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MORPHO-FUNCTIONAL AND DEVELOPMENTAL STUDY OF SWIM BLADDER IN PRE TO POST FLEXION STAGES LARVAE OF HIMALAYAN SNOW TROUT *SCHIZOTHORAX PLAGIOSTOMUS* (HECKEL) REARED IN ARTIFICIAL AND NATURAL CONDITIONS

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Abstract: Present study deals with histology, gross morphology of the larvae and its behavioural aspects to describe the progression of the development of swim bladder in pre to post flexion stages larvae of the Himalayan Snow Trout *Schizothorax plagiostomus* (Heckel) reared in laboratory as well as in natural site. It reveals a very significant information in respect to derivation, tissue differentiation, initial inflation as well as factors responsible for non-inflation of swim bladder especially among laboratory reared larvae. At hatching (108-110 and 124-130 hours after fertilization in laboratory and natural site respectively), larvae measured 9.00 mm and 10.5 mm in laboratory and natural site respectively, and their body cavity was mostly occupied with a huge amount of yolk. The mouth, pharynx and esophagus were impervious till 3rd day post hatching (dph). Primordial swim bladder appeared on 3rd-dph as a cluster of mesenchymal cells evaginating from the posterior dorsal surface of the differentiating esophagus. Later on it grows toward the caudal direction below the differentiating vertebral column and kidney. Initial swim bladder inflation occurred by 6-dph when larvae attained 12.50–13.50 mm length, with the onset of first exogenous feeding. During this phase a mucous like substance was also observed in the pneumatic duct as well as lumen of swim bladder of some laboratory reared larvae and later on they got mortality. By 8-dph well differentiated and inflated swim bladder becomes externally visible and pneumatic duct extended to join the dorsal wall of esophagus. A number of biotic and abiotic factors were found associated with preventing swim bladder inflation during Pre flexion to Post flexion stages.

Key Words: Swim Bladder inflation, Embryonic development, Ontogeny, *Schizothorax plagiostomus*, Flexion stage.

Introduction

Swim bladder is an internal hydrostatic organ which contribute to the ability of a fish larvae to regulate its buoyancy and thus to stay at the particular water depths, ascend and descend without wasting energy in swimming. It also plays a significant role in sound production, hearing by amplifying the sound registered by Weberian apparatus, detection of pressure changes. Proper swim bladder inflation is essential for further development of the fish. The incorrectly developed or non-inflated swim bladder disturbs arrangement of other internal organs and ultimately results in deformed notochord and vertebral malformations or skeletal deformities, decreased growth and survival rate etc. (Kitajima et al., 1994; Sarnowski, 2004, Grotmol, 2005). Non inflation is common in hatchery-reared species, but is also observed in wild populations.

Extensive variation exists in the morpho-funtional development of swim bladder among species, reflecting the phylogeny, reproductive strategy, and habitat or rearing conditions of the species (Govoni and Hoss, 2001). Detailed information of various aspects concerned with the morpho-functional development of swim bladder is essential for understanding why this critical stage of ontogeny often fails. In this respect the contribution of Kaji (2000), Govoni and Hoss (2001), Trotter et al. (2004), Sarnowski (2004), Rayal and Bahuguna (2006); Hirata et.al. (2009), Clayton et al., (2010), Honryo et al. (2018) etc. covers comprehensive account of various aspects of the early development of fresh and marine fishes.

Himalayan Snow trout *Schizothorax plagiostomus* inhabits coldwater 8-19^{0}C of the fast running snow fed streams and is considered to have great aquaculture potential. However, cultured fish often have high mortality due to the malformation and non-functioning of some organs. The natural stock of fish is decreasing day by day due to the over fishing, erosion and shrinkage of fish breeding grounds (Bahuguna and Rayal, 2006). Present study was aimed to describe the morpho-functional development of swim bladder in the snow trout *Schizothorax plagiostomus* (Heckel) larvae as well as to relate the findings to the culture technique. In this respect study will be very useful for the improvement of aquaculture practice for the socio-economic growth in Himalayan region.

Material and Methods

To conduct artificial breeding and fertilization, live brooders of *Schizothorax plagiostomus* (Heckel) were collected from snow fed river Alaknanda during the breeding season September-October by using Cast Net. After stripping the mature brooders, milt and eggs were obtained and mixed with the help of bird feather for 5-10 minutes. Fertilized eggs were place in hatching trays, glass jar hatchery and hatching tub in the laboratory with proper aeration, temperature (17-20^{0}C) and regular water supply. Some of the fertilized eggs were also placed in hatching trays on small stream of river to ensure the proper and natural development.

