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Habit and habitats responsible for Adaptive modification of hill stream fishes of Kumaun Himalaya region with special reference to Botia almorhae, Homaloptera brucei and Schizothorax richardsonii

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Abstract
The hill stream water has different type of fish species owing to different habitats; as such they have developed and adapted for different types of food and feeding habits. It is well assumed that morphological differences in fishes are due to the action of several abiotic and biotic factors which are related to habit and habitat of fish species. The hill stream fishes *viz*. *Botia almorhae*, *Homaloptera brucei* and *Schizothorax richardsonii* collected from the Kosi river at Kakrighat, Distt. Nainital, West Ramganga river; at Chaukhutia, and Kosi river at Hawalbag, Distt. Almora, Uttarakhand have been selected for the studies. The body modification with respect to food, feeding and dwelling habits has been explored which is helpful in understanding the trophic level of these fishes. During the studies it has been analyzed that in case of *Botia almorhae* belong to the category of bottom feeder and surface feeder omnivore fish. *Homaloptera brucei* adopted for Botom-column-omnivore or carnivore feeding habit whereas *Schizolhorax richardsonii* is a bottom feeder and predominantly herbivorous.

Keywords: Hill stream fishes, habit, habitats, abiotic and biotic factors.

1. Introduction
The present study aims to examine the modifications of *Botia almorhae*, *Homaloptera brucei* and *Schizothorax richardsonii*, a common stream fish in the Kumaun Himalayas. Streams originating from the Himalayas are unique by virtue of their high altitude; fast-flowing waters, heterogeneous substrate, low water temperatures and high dissolved Oxygen due to snow melt or glacier fed water. The relationship between morphological modifications related to body, fins and the habit and habitat in *Botia almorhae*, *Homaloptera brucei and Schizothorax richardsonii*. These fishes represent the native dominant group of Pisces in the snow fed-rivers of Kumaun Himalaya, Uttarakhand. In the Hill-stream, fishes have to face several interrupt in their movement because of pouring water current associated with slopes, rocky beds and shores.
According to[23] studies on food and feeding habits are an important aspect of the biology of fishes, which shows the requirements at different stages in their life cycle differ with space and time. [54] Classified the food of fishes into main food, occasional food and emergency food. Later, [43] studied this objective and divided the food of fishes into basic food, secondary food, incidental food and obligatory food. He also categorized the fishes into euryphagic that feeds on a variety of food; Stenophagic that feeds on a few selected foods and monophagic that feeds on a single type of food.
[18] Classified the fishes on cultivable importance into surface feeders, column feeders and bottom feeders. Later, [10] classified the fishes into herbivores, carnivores and omnivores that feed on plant materials, animal materials and both animal and plant materials respectively. Depending on the percentage of the type of food, fishes are classified into piscivorous that feeds mainly on fishes; carnivorous, feeds mainly on crustaceans, planktivorous, feeds on plankton, detritivorous, feeds on detritus and cannibalistic feeder that feeds on their own kind [45].

Materials and Methods
The live fishes viz. *Botia almorhae* (Teleostei: Cobitidae), (approximately 5-7 inches in length) were collected from the Kosi river at Kakrighat of Distt. Nainital (elevation-1200m. above mean sea level), *Homaloptera brucei* (Teleostei: Balitoridae), (approximately 3-4 inches in length) from West Ramganga at Chaukhutia in Distt. Almora (elevation-1200m. above mean sea level) and *Schizothorax richardsonii* (Teleostei: Cyprinidae), (approximately 6-8 inches in length) from the Kosi river at Hawalbagh in Distt. Almora (elevation- 1194m. above mean sea level) Uttarakhand. The water current is very fast having the velocity between 0.5 to 2.0 m/sec [7]. and the river bed is rocky. The fishes were transferred from the site of collection to the laboratory in well ventilated plastic containers and were kept for a period of about 5-6 days in glass aquaria having an artificially prepared rocky bed with aquatic vegetation grown therein. The aquaria were cleaned and supplied with fresh spring water on alternate days. The fishes were fed on aqua feed (tropical fish food). To study the details of the morphological adaptations in some fishes, SEM was done. The following procedure was adopted for the preparation of the specimen for SEM. The specimen was maintained in laboratory at 25 ± 2^0C. The fishes were cold anesthetized following [41], for SEM preparation. Skin fragments of about 10×10 mm were cut from their dorsal

sides just behind their heads. Tissue were excised and rinsed in 70% ethanol with one change of saline solution to remove debris and then fixed in 3% Glutaraldehyde in 0.1M phosphate buffer at pH 7.4 overnight at 4^0C in a refrigerator. The tissues were washed with 2-3 changes in phosphate buffer and dehydrated in ascending series of ice cold Acetone (30%, 50%, 70%, 90% and 100% approximate 20-30 mins.) and dried at critical point using a critical point dryer (BIO-RAD England) with liquid carbon dioxide as the transitional fluid. Tissues were glued to stubs, using conductive silver preparation (Eltecks, Corporation, and India). The samples were coated with gold using a sputters coater (JFC 1600) and examined under (JEOL, JSM- 6610 LV) scanning electron microscope and the images were observed on the screen.

