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The Early Organogenesis of *Carassius auratus* (Cyprinidae): A Histological Perspectives

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Abstract: The present study was aimed to larval organogenesis of *Carassius auratus* with respect to histological aspects. The eggs and different stages of *C. auratus* larvae were fixed in Bouin's fluid. Histological studies were carried out from 1st day after fertilization (DAF) to 12th DAF on every day and 16th, 24th, 32nd, and 40th DAF. Histological studies of *C. auratus* egg showed embryonic disc formation, followed by neural tube and embryonic eyes on the 2nd DAF. The newly hatched larvae have well-defined eyes and a neural tube was differentiated into the brain. Subsequently, digestive system, swim bladder, gills, skin, and fins formation were studied. The ontogeny developments were divided into 5 stages. During the larval development, the first two stages have differed in their organ development. Their head was straight and free from the yolk and their body was attached to the yolk sac and gill arches which appeared between the 3rd and 5th DAF. The digestive system was straight, increased in the dorsal and the ventral part of the body on the 9th DAF. In between the 10th to 17th DAF, operculum, gill lamella, and dorsal fin were developed. The larvae fin, coiled intestine, and well-developed accessory glands were formed during 24th to 40th DAF.

Keywords: *Carassius auratus*, Organogenesis, Histology, Embryonic disc, Development

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Introduction

The goldfish, *Carassius auratus* is one of the most common aquarium fish, and its ability to thrive in a variety of environments makes them a popular choice (Küçük, 2013). Fish larvae development is the most crucial stage of the life cycle. There are many reports on the histology of many organs and organ systems of fishes, which provide a better understanding of the functioning of the specific system (Groman, 1982; Hibiya, 1982; Fukuhara, 1987; Chinabut *et al*., 1991).

The majority of freshly hatched freshwater fish larvae are not fully grown (Sulaeman and Fotedar, 2017) and morphological changes do not occur in a linear pattern throughout ontogenesis, since they might occur quickly in one or more body regions and often correlate with anatomic,

physiological, behavioral, and environmental changes. (Sagnes *et al*., 2005; Kupren *et al*., 2015a, b). While the majority of fish larvae research focuses on morphological development about function, (Osse, 1990, Moioka *et al*., 2009), some research has been carried out with the goal of structural, anatomical, and histological aspects of fish organ systems to their habits and behaviors. (Santos *et al*., 2015).

The embryonic and early larval development of genus *Leuciscus* were studied on variable and constant thermal conditions (Kupren et al. 2011) and allometric and ontogenic development of common barbel were studied under optimal conditions (Nowosad *et al*., 2021). The anatomical elements of larval dentex development, as well as anomalies in response to larval rearing circumstances, have been thoroughly characterized (Koumoundouros *et al*., 2001). Santamaria *et al*. (2004) described the major histological changes that occur during the development of dentex larvae. They also reported the structures involved in general metabolic processes, defense mechanisms, and feeding in particular. In the present study we investigated the histological aspects of ontogenic development of *C. aureatus* for successful larval rearing which is necessary for culture conditions and feeding tactics.

Materials and Methods

Breeders and maintenance conditions:

Breeding and spawning of the common goldfish, *C. auratus* was carried out in the laboratory. Broodstock of this species was procured from Kandhan hatchery, Chennai, India. They were maintained in concrete tanks, and fed with live feed (tubifex worms and *Chirnomous* larvae). The breeding and spawning tanks (10 x 4 x 5 ft) were cleaned well and filled with filtered tap water. Water with 6.7 pH, the temperature of 29.67±1.68 C, and an ammonia level less than 0.5 mg/l are desirable for breeding these fishes. Water has to be continuously aerated. The breeding tanks were provided with aquatic weed (*Chara* sp.). The breeders were introduced late in the evening hours into the breeding tanks. The usual male: female ratio of *C. auratus* introduced was 4:1.

The female swam through the plants at considerable speed and the eggs were spontaneous spawned and laid on the leaves of the plants, which is followed by the male which released the milt and in turn fertilized the eggs. Spawning in *C. auratus* usually takes place during early morning hours and ranged between 2 to 4 h. After the completion of spawning, the breeders were carefully transferred to another tank. The aquatic weed with attached eggs was carefully removed from the breeding tank and was uniformly distributed into a hatching hapa (2.5 x 1 x 1.5 m) made up of thin cloth and maintained in the water having a quality similar to that of a breeding tank. The hapa with developing eggs were maintained undisturbed until the eggs hatch out into larvae.

Larval rearing:

For larval rearing, freshly hatched out larvae (first day) were carefully selected. Larval rearing of *C. auratus* was fed with pelletized feed and mixed live feed (*Moina micrura* and *Thermocyclops decipiens*). A batch of 100 first day larvae of *C. auratus* of similar length was introduced into the concrete experimental tank (75 cm (length) x 40 cm (dia.) in 35 l of water). The larval tank were aerated and maintained without feed during the non-feeding stage of the larvae with natural photoperiod. The larvae of *C. auratus* commenced their exogenous feeding on the 7th DAF. Food was offered to the larvae *ad libitum* and experiments were conducted for 40 days. Every day morning hours fecal matter and excess feed were removed from the larval rearing tank and 50% of water was replenished. The size of eggs and larvae were measured using micrometer. The fishes were maintained as per the guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Karpaga Vinayaga Educational Group, Chennai, India.

Histology of eggs and larvae:

To study histology, eggs and larvae of *C. auratus* were selected from just spawned eggs to larvae grown up to 40 days. The larvae were anesthetized with 50 mg/l of benzocaine and fixed every day from 1st DAF to 12th DAF and also fixed on the 16th, 24th, 32nd and 40th DAF. The eggs and the larvae were euthanized and fixed in aqueous Bouins's fluid for 12 h and washed in running tap water until the yellow colour of picric acid is removed.

The specimens were dehydrated in ascending series of ethyl alcohol (30%, 50%, 70%, 90%, and absolute alcohol), and then cleared with xylene. They were embedded in paraffin wax (52 C). Longitudinal section (L.S) and cross section (C.S) of the specimens were cut at 8 µm thickness using an Erma rotatory microtome. The sections were deparaffinized in xylene and hydrated in descending series of ethanol. They were treated with water and then stained with hematoxylin for 30 min. They were washed with tap water and treated with 30%, 50%, and 70% ethanol and counter stained with alcoholic eosin for about 5 min. Further, dehydration was carried out by passing them through 90% and absolute alcohol. After dehydration sections were cleared with xylene and permanent mounts were prepared using Canada balsam.

Structure of egg, development of larvae about eye, nervous system, digestive system, gills, pigmentation, and fins were observed. They were photomicrographed using Samsung (CCD) camera attached to Leica ATC 2000 microscope at different magnifications.

Results

Growth of C. auratus:

The freshly spawned eggs of *C. auratus* measured 1.03 ± 0.04 mm in diameter. During embryonic development on the second day, the diameter of the egg was 1.13 ± 0.04 mm. The newly hatched out larva measured 3.10 ± 0.21 mm in length on the third day. On the 4th, 5th, and 6th DAF the larva remained in the non-feeding stage and utilized reserved yolk. From the 7th DAF, the larvae commenced exogenous feeding. Different types of feeds were provided for forty days. The 4th DAF to 12th DAF old larvae showed a linear increase in the length and they measured 4.87 ± 0.24 mm to 10.67 ± 0.27 mm. 40th DAF old larvae measured 20.77 ± 0.35 mm (Fig. 1).

