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Ontogeny of feeding and digestive system in cobitidian fish *Noemacheilus montanus* (McClelland)

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Abstract

Noemacheilus montanus **is a bottom feeder water tracer fish of Himalayan region. The fish moves within the minute water capillaries in the mountain region and inhabited in small tributaries of hill stream. The incubation period spanned over 40-45 hour. After 1st day post-hatching the mouth was opened and upper and lower lips were distinguished. By second day post hatching taste buds were developed on both the lips. The larvae show rudiments of barbels on second day and which continue to grow and by 5th day post hatching these acquire slender and long shapes with many taste buds scattered all over the surface. The large numbers of taste buds secrete huge amount of mucous which is morpho-ecological adaptation in these larvae for movement as well as this protect the larvae in their habited zone. Various taste buds are also located on the upper and lower lips which are heterogeneous in shape. These taste buds greatly help this loach to locate the food and render this fish with carni-omnivorous habit enabling them to be the scavengers of the water body where they live. The pharyngeal region was distinguished from the buccal cavity by the development of the gill structure evident by 1st days post-hatch which are also having a large number of taste buds and mucous cells. These are helpful to take movement as well as respiration in the environment having less amount of water.**

Keywords*: Ontogeny, taste buds, mucous cells, buccopharynx, hatching*

Introduction

Fishes are the dominating vertebrate group as far as number of species is concerned and in their immense variety have adopted many nutritional habits. Some species are extremely specialized in their feeding habits while others are omnivorous. The alimentary tract of teleostean fish has been studied widely and described morphologically, to determine the function of many specialized anatomical structures in relation to the different feeding adaptations (Hirji, 1983; Rombout et al., 1983; Loewe and Eckmann, 1988). The alimentary canal in a teleost is composed of "Kopfdarm" (mouth, buccal cavity and pharynx) and "Rumpfdarm" (remainder of the alimentary canal), the latter is efficiently equipped with sphincters and valves at various regional junctions. The mouth, buccal cavity and the pharynx are associated with the selection, seizure, orientation and predigestive preparation of the food. The form and position of the mouth, dentition on the jaws and in the buccopharynx and the gill rakers show a close relation with the mode of feeding and the kind of food (Al-Hussaini, 1947b; and Kapoor et al., 1975b).The buccal cavity and pharynx in fishes form single unit of structure and function, therefore, it is termed as "buccopharynx". The other organs like lips and barbels are the associated parts of the buccopharyngeal region. The barbels which are the house of taste buds act as taster to locate the food material while the lips help to scrap the food items from the bottom or substratum of the environment (Singh *et al*., 1993; Bahuguna and Maithani, 2005). Taste buds are the peripheral sensory organs of the gustatory system. Although not directly involved with the digestion and absorption of food, buccopharynx affect the processing and transport of food. At yolk absorption, the buccopharynx is lined with squamus epithelium along with scattered mucus cells and taste buds (Govoni, 1980). In relation to changes in the diets of fish larvae, taste

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buds become more numerous and functional as larvae grow (Twongo and MacCrimmon 1977,). Teeth develop in the areolar connective tissue underlying the buccopharyngeal epithelium, subsequently erupting during the larva period (Twongo and MacCrimmon 1977, Govoni 1980).

Many workers have done enormous work on the ontogenetic development of the digestive tract including the buccopharynx in many fishes viz., Boulhic and Gabaudan (1992), Baglole *et al*. (1997), Green and McCormick (2001), Unal *et al.* (1999), Ostaszewska and Wegiel (2002), Gisbert *et al.* (1999, 2004), Pena *et al*. (2003), Makrakis *et al.* (2005), Abol Munafi *et al*.(2006).

N. montanus is a vermiform cobitid usually worm-shaped, long and thin having a ventrally placed, bottom facing mouth encircled with barbels. The mouth is well suited for its scavenging benthic lifestyle. *N. montanus* is carni- omnivorous in its feeding habitat (Singh and Bahuguna, 1983) and hence acts as a scavenger in the aquatic environment. It is not very picky about its food. In the present study the development of the anterior portion of the digestive tract in *N. montanus* larvae i.e the buccopharynx and the associated organs like lips, barbels, teeth and of course a brief account of respiratory organ, the gills have been studied.

