AQUATIC RESEARCH

E-ISSN 2618-6365

Aquatic Research 8(1), 39-51 (2025) • https://doi.org/10.3153/AR25004 *Research Article*

Studies on organogenesis of common carp *Cyprinus carpio* var. *koi* with reference to histological perspectives

Sivakumar KANDHASAMY[1], Muthupriya PALANICHAMY[1], Meenakshi Sivalingam VALLIAPAN[1], Altaff KAREEM[2,3]

Cite this article as:
Kandahasamy, S., Palanichamy, M., Valliapan, M.S., Kareem, A. (2025). Studies on organogenesis of common carp *Cyprinus carpio* var. *koi* concerning histological perspectives. *Aquatic Research*, 8(1), 39-51. https://doi.org/10.3153/AR25004

[1] Karpaga Vinayaga College of Engineering and Technology, School of Aquaculture, Department of Biotechnology, GST Road, Chinna Kolambakkam, Chengalpattu 603308, Tamil Nadu, India

[2] AMET University, Department of Marine Biotechnology, Kannathur, Chennai 603112, Tamil Nadu, India

[3] The New College, Department of Zoology, Chennai 600014, Tamil Nadu, India

ORCID IDs of the author(s):

S.K. 0000-0001-8971-869X

M.P. 0000-0002-7859-9290

M.S.V. 0009-0007-7714-1732

A.K. 0000-0001-9178-2519

Submitted: 17.06.2024

Revision requested: 12.08.2024

Last revision received: 13.08.2024

Accepted: 14.08.2024

Published online: 11.12.2024

Correspondence:

Sivakumar KANDHASAMY

E-mail: ksivakumar76@gmail.com

Available online at
http://aquatres.scientificwebjournals.com

ABSTRACT

The objective of the current study was to investigate the histological characteristics of larval organogenesis in *Cyprinus carpio* var. *koi*. The eggs and several developmental stages of *C. carpio* var. *koi* larvae were preserved in Bouin's fluid. Histological examinations were conducted daily from the first day after fertilisation (DAF) to the 12th DAF and the 16th, 24th, 32nd, and 40th DAF. Histological examinations of *C. carpio* var. *koi* eggs revealed the development of an embryonic disc, followed by the formation of the neural tube and embryonic eyes on the second day after fertilisation (DAF). The just-emerged larvae have distinct eyes and a neural tube that has undergone differentiation into the brain. Following that, an examination was conducted on the development of the digestive system, swim bladder, gills, skin, and fins. The ontogeny developments were categorised into five distinct stages. During larval development, the initial two stages have exhibited variations in their organogenesis. The larvae head was aligned and devoid of the yolk, but their body was connected to the yolk sac and gill arches, which were visible between the 3rd and 5th days after fertilisation (DAF), and their total length range was 4.35-6.83mm. The digestive system had a linear configuration, with an enlargement in both the dorsal and ventral regions of the body on the ninth day after fertilisation (DAF). During the 10th to 17th developmental stages, larvae have a total length range of 11.23-14.35mm. The operculum, gill lamella, and dorsal fin were developed in this stage. Between the 24th and 40th days after fertilisation (DAF), the larvae acquired a fin, a coiled gut, and well-developed accessory glands and their total length was measured between 15.01 and 23.68mm.

Keywords: *C. carpio*, Ontogeny, Histology, Larval rearing

Introduction

The embryonic development and organogenesis of common carp (*Cyprinus carpio* var. *koi*) are vital processes that significantly impact the growth and maturity of this economically valuable fish species. Gaining a comprehensive understanding of the complexities involved in the development of organs and the growth of embryos in common carp is crucial for maximising the efficiency of aquaculture production and guaranteeing the long-term viability of fish populations. Ługowska & Kondera (2018) provide a detailed account of the initial growth stages of vimba under varying temperature conditions. Muthupriya et al. (2022) studied the account of the ontogenic development of Carassius auratus in detail. This data enhances our comprehension of the various stages of growth in this particular species. In addition, the research conducted by Burggren and Pinder (1991) emphasised the significant growth in size that occurs throughout the development of lesser vertebrates such as common carp. This study showed the astonishing metamorphosis from a small larva to a fully mature adult. In their study, Milan et al. (2006) examined the zebrafish as a prominent model organism for studying development, particularly about the formation of organs. Common carp's embryonic development and organogenesis are intricate processes impacted by genetic, environmental, and ecological variables. By deciphering the intricacies of these developmental processes, scientists can improve aquaculture methods, preserve fish populations, and minimise the effects of invasive species such as common carp on aquatic environments. The stages of embryonic development, ontogenesis, organogenesis, and early larval stages play a vital role in the life cycle of fishes, affecting their growth, survival, and ecological interactions. Understanding these processes is crucial for knowing fish biology's intricacies and enhancing aquaculture methods. The study conducted by China and Holzman (2014) focused on the difficulties larval fish encounter when they experience hydrodynamic famine during the initial feeding stage. The research highlighted the crucial shift from limited success in capturing prey to enhanced eating behaviour throughout early growth.

In addition, Zimmer et al. (2017) conducted studies that examined the molecular and transcriptional characteristics associated with the processing of ammonia and the development of larvae in fish. These studies offer valuable information about the physiological adjustments that occur throughout the early stages of life. Nowlin & Drenner (2000) and Nebeker et al. (1985) studies emphasise the intricate relationship between fish development, environmental conditions, and species interactions in aquatic habitats. The initial phases of fish development encompass a variety of processes that impact their growth, behaviour, and ecological functions. By clarifying the processes involved in embryonic, ontogenic, and organogenetic events, scientists can improve conservation initiatives, aquaculture administration, and our comprehensive comprehension of fish biology.

Organogenesis is a crucial step in the development of organisms that significantly impacts the structure and function of different organs. When studying fish, having knowledge of the histological aspects of organogenesis helps us gain a significant understanding of the complex cellular and tissue-level transformations that take place during the development of embryos and larvae. Boulhic & Gabaudan (1992) studied the histological examination of organogenesis in the digestive system and swim bladder of the Dover sole (*Solea solea*). This research provided insights into the structural growth of these crucial organs in fish. It explored the intricate aspects of tissue development, providing a thorough understanding of how organs are formed in a particular fish species. In addition, Behrouz et al. (2014) studied the histological aspects of larval organogenesis in *Schizothorax zarudnyi*. Their research provided an in-depth understanding of the development of important organs like the gills, heart, kidney, bladder, and spleen during the initial phases of life. This study provided significant insights into the structural condition of these organs during their development. Histological examinations, as shown in this research, are crucial for understanding the cellular mechanisms and tissue structure involved in fish organ development. By analysing minute alterations that occur throughout growth, scientists can better understand the processes by which organs are shaped and operate in various fish species. This knowledge contributes to the broader field of developmental biology and aids in advancing aquaculture operations. The present study examined many stages of organogenesis in *C. carpio* var. *koi*, specifically focusing on the development of various organs.