Results

The Cyprinid fishes found in the hill-streams of Uttarakhand are represented by genera belonging to the families; Cobitidae, Homalopteridae (Blitoridae) and Cyprinidae. These fishes show a remarkable uniformity in their body surface. There are two subfamilies of Cobitidae; Cobitinae and Botiinae, which means it's more correct to refer to these loaches as Botiinae or botine loaches. *Botia* (Indian loaches) is a genus of fresh water fish in the family Cobitidae. Yoyo loach is the common name of *Botia almorhae* (once called *Botia lohachata*) that belongs to the botinae species.

The mouth of the *Botia almorhae* points downward and sports four pairs of barbells**.** *Botia almorhae* is more active during the night. It being a shy fish, it is better to avoid the use of strong lighting and to use some floating plants to shade the aquarium. Another interesting character exhibited by the *Botia almorhae*, is the clicking sound it makes when feeding on the surface. It does so by swallowing air and forcing it through the gills.

Botia almorhae is an omnivorous species that feeds primarily on small, bottom-dwelling invertebrates such as worms, insect larvae and snails. They also consume plant material and algae. Being omnivore, the *Botia almorhae* will accept virtually any type of food, flakes, pellets, shrimp wafer, catfish wafers etc. like all loaches they have a gluttonous appetite for snails and for live and frozen foods.

The *Botia almorhae* is a riverine species; this species prefers still and slow running waters. It usually congregates in pools and still areas with rocky substrates.

Homaloptera brucei also require similar conditions as the other hill-stream species. *Homaloptera spp*. is specialised grazers feeding on biofilm, small crustaceans, insect larvae and other invertebrates. In captivity some sinking dried foods may be accepted but regular meals of live or frozen *aphnia*, *Artemia*, bloodworm, etc. are essential for the maintenance of their good health and it's highly preferable if the tank contains rock and other solid surfaces with growths of algae and other overgrowth.

Homaloptera brucei is found mostly attached to the boulders in fast flowing waters, especially on stones covered with fine algae. All *Homaloptera spp*. have morphology, specialized for their life in fast-flowing water; the fish can tolerate high current waters with algae covered boulders and pebbles. The paired fins are orientated and extended horizontally; paired fin and rays help the fish to anchor to the substratum. *Homaloptera* have large pectoral fins which they use as aerofoils to wing themselves down onto rocks in the water flow. They utilize the passing water and by angling the fins they create reverse lift and fly downwards. Without the sucker adaptations, their ability to maintain station, in a really fast flow, is reduced. The perching habit of the fish has brought about the ventral attachment of the paired fins to provide a large area for adhesion. Spines on the ventral surface of the first two rays further add to the mechanism of adhesion. The hill-stream fishes; *B. almorhae, H. brucei and S. richardsonii* prefer a river basin with a rocky bed because it furnishes them a place for adhesion and is of great advantage for the fish.

Head and body flattened, belly depressed, they feed on fine algae on the substratum and entangled minute sand particles. These features form a powerful sucking cup which allows the fish to cling tightly to solid surfaces. The ability to swim in open water is greatly reduced and they instead appear to crawl and hop their way over rocks and other such surfaces. *Homaloptera brucei* inhabits swiftly-flowing streams and headwaters containing clear, oxygen-saturated water.

The *Schizothorax richardsonii* belongs to subfamily Schizothoracinae, family Cyprinidae under an order Cypriniformes of class Pisces. *Schizothorax richardsonii* is one of the indigenous coldwater dominant genera in Schizothoracinae subfamily in Uttarakhand and is one of the important food and game fish. Schizothoracinae are the specialized group of fishes which inhabit snow fed torrential streams of Himalayas. *Schizothorax* is commonly known as "snow trout" because it inhabits the snow fed streams. The fish inhibits in streams and rivers of Himalayan and Sub-Himalayan regions.

Morphologically *Schizothorax richardsonii* has been observed that cylindrical or approximately cylindrical body form that helped to brows well under fast water currents. *Schizothorax spp*. and *Tor spp*. are the most important fish, from the economic and sport-fishery, point of view. These are also an excellent food fish. They undergo migration during winter months when the temperatures in the greater Himalayan waters nearly reach the freezing point. This induces them to migrate downstream and frequent the warmer spring-fed streams in search of suitable spawning grounds. The *Schizothorax richardsonii* is one of the most important and common palatable fish of the Kumaun Himalaya. The *Schizothorax* is an indigenous herbivorous cold fresh water teleost, belonging to the family Cyprinidae proves to be morphometrically, meristically and economically the most variable and valuable promising food species. The population of the *Schizothorax* contributes towards the major fisheries of this upland region, but a very low quantum of food resources is available for them to maintain a good growth in the hill-streams.

Schizothorax richardsonii inhabits rivers, prefers to live among rocks and is primarily a bottom feeder, preferably near big submerged stones. The *Schizothorax* thrives very well in crystal clear water bearing high dissolved oxygen (8.9-17.8 mg/l). Enhancement of food resources or application of artificial fish food is an urgent requisite for obtaining a better growth of the fish.

The importance of the study of food and feeding habits is that it indicates the trophic segregation pattern among the members of the fish community in that area [20]. The study of the feeding habits would help to determine the ecological condition of the fish, niche in the eco-system and preferred food [46]. Fishes exist in various body forms. Depending upon the various sizes of fishes, a wide variety of feeding habits exist from herbivores to tertiary predators, to

decomposers representing every trophic level [19, 68].