Material and Methods

Noemacheilus montanus breeds naturally once in a year during the months of August and September. The brooders of *N. montanus* were collected from River Alaknanda and its tributaries. They were released in three different tanks in the hatchery where they spawn naturally by providing sandy bottom. A constant flow of water current was also maintained in these tanks. The brooders spawn within 2-3 days. After spawning the brooders were carefully removed and shifted to another tank. The fertilized eggs were transferred to the flour sieves which were made to float in the glass aquariums properly aerated and kept in air- conditioned laboratory. The fertilized eggs and the subsequent developing embryos were photographed in live condition with the help of photo micro system (Olympus CX41) in different magnifications to study the morphological development of the fish. The larvae hatching were started after 40 hrs of fertilization. After hatching, the observations were made, daily, until the larvae reached post flexion stage. The newly hatched larvae were fixed in different fixatives viz. Bouin's fluid (aqueous and alcoholic), calcium formol, 70% alcohol and 4% formalin taking a time interval of 4-8 hours till post flexion stage for carrying out histological evaluations. The samples are kept in fixative for about 18-24 hours and then washed in 70% alcohol until no more colour comes away. The samples were then preserved in 70% alcohol for further processes. After removal of fixative samples were dehydrated by using alcoholic series. After dehydrating, clearing was done in order to remove the dehydrating agent from the larvae. This was accomplished by use of xylene. The cleared samples were soaked in molten wax (E Merck Histo Paraffin Wax, 54-56 ^{0}C melting point) long enough to ensure that they were completely impregnated. Now the wax impregnated samples were embedded in wax blocks made with the help of thick L-shaped metal pieces. The tissue blocks were trimmed to the correct shape and attached to the object holder of the microtome. Now the attached blocks were cut into ribbons of sections of 5-6 µ thickness (both transverse and longitudinal sections) with the help of an Erma rotary microtome (Japan). A thin smear of Mayer's albumen was applied on clean slide for adhering the sections of the slide and then the ribbon of sections were put on these slides. Water was applied over these and then the slides were warmed on a hot plate (35-40 ^{0}C) in order to flatten the sections.The flattened and dried sections were first freed from wax by immersing them in two successive jars of xylene for 5-10 minutes each. Now staining was done and the methods given by Gray (1964), Taylor (1967), Pearse (1975) and Kaji *et al.* (1996) was followed. The slides were stained in Ehrlich's acid alum haematoxylin and Eosin stain (Double staining), Mallory's triple staining, Heidenhain's iron haematoxylin, Mercuric bromophenol blue, PAS reagent. The stained slides were again dehydrated in graded alcohol series, cleared in xylene and drained. Now DPX was used as a mountant and dropped liberally over the sections which were then covered with the help of suitable sized cover slips. The prepared slides were then

examined under the Olympus PM-6, PM-10 and CX 41 microscopes and photographed at varying magnifications for histological study of the desired organs.

Results and Discussion

In the present study the development of the buccopharynx and also the associated parts like lips, barbels and teeth in the larvae of *N. montanus* from pre to post flexion stage has been studied and the results are stated as follows.

Lips: In newly hatched larvae the digestive system was still not differentiated. After 1st day post hatching the mouth was opened and upper and lower lips were distinguished. By second day post hatching taste buds were developed on both the lips (Fig 4 and 5). The transverse section showed that the lips had an epidermis and a dermis layer.

Barbels: Barbels showed their appearance on the 2nd day post hatching in the form of rudimentary outgrowths (Fig 2 and 3). Thereafter as the fish grew the barbels increase in length and also the number of taste buds increased on their surface. Three pairs of barbels were present in the *N. montanus* fish. Histology of the barbels showed that they were composed of epidermis and dermis. The dermis was composed of polygonal oval cells which were compactly arranged (Fig 6 and 7). The barbel's epithelium was containing many taste buds exhibiting their gustatory function.