Materials and Methods

Breeding of Common Carp C. carpio var. *Koi*

The experiment was conducted at the School of Aquaculture, Department of Zoology, The New College, Chennai, Tamil Nadu, India, in the laboratory, breeding and spawning of the common carp. The broodstock of this species was obtained from a Tamil Nadu hatchery located in Poondi, Tamil Nadu, India. The organisms were housed in concrete tanks and nourished with live feed consisting of tubifex worms and chironomids larvae. The breeding and spawning tanks, measuring 10 x 4 x 5 ft, were thoroughly cleaned and then filled with tap water that had been filtered. For optimal breeding conditions,

it is preferable to have water with a pH of 6.8 and a temperature ranging from 28.67±1.92C. A continual aeration process is necessary for water. Aquatic weed (*Chara* sp.) was supplied in the breeding tanks. The breeders were introduced into the breeding tanks during the late evening hours. The typical male-to-female ratio of introduced fish was 4:1 (Mohale et al., 2020).

The female swam rapidly through the plants and laid the eggs directly on the leaves. The male then releases milk to fertilise the eggs. Spawning in fish often occurs in the early morning hours and lasts 2 to 4 hours. Once the spawning process was over, the breeders were delicately relocated to a different tank. The aquatic weed with attached eggs was delicately extracted from the breeding tank and evenly dispersed into a hatching hapa, measuring 2.5 x 1 x 1.5 meters, constructed from thin fabric. The hapa was then placed in water with qualities comparable to the breeding tanks. The hapa containing developing eggs was undisturbed until the eggs hatched into larvae (Sivakumar, 2005).

Process of Larval Rearing

We chose newly born larvae that were just one day old for larval rearing. The larval rearing of *C. carpio var. koi* was nourished with pelletised feed and a combination of live feed consisting of cladoceran (*Moina micrura*) and cyclopoid (*Thermocyclops decipiens)*. A group of 100 newly hatched larvae of *C. carpio* var. *koi*, all of the same size, were placed into a concrete experimental tank of 75 cm in length and 40 cm in diameter, containing 35 litres of water. The larval tank was aerated and kept without food during the non-feeding stage of the larvae, following the natural light-dark cycle. The larvae of *C. carpio* var. *koi* began feeding on external food sources on the seventh day after fertilisation. The larvae were provided unlimited food, and studies were carried out for 40 days. Each morning, the larval rearing tank was cleaned by removing faecal matter and surplus feed, and 50% of the water was replaced. The dimensions of eggs and larvae were assessed using a micrometre (Qin & Fast, 1997; Sivakumar, 2005).

In order to conduct histological analysis, *C. carpio* var. *koi* eggs and larvae were chosen from the earliest stage of freshly hatched eggs to larvae that had reached a maturity of 40 days. The larvae were rendered unconscious using a concentration of 50 mg/l of benzocaine and preserved daily from the first day after fertilisation (DAF) to the 12th DAF. They were also preserved on the 16^{th}, 24^{th}, 32^{nd}, and 40^{th} DAF. The eggs and larvae were euthanasia (0.4 ml of clove oil/litre) and immersed in aqueous Bouin's fluid for 12 hours. They were rinsed in flowing tap water until the yellow hue of picric acid was eliminated.

Histological Study

The samples were dehydrated using a progressive sequence of ethyl alcohol concentrations (30%, 50%, 70%, 90%, and absolute alcohol), followed by clearing with xylene. The specimens were immersed in paraffin wax at a temperature of 52ºC. The specimens were sliced into longitudinal sections (L.S.) and cross sections (C.S.) at a thickness of 8 µm using an Erma rotatory microtome. Tissue sections were stained using the hematoxylin and eosin staining method. The sections underwent deparaffinisation in xylene and were then hydrated in a declining ethanol series. The specimens underwent water treatment and a 30-minute staining process using hematoxylin. The specimens were rinsed using tap water and subsequently treated with 30%, 50%, and 70% ethanol. Following this, they were stained with alcoholic eosin for around 5 minutes. Additionally, dehydration was conducted by subjecting them to 90% absolute alcohol. After drying, the sections were treated with xylene to remove any remaining moisture. Subsequently, permanent mounts were created using Canadian balsam (Martins et al., 2018).

Observations were made on the structure of the egg and the development of larvae, including the eye, nervous system, digestive system, gills, pigmentation, and fins. Photomicrography captured the specimens using a Samsung (CCD) camera connected to a Leica ATC 2000 microscope. The images were taken at various levels of magnification.

Results and Discussion

Fertilised Eggs

The fertilised eggs of *C. carpio* var. *koi* have a sticky surface, and at some particular points, they have adhesive material consisting of hexagonal compartments. The fertilised eggs are attached to the leaves of the aquatic plants. The fertilised eggs of koi carp are either spherical or oval, with a light yellowish colouration, while unfertilised eggs appear whitish. Scanning electron microscopy of the eggs (Fig. 1) reveals irregular folding of the outer membrane with depressed regions that aid in attaching the eggs to vegetation. The breeding tank water temperature for koi carp should be maintained between 25ºC and 30ºC to facilitate breeding (Wu et al., 2007). Regarding aquaculture, koi carp are raised globally, especially in Japan, as an ornamental variety of common carp. During further development, embryonic tissue rises from the yolk surface, constricting the broad connection between the body of the embryo and the yolk into a narrow zone. Through this narrow zone, yolk material, after enzymatic breakdown, is transported to the embryo. As the embryo grows, the connection between the body and the yolk is further constricted,

forming a stalk with which the embryo proper is connected to the yolk sac.

When hatching, the larva has a small yolk sac attached to it ventrally. The newly hatched-out larva is transparent and has a laterally compressed body. The yolk sac is oval. Within two days after hatching, the yolk is completely utilised, and the yolk sac is absorbed ventrally. The eyes are dark, and there is a faint black pigmentation near the lateral line of the anterior part of the larva. As growth proceeds, the larva shows morphological changes such as the formation of distinct head and body regions, the development of eyes, the appearance of buccal invagination, and the formation of upper and lower jaws. During further development, the caudal fin is fully distinct from the embryonic fin fold, and the pectoral fins appear as flaps just behind the operculum. The morphological structures of the head and body of the larva resemble those of the juvenile koi carp. The larva starts surfacing and swims actively in the water column. It feeds voraciously on zooplankton and grows into a juvenile, showing most adult characteristics (Table 1).