It is interesting to notice the differences exhibited in the patterns of microridges on epithelial cells. The distribution of mucous cells on the general body epidermis of *B. almorhae, H. brucei and S. richardsonii* may be considered as modifications relating to possible difference in the functional requirement at the different locations and their habitat (Fig: 01,02, 03,04,05 and 06).

Noticeable difference exhibited in the distribution of mucous cells may be considered as modifications relating to possible difference in the functional requirement at the different locations. A large number of mucous cells are present in *B. almorhae* and *S. richardsonii* a compared to *H. brucei* (Fig: 07, 08 and 09); This may provide sufficient lubrication to reduce the friction between the body surface and water current and protect the epidermis from wear and tear. It means *B. almorhae* (bottom feeder and surface feeder) and *S. richardsonii* (bottom feeder) live against high velocity currents of water as compare to *H. brucei* (Botom-column feeder*)*.

In *H. brucei* well developed papillae/tubercles are present on the rostral cap with the taste buds (Fig.10 and 11). The anterior lip is clearly divided into two regions; anterior and posterior, the anterior region surrounds the mouth opening. The anterior region of the anterior lip has a large number of papillae or tubercles, characterized by their disc shape (Fig.12 and 13) and each papilla bears numerous glandular secretive devices, which are well developed taste buds (Fig. 14). On the other hand the posterior region of the anterior lip is encircled with two rows of uneven papillae with a large number of taste buds (Fig.15). The outer row comprises of eight to ten bigger papillae/tubercles, while the inner row with ten smaller ones. The outer-most papillas of the outer row are slightly elongated and bear taste buds (Fig.15). The taste buds on the body surface are probably essential to inform the fishes about the various substances in the surrounding media. This may help the fishes to direct it towards food sources. The presence of a large number of taste buds increases the probability of detecting and locating prey concealed by darkness or turbidity accurately and may also permit the accurate location of small food particles, which could be missed otherwise.

The adhesive pad is characteristically large and an arched structure that appears like a shallow plate divided into a region and a relatively wide peripheral region. Adhesion results due to friction between tubercles and surface of the substratum. We concentrate here mainly on its role in adapting the fish to its peculiar mode of life in the mountain torrents.

Discussions

Stream ecosystems are generally characterized by various geomorphological conditions such as channel slope and bed morphology that can profoundly influence the characteristics of resident fishes [29]. In addition to these geomorphological conditions, environmental parameters such as water velocity, depth, substrate diversity and water temperature also influence the structural and functional morphology of fishes [36]. Studies on the food and feeding habits are important aspect in the study of biology which shows the requirement of food in different stages of life with space and time [23] and significant importance in aquaculture practice.

Advanced microscopical techniques like Scanning Electron Microscopy have enabled us to study structural peculiarities in organs like the fins, skin, barbels, lips and adhesive apparatus. Most of these organs are composed of some horny tubercular projections with a central pore. These structures are known as taste buds (TBs). These taste buds are used for chemosensing, thermosensing, and mechanosensing activities [20].

The specific structures, in different groups of fishes, are modified in relation to the habit, habitat and mode of feeding, food preference and the mode of life exhibited by the fishes [50]. All the fish species found in the river were characterized with some special adoptive organs to survive in hill streams. The presence of these fish species indicates that the ecosystem of Kosi River is suitable for the survival of fish diversity. Walker [66] recorded five species from the Nainital Lake. [26, 27, 28] recorded seventeen species of fishes belonging to different families from Kumaon hills. His collections were mainly from lower waters of Nandhaur and Kalaunia rivers and from Kosi River near Khairna. [40, 41] gave a list of twenty three species of fishes from the lake and rivulets of Nainital. He also collected fishes from Kosi River (Khairna) and from Almora proper.

Most of the hill stream fishes possess structural integumental modification [13] also noticed adaptive modification in there fishes. [24, 25] described a large number of hill stream fishes with respect to their adaptive modification and evolutionary point of view. In various hill stream fishes like *Garra annandalei, Glyptothoray madraspatnum, Garra lamta, Glyptothorax mullya, Glypothorax telchilta* and *Pseudoechinesis sulcatus* modified adhesive apparatus has been studied by [49, 8, 53, 30]. [63] Also work on diversity of hill stream fishes of Madhya Pradesh.

Botia almorhae is demarsal and oviparous and feeds on worms, snails, small fish etc. and inhabits creeks with rocky and sandy bottoms. It plays an important role in controlling the snail population [46]. In the aquarium trade, this fish is usually known as the *Pakistani loach*. [64] Said that, this species is said to be sociable, as well as less shy and pugnacious than other *Botia spp*. True loaches are mostly scavengers and are omnivorous and are usually not very picky about their food. They may eat aquatic crustaceans, insects and other small invertebrates as well as scraps of organic detritus. Some of these loaches have adapted themselves to low oxygen levels in warm, muddy rivers or dirty ponds by being able to gulp up atmospheric oxygen from the air.