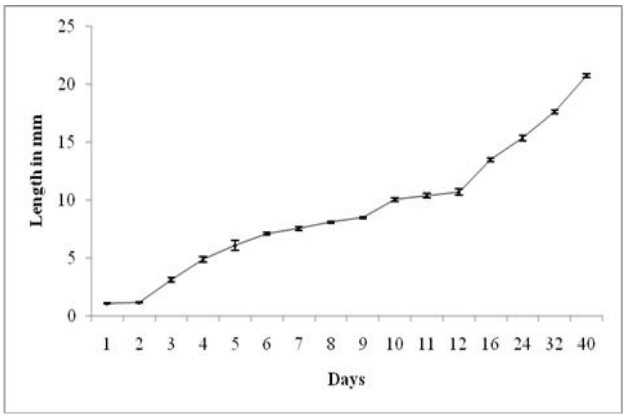

Fig.1: Length of larvae of *C. auratus* at different days of experimental period.

The larval development of *C. auratus* was divided into 5 stages. These stages were divided based on feeding strategies, morphological characteristics and organ development. Stage I and stage II has exclusively endogenous feeding larvae. In this stage, the embryo was visible and well formed yolk sac was present around the embryo. The head was straight and the yolk body attached to the yolk sac. Stage III, mouth and anus were opened and the digestive system was straight and elongated. They have a small amount of yolk present, which was endo and exogenous feeding stage. Stage IV and V were exclusively exogenous feeding conditions. In this stage, the digestive system became coiled, and well developed accessory glands formed. The gill lamella and caudal fin was developed and the swim bladder was inflated (Table 1).

Histological aspects of egg and larvae of C. auratus:

Egg:

The whole mount and histological studies on fertilized eggs undergoing embryonic development indicated accumulation of active cytoplasm at the animal pole and initiation of cleavage (Figs. 2a-2d). At the beginning of

Table 1: Larval developmental stages of *C. auratus* classified based on external morphological observations

Stage	External morphological observation	Duration (Days)	Standard length (mm)	Total length (mm)	Food source
1	The egg has a smooth surface, yolk yellowish devoid of oil globule, rudimentary embryo visible on day 1, advance stage embryo with the well-formed body around yolk sac, clearly visible on day 2.	0 - 2	-	1.03 - 1.13 in diameter	Exclusively endogenous
2	Head is straight and free from the yolk, body attached to a large oval yolk sac, mouth and anus closed, gill arches appear, buccal invagination observed on day 4.	3 - 5	3.10 - 6.07	3.60 - 7.01	Exclusively endogenous
3	Mouth and anus open, pigmentation of the eye, pectoral fins observed, caudal fin distinguished from embryonic fin, digestive system straight and elongate pigmentation increased on the dorsal and ventral part of the body.	6 - 9	7.10 - 8.46	7.70 - 9.30	Endo and exogenous
4	Digestive system looped, nostrils formed, operculum, gill lamellae, caudal fin developed, swim bladder inflated, resorption of primordial fin folds pigmentation of the whole body, dorsal fin originate.	10 - 17	10.03 - 12.80	10.95 - 13.60	Exclusively exogenous
5	Fin fully formed, coiled intestine and well-developed accessory glands, intense pigmentation of the body.	18 - 40	13.20 - 20.55	13.90 - 21.38	Exclusively exogenous

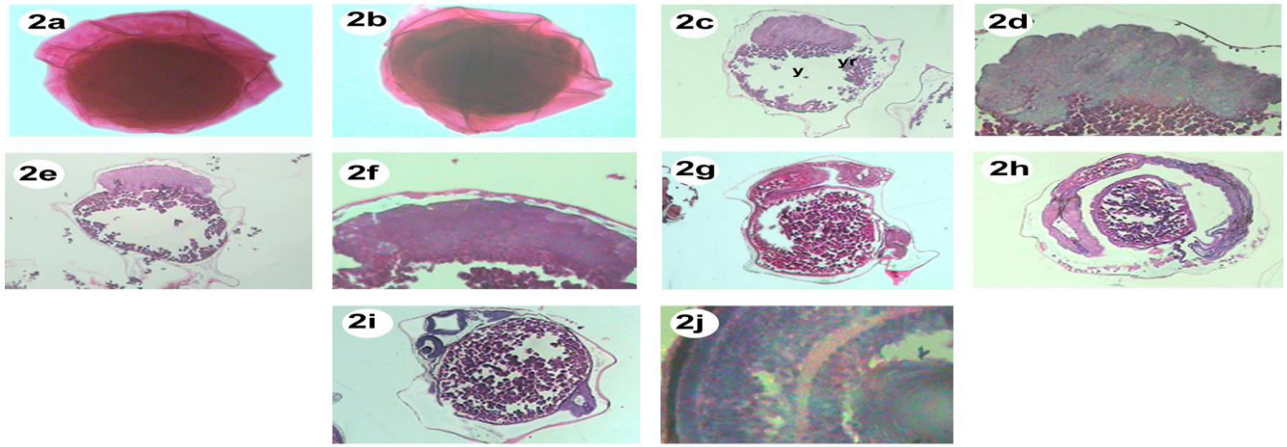

Fig. 2: **(a)** Egg with cytoplasmic mount; **(b)** Egg showing cleavage; **(c)** Histology of egg showing active cytoplasm and yolk y- yolk; yg- yolk granules; **(d)** Higher magnification of active cytoplasm in cleavage; **(e)** Histology of egg showing blastodisc; **(f)** Histology of egg showing gastrulation; **(g)** Histology of egg showing head formation; **(h)** Histology of egg showing metamerism of embryo and body; **(i)** Histology of egg showing neural tube and eye formation; **(j)** Higher magnification of embryonic eye

cleavage, cytoplasm occurred as a semi-spherical condensed mass and then it gradually spread over the yolk material by epiboly. The yolk material was granular and stained as blue and pink granules with hematoxylin and eosin. Vitelline envelop was formed around the yolk and associated with the embryonic disc, at the interface between embryo and yolk sac, yolk granules break down before they passed on to the embryo. During the process of epiboly, gastrulation occurred in the embryonic disc in which the head, body, and tail regions of the embryo were distinguished (Figs. 2e – 2h). Cross section of the egg at this stage showed the formation of the neural tube and embryonic eyes (Figs. 2i, j).

Different stages of larvae:

Whole mounts of the different stages of larvae of *C. auratus* with the exclusively endogenous source of food, with endo and exogenous food as well as with exclusively exogenous food are presented in Figures 3a, 3c and 3e. Histological details of these larval stages through their longitudinal sections are presented in Figures 3b, 3d and 3f and longitudinal section of *C. auratus* metamorphosis and juveniles' stages are presented in 3g and 3h.

Nervous system:

In the newly hatched out *C. auratus* larva, the neural tube was differentiated into the brain (encephalon) and spinal cord. The nervous system was formed of neurons and non-neural components such as ependymal cells and neuroglia (Figs. 4a, b). The brain was differentiated into five divisions, the telencephalon, the diencephalon, the mesencephalon, the metencephalon, and the myelencephalon in 7th day old larva (Figs. 4c, d). The telencephalon has paired olfactory tracts, olfactory bulbs, and olfactory lobes. The olfactory lobes were connected centrally. The diencephalon included three regions, the epithalamus, thalamus, and hypothalamus. The dorsal and ventral regions of the diencephalon were associated with the pineal gland and pituitary gland, respectively. The mesencephalon was chiefly formed of optic lobes. The metencephalon constitutes the cerebellum and was located behind the optic lobes as a prominent structure on the dorsal surface of the brain. The myelencephalon constituted the expanded posterior region of the brain. It has anterior vagal lobes and posterior medulla oblongata. The spinal cord extended from the myelencephalon in the vertebral canal and distally terminated at the end of the vertebral column as urophysis (Fig. 4e).