Histological Aspects of Eggs and Larvae of C. carpio var. Koi

Eggs

The incubation period of koi carp eggs typically ranges from four to seven days, a duration influenced by the temperature of the medium. Approximately 80 to 90% of these eggs hatch into larvae, with the fertilised eggs having a diameter ranging from 1.15 to 1.51 mm. Unlike some species, koi carp eggs do not contain oil globules. The development of fertilised koi carp eggs begins with the accumulation of active cytoplasm towards the animal pole, followed by cleavage (Adamek et al., 2017). During this process, the yolk granules and plates get more compactly arranged at the centre of the egg. Blastoderm is formed at the cytoplasmic cap, and the yolk and a thin layer of cytoplasm surrounding it remain unaffected. The periblast did not contribute to the formation of the embryo. The blastoderm cells undergo epibolic and embolic movement to form a gastrula. Further, development leads to the blastodisc formation, which thickens to become the embryonic shield (Fig. 2a). The primary organ rudiments, such as the neural plate, neural tube, notochord, and lateral somites, are formed in the embryonic shield. The formation of primary organ rudiments starts at the anteriormost part and progresses backward (Fig. 2b). The lateral edges of the blastodisc develop progressively to form the embryo's body. The lateral edges of the blastodisc are drawn towards the midline, forming more posterior parts of the body and the tail. At this stage, the body is formed completely around the yolk sac, and the head and eyes are visible (Fig. 2c).

Histology of the egg undergoing embryonic development shows granular yolk, borderer cells, and active cytoplasm (Fig. 2d). The development of the fertilised egg commences with the accumulation of cytoplasm at the animal pole of the egg. In this species, the active cytoplasm forms a mount on the yolk and undergoes meroblastic cleavage. The active cytoplasm stains blue with hematoxylin and eosin, while yolk granules stain pink and bluish-brown with these dyes. The border cells are large and highly acidophilic, appearing bright pink with haematoxylin and eosin. These cells have a centrally located, prominent nucleus. After cleavage, a blastodisc is formed, which lies over the yolk at the animal pole. Gastrulation proceeds with the epibolic and embolic movement of the blastomeres in the spreading of the embryo over the yolk and the commencement of organogenesis. At this stage, the vitelline envelope is formed, leading to the formation of the yolk sac. The embryo now has a curved body distinguished into a head and trunk (Fig. 2e). As development proceeds, the embryo grows over the yolk sac to the extent that the head and tail end come into proximity (Fig. 2f). Initially, the head region of the embryo is acidophilic, and the trunk appears to be basophilic. When embryonic development is complete, the entire embryo becomes basophilic. At the end of embryonic development, the tail region gradually detaches from the yolk sac and moves away; however, the head is directed downwards and still attached to the yolk. The release of the embryo's body from the yolk and its straightening narrow the contact point between the embryo and the yolk sac. Yolk material is broken down at this contact point, and nutrients are transferred to the embryo. Sections passing through the head region of the embryo indicate differentiation of the anterior part of the neural tube into the cephalon. At this stage, the optic vesicles are differentiated; however, the eyes are not pigmented. The duration of embryonic development is about three days, and the larva with an elongated yolk sac hatches out.

Nervous System

During gastrulation, neural cells differentiate from blastomeres to form the neural plate, which subsequently folds to give rise to the neural tube. By the second day of embryonic development, the neural tube differentiates into the brain and spinal cord (Fig. 3a), with histological sections indicating the division of the brain into the forebrain, midbrain, and hindbrain (Fig. 3b). The process of neural tube formation and subsequent brain and spinal cord development is crucial for the proper functioning of the central nervous system. An interplay between molecules like Robo and N-cadherin plays a role in sorting spinal commissural axons within the spinal cord, facilitating their targeting to the brain (Sakai et al., 2012). In the hatched-out larva, differentiation of the brain

into the telencephalon, diencephalon, mesencephalon, metencephalon, and myelencephalon is evident (Figure 3c). In fifth-day-old larvae, olfactory bulbs and olfactory lobes are formed. The diencephalon shows the formation of the thalamus and hypothalamus. The optic lobes, cerebellum, and medulla oblongata take shape in this stage. The pineal and pituitary glands are observed in the tenth-day-old larval olfactory tract. In twelfth-day-old larvae, brain structures are fully formed. The spinal cord is enclosed in the vertebral canal.

Eye

During the second day of embryonic development, a critical stage in eye formation, a pair of optic vesicles emerges in the anterior region of the embryo. These optic vesicles are spherical and darker than other regions of the embryo. As the larva hatches, the eyes are located dorso-laterally as dark spherical structures, increasing their size the following day. Pigmentation is not initially observed during these early stages of eye development (Fig. 4a). By the time the larva is two days old, differentiation of eye structures such as the sclera, choroids, cornea, and lens become apparent. Subsequent stages show intensified pigmentation and differentiation of the cornea. By the fourth day, distinct layers of the retina are distinguishable, including the pigment layer, visual cell layer, outer plexiform layer, nuclear layer, inner plexiform layer, ganglionic cell layer, and nerve fibre layer (Fig. 4b). Further development leads to the formation of a compact retina lining the choroid coat, with the completion of lens formation dividing the eye into aqueous and vitreous chambers. By the fifth day, eye development is complete. The development of the eye has highlighted the crucial roles of various signalling molecules and transcription factors. Bone Morphogenetic Protein 7 (BMP-7) has been identified as an inducer of nephrogenesis and is required for eye development and skeletal patterning, emphasising its significance in early eye development in both flies and vertebrates (Luo et al., 1995). Proper patterning of the optic fissure has been shown to require the sequential activity of BMP7 and Sonic Hedgehog (SHH), underscoring the importance of lens-derived signalling in the regionalisation of the optic vesicle (Morcillo et al., 2006).

Furthermore, the differentiation of the optic vesicle into distinct eye structures involves complex molecular interactions. Studies have indicated that dorsal and ventral specification in the early optic vesicle is crucial in proper eye development (Uemonsa et al., 2002). The transcription factor Six3 has been identified as necessary for neuroretinal specification by regulating cell signalling and survival, particularly in a small population of progenitors during early eye formation (Liu & Cvekl, 2017). Moreover, retinoic acid signalling, generated from the optic vesicles and retina, has been implicated in eye development, highlighting the intricate molecular mechanisms involved (Duester, 2022).