Homaloptera brucei was originally described as *Balitora brucei* by [21, 17]. The first species of the genus *Balitora* was described by [21], and named it as *Balitora brucei*. It belongs to the family Balitoridae [21]. [11, 12, 13, 14, 22]. Reported *Balitora brucei* as *Homaloptera brucei* from Southern India. Balitoridae fishes are the loaches generally found in fast flowing waters of the hill stream. These stone loaches are characterized by depressed body; ventrally flattened head and abdomen, ventrally situated mouth, arched, jaws covered with horny tubercles; lips are papillated; gill opening extend to the ventral side of the head, 2-3 pelvic fin rays, 8-10 pectoral fin rays and paired fins placed ventrally [31]. Balitoridae family of fish eat small invertebrates, algae and detritus from the bottom of the river [32].

The *Homaloptera* species has adaptive features like much larger pectoral fins with more simple rays. These fishes depress their head and force it down onto their body to

balance against strong water currents. [47] Noted this shape to be common among Asian hill-stream fishes which attach themselves to the hard substrate in high gradient streams (e.g. *Garra*, *Homaloptera* and *Gastromyzon*). *Balitora ludongensis* sp swim slowly and usually inhabit the bottom of water [59]. *Schizothorax richarsonii* is most common and widely distributed species in Himalayan belt [56]. This species has been considered to be a valuable source of fish protein for the hill community. *Schizothorax* is a phytophagous fish and has developed a special mouth, adapted to scraping attached algae, from the surface of stones. The feeding habits of *Schizothorax* have been reported by [58, 35, 65, 57]. It feeds on attached algae including *Spirogyra, Ulothrix and Oedogonium*. The recent emergence of Tehri dam reservoir has resulted into the large destruction of natural habitat of snow trout species (*viz. Schizothorax richardsonii, Schizothorax plagiostomus, Schizothorax curvifrons*) that are basically bottom feeder and lithophil spawner, thrive in the snow fed river habitat of clear, shallow water of stony substratum with a average depth from 1 to 3 meters, and river flow not less than 0.5 meter per sec [61, 60, 2, 3].

Body depth and fin size are the two important morphological characters of stream fish which affect static location and moving manipulation [15]. On the basis of morphological data, Indrawati and Khudi River populations were the most divergent. Compared to the six river populations of *S. richardsonii*, the two populations from relatively deeper Indrawati and Khudi Rivers had the most large fins, head, wider eyes and deeper body. This pattern of deeper body is consistent with the observation made on *Atherinops affinis*, in lakes of California [51]. that the body depth of fishes increases in response to warmer water temperature. Fishes in low velocity and deep water are more often deeper bodied with larger caudal areas for improved burstswimming performance and increased maneuverability [33]. Shorter pectoral fin length of *S. richardsonii* populations measured in Tadi, Sabha and Melamchi Rivers associated with colder water temperature and faster flowing did support the findings of [5]. Fishes that evolved in faster flowing water tend to be more streamlined to reduce drag [34]. [4] Also reported that high water velocity leads to slender body shape in a Caspian cyprinid (*Alburnus chalcoides*, Guldenstadt 1772). Thus, the different current pattern of these water bodies may have been playing an important role in modifying the morphology of *S. richardsonii* among these water bodies. A more cylindrical body shape paired with short pectoral fins length of *S. richardsonii* in Sabha and Tadi Rivers measured in this study might have been the fish plasticity to allow individuals to better confer swift flowing habitats with high substrate heterogeneity. Environmental parameters such as water temperature, conductivity and substrate heterogeneity influence morphological traits of *S. richardsonii* and other fish systems have been well documented [9, [33, 48].

Schizothorox richardsonii inhabiting Bhagirathi River (a cold water hills-tream river) is a periphytonic feeder, feeding on Bacillariophyceae, Chlorophyceae, Cyanophyceae, detritus, and sand in this very preferential order. *Schizothorax richardsonii* have very small scales on their body. The reduction or degeneration of scales in *Schizothoracinae* is a character shared by high altitude fishes of the family Cobitidae and Salmonidae. The reduction in size or absence of scales on the body is the distinguishing character of *Schizothorax richardsonii* from other species. *Schizothorax richardsonii* is a bottom feeder, mainly herbivorous and their horny jaws are helpful in scraping off algae from stones and rocks in the fast running water. This ventral position of the mouth with hard papillae plate is helpful for the scraping of algae and diatoms from the surface of rocks in torrential streams [37].

From the nature of the food available in the hill-streams it is obvious that it has to be scraped off from rocks and stones. For this purpose the lower jaw is hard and strong, and its free end is sharp and shovel-like. The posterior lip does not cover the jaw entirely, but leaves the rasping edge bare. The anterior jaw does not bite against the posterior but is modified to form a vertical plate in front of it. In this way the scraped off algae and slime are prevented from being washed away by the current and are ingested with the help of the respiratory current that probably flows into the mouth from its corners. The mechanism of feeding seems to be partly responsible for the grooves that are found round the mouth, for, if a "current were to enter the mouth directly from the anterior end, it would tend to carry away with it a part of the food turn off the rocks.

