

Morphological Study of the Digestive Organs (Oesophagus, Intestinal Bulb, Intestine, Rectum) in Post Flexion to Finger Ling Stages of the Himalayan Snowtrout *Schizothorax Plagiostomus* (Heckel)

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The morphology and relative length of gut (RLG) of the digestive tube among the larvae of *S. plagiostomus* is directly related to the age and size of the larvae. The digestive tube is almost straight when larvae are less than 1.5 cm in length, but as the size of larvae increases the gut increases in length and The RLG value also increase as 1.5 cm long larvae are having RLG value 1.0. While in 6.7 cm larvae RLG value was 3.34. When the larvae of *S. plagiostomus* become more than 6.0 cm long, they move towards the mainstream. Again, if the size of the fish is about 7.0 - 8.0 cm the relative length of gut (RLG) decreases (3.16). This is the size when most of the larvae move towards the mainstream (deep water) where the quality of food is different from the shallow water. Also the temperature is less than that is shallow water. The 1.0-8.0 cm *S. plagiostomus* larvae are having short oesophagus, which shows four walls viz. mucosa, submucosa, muscularis, and serosa layers. In 1.0 cm larvae the oesophagus lumen is very narrow and short and has a single layer of cuboidal epithelial cells. The lumen is also lined with a stratified squamous epithelium. The mucous cells are continuously arranged at the corners of the mucosal layer, helping in the food movement. When the size of fingerling stage larvae of *S. plagiostomus* is about 20 6.0 cm the submucosa reduces due to invasion of striated muscle fibres and projects into the folds forming the lamina propria. Also the same has been seen in the 7.0 cm larvae. Here the T.S. and L.S. are showing muscularis, which is having an inner longitudinal and outer circular muscle fibers. In quite healthy fingerling larvae which are having size about 8.0 cm, the fully developed oesophagus with all four layers and well organized mucosal folds, mucous cells and epithelial cells are present. In *S. plagiostomus* larvae, the walls of intestine consist of mucosa, submucosa, muscularis, and serosa in all the stages of the development viz. post-flexion to fingerling stage larvae. Only the difference was recorded in their thickness. This is related to the feeding quality (micro-phytoplankton to macro-phytoplankton). The intestine started to form coils as the age and size increases and it's divided into three regions (1st, 2nd, & 3rd) based on coiling formation. The mucosal folds become shorter as the intestine diameter becomes 30 narrow towards the rectum. They were uniform in shape in the 2nd region, while in the 3rd region they become shorter in post-flexion and fingerling stage. The number of goblet cells is more in the 2nd region, but it decreases towards the 3rd region, which is showing more absorption of food in the 2nd region than the 3rd region. The last region of the intestine through which the gut opens to the outside is the rectum which is comparatively narrower than the intestine.

35 Introduction

In many fishes there are "critical periods" in development whereby they must reach certain functional threshold in order to procure and digest food [1-3]. The onset of exogenous feeding represents a period of high mortality for fish larvae, particularly 40 those in tropical and sub-tropical waters, where the transitional period between yolk absorption and first feeding is short (1,4-5). The same has also been reported in the larvae of snow trout *Schizothorax species* by Bahuguna (6-7). Fish larvae must be competent at procuring and assimilating food before the yolk sac and oil globules are depleted, or risk starvation (8-9). A second 45 'Critical period', at the end of the larval period during the transition to a benthic juvenile form, has been proposed for demersal fish species (2). For many species a complex

metamorphosis occurs at this time involving rapid changes in 50 morphology (10), Sensory systems (11-13), locomotory ability (9), respiration and digestive and hormonal physiology (14). Studies on the ontogeny of fish larvae have been often focused on only one area of development, either osteological (15-16) or changes in the digestive system (17). However, an integrated 55 consideration of different structures during ontogeny is important to understand how structural changes may influence functional capability as the fish change its environments. The intestinal tract must grow and differentiate with changes in the feeding and metamorphosis. The transition from endogenous to exogenous 60 nutrition is a major change requiring the development of organs that can digest food. During embryonic or larval development the swim bladder also develops from the anterior portion of the alimentary tract (18-19). Histology of the digestive system during

post-hatching development of the *carp*, *Cyprinus carpio*, has been described (20-21). The morphology of the digestive system and the food habits of the *carp* are quite different from those of the many other fishes as described by Turner (22) & Thomas (23).