Digestive System

The development in larval fish is a critical process involving transforming simple structures into fully functional organs. During the early stages of larval development, the digestive tract consists of a basic tubular structure without a mouth or anal opening. The oral cavity, pharynx, and oesophagus are lined with squamous epithelium, and the stomach and intestine exhibit infoldings along their inner surfaces. As the larva progresses, the formation of the mouth and anal opening occurs, with the appearance of an oral invagination on the first day and the development of a buccal opening by the second day. Exogenous feeding becomes evident as the larva consumes cladocerans and copepods, showing the initiation of feeding behaviour. Histological studies have revealed the differentiation of various digestive organs around the stomach region, including the liver and pancreas. The stomach transitions into a sac-like structure with deep infoldings, while the intestine becomes coiled with a wider lumen. Taste buds are observed in the oral cavity and pharyngeal region, indicating sensory development (Fig. 5). By the fourth day, the stomach transforms into a round muscular structure, and the liver becomes well-developed with vacuolated hepatocytes. The pancreas forms acini, contributing to digestive enzyme production. By the twelfth day, the digestive system is fully formed, with taste buds in the pharynx and well-developed gastric glands in the stomach. The intestine differentiates into anterior and posterior regions, and mucous cells line the inner surface of the digestive tract. Understanding the ontogeny of the digestive system in larval fish is crucial for optimising feeding practices and rearing protocols to enhance larval survival and growth. Research on the histological development of the digestive system in various fish species provides valuable insights into the maturation of digestive organs and enzymatic activities during larval development, aiding in the formulation of appropriate diets and feeding strategies tailored to larval fish's nutritional requirements and digestive capacities.

Swim Bladder

The development in larval fish is a complex process that involves the differentiation of epithelial cells from the anterior part of the stomach to form the primordial cells of the swim bladder. As the larva progresses, these cells develop into a swim bladder, enclosing a cavity with one or two layers of epithelial cells. The swim bladder exhibits thickened regions at the anterior and posterior ends, with the thickened region at the anterior end constituting the gas gland. The swim bladder inflates at the onset of the exclusively exogenous feeding

stage, and its size increases during further larval development. By the twelfth day, the swim bladder takes on a round and large shape anteriorly and an elongated and narrow shape posteriorly. Research on swim bladder morphology and development in fish species has provided valuable insights into the functional significance of swim bladders (Fig. 6). Studies have shown that swim bladder morphology can influence hearing sensitivity in cichlid species, highlighting the relationship between swim bladder structure and auditory abilities (Schulz-Mirbach et al., 2012). Additionally, the swim bladder has been implicated in expanding the frequency range of sound detection in sciaenid fishes with different swim bladder-inner ear configurations, underscoring the importance of swim bladder morphology in auditory functions (Ramcharitar et al., 2006). Understanding the ontogeny of the swim bladder in larval fish is crucial for elucidating its role in buoyancy regulation and sound detection. Studies have demonstrated that swim bladder inflation affects larval density and buoyancy, with larvae exhibiting diel changes in swim bladder volume to adjust their vertical distribution in the water column (Leis, 2007). Furthermore, the swim bladder has been linked to larval survival and growth, with swim bladder inflation influencing larval behaviour and feeding activity (Witeska et al., 2013).

Gill

The development of gill structures in larval fish is a crucial process that involves the differentiation of primordial gill arches into functional respiratory organs. In freshly hatched larvae, primordial gill arches appear on either side of the branchial region. By the second day, these gill arches differentiate into conical structures within the branchial cavity (Fig. 7a). Subsequent stages show the formation of undifferentiated cells at the base of the gill arches, which give rise to gill filaments in later stages. The development of gill filaments involves the formation of lamellae composed of pillar cells, with chloride cells observed at the base of the lamellae. Blood supply becomes evident in the gill filaments of five-day-old larvae, with further development leading to an increase in the length of gill lamellae and filaments (Fig. 7b). The gill development in fish species has provided insights into the morphological and physiological adaptations of gills for respiratory and ionoregulatory functions. Studies have shown that neuroepithelial cells in the gills of zebrafish play a role in oxygen sensing, contributing to the detection of changes in oxygen tension in embryos and larvae (Jonz & Nurse, 2003; 2005). Additionally, chloride cells in gill structures have been linked to ion regulation and osmoregulation in fish larvae, highlighting the importance of gill function in maintaining internal homeostasis (Saltys et al., 2005).

Heart

During embryonic development, the heart transforms from primordial cells located ventrally beneath the pharyngeal region to a fully functional organ. The initial organisation of these cells into a simple tube marks the onset of heart development, with the observation of the heartbeat preceding hatching. The heart exhibits a slightly broader posterior and narrow anterior tube in newly hatched larvae. As larval development progresses, the tubular heart undergoes intricate foldings, leading to the formation of distinct cardiac structures, including the sinus venosus, atrium, ventricle, and bulbous arteriosus (Fig. 8). Subsequent development involves the formation of blood vessels that initially vascularise the gills, highlighting the intricate process of heart development and vascularisation in larval fish. The study of heart organogenesis in vertebrates has shed light on the regulatory mechanisms and morphogenetic processes that drive the formation of cardiac structures. Studies have shown that regulated patterns of gene expression and proliferation within the embryonic heart play a crucial role in the morphogenesis of atrial and ventricular chambers. At the same time, the formation of cardiac cushions contributes to the development of definitive valves in the heart (Miquerol & Kelly, 2012). The developmental processes of the heart in larval fish are essential for elucidating the molecular and cellular events that govern cardiac morphogenesis and function. Research on heart development in fish species provides valuable insights into the evolutionary conservation of cardiac developmental pathways and the adaptive mechanisms that enable efficient cardiovascular function in aquatic environments.

Kidney

Kidney development in larval fish involves a series of intricate processes leading to distinct renal structures. In a freshly hatched larva, the kidney appears as an elongated structure below the notochord, distinguishable into the head kidney and trunk kidney, consisting of pronephros, internal lymphoid, and hematopoietic tissue. By the fifth day, renal corpuscles and tubules become distinguishable, and the urinary bladder opens directly to the exterior. By the twelfth day, the mesonephros are well developed, marking the maturation of the kidney in the larval fish (Figs. 9a and 9b). Research on kidney development in various species has provided insights into the molecular and cellular mechanisms underlying nephrogenesis and renal maturation. Studies have shown that mesonephric nephrons in teleosts are derived from precursor cells within the nephrogenic zone, with the nephrogenic capacity of cells maintained throughout life, allowing for neonephrogenesis (Zhou et al., 2010). Understanding the developmental processes of the kidney in larval fish is essential for

elucidating the evolutionary conservation of renal structures and their functional adaptations in aquatic environments. Furthermore, investigations into the ontogeny of the kidney in fish larvae have implications for understanding renal physiology and osmoregulation in early life stages.