The head morphology reflects a species' feeding habits [62]. *S. richardsonii* is known to be planktivore species, as adult it feeds upon aquatic plants, algal slime, and slimy deposits on rocks [58]. The first principal component consisted of head region, being a strong classificator, indicate the foraging habits of the studied populations. Relatively large heads of Indrawati and Khudi River populations found in the present study may enhance the capture of small prey [6].

The adaptation of *S. richardsonii* populations from the relatively large rivers Indrawati and Khudi reflects their body morphology; they are relatively strong with long and distant apartness of fins, which are related to slow and precise movement [16] large fins are also of advantage in maintaining ones position in the river [52]. In a study[42] reported that 90% variation in morphometric characters of *S. richardsonii* populations from Uttar Kashi, India are genetically controlled and environmentally controlled characters are a few (10%).

[55] Has shown that the cave dwelling race has a larger number and a more extensive distribution of external buds than the river dwelling race, corresponding to acute gustatory feeding behavior. The projected taste buds are the first to come in contact with the surface water and enable the fish to sense the nature of food, available in a particular feeding zone, before it opens its mouth to form a temporary feeding tube to gulp in large quantities of water along with available food particles [1].

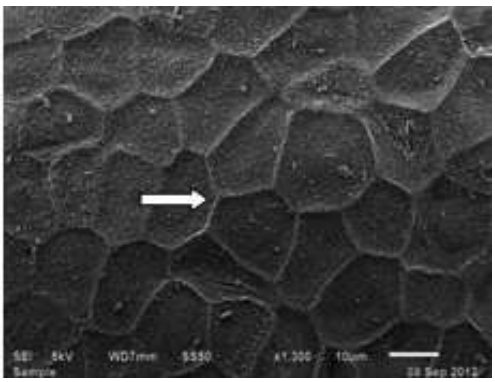

Fig 1: SEMPH of the GBE of *B. almorhae* epidermis showing polygonal epithelial cells (marked by

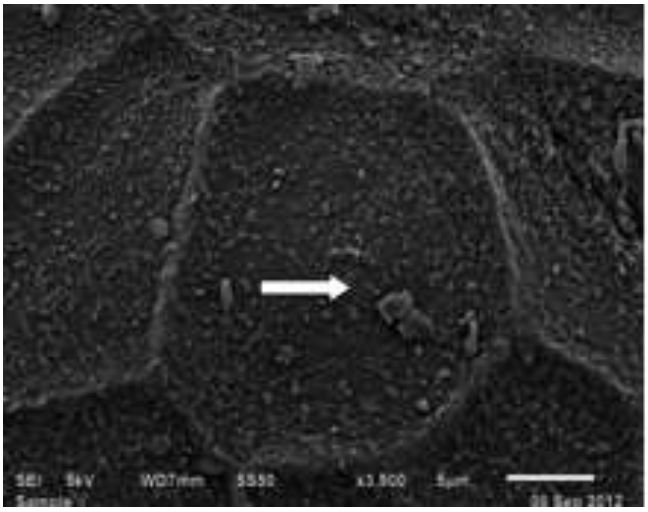

Fig 2: SEMPH of the GBE of *B. almorhae* epidermis showing microridges at the surface epithelium (Scale bar- 5µm).

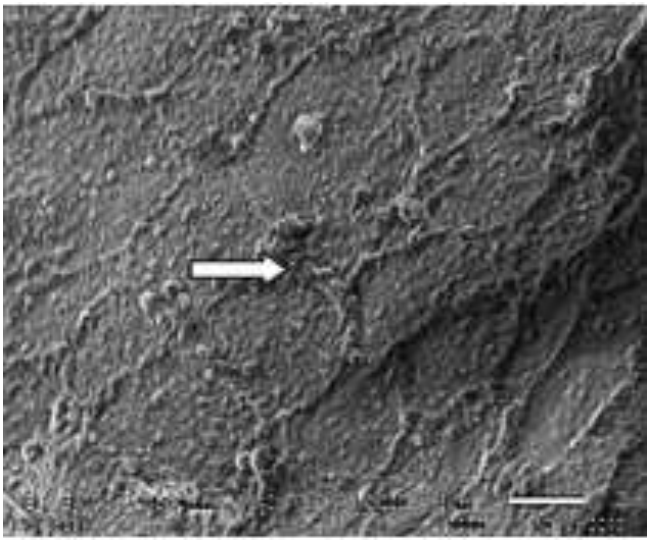

Fig 3: SEMPH of the GBE of *H. brucei* showing polygonal epithelial cells (marked by arrow) (Scale bar- 5µm).

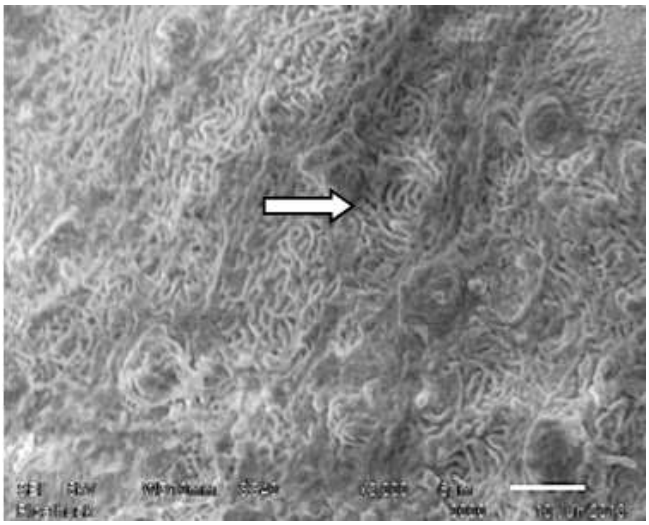

Fig 4: SEMPH of the GBE of *H. brucei* showing that the microridges are generally; finger print- like, and are often arranged in the form of small groups (Marked by arrows) (Scale bar- 5µm).