The behaviour and developmental changes among the larvae at both the laboratory and natural sites were monitored regularly. For subsequent morpho-histological study, from hatching to post flexion stages at 4-8 hrs intervals, 5-10 larvae were fixed in different fixatives viz. aqueous bouin's, alcoholic bouin's, 4% formalin, calcium formol, 70% alcohol etc. with some modifications according to local conditions. For light microscopy after completion of fixation (18-24 hrs) in Bouin's fluid, sample were washed and then dehydrated in an ascending series of ethanol for embedding in paraffin (E. Merck, 54-56^{0}C melting point paraffin wax). Following embedment in paraffin, transversal and longitudinal sections of 5-6µm were cut on an Erma rotary microtome. Serially arranged sections of paraffin embedded material were stained with haematoxylin (nuclear stain), Iron haematoxylin, Eosin (cytoplasmic stain) and Malory triple stain. The stained slides were mounted in DPX and then photomicrographs of prepared slides were taken with the help of Olympus- photo-microscope.

Result

Just after hatching mostly the body cavity was occupied with a huge amount of yolk and digestive tract as well as the other visceral organs were not visible at both the natural and laboratory sites larvae (Fig.1). On 2nd dph (day post hatching) the pharyngeal region was easily distinguishable from other parts by means of rudimentary gills structure on its epithelial wall. During this period larvae were unable to swim and rested at the bottom.

Primordial swim bladder appeared on 3-dph as a cluster of mesenchymal cells evaginating from the posterior dorsal surface of the differentiating esophagus. (Fig. 2). Later on it grows toward the caudal direction below the differentiating vertebral column and renal system. During this stage (3-4th dph) occasional swimming was also observed among the larvae, but usually they rested

on the bottom. By 5th dph cellular differentiation of swim bladder primordium proceeded further and becomes increasingly separate entity.

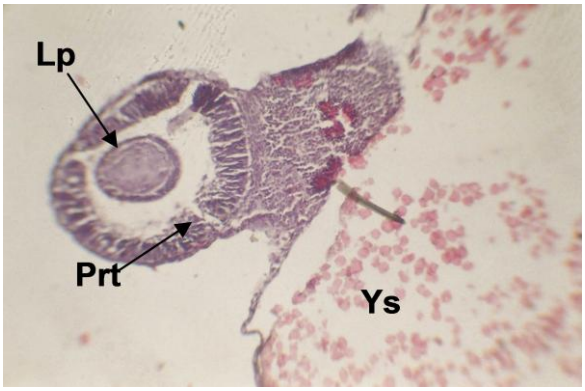

Figure 1: L.S. of 1st DPH larvae showing undifferentiated cells of eye, yolk sac, and rudimentary pharyngeal cavity etc. (H.E. 70X).

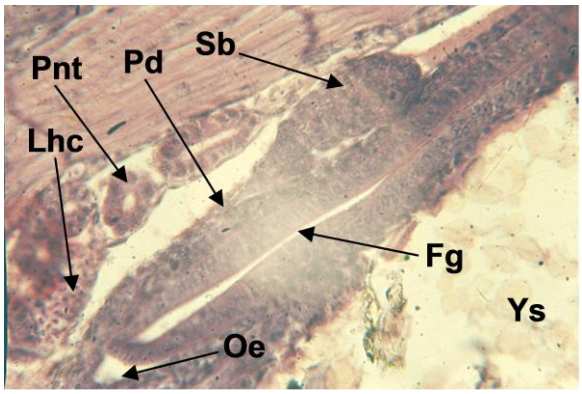

Figure 2: L.S. of 3rd DPH larvae showing differentiation of swim bladder anlage as a cluster of mesenchymal cell from the dorsal wall of esophagus, pronephric tubule with rudimentary hemopoietic and lymphatic tissues, yolk sac etc (H.E. 280X).

Morphologically the epithelial cells of the inner primordium were very similar to that of columnar cells of the incipient digestive tract.

Further differentiation of the mesenchymal cells into a more organized connected tissue sheath started by 6-7th dph (around the beginning of flexion stage). In comparison of the connective tissues of gut, the tunica externa surrounding the swim bladder appeared noticeably thick in transverse and longitudinal sections. At the same time initiation of swim bladder inflation occurs in both the laboratory and natural site larvae (Fig. 3-4).