Muscle

The skeletal muscle in larval fish is a complex structure composed of muscle bundles, or myotomes, formed of myofibers. These myofibers exhibit striations and contain multiple nuclei within them. The muscle bundles are separated by the myoseptum, contributing to the organisation and segmentation of the skeletal muscle. In a 7-day-old larva, the notochord is well flexed, indicating the ongoing musculoskeletal development in the larval fish (Figs. 10a and 10b). The development of skeletal muscle in vertebrates has highlighted the role of signalling pathways and transcription factors in regulating myogenesis and muscle fibre differentiation. Studies have shown that hedgehog signalling plays a crucial role in the regulation of muscle fibre types, including slow-twitch and fast-twitch fibres, contributing to the diversity of muscle function in vertebrates (Grimaldi et al., 2004; Du et al., 1997). Establishing the epaxial-hypaxial boundary in the myotome also segregates trunk skeletal muscles into distinct regions, reflecting the spatial organisation of muscle groups in the larval fish (Ahmed et al., 2006). The morphological and functional aspects of skeletal muscle development in larval fish are essential for elucidating the mechanisms underlying muscle growth and locomotor abilities. The myotome organisation, myoseptum structure, and muscle fibre differentiation provide valuable insights into the evolutionary conservation of musculoskeletal systems and their adaptations to diverse environmental conditions.

Fin

The development in larval fish is a dynamic process that involves the differentiation and morphogenesis of various fin structures. In the hatched-out larva, the presence of marginal fin folds and pectoral fins marks the initial stages of fin development. By the second day after hatching, differentiation of the caudal fin is observed, leading to the formation of a homocercal caudal fin in five-day-old larvae, which subsequently differentiates into a homocercal caudal fin. By the twelfth day, the larva exhibits a well-developed caudal fin, with fin rays formed in the pectoral, pelvic, and caudal fins (Figs. 11a–11b). The dorsal fin begins its differentiation around the tenth day and is fully formed by the twentieth day in larval fish. The fin development in fish species has provided insights into the genetic and molecular mechanisms that govern fin morphogenesis and patterning. Investigations into the ontogeny of fins in larval fish have implications for understanding the evolutionary origins and functional adaptations of fins in aquatic organisms (Thorsen & Hale, 2007; Cajado et al., 2021). The developmental processes of fins in larval fish are essential for elucidating fins' structural diversity and functional roles in locomotion, stability, and manoeuvrability. Research on fin differentiation, fin ray formation, and fin morphology provides valuable insights into fins' evolutionary and ecological significance in fish species.

Skin

Histologically, the skin of larval fish is composed of three main layers: the epidermis, dermis, and hypodermis. The epithelium of the skin consists of layers of squamous and columnar cells, with club-shaped cells and goblet cells interspersed between them. In the dermis, two distinct layers, the stratum spongiosum and stratum compactum, are observed, with scales originating from this layer. The hypodermis is present as a thin layer. In a two-day-old larva, pigment cells are observed in the dermis and hypodermis, with the intensity of pigment cells and pigments progressively increasing during further larval development. The histological characteristics of the skin in larval fish are essential for elucidating the skin's structural composition and functional adaptations in early life stages. Research on skin histology provides insights into the cellular organisation, pigment distribution, and developmental changes in the skin of larval fish, contributing to our understanding of skin biology and physiology in aquatic organisms.

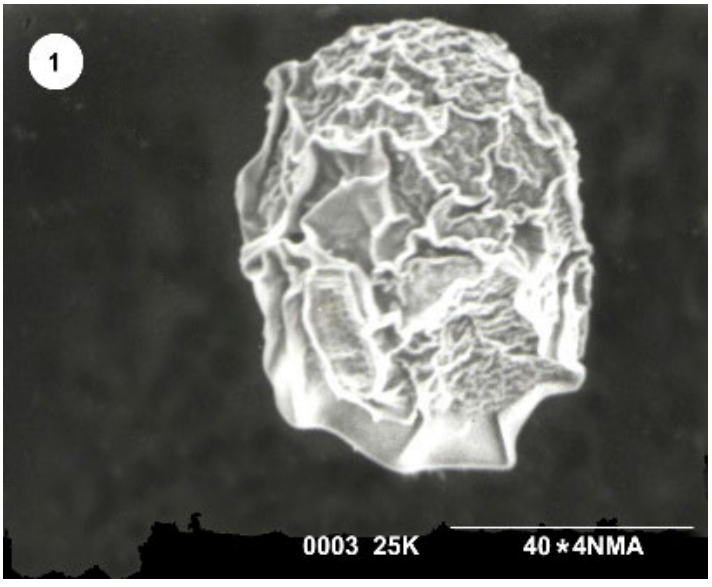
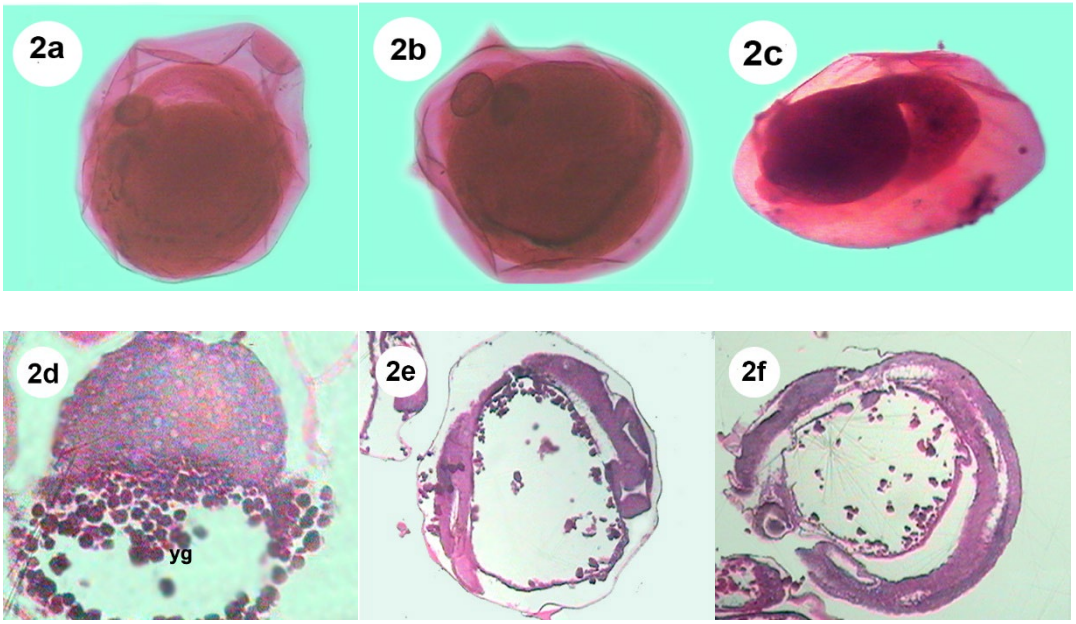
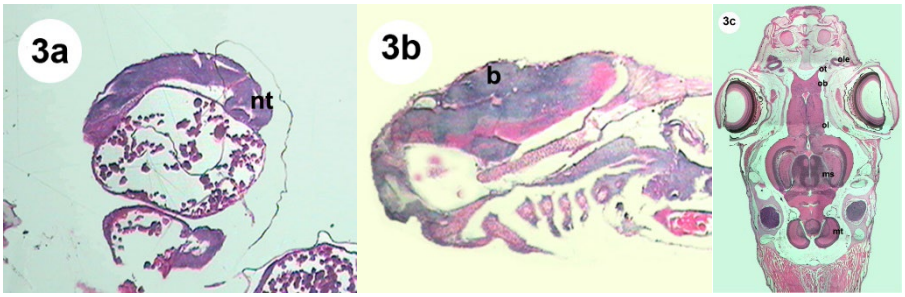