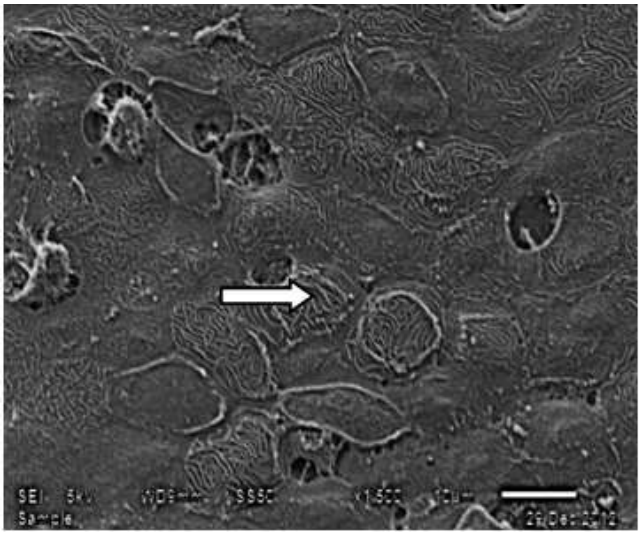

Fig 5: SEMPH of the GBE of S. *richardsonii* showing finger print-like patterns of microridges (Marked by arrows) and also showing the mucous openings and their secretory contents profusely at the surface through a small pore. (Marked by arrows) (Scale bar- 10 µm).

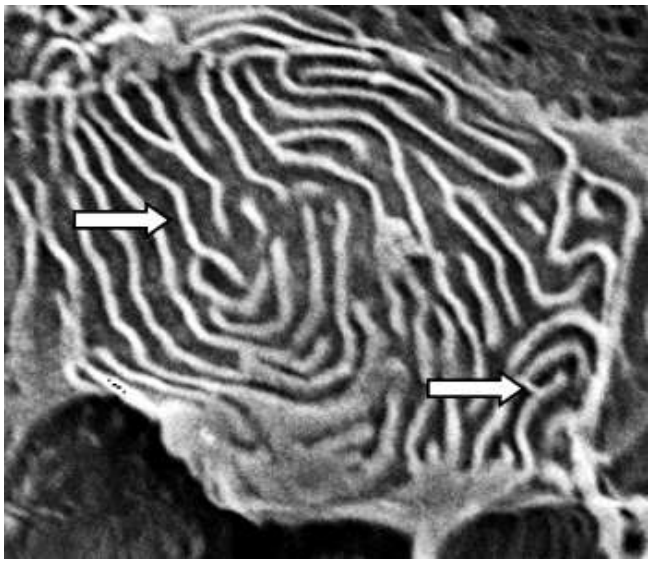

Fig 6: SEMPH of the GBE of S. *richardsonii* showing finger print-like patterns of microridges that have canaliculi and microbridges (Marked by arrows) (Scale bar- 5µm).

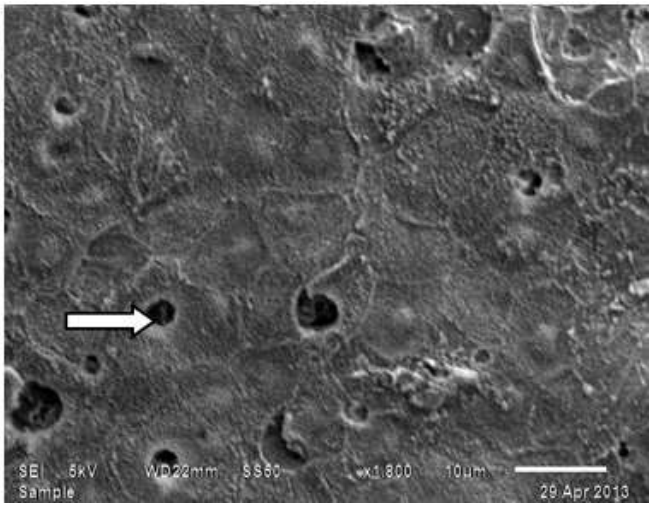

Fig 7: SEMPH of the snout epidermis of *B. almorhae* showing mucous cells opening (Marked by arrow) (Scale bar- 10 µm).