Eye:

During embryonic development, the formation of the eye commenced in the form of an optic vesicle at the anterior region immediately after the differentiation of the brain from the neural tube. The optic vesicle at this stage was unpigmented. During further development of the embryo, primordial tissues for eyeball and lens were differentiated. The newly hatched out larva has a pair of dark eyes. In the case of two days old larva, pigmentation in the eye was observed. However, layers such as sclera, choroids and retina were not differentiated at this stage (Figs. 5a-5d). The anterior and posterior chambers of the eye were not distinguishable. As the larva grows pigmentation of the eye intensified and the different retinal layers get closely associated to become a compact retina. Simultaneously, vitreous humor and aqueous humor were formed and lens development was also completed (Figs. 5e, f). The retina has an outer limiting membrane, pigment layer, visual cell layer, basement layer, outer plexiform layer, nuclear layer, inner plexiform layer, ganglion cell layer, nerve fiber layer and inner limiting membrane (Fig. 5g). The visual layer has rods and cone cells. Four days after hatching the larva attained complete development with all typical teleost eye structures such as sclera, choroids, retina, iris, lens and cornea.

Digestive system:

The digestive tract of the just hatched out larva was a simple straight tube without oral and anal apertures. It was attached dorsally to the yolk sac.

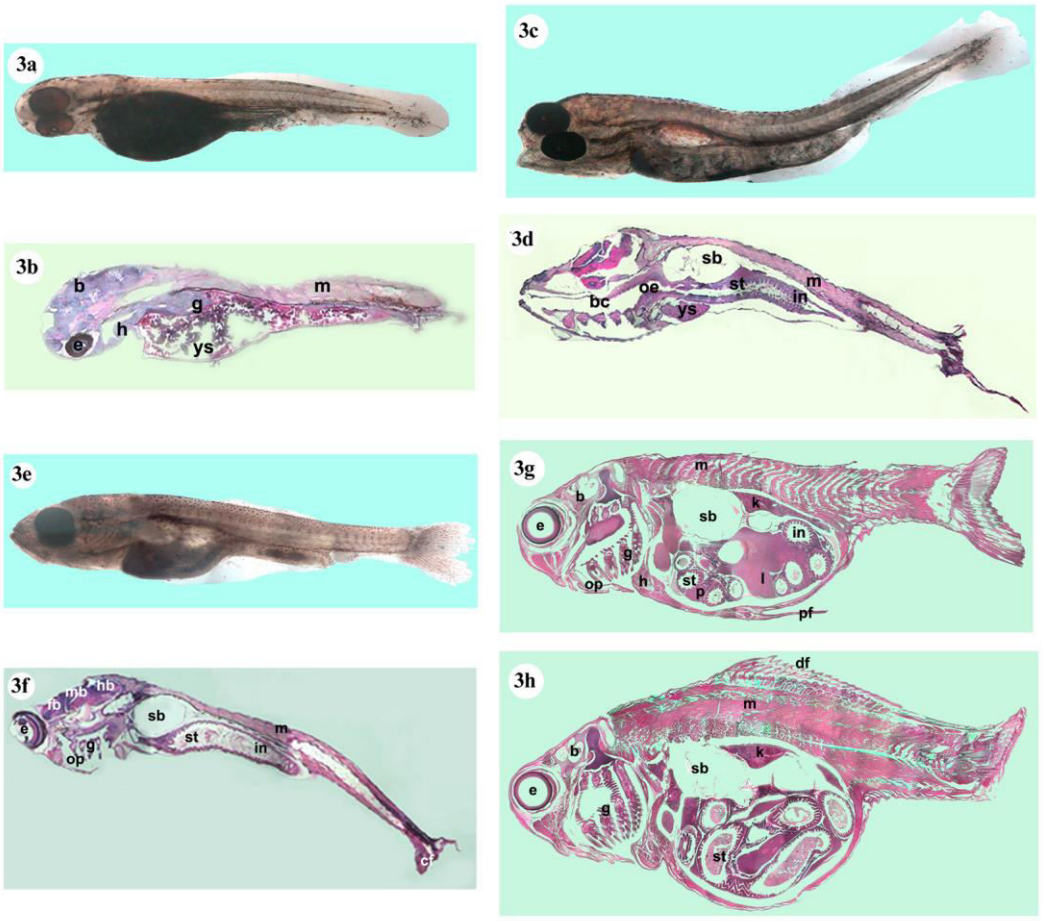

Fig. 3: **(a)** Freshly hatched *C. auratus* larva; **(b)** L.S. of freshly hatched *C. auratus* larva b- brain; g- gills; m- muscle; h- heart; e- eye; **(c)** Endo and exogenous feeding *C. auratus* larva; **(d)** L.S. of endo and exogenous feeding *C. auratus* larva oe- oesophagus; bc- buccal cavity; ys- yolk sac; in- intestine; sb- swim bladder; **(e)** *C. auratus* larva in metamorphosis; **(f)** L.S. of exclusively exogenous feeding *C. auratus* larva; e- eye; b- brain; l- liver; op- operculum; g- gills; p- pancreas; dt- digestive tract; fb- fore brain; mb- mid brain; hb- hind brain; sb- swim bladder; m- muscle; cf- caudal fin; in- intestine; **(g)** L.S. of metamorphosing *C. auratus* larva e- eye; b- brain ; m- muscle; k- kidney; sb- swim bladder; in- intestine; pf- pelvic fin; l- liver; p- pancreas; h- heart; op- operculum; g- gills; **(h)** L.S. of juvenile *C. auratus*e- eye; b-brain; k- kidney; dt- digestive tract; in- intestine; sb- swim bladder; g- gills.

It has an anterior straight blunt end while the posterior region is slightly bent ventrally. It was formed of a single layer of columnar epithelial cells. Three days after hatching, mouth and anus open and the digestive tract could be divided into mouth, oral cavity, pharynx, oesophagus, and intestine (Figs. 6a, b). The buccal cavity was lined by many layers of squamous epithelium. The buccal cavity leads into the pharynx, which was lined by stratified squamous epithelium. The ventrolaterally pharynx communicated with the branchial chamber through four pairs of gill slits. The pharynx leads into the muscular oesophagus, which has a wide proximal and narrow distal region. This region has longitudinal folds, which were deeper towards the posterior region (Fig. 6c). The narrow posterior part of the oesophagus opened into the muscular intestine. The intestine has an anterior gastric part and posterior pyloric part (Figs. 6b, c). The pyloric region communicated with the intestine, which in turn opened to the exterior through a wide anus.