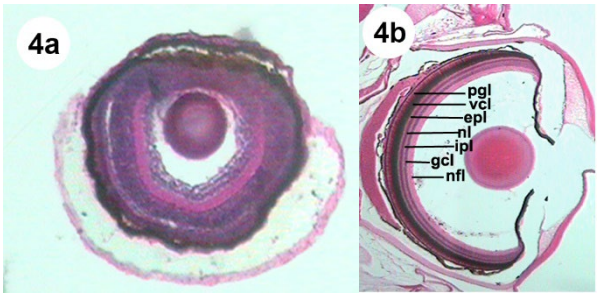
Figure 1. SEM of *C. carpio var. koi*



Figures 2. a. Fertilized egg of *C. carpio var. koi;* b. Egg showing the development of head and eye; c. Advanced stage embryo; d. C.S. of egg showing cleavage; e. C.S. of egg showing the formation of the head; f. C.S. of egg showing fully formed embryo



Figures 3. a. Section of the egg showing neural tube (nt- noto cord); b. L.S. of an embryo showing brain (b- brain); c. V.S. of the brain showing differentiation of different regions of forebrain, midbrain and hindbrain (ole- olfactory epithelium, ot- olfactory tract, ob- olfactory bulb, ol- olfactory lobe, ms- mesencephalon, mt- metencephalon)



Figures 4. a. Histology of embryonic eye; b. 168 Histology of the eye showing different layers of the retina (PGL- pigment layer, vcl- visual layer, epl- external plexiform layer, nl- nuclear layer, ipl- internal plexiform layer, gcl- ganglion cell layer, nfl- nerve fibre layer)

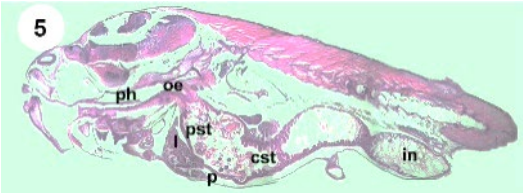


Figure 5. L.S. of larva showing differentiation of stomach (ph- pharynx, oe- oesophagus, l- liver, pst- pyloric stomach, cst- cardiac stomach, in- intestine, p- pancreas)

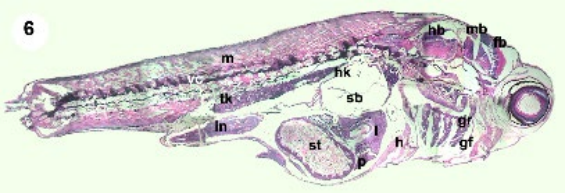
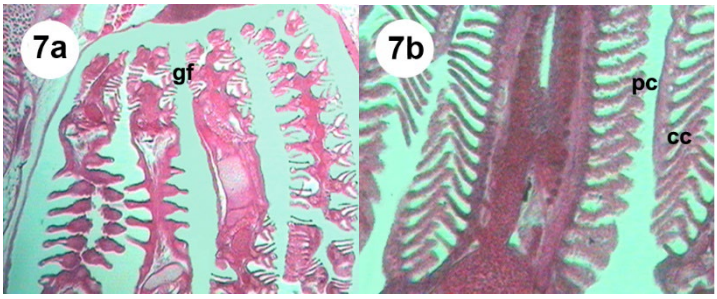


Figure 6. 153 L.S. of larva showing exclusively exogenous feeding (fb- forebrain, mb- midbrain, hb- hindbrain, hk- head kidney, tk- trunk kidney, gr- gill racker, gf- gill filament, h- heart, l- liver, p- pancreas, st- stomach, sb- swim bladder, in- intestine)



Figures 7. a. C.S. of the branchial region showing differentiation of gill filaments (gf- gill filament); b. Higher magnification of gill lamellae showing pillar cells and chloride cells (cc- chloride cells, pc- pillar cells)

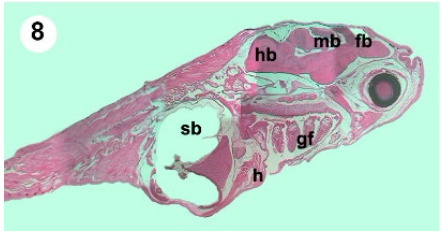
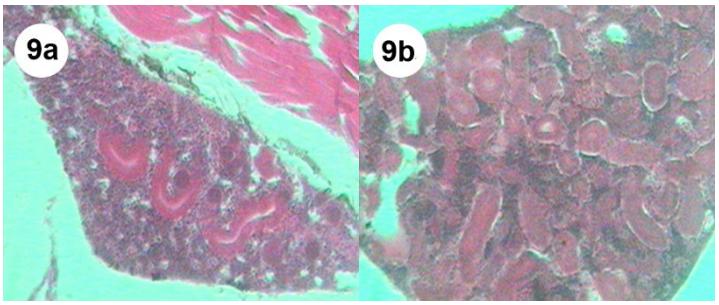
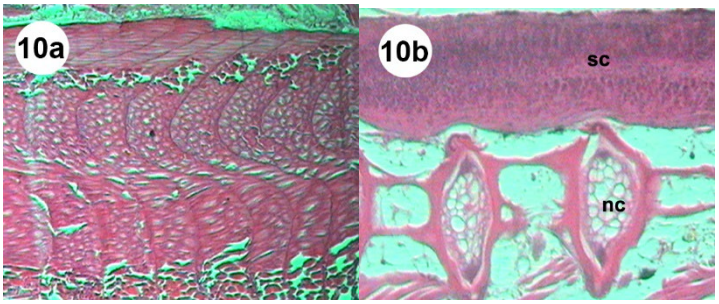