Buccal Cavity: A small buccopharyngeal cavity appeared on the first day of hatching (Fig 1). The mouth showed opening by day 1st after hatching. The buccal cavity was composed of simple squamus epithelium. By day 2 post hatching the fish larvae developed taste buds in the buccal cavity (Fig 3 and 5). A few goblet cells were also seen interdispersed within the epithelium (Fig 5). Oral valves were present on the day 3 post hatching and were defined by dorsal and ventral epithelial folds that were evaginations of connective tissue. They were also composed of simple squamus epithelium .Teeth started to develop on upper and the lower jaw by day three. The shape of the teeth in this fish larva was broad with a sharp cutting edge. On day 5 post hatching, three pairs of teeth were seen on the lower jaw and five pairs were present on the upper jaw (Fig 8). The number increased as the larvae grew further. The numerosity of taste buds and mucus cells on the buccal epithelium increased progressively with the development of the fish larvae. No stratification of the oral mucosa was seen till the post flexion stage.

Pharynx: The pharyngeal region was distinguished from the buccal cavity by the development of the gill structure evident by 1st days post-hatch (Fig 1). By day 2 post hatch the taste buds and mucus cells were seen in the pharyngeal epithelium on the roof of pharynx as well as the gill arch. Taste bud cellular components included marginal cells, light receptor cells, dark receptor cells, and basal cells. These were identical in all taste buds. On the day 5 post hatching pharyngeal teeth appeared in the post gill region in the larvae of *N. montanus* (Fig 8). The teeth were of same structure and type as seen in the buccal cavity. The gill rakers showed development from pre to post flexion stage. They were unadorned and stubby at this stage.

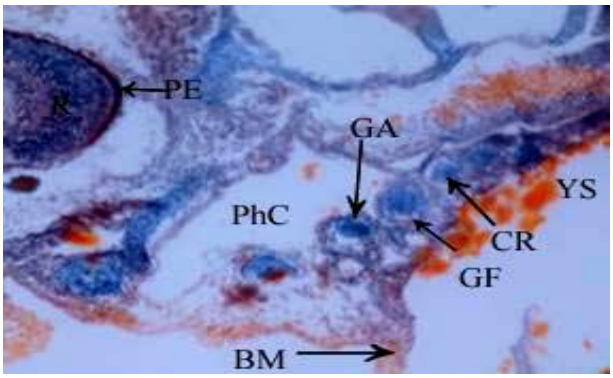

Fig 1: L.S. of 1 dph larva through the head region showing pharyngeal cavity, rudimentary gills, basal membrane and the pigmented retina (400X).

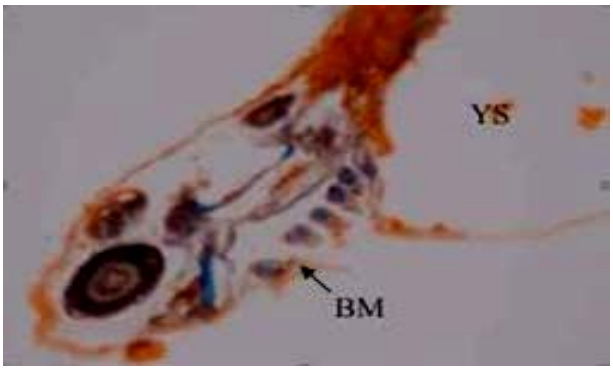

Fig 2: L.S. of 2 dph larva through the anterior half of the body (100X).