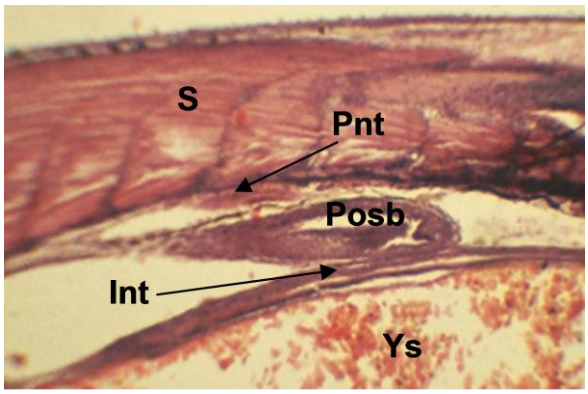

Figure 3: L.S. of 6th DPH larvae showing lumen formation or inflation in posterior swim bladder, yolk sac and differentiating cells of the gut etc (H.E. 100X).

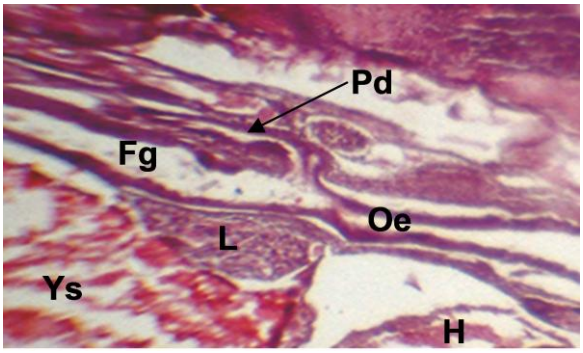

Figure 4: L.S. of 7th DPH larvae showing connection between pneumatic duct and for gut (ostium), liver and yolk sac etc (H.E. 100X).

During this period initially swim bladder appeared as a long tubular structure with a narrow lumen and was not differentiated into anterior and posterior chamber. Later on, at its cephalic end the wall becomes thick and elongated anteriorly to form second/anterior chamber (Fig. 5). In comparison to the posterior region of swim bladder its anterior wall was thicker and poorly differentiated into tunica externa, sub-mucosa, muscularis and inner epithelium. The internal epithelial layer had different height in different part of the bladder. In this duration larvae were able to swim actively as well as most of them began to feed on exogenous food. In case of some laboratory reared larvae a mucous like substance was also observed during this period.

By 8-dph well differentiated and inflated swim bladder becomes externally visible and pneumatic duct extended from anterior side of posterior chamber to join the dorsal wall of esophagus. The

lumen of anterior chamber (termed as first and second chamber respectively) was well differentiated from posterior one with a narrow inter connection but it was comparatively less inflated in case of laboratory reared larvae (Fig. 5-6).

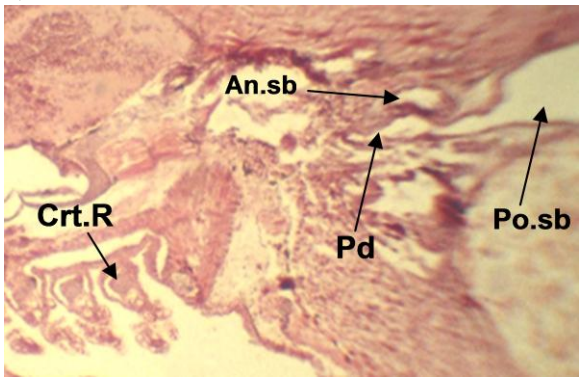

Figure 5: L.S. of 8th DPH larvae showing inflated posterior chamber of swim bladder and its connection with pneumatic duct, differentiation anterior chamber, and cartilaginous rods in gills arches, yolk sac, etc. (H.E. 100X).

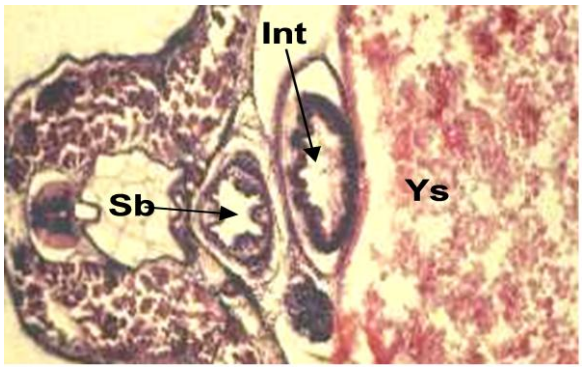

Figure 6: T.S. of 8th DPH larvae through mid-portion showing mucosal folding in posterior swim bladder and mid gut. (H.E. 70X).

