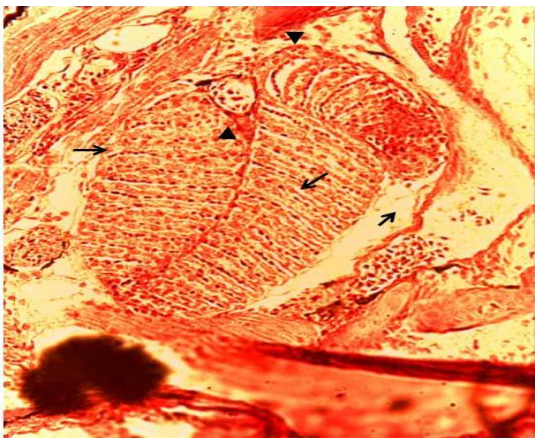

Fig.12: Differentiated gill arch containing cells(→) and chloride cells (►)

In the newborn youngs, the fourth gill arch is still represented by a basal part, whereas the three primary arches have no perfectly developed lamellae, in spite of apparent blood vessels that indicate gas exchange. Also, no well-done cartilage appeared which seems well-marked in cranium during this stage of development. The length and width of first, second and third gill arches were 262 x117, 300x84 and 250x105 µm, respectively. The pinnate gill filaments in molly embryos were observed also in the embryos of livebearer *Gambusia affinis* , as the pinnae contain loops of blood vessels (Kuntz ,1913) . The extradevelopment of gill shown in newborn individuals appears analogous to that observed at early post-hatch at Atlantic salmon *Salmo salar* , where the timing of gill development is related to developmental stage than to body size(Wells and Pinder ,1996) . However, the scorpaenid viviparous black rockfish *Sebastes schlegeni* attained full development of gill rakers and gill teeth by 15 mm T.L (Omeri *et al*., 1996).

It seems that gill arches play their role in feeding for they prevent entry of undesirably large food items into pharynx, where molly searches for planktonic items. Besides, the youngs need no tongue to aid in food grasp. Morris and Gaudin (1982) demonstrated that the earliest bones to appear are those dermal elements of branchial skeleton involved with feeding.

Conclusion

The pharynx, esophagus and intestine started as solid structures, then they open and connected to each other as continuous tract. It was observed that the rostral digestive tract created prior to hind gut formation. However, there is maternal nutrition during embryological stages before external nutrition at birth. The branchial system started at early

stages of organogenesis and developed along with digestive system to perform its role in feeding, in addition to respiratory function. So, it looks necessary in nutrition of molly.

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