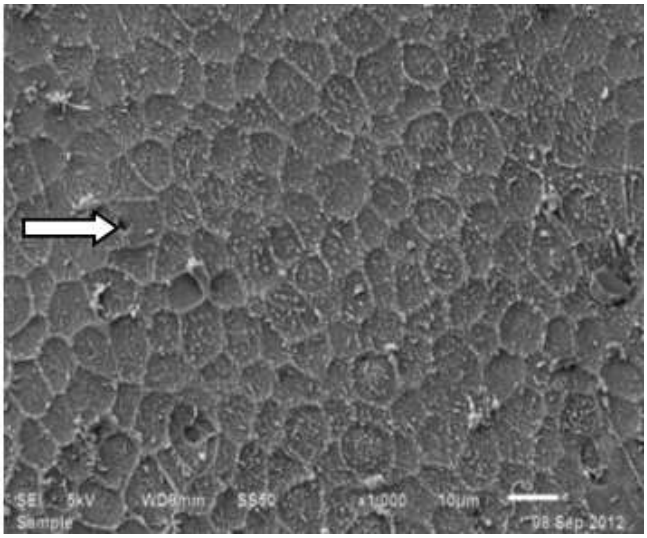

Fig 8: SEMPH of *H. brucei* of showing mucous openings (Marked by arrow) (Scale bar- 10µm).

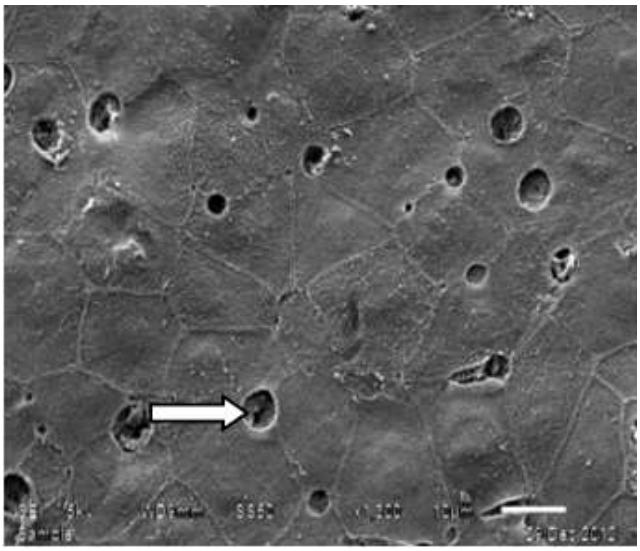

Fig 9: SEMPH of *S. richardsonii* showing mucous openings (Marked by arrows) (Scale bar- 10 µm).

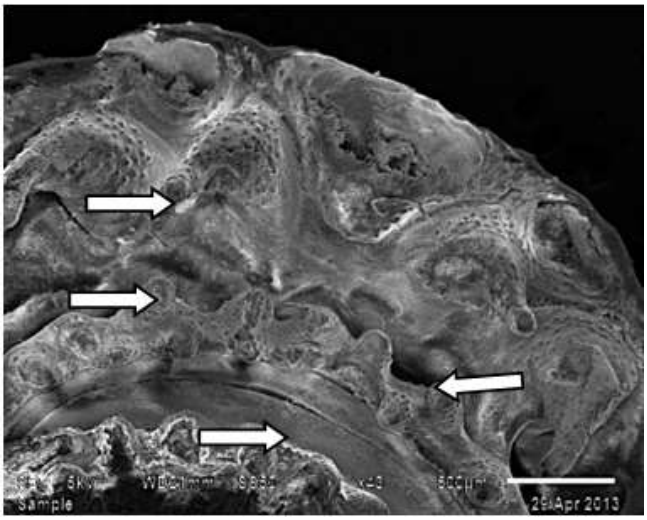

Fig 10: SEMPH showing the deep groove between the anterior lip and rostral cap; marked by arrow and the elongated papillae with the taste buds on rostral cap of *H. brucei* (Scale bar- 500 µm).

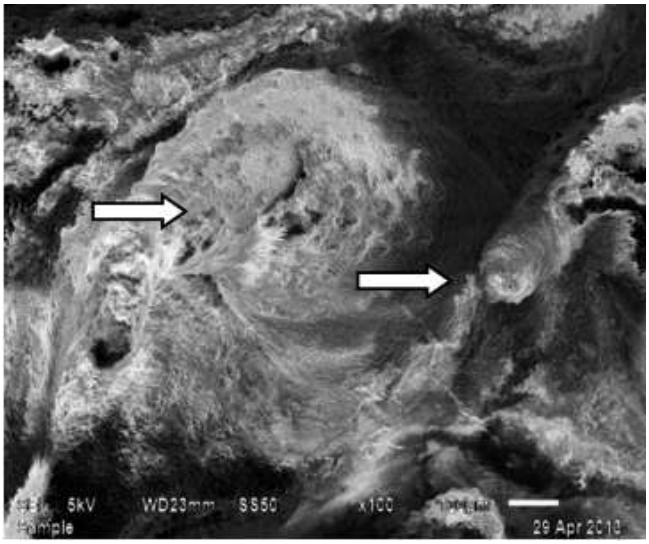

Fig 11: SEMPH of the anterior portion of the anterior lip and posterior lip showing the papillae or tubercle of *H. brucei* (Scale bar- 100µm).