At natural site the bladder turned to be oval shaped but still it was somewhat elongated in laboratory site. Histologically the lumen of inflated swim bladder was ellipsoidal and the epithelium was squamous (Fig. 5-6). On 9-11th dph the swim bladder was well differentiated with squamous epithelial cells and its lumen was more inflated in compare to 8th dph larvae. During this stage slight variation was also noticed in the size of swim bladder which was more in the laboratory site (Fig. 7, 8, 9, 10).

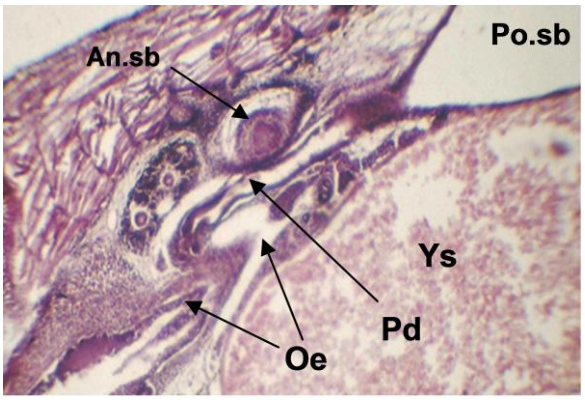

Figure 7: L.S. of 9th DPH laboratory larvae showing inter connection of swim bladder and gut by pneumatic duct, wide lumen of posterior chamber, slightly inflated anterior chamber, yolk sac etc. (H.E. 100X).

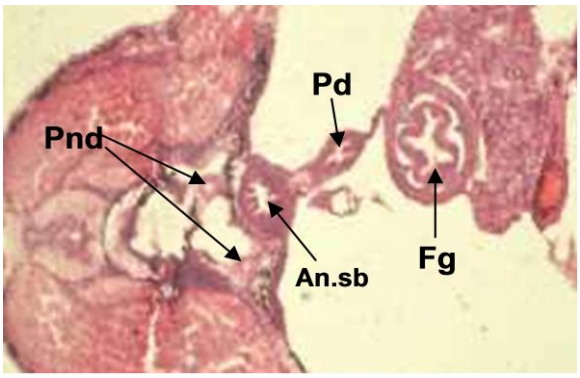

Figure 8: T.S. of 9th DPH larvae showing anterior chamber of swim bladder, pneumatic duct, fore gut surrounded by liver etc (H.E. 100X).

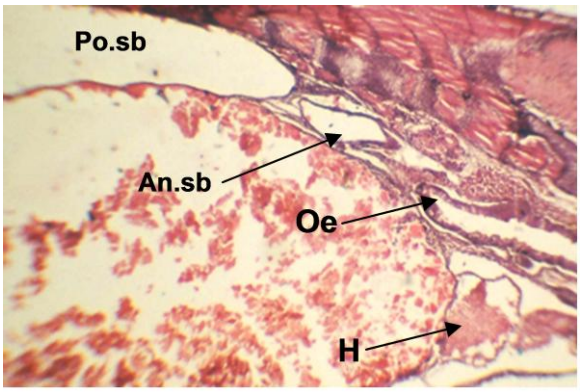

Figure 9: L.S. of 10th DPH natural site larvae showing both chamber of swim bladder, yolk sac, oeso-pharyngeal region of gut etc (H.E. 100X**).**

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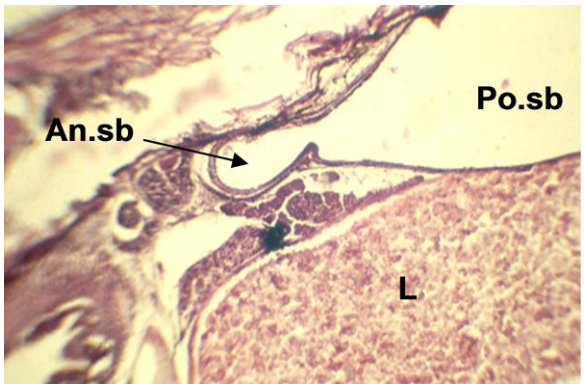

Figure 10: L.S. of 11th DPH laboratory larvae showing inter connection of the anterior and posterior chamber of swim bladder, yolk sac etc. (H.E. 100X).