Five days after hatching, the digestive system of the larva showed clear constrictions between oesophagus and stomach. The gastric and pyloric regions were also distinguishable at this stage. The intestine remained as a simple tube. The inner surface of the digestive tract from oesophagus to

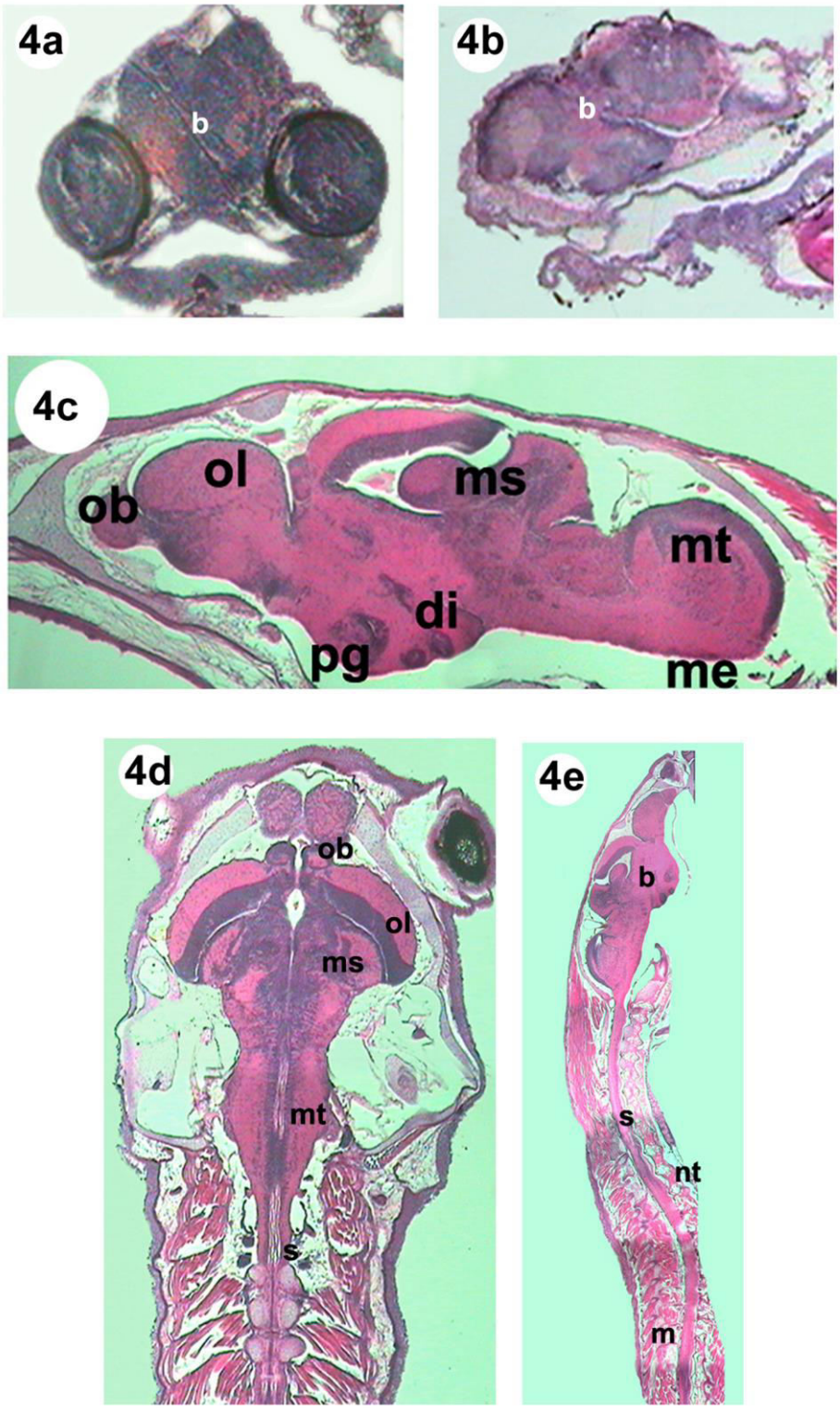

Fig. 4: **(a, b)** C.S. and L.S. of egg showing formation of encephalon b- brain; **(c, d)** L.S and V.S. of brain showing differentiation of different region of forebrain, midbrain and hindbrain ob- olfactory bulb; ol- olfactory lobe; ms- mesencephalon; mt- metancephalon; me- myeloncephalon; sc- spinal cord; di- diencephalon; pg- pituitary gland; ole- olfactory epithelium; ot- olfactory tract; **(e)** L.S. of *C. auratus* larva showing fully formed brain and spinal cord b- brain; sc- spinal cord; nc- notochord; m- muscle.

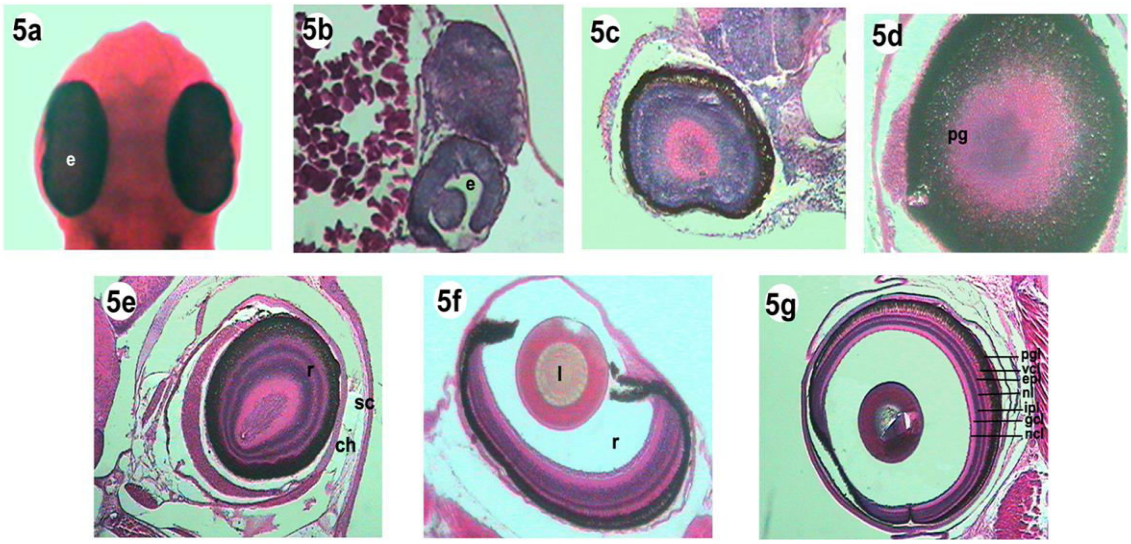

Fig. 5: **(a)** Formation of eye e- eye; **(b, c)** Histology of embryonic eye e- eye; **(d)** C.S. of larva showing pigmentation of eye pg- pigments; **(e)** Histology of eye of larva showing differentiation of sclera, choroids and retinal layer r- retina; sc- sclera; ch- choroid; **(f)** Histology of eye showing formation of retina and lens l- lens; r- retina; **(g)** Histology of eye of larva showing different layers of retina pgl- pigment layer; vcl- visual layer; epl- external plexi form layer; nl- nuclear layer; ipl- internal plexi form layer; gcl- ganglion cell layer; nfl- nerve fibre layer.