Knowledge of gastrointestinal tract (GIT) developmental changes associated with the process of food assimilation is essential for understanding the nutritional physiology in larval stages (6-7). In some fishes when larvae initiate feeding, they possess an anatomically complete digestive tract (24-25) and an enzyme complement which is similar to that of juveniles and adults (26). As a consequence, artificial larval diets have been used for the intensive commercial culture of several acipenserid species from the onset of exogenous feeding (27-30). However, the end of the lecithotrophic stage and transition to exogenous feeding still represents an important source of larval mortality (28,30-32) suggesting some nutritional problems associated with the digestion and assimilation of artificial diets, which are normally formulated for salmonids or marine fish species (29-30).

Detailed anatomical, histological, and physiological descriptions of the digestive tract of the coast rainbow trout have been performed (33-34). The ontogeny of the digestive system and the effect of growth conditions on this process have also been studied (35-36).

Phylogenetic predeterminations of the structure of the oesophageal epithelium and the characteristic features of the intestinal morphology have been suggested within the salmonidae (37). The oesophagus wall in fish is very muscular with interweaving skeletal muscle fibers, which may extend as far as the stomach (38). Therefore, a histological comparison of the tunica muscularis externa in the oesophagi of fish that consume different food was made (39). Considerable work has been done on the food and feeding habits of fresh water teleost fishes by some authors (7,40-45). However, very little information is available on the food and feeding habits of hillstream fishes (46-49). But there is no any information related to the early development of digestive organ among the Indian fresh water fishes including snowtrout *Schizothorax Species*.

Hence, an effort has been made to know how the development of digestive organs takes place in the larval stages of a Hill stream snow trout fish *Schizothorax plagiostomus*. This is an indigenous commercially as well as economically important food fish in the snow fed aquatic environment.

Methodology

Live brooders of *Schizothorax plagiostomus* (Heckel) were collected from the Glacierfed River Alaknanda located at an altitude of 540 m, longitude 78° 47' 26" and latitude of 30° 13' 16", 38.8 Km near the Chauras-Jhulapul, near Shrikot, Srinagar (Garhwal) during September-October 2006 by using "cast gill net". The mature brooders were stripped for taking out the egg and milt by applying slightest pressure on the abdomen. The eggs and milt were mixed with the help of birds feathers in a cleaned enameled tray for 5-10 minutes (6-7). After fertilization, the eggs were placed in the hatching trays and kept in the laboratory with proper aeration and controlled aeration. The post flexion stage is the formation of the caudal fin (hypural elements vertical) to attainment of full external meristic complement (fin rays and

scales) and loss of temporary specializations for pelagic life. In *Schizothorax plagiostomus* this stage begins after 12-15th days of hatching. At 12-15th days of hatching when caudal fin was fully flexed and forked most of the internal organs were developed morphological as well as functionally. At the end of this stage larva swim actively and move towards the main streams from the shallow water (this is seen among the natural site larvae). The fingerling stage is an active feeding stage when a quick growth in the body takes place. In this stage larvae were seen very active in their movement and later they become adult, means the development of gonads was seen. For the Morphological study of various organs, the different developmental stages have been fixed in Alcoholic Bouin's, Aqueous Bouin's, as well as in the 4% Formalin solution from post flexion to fingerling stages with 4-8 hrs intervals 5-10 as given by the authors (50-53) with some modifications were applied according to the local condition. After completion of fixation (18-24 hrs), the larvae were washed 2-3 times in distilled water than alcohol, till the excess of fixative comes out from the tissue. The photomicrographs were taken with the help of PM-6 and PM -10 Olympus- photomicroscope systems for detailed study.