Morpho-histological observations reveal that with the beginning of post flexion stage (12th dph) both the chambers of swim bladder were more elongated, demarcated and differentiated from each other (Fig. 11-15).

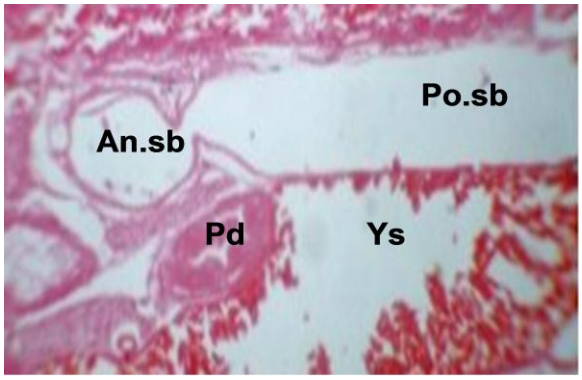

Figure 11: L.S. of 12th DPH larvae showing well differentiated and inter connected chamber of swim bladder, mucosal fold in pneumatic duct etc. (H.E. 100X).

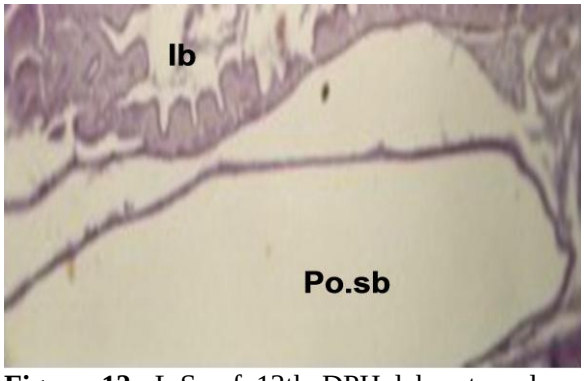

Figure 12: L.S. of 13th DPH laboratory larvae showing irregular and wide lumen of post. chamber. (M.T.S. 100X).

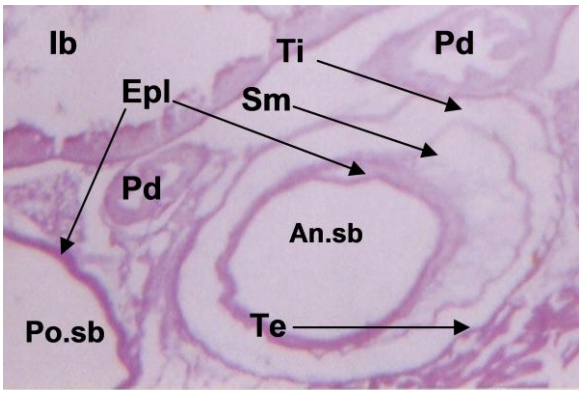

Figure 13: L.S. of 13th DPH laboratory larvae showing swim bladder with lathery outer tunica external and glassy thin inner tunica internal, sub-mucosa; consists of thin layer of smooth muscles fibers and inner most stratified epithelial layer. (M.T.S. 100X).

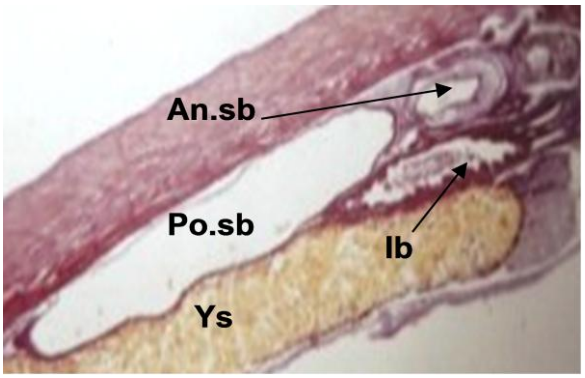

Figure 14: L.S. of 13th DPH natural larvae showing both chambers of swim bladder, yolk sac, liver, fore gut etc. (M.T.S. 70X).