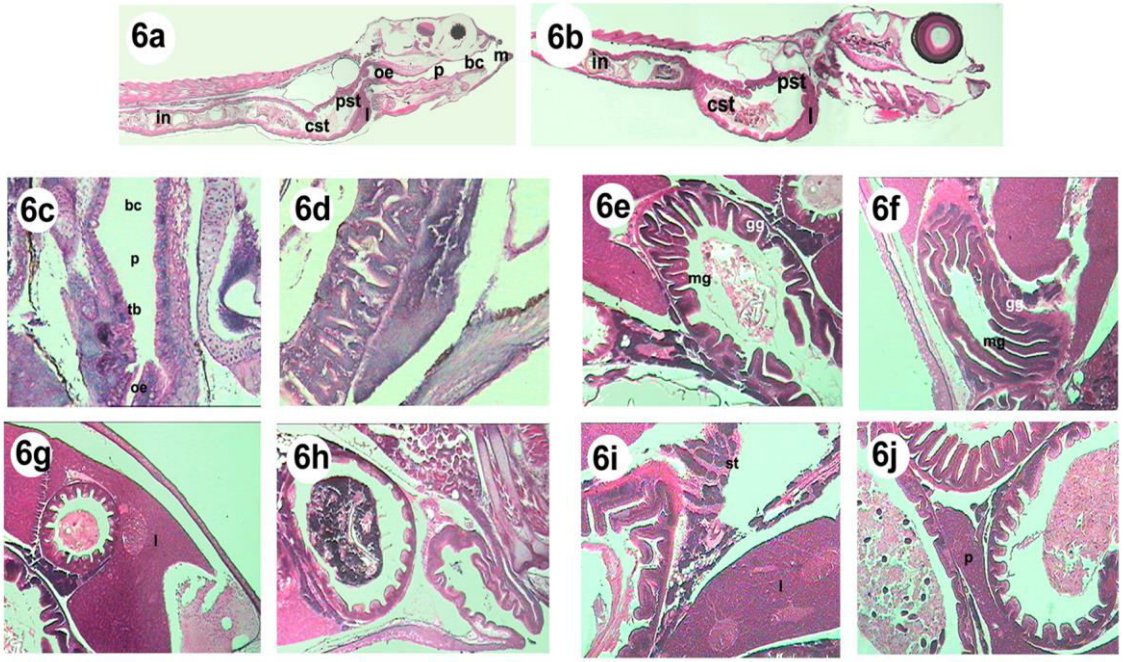

Fig. 6: **(a)** L.S. of larva showing functional mouth m- muscle; bc- buccal cavity; p- pancreas; oe- oesophagus; l- liver; pst- pyloric intestine; cst- cardiac intestine; in- intestine; **(b)** L.S. of larva showing differentiation of intestine pst- pyloric intestine; cst- cardiac intestine; l- liver; in- intestine; **(c)** L.S. of larva showing bucco pharyngeal region bc- buccal cavity; p- pancreas; tb- taste buds; oe- oesophagus; **(d)** L.S. of digestive tract of early larva showing infoldings; **(e, f)** L.S and C.S. of intestine showing mucous glands and gastric glands gg- gastric gland; mg- mucous gland; **(g)** C.S. of glandular part of intestine l- liver; **(h)** C.S. of absorptive part of intestine; **(i)** L.S. of abdomen showing liver and spleen l- liver; s- spleen; **(j)** L.S. of abdomen showing pancreas p- pancreas.

intestine has infolding which was lined with columnar epithelium. During the next stage, increased complexity was observed in all the regions of the digestive tract. The oral cavity and pharynx showed an increase in the layers of squamous epithelium. Oesophagus also showed increased epithelial layers and thicker layers of musculature. The infoldings of the stomach and intestine increased considerably and further branching was observed in them. At this stage, mucous cells were evident (Fig. 6d). In the fifth stage, the larva stomach was enlarged and showed the occurrence of many gastric glands (Figs. 6e, f). In this stage, the intestine was distinguishable into anterior and posterior intestine. The anterior intestine showed high secretory activity while the posterior region was absorptive in function.

When the larva was in the exo- and endogenous feeding stage, accessory glands of the digestive system were formed as a cluster of hepatic and pancreatic cells. These cells were round in shape and occur between the digestive tract and yolk sac (Figs. 6g – 6j). The liver occurred at an anterior position to the stomach. It was formed of hepatic cells, which were radiating from a central sinusoid. As the larva grows, vacuolization was observed in the hepatocytes. In the third stage larva bile canaliculi, bile duct and swim bladder were evident. The hepatocytes were highly basophilic and appear bright pink with hematoxylin and eosin. Three days old larva showed conversion of primordial pancreatic cells into pancreatic acini. The pancreatic cells also were basophilic with a prominent nucleus. During further development of larva, exocrine and endocrine regions were differentiated in the pancreas.

Swim bladder:

At the end of the endogenous feeding stage of the hatched out larva, swim bladder cells were differentiated from the dorsal wall of the intestine. These cells appeared to be similar to those of the columnar epithelium of the intestine. In the second stage larva, the swim bladder appeared as a small chamber lined by simple cuboidal epithelial cells. During the third stage larva, the swim bladder has a gas gland, which spread all over the surface of the swim bladder. During further development of the larva, the swim bladder was inflated and occurred as an elongated structure in between the vertebral column and digestive tract (Figs. 3d, 3f- 3h).

Gills:

Four pairs of gills constituted the respiratory organ of *C. auratus*. In addition to respiratory function, gills perform osmoregulation (maintenance of water and salt content of the body fluid and also the excretion of ammonia). At the time of hatching, larvae have primordial gill arches on either side of the pharyngeal region. At this stage, the branchial cavity was closed. In the third stage larva, four pairs of gill arches were evident (Fig. 7a). During this stage, undifferentiated cells surrounded the central chondroblast and then proliferated towards the pharyngeal cavity forming the primordial filaments (Fig. 7b). In the fourth stage larva, gill filaments were well developed and lamellae were differentiated from the gill filaments on either side. The lamellae have pillar cells and chloride cells at their bases (Figs. 7c-7e). During this stage, all four pairs of gills have lamellae on either side (Figs. 7f, g). Vascularisations of the gill were evident in this stage. Gill structure of the fourth stage larva was similar to that of juvenile fish. It showed the appearance of mucous cells. During the fifth stage of larva, gill filaments and lamellae progressively increased in their length (Fig. 7h).

Kidney:

The kidney was located beneath the notocord in the body cavity. It consisted of a paired oval anterior pronephros (head kidney) and a long posterior kidney (trunk kidney) (Fig. 8a). The urinary bladder opened directly into the gut at the anal region. The head kidney consisted of lymphoid, hemopoietic tissue, and inter-renal tissue. Renal tubes were also observed in the head

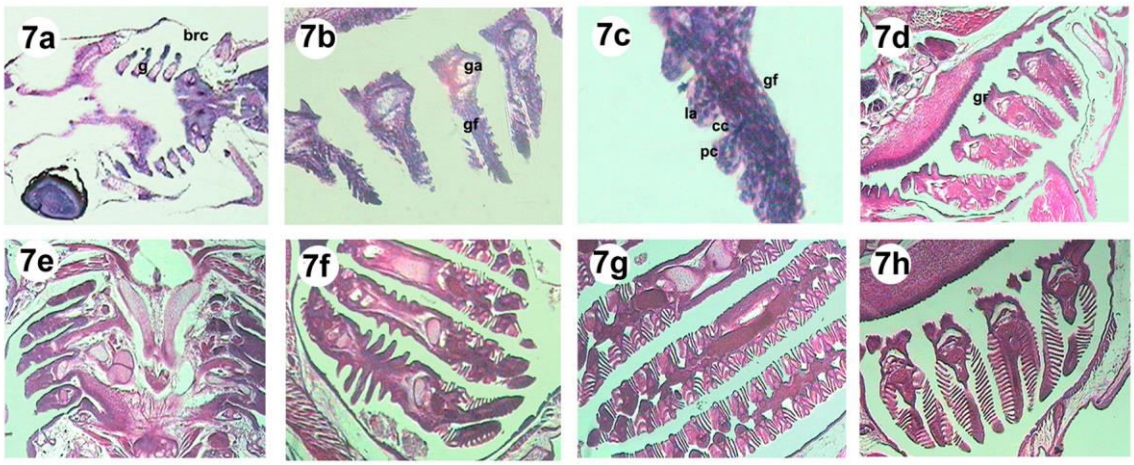

Fig. 7: **(a)** V.S. of larva showing branchyal cavity and gill arches; **(b)** L.S. of branchial cavity showing gill arches and gill filaments gf- gill filament; ga- gill arch; **(c)** Gill filaments showing origin of lamella gf- gill filament; la- lamella; cc- chloride cells; pc- pillar cells; **(d, e)** L.S. and V.S. of branchyal region showing development of gill gr- gill racker; la- lamella; **(f, g)** Differentiation of gill lamellae and their cell types; **(h)** C.S. of fully developed gills of juvenile fish.