Observation

Intestinal tract is the most important organ of the coelomic cavity of the larvae. It consists of four important parts viz. Oesophagus, Intestinal bulb, Intestine and Rectum.

The alimentary canal of an adult *S. plagiostomus* fish is longer than the total body length, while among the hatched larvae gut length increases as the age and size of the larvae increases. In larvae the pharyngeal bone and teeth are absent while the pharynx is a narrow passage. Overall the gut in hatched larvae is a short straight tubular structure. The lumen wall of the oesophagus is lined with mucous folds which are thrown into many thick longitudinal folds. These folds increase in number as the size of the larvae increases i.e. from 1.0-8.0 cm. The number of mucosal folds also increases from 2.0-4.0 cm larvae reaching up to the fingerling stage. True stomach is absent in these fish larvae which were having length 2.0-8.0 cm. The oesophagus leads directly into straight and short intestine. The anterior part of the intestine is slightly swollen in the later stages of the larvae (6.0-8.0 cm larvae) which may be called intestinal bulb. The mucosal folding has also been noticed inside the lumen of intestinal bulb/ swollen intestine in the form of zig zag linings. The rest of the intestinal tube increases in length as fish larvae increases in size. The coiling or the folds in the intestinal tube also increase from 2.0-8.0 cm fish larvae from 2-4 folds respectively. The thin zig-zag mucosal folds are in the lumen of intestine in the form of irregular lining. Rectum is comparatively very short and with a tubular structure. The number of mucosal folds in the lumen is shorter and less in comparison to the other parts of the gut. Overall, the morphology and relative length of gut (RLG) of the digestive tube among the larvae of *S. plagiostomus* is directly related to the age and size of the larvae. Table 1 showing the different parameters of the digestive tube and their size, etc. The morphological changes during the development of the gut is directly related to the age of the larvae. The digestive tube is almost straight when the larvae are less than 1.5 cm in length, but as the size of larvae increases the gut increases in length. The

RLG value also increases as shown in Table 1 as 1.5 cm long larvae are having RLG value 1.0, while in 6.7 cm larvae the RLG value was 3.34 when the larvae of *S. plagiostomus* becomes more than 6.0 cm long, they move towards the mainstream. Again, if

(RLG) decreases (3.16). This is the size when most of the larvae move towards the mainstream (deep water) where the quality of food is different from the shallow water. Also the temperature is less than that in shallow water.

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Table 1 Showing the length of the different parts of the digestive tract in postflexion to fingerling stage of *S. plagiostomus* larvae.

S. No.	No. of Observed Larvae	Age of the larvae in days	Larvae length (Mean value) (cm.)	Mean length of the digestive tract (cm.)				RLG
				Oesophagus (cm.)	Intestinal bulb (cm.)	Intestine (cm.)	Rectum (cm.)	
1	25	15	1.5	0.1	0.2	1.0	0.2	1.0
2	20	20-22	2.7	0.2	0.5	2.5	0.2	1.25
3	15	35-37	3.0	0.2	1.0	7.0	0.2	2.8
4	12	45-47	3.8	0.2	1.4	10.2	0.3	3.18
5	10	60-62	4.7	0.3	2.0	12.7	0.3	3.25
6	10	75-77	5.5	0.3	3.1	14.5	0.3	3.30
7	10	76-87	6.7	0.3	4.0	17.8	0.3	3.34
8	8	95-98	7.8	0.3	4.2	19.8	0.4	3.16

