



## Morpho- Functional Observations on Ontogenic development of the Renal excretory system in pre to post flexion stages larvae of Himalaya Snow trout *Schizothorax plagiostomus* (Heckel)

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Received: 7.10.2021; Revised: 2.11.2021; Accepted: 7.11.2021

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**Abstract:** *Schizothorax plagiostomus* is one of the most important food fish inhabiting the snow-fed tributaries of the Himalayan region. Due to nutritional value, good growth rate, high economic prospects as well as the decline in the natural habitat its commercial production is desired. Keeping in view this fact, during the present investigation by following the artificial breeding and rearing experiments an attempt has been made to understand the morpho-functional development of the renal excretory system in pre to post-flexion stages larvae. The study reveals that all the essential basic structures of the renal excretory system (pronephros) were established before the initiation of external feeding. During the post-flexion stage when the yolk sac was almost reabsorbed and larvae exclusively depend on exogenous feeding the mesonephric tubules were forming and some of them become functional i.e. presence of glomerulus to maintain the internal environment as well as efficient removal of gradually increasing amount of nitrogenous wastes. The presence of lymphoid tissues was suggesting that cell-mediated immune responses develop progressively in the Himalayan snow trout *S. plagiostomus* larvae.

**Keywords:** Himalayan Snow trout, *Schizothoraxplagiostomus*, flexion stage, Kidney, renal system, Ontogeny, Organogenesis

### Introduction

The renal excretory system is one of the most complex organs of the vertebrates and its function in regulating blood composition necessitates the coordinated activity of different cell types, which are meticulously arranged in the nephron and supporting tissue (Seldin and Giebisch, 1992; Drummond, 2000). In teleosts, differences in kidney form have been observed both externally and internally among various species. The head kidney is entirely made up of lymphoid tissue and lacks a nephron (Kapoor and Khanna, 2004). During the organogenesis of the larval kidney/renal system in teleosts, four phases are recognized: commitment of undifferentiated mesodermal cells to a nephrogenic fate; formation of the primordium, as well as the caudal growth and epithelialization of the pronephric duct; induction and formation of the nephron; and ingrowth of endothelial cells and the formation of the glomerular capillary tuft & blood filter (Saxen, 1987; Drummond, 2000). During ontogeny, proper organogenesis and morpho-functional development of the

organ systems are essential for the growth and survival of the larvae (Bahuguna and Rayal, 2006a,b,c; Rayal and Bahuguna 2006; Rayal et al., 2011; Rayal, 2020, 2021; Rayal et al., 2020, 2021); the kidney is one of them as it is associated with the excretion of waste, osmoregulation, acid-base balance, secretion of hormones, reabsorption of metabolites, immune response, etc. In light of this, an attempt has been made to comprehend the mechanism of organogenesis of the kidney/renal excretory system during ontogeny in Hillstream Himalayan snow trout *S. plagiostomus* pre to post-flexion stages larvae.

### Material and Methods

To obtain the desired pre to post-flexion larvae, artificial fertilization was carried out on the bank of the Alaknanda River in Srinagar Garhwal, Uttarakhand, India, during the breeding season (September-October). Fertilized eggs were transferred to the laboratory and placed in incubation and hatching trays, glass jar hatcheries, and hatching tubs with sufficient aeration, suitable temperature (17-20°C), and consistent water supply. Some of the fertilized eggs of the same



batch were also placed in incubation & hatching trays on a small stream of river to ensure proper development in natural habitat. The behavior and developmental changes among the larvae at both the laboratory and natural sites were monitored up to the post-flexion stage. For the subsequent morpho-functional and histological analysis, 5-10 larvae were fixed in different fixatives, viz. aqueous bouins, alcoholic bouins, 4% formalin, calcium formol, and 70% alcohol, etc. from hatching to post-flexion stages at 4-8 hrs intervals. After completing fixation (18-24 hours) in bouins, samples were rinsed and then dehydrated in an increasing series of ethanol for embedding. Following the embedding in paraffin wax (E. Merck, 54-560C melting point paraffin wax), transverse and longitudinal sections of 5-6µm were obtained using an Erma Rotary Microtome. After dewaxing, the serially ordered stained sections were mounted in DPX, and photomicrographs were made using an Olympus-photo-microscopic system.