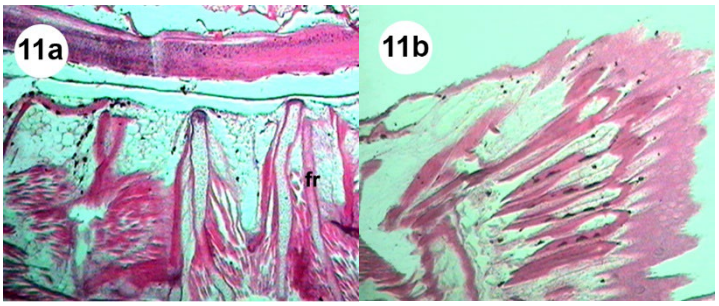
Figure: 8. L.S. of larva showing differentiation of brain lobe, gill filament and caudal fin lobe (fb- forebrain, mb- midbrain, h- heart, hb- hindbrain, gf- gill filament, sb- swim bladder)



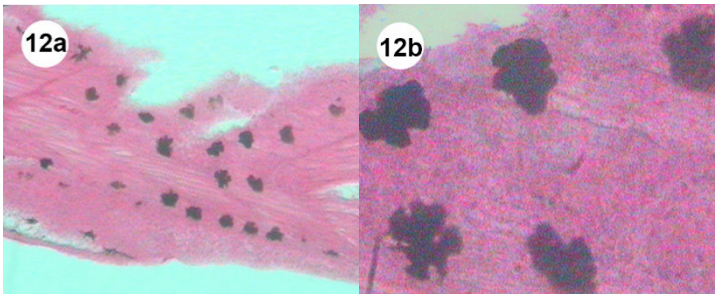
Figures 9. a & b. L.S. of larva showing mesonephric kidney



Figures 10 a. L.S. of longitudinal and circular muscle; L.S. of larva showing notochord (nc- noto cord, sc- spinal cord)



Figures 11. a. L.S. of larva showing fin rays; L.S. of larva showing differentiation of caudal fin



Figures 12. a & b. The skin of different body regions of a larva shows pigmentation

Conclusion

The current study on larval organogenesis in *C. carpio var koi* demonstrates species-specific variation in the incubation duration and distinct ontogenic processes occurring in larvae at different trophic levels. Key developmental milestones include the maturation of the brain, digestive tract, liver, pancreas, gills, kidney, muscles, skin, and fins. Coordinated development is crucial for achieving the functions of eating, breathing, osmoregulation, and behaviour. The transition from an internal to an external energy source was recognised as a crucial phase that could result in significant mortality rates throughout the early stages of life. Investigating the variation in the timing of start feeding among different fish species, the yolk sac larvae that exhibit successful prey consumption are more likely to survive than those that commence feeding later. The transition phase in which tropical fishes switch from relying on internal food sources to external food sources for energy is brief. A wait of more than 24 hours in initiating feeding, either after eye pigmentation or after yolk absorption, is considered crucial for the survival of these species. The larvae are well adapted for this transition in the energy source since they can be raised with a greater survival rate when provided with suitable live feed.

Compliance with Ethical Standards

Conflict of interest: The author(s) declare no actual, potential, or perceived conflict of interest for this article.

Ethics committee approval: The experiments were conducted in accordance with the guidelines and regulations established by the Committee for Control and Supervision of Experiments on Animals (CCSEA), Department of Animal Husbandry and Dairying, Ministry of Fisheries, Animal Husbandry and Dairying, Government of India and the experimental protocol was approved by Institutional Animal Ethical Committee (Karpaga Vinayaga Institute of Medical Sciences and Research Institute, Tamil Nadu, India) (No: 181GO/ERE/S/15/CPCSEA dated 04.12.2018).

Data availability: Data will be made available on request.

Funding disclosure: No funding provided.

Acknowledgements: We would like to the Managements, the Principals of Karpaga Vinayaga College of Engineering and Technology, Chengalpattu – 603308 and The New College, Chennai - 600014 for providing laboratory facilities to complete the work.

Disclosure: -

References

Adamek, M., Oschilewski, A., Wohlsein, P., Jung-Schroers, V., Teitge, F., Dawson, A., Gela, D., Piackova, V., Kocour, M., Adamek, J., Bergmann, S.V., Steinhagen, D. (2017). Experimental infections of different carp strains with the carp edema virus (CEV) give insights into the infection biology of the virus and indicate possible solutions to problems caused by koi sleepy disease (KSD) in carp aquaculture. *Veterinary Research,* 48(1). https://doi.org/10.1186/s13567-017-0416-7

Behrouz, A., Moghadam, M., Rezaei, S., Abbasi, H. (2014). Larval organogenesis of *Schizothorax zarudnyi* (Nikolskii, 1897) (Cyprinidae): histological aspects. *Journal of Fisheriessciences.com.* https://doi.org/10.3153/jfscom.201416

Boulhic, M. and Gabaudan, J. (1992). Histological study of the organogenesis of the digestive system and swim bladder of the dover sole, *Solea solea* (Linnaeus 1758). *Aquaculture,* 102(4), 373-396. https://doi.org/10.1016/0044-8486(92)90190-v

Burggren, W. and Pinder, A. (1991). Ontogeny of cardiovascular and respiratory physiology in lower vertebrates. *Annual Review of Physiology,* 53(1), 107-135. https://doi.org/10.1146/annurev.ph.53.030191.000543

Cajado, R., Oliveira, L., Silva, F., Zacardi, D. (2021). Early development of the neotropical fish known as long sardine *Triportheus auratus* (Valenciennes 1850) (Characiformes, Triportheidae). *Journal of Applied Ichthyology,* 37(5), 759-769. https://doi.org/10.1111/jai.14228

China, V. and Holzman, R. (2014). Hydrodynamic starvation in first-feeding larval fishes. *Proceedings of the National Academy of Sciences,* 111(22), 8083-8088. https://doi.org/10.1073/pnas.1323205111

Du, S., Devoto, S., Westerfield, M., Moon, R. (1997). Positive and negative regulation of muscle cell identity by members of the hedgehog and TGF-β gene families. *The Journal of Cell Biology,* 139(1), 145-156. https://doi.org/10.1083/jcb.139.1.145

Duester, G. (2022). Towards a better vision of retinoic acid signaling during eye development. *Cells,* 11(3), 322. https://doi.org/10.3390/cells11030322

Grimaldi, A., Tettamanti, G., Martin, B., Gaffield, W., Pownall, M., Hughes, S. (2004). Hedgehog regulation of superficial slow muscle fibres inxenopusand the evolution of tetrapod trunk myogenesis. *Development,* 131(14), 3249-3262.
https://doi.org/10.1242/dev.01194