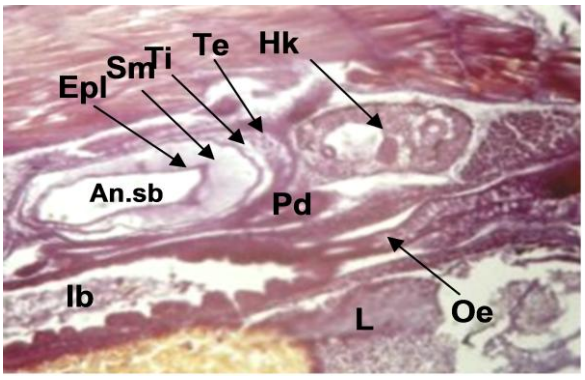

Figure 15: L.S. of 13th DPH natural larvae showing swim bladder with lathery outer tunica external and glassy thin inner tunica internal, sub-mucosa; consists of thin layer of smooth muscles fibres and inner most stratified epithelial layer, fore gut, esophagus.

Anterior chamber of SB was roughly oval and nearly one-fourth of posterior one. Its wall

comprises of a relatively thick and lathery outer tunica external and glassy thin inner tunica internal which is made up of dense collagenous fibrous material, sub-mucosa consists of thin layer of smooth muscles fibers and inner most was stratified epithelial layer (Fig.14-15). In laboratory reared larvae lumen of SB was comparatively more expanded and irregular in shape as well as in size. Its sub-mucosa was thicker at anterior side and decreasing toward posterior direction and finally shrieked at mid posterior region. Both the tunica external and internal also thick and irregular (Fig. 12-13). Pneumatic duct enlarged markedly and its lumen was occupied with the numerous folds composed of squamous epithelial cells (Fig. 11, 13). Dilation of pneumatic duct was greatest toward the gut and it gradually decreased toward swim bladder.

During 12-15th dph most of the surviving larvae were actively feeding and swimming in water column or near bottom but in laboratory site still so many larvae often rested on the bottom and swim occasionally near bottom. Later on such types of larvae also got mortality. At natural site some larvae were found chasing the air/gas bubbles releasing from the shallow bottom through the process of photosynthetic activities or by the increase in temperature. Histological observations reveal that during this period there was two conditions of swim bladder (especially in laboratory reared larvae); one with well inflated and others having narrow or non-inflated lumen. Congestion of pneumatic duct was also common among such type of larvae in which swim bladder was partially inflated or non-inflated (Fig. 16).

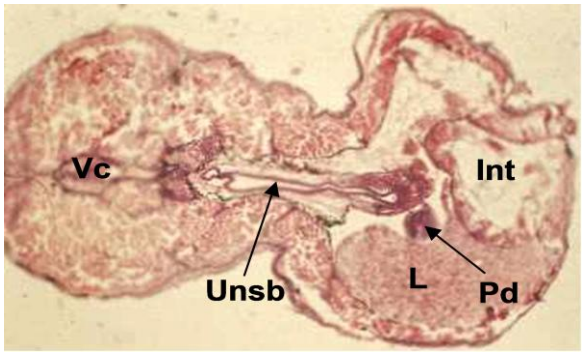

Figure 16: T.S. of 13th DPH laboratory site larvae showing uninflated swim bladder and intestine etc. (H.E. 70X).

Abbreviations of figures

An.sb	anterior chamber of swim bladder
Crt.R	cartilaginous rod
Epl	epithelial layer
Fg	fore gut
Fg	fore gut
H	heart
Hk	head kidney
Ib	intestinal bulb
Int	intestine
L	eye lens
Lhc	lymphatic and hemopoietic cells
Lp	lens placode or rudimentary or primordial lens
Oe	oesophagus
Pd	pneumatic duct
Pd	pneumatic duct
Pnd	pronephric duct
Pnt	pronephric tubules
Pnt	pronephric tubules
Po.sb	posterior chamber of swim bladder
Prt	presumptive retina
S	somites
Sb	swim bladder
Sb	swim bladder
Sm	sub-mucosa
Te	tunica external
Ti	tunica internal
Unsb	uninflated swim bladder
Vc	vertebral column
Ys	yolk sac
Ys	yolk sac

Discussion

During the larval development of *Schizothorax plagiostomu*, (Heckel), the change from pre-flexion to flexion and flexion to post-flexion stage began by 7th and 12th day post hatching (dph) respectively. Duration this period various histological and morpho-functional developmental changes takes place in the larval swim bladder and other visceral organs, which ultimately determine its survival. These changes varied as per their environmental and rearing conditions, nutritional status or qualities, genetic potential etc.