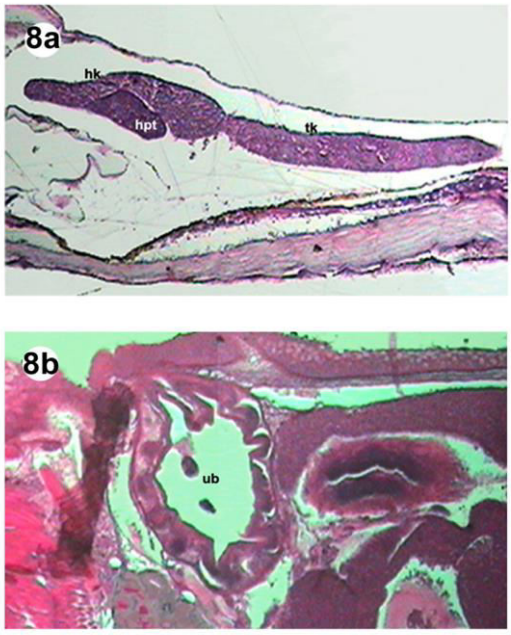

Fig. 8: **(a)** L.S. of larva showing head and trunk kidney hk- head kidney; tk- trunk kidney; **(b)** L.S. of larva showing urinary bladder ub- urinary bladder

kidney in the just hatched out larva. The trunk kidney has nephrons, each of which is composed of a renal corpuscle and a renal tubule. Four days after hatching pronephric kidney showed more convoluted tubules anteriorly. The urinary bladder opened directly to the exterior (Fig. 8b). At 12th day (DAF), the kidney of the larvae showed the appearance of the mesonephros. The glomeruli and convoluted tubules of mesonephrons were well formed in the 20 days old larva.

Skeletal muscle:

The skeletal muscle occurred in the form of the striated muscle fibre bundle. Each muscle cell

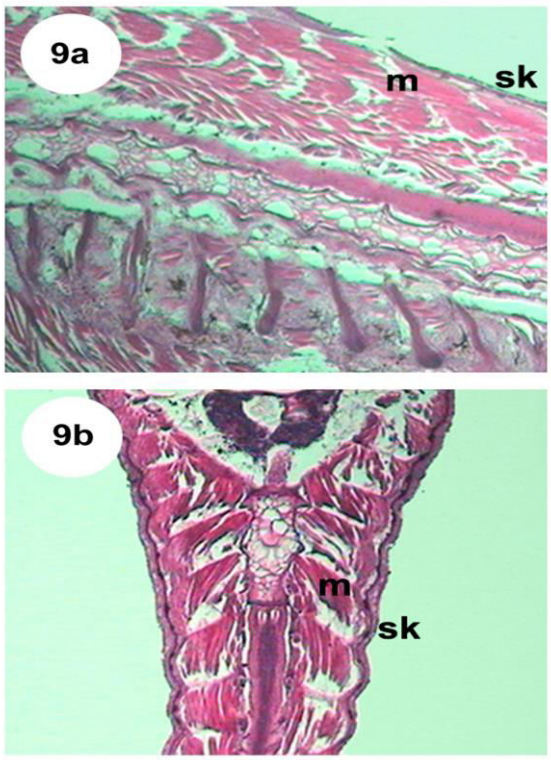

Fig. 9: **(a, b)** L.S. & V.S. of larva showing muscle bundles m- muscle; sk- skin.

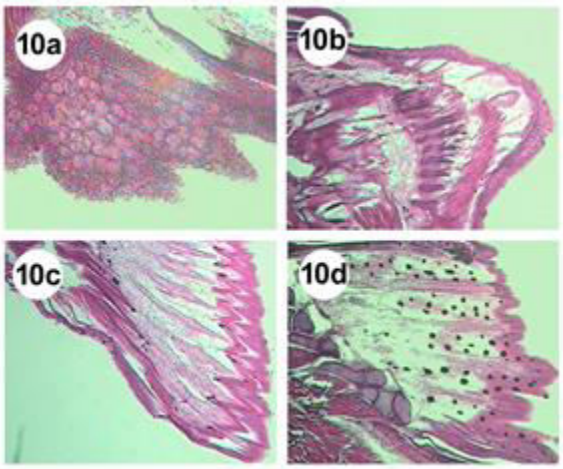

Fig. 10: **(a)** L.S. of larva showing development of fins; **(b)** Development of pectoral fin; **(c)** Development of caudal fin; **(d)** L.S. of caudal fin showing pigmentation.

consisted of several myofibrils and peripheral nuclei. The muscle bundles were arranged in the form of myomere or myotome which was separated from the adjacent myomere by myoseptum. The skeletal muscle was attached to the skeletal structure and controls the movement of fins, opercula, gills, and jaw (Figs. 9a, b).

Fins:

The newly hatched out larva has well developed primordial marginal fin folds and pectoral fin folds (Fig. 10a). During further growth of larva, caudal fin began to differentiate with fin fold and rays. This was followed by the pectoral and pelvic fins (Fig. 10b). 15th day (DAF) larva has well developed

paired fins and caudal fins. At this stage, the dorsal fin begins its origin and after 30 days of hatching, the larva has well formed dorsal and anal fin (Figs. 10c, d). At this stage, the primordial fin fold was completely resorbed.

Discussion

In the present study, larval organogenesis of *C. auratus* was described from histological perspectives. The larval development was categorized into 5 stages and 1st and 2nd stages were last on 2nd DAF and 5th DAF, respectively. The morphological changes and size variation in different stages of larval development is similar to the European Chub *Leuciscus cephalus* (Kupren *et al*., 2015a). The major developmental events of *Dentex dentex* (Santamaria *et al*. 2004) staging of larval form after hatching resembles those recorded for *C. auratus* larvae in the present study. Nevertheless, there was a variation in the total length of the different larval stages of these fishes and also with regard to the duration of different stages. The variation in the duration of different larval stages can be attributed to the ontogenic events in general and functioning of the digestive system in particular on which endo and exotrophic or exclusively exotrophic nutrition was depended. The ontogenetic larval development of common barbel (Nowosad *et al*., 2021) has dramatic increased head and trunk in the early stages of embryo development.

The development of the nervous system in the *C. auratus* larvae indicated that the brain was well differentiated into five divisions in 7 day old larvae. The differentiation of the brain regions was evident in 5 days old larvae. In general, the central nervous system of 36 days old larvae is similar to all other teleosts (Bernstein, 1970). It contains neurons and non-neuronal elements such as ependymal cells, neuroglia, endothelia, and periocytes of blood vessel walls. (Chinabut *et al*., 1991). Occurrences of such cell types are also observed in the present study.