## Results

The microscopic examination of the serially arranged sections (L.S. & T.S.) of hatchlings shows that the renal excretory system and other visceral organs were not differentiated at hatching. It proceeds gradually in an anterior-posterior direction after eight hours of hatching.

The pronephric duct differentiates from the renal primordial cells or primordia by the second dph. Between the yolk sac and the notochord, it runs anterior-caudally (Figure-1). Pronephric nephrons and tubules form shortly after the pronephric duct and were connecting the anterior-most tips of pronephric ducts. On 3-4<sup>th</sup>dph convolution in the anterior portion of pronephric tubules, and some of the erythroid and lymphoid tissue were observed associated with this region (Figure-2). At the same time, the caudal portion of the pronephric duct (near cloaca) starts to become broader than the comparison of other parts.

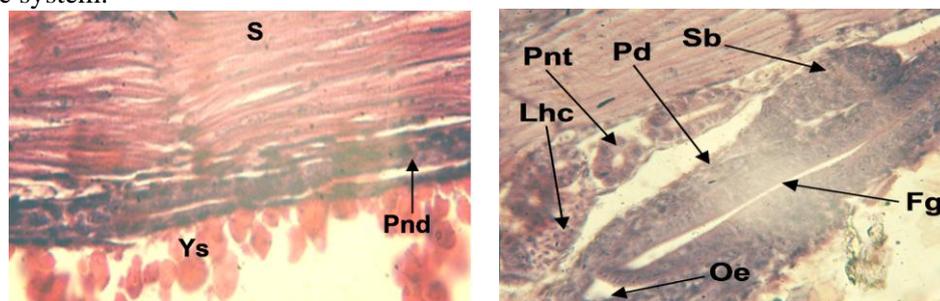


Figure-1. L.S. of 2<sup>nd</sup>dph larvae showing pronephric duct running anterior-caudally, between yolk sac and notochord (H.E. 280X).

Figure-2. L.S. of 3<sup>rd</sup>dph larvae showing differentiation of swim bladder anlage as a cluster of mesenchymal cell from the dorsal wall of the esophagus, pronephric tubule with rudimentary hemopoietic and lymphatic tissues, yolk sac, etc (H.E. 280X).

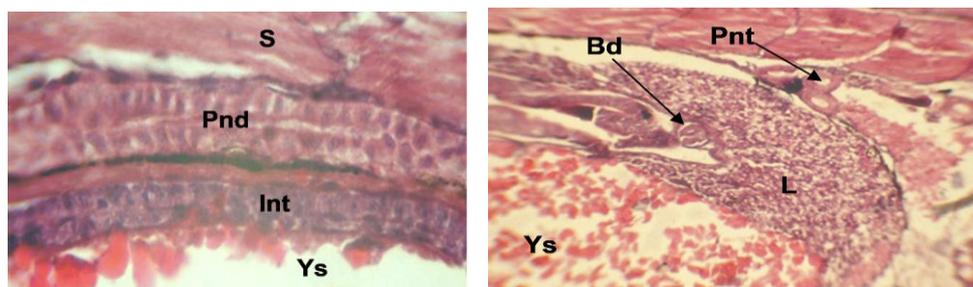


Figure-3. L.S. of 7<sup>th</sup>dph larvae showing pronephric duct at the trunk kidney, midgut portion, and yolk sac, etc (H.E. 100X)

Figure-4. L.S. of 9<sup>th</sup>dph larvae showing the opening of the bile duct in the fore gut, liver cells around the bile duct, and foregut, Kidney, etc. (H.E. 100X).

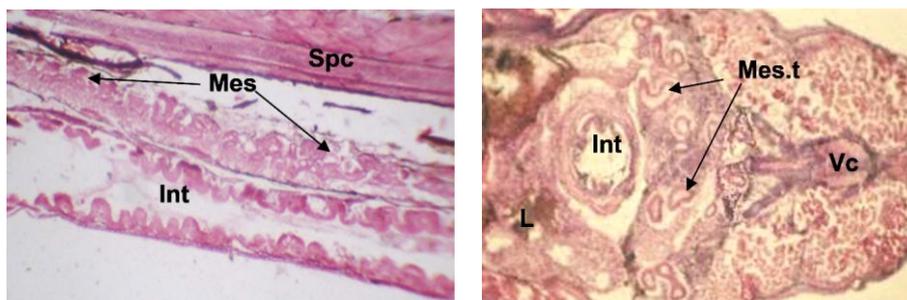


Figure-5. L.S. of 12<sup>th</sup>dph larvae showing the development of the segmented tubules of mesonephros (H.E. 100X).