The present study reveals that the primordial swim bladder differentiates during the yolk sac period; by third day post hatching as a cluster of mesenchymal cells evaginating from the posterior

dorsal surface of the differentiating esophagus and later on it grows toward the caudal direction below the differentiating vertebral column and kidney. In case of some other fishes its evagination and differentiation takes place from the posterior wall of the rudimentary stomach Makino et al. (1995), Hamlin et al. (2000), Trotter et al. (2004), Sarnowski (2004) etc. The basic pattern of cellular differentiation of swim bladder tissues in *S. plagiostomus* larvae from evagination (3rd dph) until its inflation (6-7th dph) was found similar to those of reported in *L. japonicus* by Makino et al. (1995), gilthead sea bream, *Sparus aurata* (Sarasquete et al. by (1995) etc. but it differs in timing or onset as well completion of the process.

By the early flexion stage (6-7th dph), due inflation of swim bladder larvae was able to regulate its buoyancy and thus to stay as well as capture a prey/food item at the particular water depths, ascend and descend without wasting energy in swimming; that's why there was a good growth and survival rate especially among the larvae reared at natural river site. On other hand, in laboratory rearing condition during the same period due to the presence of a mucous like substance in the lumen of swim bladder as well as in pneumatic duct ultimately larvae were unable to regulate the buoyancy. Such types of larvae weren't able to stay or capture a prey/food item at the particular water depths, ascend and descend easily without wasting energy in swimming. Consequently, reduced growth and survival rate was noticed. Similar findings were observed and suggested by Trotter et al. (2001) and Morrison et.al. (2001) in other teleost fish. Linkage between esophagus and swim bladder by means of the establishment of pneumatic duct and subsequent initial air filling or inflation was the most important events in functioning of the swim bladder. Initial inflation of larval swim bladder was accomplished by ascending the surface or the larvae penetrate the air-water interface to engulf and force the air through a persistent pneumatic duct into the lumen of swim bladder. As noticed during the present study, the air/gas bubble released from the shallow bottom through the process of photosynthetic activities or by the increase in temperature of shallow bottom may be the of air/gas source for initial swim bladder inflation. The deflation is done by releasing the gas through the pneumatic duct along with expulsion through the anus or mouth (John et.al., 1978; Rieger and Summerfelt, 1998; Govoni et.al. 2001; Sarnowski, 2004). In *S. plagiostomus* larvae rapid differentiation of pneumatic duct with the appearance of swim bladder anlage was associated with the preparation for initial inflation by 6-7th dph; almost with the onset of exogenous feeding. Posterior chamber of swim bladder differentiates and inflate first (6-7th dph) so that termed as first chamber; anterior chamber differentiates and inflate later on (7-8th dph) by receiving the air from first chamber, so that termed as second chamber in present study. In this respect the observation and opinion of Morrison et al. (2001) in *Oreochromis niloticus* also supports our findings and views.

During the present study it was observed that, malformation and non-inflation of swim bladder was very common in case of laboratory reared larvae while it was rare in natural site. A number of biotic and abiotic factors have been associated with preventing swim bladder inflation; lack of access to the water air interface due to turbulence, surface film of food and oil preventing the larvae from gulping air (Hoss and Phontor, 1984), water temperature (Bailey and Doroshove, 1995), some heavy metal and pH (Sarnowski, 2004), lower light intensity (Martin and Peterson, 1998; Uotani et al. 2000), pesticides (Hamm, 2000).

The current findings and facts indicates that the most probable causes behind the malformation and failure of swim bladder inflation in the larvae of *S. Plagiostomus* and consequently reduced growth and survival rate especially around the transition from pre flexion to flexion (6-7th dph with the onset of exogenous feeding) and flexion to post flexion stage (12-13th dph with the embellishment of exclusive exogenous feeding) may include;

(i). Various factors directly and indirectly inhibit or retards the inflation of swim bladder, due to which larvae were unable to detect and capture the

prey /food items efficiently or they have to spend a lot of energy to do this. They mostly rest on bottom, or crawl occasionally, during which due to lack of natural softness/ sponginess on the bottom surface their yolk sac also gets punctured. (ii). Malformation and failure of inflation of swim bladder may affect or disturb the embryonic development as well as the arrangement of other visceral organs.

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