The development of eye structures such as sclera, choroids, retina, cornea, and lens in *C. auratus* is similar to the development of the eye of other teleost fish. The sclera and choroids comprise the outer fibrous coat of the eyeball. The transparent cornea was thicker at the periphery than in the central region. It has several layers of epithelial cells that are continuous with the integument. The iris was a continuation of the choroidea and extends out under the cornea. The spherical lens was suspended within the eyeball by a dorsal suspensory ligament. The retina was divided into 10 layers. The visual cell layer has rod and cone cells. The visual cell layer was covered by a pigment layer. The other layers of the retina have synaptic layers (minor and outer plexiform layers) nuclear layer, the ganglion cell layer, and the nerve fibre layer (Chinabut *et al*., 1991). The freshly hatched larva has a pair of dark eyes, which are unpigmented. Nevertheless, all typical structures of the teleost eye were formed in five days old larva. The olfactory organs of the larva were placed in the olfactory sacs in the nasal pits. They have sensory epithelial cells, which might perform the chemosensory function. Hara (1971) had attributed such functions to the olfactory organs of many teleosts.

Anatomical and histological studies of the alimentary canal of *C. auratus* larvae indicate general similarity with other teleosts. The digestive system of just hatched larva occurs as a simple tubular structure without mouth and anal opening. However, the mouth and the anal opening were formed on the 3rd or 4th day (DAF), it was evident that exogenous food was observed in the gut. The alimentary canal even though gets differentiated into the buccal cavity, pharynx, oesophagus, stomach, and intestine during endo and exogenous feeding, was not developed functionally to digest the prey completely. Higher ingestion of live prey during the beginning of exogenous feeding was observed in the larvae as evidenced by the gut content. It was quite interesting to note that the ontogenic events in *C. auratus* larvae leading to the formation of the alimentary canal and associated glands such as the liver and pancreas show the temporal relationship with the availability of endogenous store of food.

Further, the period of the endo-exogenous feeding stage appears to be critical in the larval life, and those species in which there were smooth transitions from this stage to exclusively exogenous feeding appear to have a higher rate of survival. Events of development of the digestive system in the larva of *C. auratus* was also synchronized with the development of the eye musculature.

The development of the liver and pancreas, which commences in the freshly hatched larva attains a functional state during the endo-exogenous feeding stage. This suggests that the larva develops associated glands of the digestive system for the chemical digestion of ingested food during the endo-exogenous feeding stage. Development of digestive tract and accessory organs during endogenous food source is reported in many teleosts (Guyot *et al*., 1995; Sarasquete *et al*., 1995; Roo *et al*., 1999; Ortiz-Delgado *et al*., 2003; Sivakumar, 2005). Govoni *et al*. (1986) and Segner *et al*. (1989) have reported that in *Dentex dentex* as resorption of endogenous reserves (mainly yolk) proceeds the fish larva acquires morphological and enzymes, which will ensure digestive and absorptive process before the mouth opening. This suggests that there were variations in the histology of specialization of stomach, liver, and pancreas to produce enzymes for digestion of food as evidenced in the larvae.

The gills of fish and their associated organs are extremely efficient in supplying oxygen from the water of adequate oxygen content. The gills were associated with the anterior end of the gut being on the sides of lateral openings in the pharynx, which lead from the digestive tract to the exterior. They were mostly enclosed in a branchial chamber. The water was taken in anterior to the gills through the mouth and passes laterally through the gills and out through the external branchial openings (Fry, 1957). Many of the marine fishes have well-developed gills during exclusively exogenous feeding stages of their larvae (Santamaria *et al*., 2004). However, it was reported that in most freshwater species, there was an occurrence of a well developed gill system during their first day of life (Li *et al*., 1995; Rombough and Moroz, 1997). In the present study, it was observed that in the *C. auratus* larvae primordial gill arches were present in the branchial cavity at the time of hatching and their development followed the pattern of other freshwater teleosts. In *C. auratus* larva at the time of hatching an elongate kidney was distinguishable into the head kidney and trunk kidney. During larval development hematopoietic tissue, inter-renal tissues, and renal tubes were formed from the head kidney, while the trunk kidney gives rise to nephrons. During earlier stages of larvae, the kidney has pronephros and during advanced stages of larvae, it does mesonephros constituting glomeruli and convoluted tubes. The development of the kidney follows a typical pattern as reported in many fishes (Chantanachookhin *et al*., 1991; Fukuhura, 1987; Fernandez-Palacios *et al*., 1994). Groman (1982) and Hibiya (1982) have reported the different histological details of the kidney of striped bass and common carp, respectively. The different functions of the nephros reported in common carp could also be attributed to the *C. auratus* larva.

Rombough and Moroz (1997) have opined that osmoregulation rather than respiration would be the priority function of the gill during ontogeny. It is interesting to note that there is coordinated development of the respiratory system and cutaneous structures, which might lead the larva to transform from cutaneous to gill respiration. The most striking feature reported in the advanced larval form of *Dentex dentex* was the increase in length and number of gill filaments and lamellae, which coincided with the progressive thickening of the integument and the formation of scales in the stratum spongiosum of the dermis (Santamaria, 2002).

The skeletal muscles on either side of the fish constitute the major tissue of the body. The rhythmic alternate contractions of the muscles of two sides of the body produce propulsive waves

which effect locomotion. The individual muscle was composed of numerous myofibrils or muscle cells. Each muscle fibre was interconnected through a supporting meshwork of connective tissue. The myofibrils were composed of hundreds of protein myofilaments divided into actin and myosin elements. Each myomere of the body muscle comprises a large block of myofibrils. The myomere was cone or wedge shaped and serial myomeres lie parallel to each other (Chinabut *et al*., 1991). The histology of *C. auratus* larva also showed similar structures.

About the skin, different layers such as the epidermis, dermis, and hypodermis of the larvae show similarity with other freshwater teleosts. Different types of cells observed in the skin of the *C. auratus* fishes were similar to those which were reported in fishes such as catfish (Chinabut *et al*., 1991) and common carp (Hibiya, 1982). The functioning of goblet cells, scale formation, and occurrence of lipid cells and pigment cells also showed a typical teleost pattern. The newly hatched larvae of *C. auratus* larvae have numerous melanophores in the dorsal and dorsolateral region of both the side of the body. The body surface was heavily covered with melanophores in metamorphosed fish. Further after metamorphosis, the fishes showed a distinct change in pigment development which was characteristic of the particular ornamental fish. Similar to the present study formation of melanophores, their distribution in different stages of larval development and metamorphosis of *Limanda yokohamae* is reported by Fukuhara (1988).

Newly hatched larvae of *C. auratus* have a prominent larval fin fold. The pectoral fins without rays were observed in the fish larva, it was evident on one day after hatching pectoral fins appeared. The caudal fin started its differentiation and fin rays development was first observed in its fish larvae. The development of the pectoral, pelvic, and anal fin followed next, and finally, the dorsal fin was differentiated. As the fin rays developed the primordial fin fold completely gets resorbed. Development and differentiation of fins similar to the present study are also reported in different teleosts (Kato *et al*., 1974; Minami, 1981; Fukuhara, 1988; Santamaria *et al*., 2004).

Thus the present study on the larval organogenesis in *C. auratus* indicates that there is species-specific variation about the incubation period as well as different ontogenic events that take place in larvae of different trophic stages. The most important events are the development of the brain, digestive tract, liver, pancreas, gills, kidney, musculature, skin, and fins. Co-ordinated development is essential for the attainment of feeding, respiration, osmoregulation, and behavior. The change of energy supply from an endogenous to an exogenous source was identified as a critical stage that could cause high mortalities during early life history. Examining the diversity of the onset of initial feeding in various fish species, Iwai (1972) stated that yolk sac larvae that were able to feed on prey successfully have a better survival potential than those starting later. The transitory period during which the source of energy supply changes from endogenous to exogenous food is very short in tropical fishes. A delay of initial feeding is more than 24h. after eye pigmentation or after yolk absorption is considered critical for survival in these species. The larva is well equipped for this transition in the energy supply devoting that they can be reared with a higher percentage of survival with appropriate live feed.