Figure-6. L.S. of 14<sup>th</sup>dph larvae showing formation and coiling of mesonephric tubules in trunk kidney. (H.E. 100X).

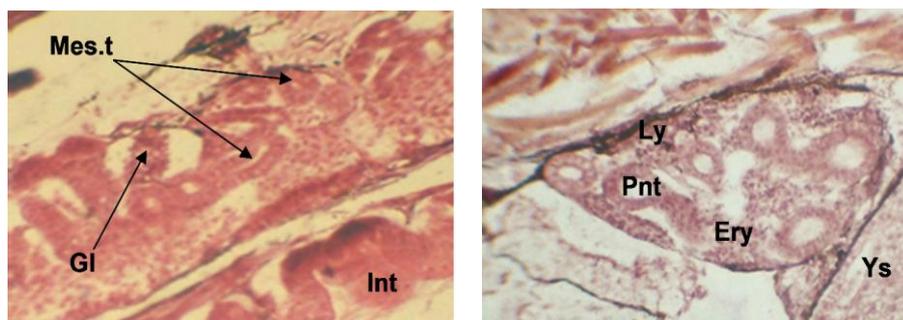


Figure-7. L.S. of 15<sup>th</sup>dph larvae showing glomerulus, proximal and distal tubules of mesonephros (Mes.t), etc. (H.E. 100X).

Figure-8. L.S. of 15<sup>th</sup>dph larvae showing head kidney: pronephric tubule consists of large columnar epithelial cells, erythroid and lymphatic tissue. (H.E. 100X).

### Abbreviations

Bd= bile duct, Ery= erythroid tissue, Fg= fore gut, Gl= glomerulus, Int= intestine, L= eye lens, Lhc= lymphatic and hemopoietic cells, Ly= lymphatic tissue, Mes= mesonephros, Mes.t= mesonephric tubule, Oe= oesophagus, Pd= pneumatic duct, Pnd= pronephric duct, Pnt= pronephric tubules, S= somites, Sb= swim bladder, Spc= spinal cord, Vc= vertebral column, Ys= yolk sac

Pronephric tubules were more convoluted in 6-7<sup>th</sup>dph larvae. Until the one-half of flexion stage (9<sup>th</sup>dph) kidney remains completely pronephric; no evidence of mesonephros at the trunk and caudal region (Figure-3). Convulsions of nephric tubules were observed in the anterior portion of the kidney. Erythroid and lymphoid tissues associated with this portion were increasing in size (9-10<sup>th</sup> dph; Figure-4). By 10-11<sup>th</sup>dph urinary duct open into a small sac (slightly broad portion) which was closely associated with the posterior digestive tract. The segmented tubules of mesonephros were forming during the commencement of exogenous feeding or post flexion stage (12-13<sup>th</sup>dph; Figure-5). During the 14-15<sup>th</sup> dph hemopoietic tissues were developing around the pronephric tubules (Figure-8). The coiling of the mesonephric tubules was becoming more extensive (Figure-

6). Some tubules were found with large blast-like cells (columnar epithelial cells) between the nephric tubules (Figure-7). As development continued the blast-like cells increased in number, and become smaller and darker in appearance. The development of mesonephric kidney was continued; distinct increase in hematopoietic tissue and a variety of cell types were evident there during the 20-22<sup>nd</sup>dph.

### Discussion

The general pattern of renal excretory system development in *S. plagiostomus* is almost comparable to that described in other fish, although the timing differs. The absence of functional mesonephric kidneys during the early stages (especially during pre-flexion and flexion stages) of larval development in most fishes, including *S. plagiostomus*, is usually the major variation in their renal system



between larvae and adults. The pronephros, also known as the pronephric kidney in teleosts, is made up of only two nephrons with glomeruli fused at the midline, pronephric tubules connected directly to the glomeruli via a neck segment, and paired bilateral pronephric ducts that transport the altered blood filtrate outside the animal (Tytler, 1988; Drummond et.al., 1998). According to Vize et. al. (1997), Drummond et.al. (1998) all functional pronephroi receive their blood supply from the dorsal aorta. During the current investigation, similar observations were obtained in *S. plagiostomus* larvae.