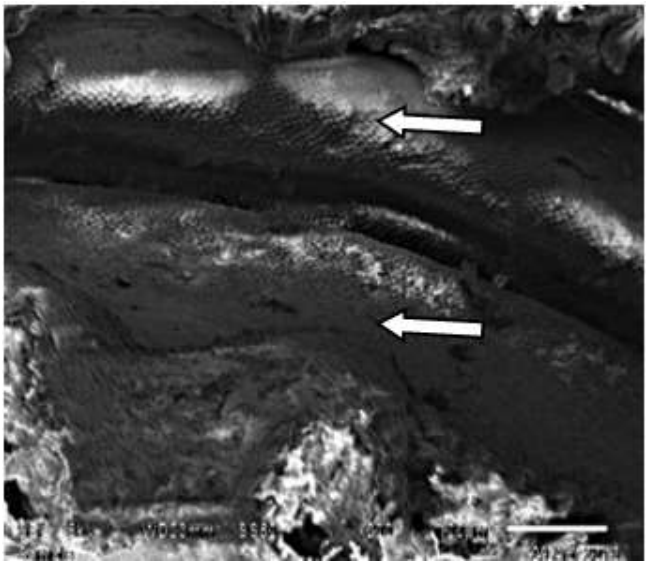

Fig 12: SEMPH of the anterior portion of the anterior lip showing the disc shape tubercle of *H. brucei* at high magnification (Scale bar- 10µm).

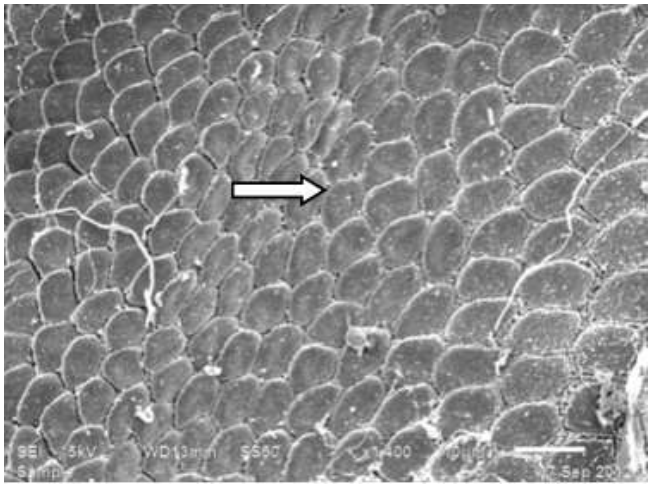

Fig 13: SEMPH of the anterior portion of the anterior lip showing the disc shape tubercle of *H. brucei* at high magnification (Scale bar- 10µm).

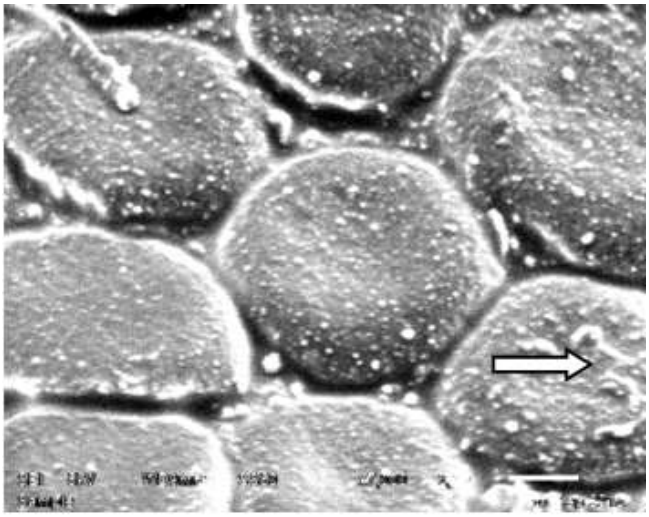

Fig 14: SEMPH showing the taste buds on the papillae or tubercle of the anterior portion of anterior lip in *H. brucei* (Scale bar- 2µm).

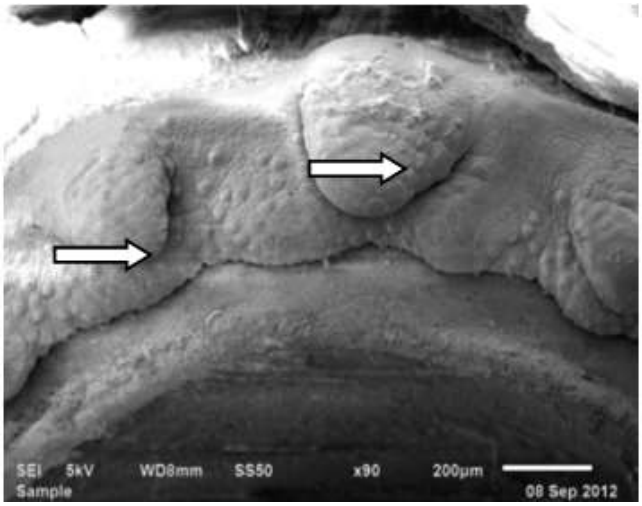

Fig 15: SEMPH of the posterior region of the anterior lip showing two rows of tubercles with taste buds of *H. bucei* (Scale bar- 200µm).

Conclusion

We found correlations between environmental and morphological variables suggesting that certain mouth, body shapes and fin shapes may be adapted for a specific habit and habitat of the fishes. These results suggest that and individual's mouth; body and fin morphology is influenced by the habit and habitat of fishes, where they live. Adaptations occur with the lapse of time in response to the environment. Hill-stream fishes too have undergone of countless adaptations through the life.

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