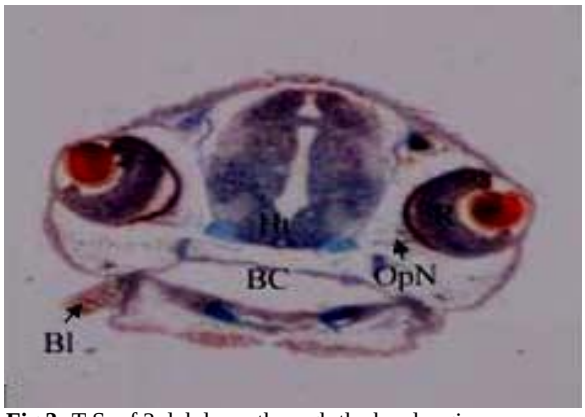

Fig 3: T.S. of 2 dph larva through the head region showing the barbel, buccal cavity, eyes and optic nerve (40X).

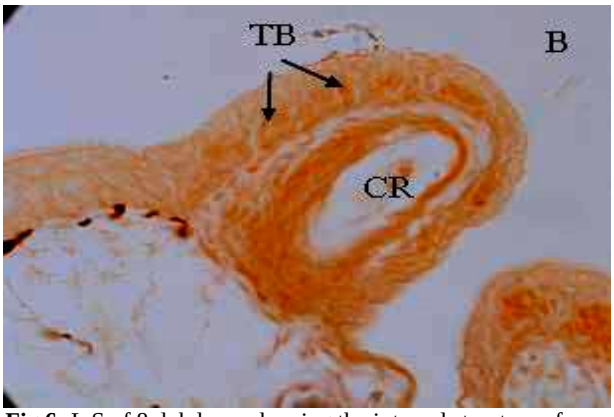

Fig 6: L.S of 8 dph larva showing the internal structure of barbel which possess numerous taste buds (1000X)

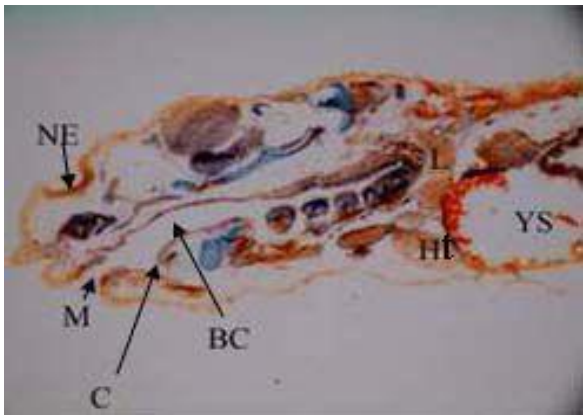

Fig 4: L.S. of 2 dph larva through the anterior half of the body showing the nasal epithelium,buccopharyngeal cavity, heart and brain (100X)

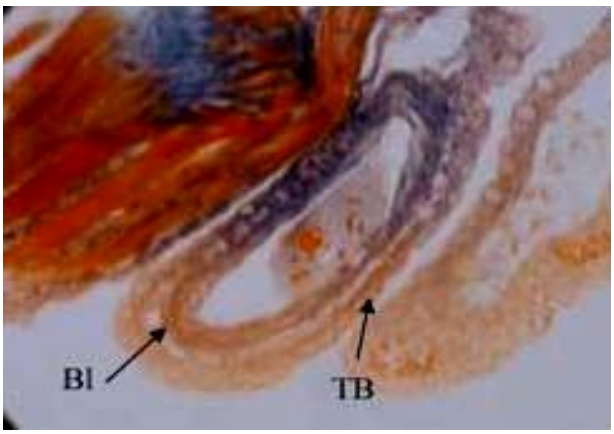

Fig 7: L.S of 9 dph larva showing taste buds on barbel and internal cartilage rod (1000X)

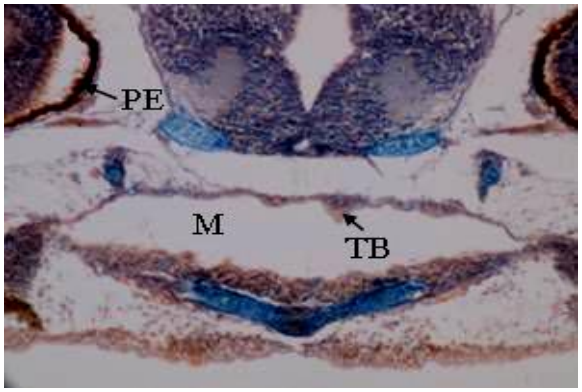

Fig 5: T.S. of 2 dph larva showing the taste buds in buccal Region (400X)