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References

Bernstein JJ. (1970) Anatomy and physiology of the central nervous system. In: "Fish physiology". Vol. IV. Academic Press, New York, pp. 1-90.

Chantanachookhin C, Seikai T and Tanaka M. (1991) Comparative study of the ontogeny of the lymphoid organs in three species of marine fish. Aquacult. 99: 143-155.

Chinabut S, Limusuwan C and Kitsawat P. (1991) Histology of the walking catfish, *Clarias batrachus.* Asian Fish Heath Network Co-ordination, Sponsored by International Development Research Center, Canada, pp. 94.

Fernandez-Palacios H, Montero D, Socorro J, Izquierdo MS and Vergara JM. (1994) First studies on spawning, embryonic and larval development of *Dentax giboosus* (Rafinesque, 1810) (Osteichthyes: Sparidae) under controlled conditions. Aquacult. 122: 63-73.

Fry FEJ. (1957) The aquatic respiration of fish. In: The physiology of fishes, Vol. I. Academic Press, Inc., New York, pp: 1-63.

Fukuhara O. (1987) Larval development and behaviour in early life stages of black sea bream reared in the laboratory. Bull Japan Soc Sci Fish. 53: 371-379.

Fukuhara O. (1988) Morphological and functional development of larval and juvenile *Limanda yokohamae* (Pisces: Pleuronectidae) reared in the laboratory. Mar Biol. 99: 271-281.

Govoni JJ, Boehlert GW and Watanabe Y. (1988) The physiology of digestion of fish larvae. Environ Biol Fishes 16: 59-77.

Groman DB. (1982) Histology of the Striped Bass. American Fisheries Society Monograph number 3, Bethesda, Maryland, pp.1-116.

Guyot E, Diaz JP and Connes R. (1995) Organogenesis of the liver in sea bream. J Fish Biol. 47: 427-437.

Hara TJ. (1971) Chemoreception. In: Fish physiology. Vol. IV. Academic Press, New York, pp. 79-120.

Hibiya T. (1982) An Atlas of fish histology: Normal and pathological features. Kodansha Ltd. Tokyo, p. 147.

Iwai T. (1972) Feeding of teleost larvae: a review. La mer (Bull. Soc. Franco-Japon. Oceanogr) 10(2): 71-82.

Kato F, Okiyama M and Tajima M. (1974) External morphology and discrimination of two species of flounders belonging to the genus *Limanda* (*L. yokohamae* and *L. herzensteini*) from the Japan sea. Bull Japan Sea Reg Fish Res Lab. 25: 63-87.

Koumoundouros G, Divanach P and Kentouri M. (2001) The effect of rearing conditions on development of saddleback syndrome and caudal fin deformities in *Dentex dentex* (L.). Aquacult. 200: 285-304.

Küçük S. (2013) The effects of salinity on growth of goldfish, *Carassius auratus* and crucian carp, *Carassius carassius*. Afr J Biotechnol. 12(16): 2082-2087.

Kupren K, Mamcarz A and Kucharczyk D. (2011) Effect of variable and constant thermal conditions on embryonic and early larval development of fish from the genus *Leuciscus* (Cyprinidae, Teleostei). Czech J Anim Sci. 56(2): 70-80.

Kupren K, Nowosad J, Żarski D, Targońska K, Hakuć-Błażowska A and Kucharczyk D. (2015a) Early development and allometric growth in laboratory-reared European chub *Leuciscus cephalus* (Linnaeus, 1758). Turkish J Fish Aquat Sci. 15(4): 391-398.

Kupren K, Żarski D and Kucharczyk D. (2015b) Early development and allometric growth patterns in ide *Leuciscus idus* (Linnaeus 1758). J Appl Ichthyol. 31(3): 509-517.

Li J, Eygensteyn J, Lock RAC, Verbost PM, Vender Heijden AJH, Wndelaar-Bonga SEW and Flick G. (1995) Branchial chloride cells in larvae and juveniles of freshwater tilapia *Oreochromis mossambicus.* J Exp Biol. 198: 2177-2184.

Minami T. (1981) The early life history of a flounder *Limanda yokohamae.* Bull Japan Soc Sci Fish. 47: 1411-1419.

Morioka S, Ito S, Kitamura S and Vongvichith B. (2009) Growth and morphological development of laboratory-reared larval and juvenile climbing perch *Anabas testudineus* . Ichthyol Res. 56: 162-171.

Nowosad J, Kupren K, Biegaj M and Kucharczyk D. (2021) Allometric and ontogenetic larval development of common barbel during rearing under optimal conditions. Animal 15(2):100107.

Ortiz-Delgado JB, Darias MJ, Canabate JP, Yufera M and Sarasquete C. (2003). Organogenesis of the digestive tract in the white sea bream *Diplodus sargo.* Histological and histochemical approaches. Histol Histopathol. 18: 1131-1140.

Osse JWM. (1990) Form changes in fish larvae in relation to changing demands of function. Netherlands J Zool. 40: 362-385.

Rombough PJ and Moroz BM. (1997) The scaling and potential importance of cutaneous and branchial surfaces respiratory gas exchange in larval and juvenile wall eye *Stizostedion vitreum.* J Exp Biol. 200: 2459-2468.

Roo FJ, Socorro J, Izquierdo MS, Caballero MJ, Hernandez-Cruz CM, Fernandez A and Fernandez-Palacios H. (1999) Development of red porgy *Pagrus pagrus* visual system in relation with changes in the digestive tract and larval feeding habits. Aquacult. 179: 499-512.

Sagnes P, Gaudin P and Statzner B. (2005) Shifts in morphometrics and their relation to hydrodynamic potential and habitat use during grayling ontogenesis. J Fish Biol. 50(4): 846-858.

Santamarıa CA. (2002). Desarrollo de la larva del dento´n *Dentex dentex* (Linnaeus, 1758). Estudio cuantitativo del crecimiento, aspectos histolo´gicos y organoge´nesis. Tesis Doctoral. Departamento de Biologı´a Animal, Biologı´a Vegetal y Ecologı´a. Universidad Auto´noma de Barcelona (Spain).

Santamarıa CA, Marı´n de Mateoa M, Traveseta R, Salab R, Grauc A, Pastorc E, Sarasqueted C and Crespoa S. (2004) Larval organogenesis in common dentex *Dentex dentex* L. (Sparidae): histological and histochemical aspects. Aquacult. 237: 207-228

Santos MLD, Arantes FP, Santiago KLB and Santtos JED. (2015) Morphological characteristics of the digestive tract of *Schizodon knerii* (Steindachner, 1875), (Characiformes: Anostomidae): An anatomical, histological and histochemical study. An. Acad. Bras. Cienc. 87(2): 867-878.

Sarasquete C, Polo A and Yufera M. (1995). Histology and histochemistry of the development of the digestive system of larval gilt head sea bream *Sparus aurata* L. Aquacult. 130: 79-92.

Segner H, Storch V, Reinecke M and Kloas W. (1995) A tabular overview of organogenesis in larval turbot (*Scopthalamus maximus* L). ICES Mar Sci Symp. 201: 35-39.

Sivakumar K. (2005) Freshwater fish and prawn larval rearing using indigenous live feed. Ph.D. Thesis, University of Madras. Tamil Nadu, p.135.

Sulaeman and Fotedar R. (2017) Yolk utilization and growth during the early larval life of the Silver Perch, *Bidyanus bidyanus* (Mitchell, 1838). Int Aqua Res. 9: 107-116.