Jonz, M. and Nurse, C. (2003). Neuroepithelial cells and associated innervation of the zebrafish gill: a confocal immunofluorescence study. *The Journal of Comparative Neurology,* 461(1), 1-17.
https://doi.org/10.1002/cne.10680

Jonz, M. and Nurse, C. (2005). Development of oxygen sensing in the gills of zebrafish. *Journal of Experimental Biology,* 208(8), 1537-1549.
https://doi.org/10.1242/jeb.01564

Leis, J. (2007). Behaviour as input for modelling dispersal of fish larvae: behaviour, biogeography, hydrodynamics, ontogeny, physiology and phylogeny meet hydrography. *Marine Ecology Progress Series,* 347, 185-193.
https://doi.org/10.3354/meps06977

Liu, W. and Cvekl, A. (2017). Six3 in a small population of progenitors at E8.5 is required for neuroretinal specification via regulating cell signaling and survival in mice. *Developmental Biology,* 428(1), 164-175.
https://doi.org/10.1016/j.ydbio.2017.05.026

Ługowska, K. and Kondera, E. (2018). Early development of vimba (*Vimba vimba*) at different temperatures and temperature-related anomalies. *Aquaculture Research,* 49(6), 2336-2344.
https://doi.org/10.1111/are.13670

Luo, G., Hofmann, C., Bronckers, A., Bradley, A., Karsenty, G. (1995). BMP-7 is an inducer of nephrogenesis, and is also required for eye development and skeletal patterning. *Genes Development,* 9(22), 2808-2820.
https://doi.org/10.1101/gad.9.22.2808

Martins, M.L., Cardoso, L., Furtado, W., Tancredo, K.R. (2018) Histopathology guide for freshwater fish. Editora UFSC, Florianopolis

Milan, D., Jones, I., Ellinor, P., MacRae, C. (2006). In vivo recording of adult zebrafish electrocardiogram and assessment of drug-induced QT prolongation. *American Journal of Physiology Heart and Circulatory Physiology,* 291(1), H269-H273.
https://doi.org/10.1152/ajpheart.00960.2005

Miquerol, L. and Kelly, R. (2012). Organogenesis of the vertebrate heart. *Interdisciplinary Reviews Developmental Biology,* 2(1), 17-29.
https://doi.org/10.1002/wdev.68

Mohale. H.P., Sarang, N., Desai, A.Y. (2020) The common carp and its culture systeme management. Academia Publications, Delhi**.**

Morcillo, J., Martínez-Morales, J., Trousse, F., Fermin, Y., Sowden, J., Bovolenta, P. (2006). Proper patterning of the optic fissure requires the sequential activity of BMP7 and SHH. *Development,* 133(16), 3179-3190.
https://doi.org/10.1242/dev.02493

Muthupriya, P., Sivakumar, K., Altaff, K. (2022). The Early Organogenesis of *Carassius auratus* (Cyprinidae): A Histological Perspectives. *International Journal of Zoological Investigations,* 8(1), 219-234.
https://doi.org/10.33745/ijzi.2022.v08i01.025

Nebeker, A., Savonen, C., Stevens, D. (1985). Sensitivity of rainbow trout early life stages to nickel chloride. *Environmental Toxicology and Chemistry,* 4(2), 233.
https://doi.org/10.1897/1552-8618(1985)4[233:sortel]2.0.co;2

Nowlin, W., Drenner, R. (2000). Context-dependent effects of bluegill in experimental mesocosm communities. *Oecologia,* 122(3), 421-426.
https://doi.org/10.1007/s004420050048

Qin, J., Fast, A.W. (1997). Food selection and growth of young snakehead *Channa striatus*. *Journal of Applied Ichthyology,* 13, 21–25.

Ramcharitar, J., Higgs, D., Popper, A. (2006). Audition in sciaenid fishes with different swim bladder-inner ear configurations. *The Journal of the Acoustical Society of America,* 119(1), 439-443. https://doi.org/10.1121/1.2139068

Sakai, N., Insolera, R., Sillitoe, R., Shi, S., Kaprielian, Z. (2012). Axon sorting within the spinal cord marginal zone via robo-mediated inhibition of N-Cadherin controls spinocerebellar tract formation. *Journal of Neuroscience,* 32(44), 15377-15387. https://doi.org/10.1523/jneurosci.2225-12.2012

Saltys, H., Jonz, M., Nurse, C. (2005). Comparative study of gill neuroepithelial cells and their innervation in teleosts and Xenopus tadpoles. *Cell and Tissue Research,* 323(1), 1-10. https://doi.org/10.1007/s00441-005-0048-5

Schulz-Mirbach, T., Metscher, B., Ladich, F. (2012). Relationship between swim bladder morphology and hearing abilities–a case study on Asian and African cichlids. *Plos One,* 7(8), e42292. https://doi.org/10.1371/journal.pone.0042292

Sivakumar, K. (2005). Freshwater fish and prawn larval rearing using indigenous live-feed. PhD thesis, University of Madras. India, 1-135.

Thorsen, D., Hale, M. (2007). Neural development of the zebrafish (*Danio rerio*) pectoral fin. *The Journal of Comparative Neurology,* 504(2), 168-184. https://doi.org/10.1002/cne.21425

Uemonsa, T., Sakagami, K., Yasuda, K., Araki, M. (2002). Development of dorsal–ventral polarity in the optic vesicle and its presumptive role in eye morphogenesis as shown by embryonic transplantation and in ovo explant culturing. *Developmental Biology,* 248(2), 319-330. https://doi.org/10.1006/dbio.2002.0737

Witeska, M., Sarnowski, P., Ługowska, K., Kowal, E. (2013). The effects of cadmium and copper on embryonic and larval development of ide *Leuciscus idus* l. *Fish Physiology and Biochemistry,* 40(1), 151-163. https://doi.org/10.1007/s10695-013-9832-4

Wu, G., Yang, Y., Zeng, L. (2007). Kinematics, hydrodynamics and energetic advantages of burst-and-coast swimming of koi carps (*Cyprinus carpio* koi). *Journal of Experimental Biology,* 210(12), 2181-2191. https://doi.org/10.1242/jeb.001842

Zhou, W., Boucher, R., Bollig, F., Englert, C., Hildebrandt, F. (2010). Characterization of mesonephric development and regeneration using transgenic zebrafish. *American Journal Physiology Renal Physiology,* 299(5), F1040-F1047. https://doi.org/10.1152/ajprenal.00394.2010

Zimmer, A., Wright, P., Wood, C. (2017). Ammonia and urea handling by early life stages of fishes. *Journal of Experimental Biology,* 220(21), 3843-3855. https://doi.org/10.1242/jeb.140210