Pronephric primordium appears as a mass of intermediate mesoderm lying beneath the anterior somites during early somitogenesis in amphibians and teleost fishes, and future pronephros is derived from this cluster of cells (Kimmel et.al, 1995; Vize et. al., 1997). By the 2<sup>nd</sup>dph in *S. plagiostomus*, the renal anlage was visible under the optical microscope, progressing from anterior to caudal. The pronephric duct develops before the pronephric nephrons and tubule from kidney primordia. Tytler (1988), Tytler et al. (1996), Drummond et al. (1998), and Drummond (2000) observed similar phenomena in other fishes.

From three to seven dph larvae of *S. plagiostomus*, the anterior side of the pronephric tubules becomes more convoluted, some erythroid and lymphoid tissue is also associated with this region, and the caudal portion of the pronephric duct becomes slightly wider than the other portions. Some researchers have noticed similar phenomena in other fishes at various phases of their early growth. While in *Oreochromis niloticus* pronephric tubules appears at 46-48 hrs of fertilization and its convolution, as well as the development of erythropoietic and lymphoid tissue, occurs before hatching i.e. 100 hrs after fertilization (Morrison et.al., 2001 ). Kato et al. (2004) reported a well-established kidney with erythropoietic and lymphoid components in *Epinephelus bruneus* at hatching in kelp grouper.

The pronephros in several fish genera, such as *Fierasfer*, *Zoarcis*, and *Lepadogaster*, remains functioning throughout their lives (Kerr, 1919; Hickman and Trump, 1969; Lagler et al., 1977). As the mesonephros develops, most

vertebrates' pronephros degenerate, and the anteriormost region becomes hemopoietic; this is referred to as the head kidney (Drummond, 2000; Reimschuessel, 2001). As with development, this process progressed anterior to the posterior end. In the present study, such type of phenomenon has been observed by 10-11th dph. The mesonephros is the functional kidney of adult teleosts, and it is also functional in the mammalian embryo at various stages of development (Davies and Routh, 1957; Leeson and Baxter, 1957; Martino and Zamboni, 1966; and Tiedemann, 1976).

In more posterior segments, the mesonephros arises from the nephrotome. The tubular outgrowths, Bowman's capsule and glomerulus, are formed like that of the pronephros (Fraser, 1950; Goodrich, 1958). Renal vesicle is formed by the formation of a cavity in the center of the cluster of nephrotomic cells located near the pronephric duct. Initially, it becomes C-shaped but later on changed into S-shape. The glomerulus and peritoneal funnel are formed respectively by the invagination and evagination of the medial end of the S-shaped vesicle. The remaining portion of the S-shaped vesicle differentiates into the primary tubule which fuses with the pronephric excretory duct by the outgrowth and ultimately becomes mesonephric duct. Secondary and tertiary tubules differentiate from the nephrogenic cluster of the nephrotome and are finally fused with the primary duct. The tertiary tubule opens into the secondary tubules. In due course of time, all the tubules elongate, become coiled, and intertwine forming the mesonephros (Reimschuessel, 2001). A similar pattern supports the developmental phases in *S. plagiostomus* larvae during pre to post-flexion stages.

The current investigation on the development of *S. plagiostomus* reveals that mesonephros differentiation continued during the post-flexion stage. Drummond (2000) made similar observations about a variety of other teleosts.

After the fourteenth to fifteenth dph, *S. plagiostomus* larvae showed a gradual rise in the volume of erythropoietic and lymphatic tissue in the kidney and other lymphatic organs, indicating the development of cell-mediated immune responses. The thymus and



kidney are the two most significant primary lymphoid organs. The kidney is the equivalent of adult mammals' bone marrow as a source of B lymphocytes (Zapata, 1979; Zapata et al., 1996; Zapata and Amemlya, 2000), suggesting that cell-mediated immune responses develop progressively in Himalayan snow trout *S. plagiostomus* larvae.

#### Acknowledgment

The author sincerely acknowledges Prof. S.N. Bahuguna, Department of Zoology and Biotechnology, Srinagar Campus, H.N.B. Garhwal University (Central University) Srinagar Garhwal, for constant supervision and encouragement.

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