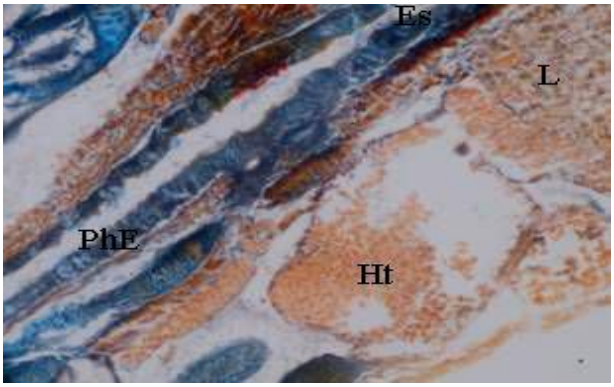

Fig 8: L.S of 5 dph larva showing the pharyngeal teeth and liver (400X)

B=Barbel; BC=Buccal Cavity; BM=Basal Membrane; Br=Brain; C=Capatulum (Valve); CR=Cartilaginous Rod; GA=Gill Arch; GF=Gill Filament; Ht=Heart; L=Liver; M=Mouth; NE =Nasal epithelium; OpN=Optic Nerve; Ph=Pharynx; PhC=Pharyngeal Cavity; PhE=Pharyngeal Epithelium; PE=Pigment Epithelium; TB=Taste Bud; YS=Yolk Sac.

The lips are the primary food procuring organs. They assume different forms in different fishes and may be also adhesive in some teleosts (Kapoor *et al.*, 1975b; Kapoor and Khanna, 1994). In *N. montanus* larvae, mouth was ventrally placed that shows its bottom dwelling habit**.** The lips did not develop any adhesive pad till the post flexion stage which suggests that the larvae till this period stays in the slow current area or shallow river water.

Three pairs of barbels were present in the larvae of *N. montanus* and their histology showed that they were well equipped with taste buds. The taste buds are the peripheral sense organs of the gustatory system. In fishes, they enable the animal to identify food by detecting distinct chemical substances on a short distance (Kasumyan, 1997). The presence of taste buds thus signifies the role of gustation in feeding in this fish.

Outstanding among the obvious adaptations for feeding in fishes are the teeth. They are thought to have arisen from scales covering the lips. There is strong correlation among kind of dentition, feeding habits and food eaten. The teeth develop at an early stage in the *N. montanus* larvae at about day 3 post hatching that suggests its carnivore feeding habit. The teeth of the *N. montanus* larvae morphologically are intermediate between the hooked and shearing teeth which suggest that this fish is an omnivore.

Many freshwater and marine fishes are equipped with oral valves behind the lips (Gudger, 1946) whose surfaces are provided with taste buds (Kapoor, 1957b). In *N. montanus* larvae crescentric maxillary valve is present along the inner margin of upper lip which contains many taste buds. This connective tissue hanging increase the sensory surface of the buccal cavity and support the results as observed in some other fishes by Girgis (1932,1952a,b) and Subla (1970) etc.

Besides protecting the tender gill filaments from abrasion by ingested materials that are coarse in texture, gill rakers are also specialized in relation to food and feeding habits. They show marked structural correlation with the feeding mechanism of fishes (Iwai, 1964; Kapoor 1965a). The gill rakers in *N. montanus* larvae are well developed and are stubby and unadorned. Long gill rakers characterize the majority of bottom feeders which stir up the mud. Long gill rakers have also been reported in other bottom feeding fishes like *Mugil auratus* and *Upeneus barberinus* (Al- Hussaini, 1947b).

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