Discussion

The digestive tract can be divided into three periods the embryonic, larval and juvenile period (Balon, 1975; Loewe and Eckmam, 1998; Unal, 2001). The digestive tract of snow trout just after hatching was poorly developed (19,49). Gradually, when the fish grow in size and weight, the digestive system and digestive organs are developed systematically. The same gradual development or organogenesis in the digestive tract has been reported in other fishes (54-55). In the *Schizothorax plagiostomus* larvae, three developmental phases have been reported for the development of the digestive system (7,19). The first stage occurs when the first feeding started i.e. at the age of 4-6 days after hatching (preflexion stage). This was the time when the digestive tract enables to ingest as well as digest the exogenous nutrients in the hatching, nutrition is provided only by yolk sac (7). The second stage (flexion stage) was around the age 7-8th days after hatching when the functional intestinal bulb, mid gut and rectum were distinguished but functionally these organs were not active (19). The 3rd stage was post flexion stage when the fish larvae were 9-12th days old. In this stage, the gastric gland was developed and in the long intestine coiling started. Therefore, the relative length of the gut (RLG) increases (19,56). This morphological change in the gut with its RLG depends on different fish species and also with their body growth in respect to their different developmental stages, as well as quality of food and their availability in nature (57). Same finding is supported in the snow trout fish, *Schizothorax plagiostomus*, which is having different development stages and a variety of food in the different stages of development. After post flexion to fingerling stage, the digestive tract was having different morphological changes. This was the stage when larvae were having 1.0 cm long or 12 days old after hatching. In this stage, the exogenous feeding was

started. Tanaka, Kaji, (54-55,58) etc. reported the presence of mucosal goblet type cells at postflexion stage when initial feeding was reported in some freshwater fish species where pharyngeal teeth also started to develop. In snow trout, *S. plagiostomus* mucosal goblet type cells were also found in the intestinal bulb just after exogenous feeding, supporting the finding in other fresh water fishes. Dabrowski (59) also reported the same finding in other teleost fishes as were seen in gut histology of snowtrout larvae just after exogenous feeding. In *Clarias lazera* larvae the third segment of gut (rectum) does not seem to play an important role in the absorption of nutrients. In the postflexion larval stage of *S. plagiostomus* small numbers of microvilli with low height are present and support the observation as proposed by Stroband and Karoon (60) in some other fishes. In Snow-trout larvae which was having 1.0-8.0 cm length the rectum was also having a small number of microvilli with low height with usual other region viz. mucosa, submucosa, muscularis and serosa layers, etc with cellular arrangement. Only the difference is in the number of mucosal cells, which were less compared to intestinal region. A long and looped intestine in 5.0-8.0 cm larvae of *S. plagiostomus* has been also described in a number of fishes studied by several authors (61-67). Histologically, it is well established fact that the teleostean intestine is provided with simple columnar absorbing cells covered by thin brush borders, although other constituents such as goblet cells, lymphocytes and various granulocytes do occur in between them (68). The histology of the larval intestine of *S. plagiostomus* having 1.0-8.0 cm length consists of the layers of serosa, muscularis, submucosa and mucosa with a large number of mucous cells, columnar cells and mucous secreting cells as were reported in other freshwater fishes by several authors (69,63,40,70-73). The last region of the intestine through which the gut open to the outside is the rectum which is comparatively narrower than the intestine. The same was observed in *S. plagiostomus* larvae from postflexion to fingerling stages. There is no distinct demarcation between the intestine and

the rectum, which is a simple developmental or organogenesis process as in other fresh water teleost fishes as reported by Tandon and Goswami (74). In the *S. plagiostomus* 5.0-8.0 cm long larvae the rectal folds are shorter and broader than the intestinal folds. The mucous secreting cells are in abundance and are distributed along the entire epithelium. In 5.0-8.0 cm larval rectum the muscularis is thicker than the intestine and is traversed with numerous blood vessels as also reported by Al-Hussaini (62); Islam (69); Chaudhary and Khandelwal (71); Dixit and Bisht (65). According to Bullock (75) the anterior rectum is the principle site for the digestion in stomachless teleosts, *Gambusia affinis*, while Western (76) states that the function of the rectum is concerned with the final explosion of the faeces. The same has been seen in *S. plagiostomus*. In this finding, it retains the food for final absorption in the long intestine with their thick muscular structure as well as low height of lamina propria.

Notes and References

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