Histological Observations and Regeneration of Barbels in Juveniles of the Chinese Longsnout Catfish *Leiocassis longirostris*

In-Seok Park¹*, Chi-Hong Kim² and Jae Wook Choi¹

¹Division of Marine Environment and Bioscience, College of Ocean Science and Technology, Korea Maritime University, Busan 606-791, Korea
²Inland Fisheries Ecological Research Institute, National Fisheries Research and Development Institute, Gapyeong 477-815, Korea

Abstract
Barbel structure and regenerated barbel length in the juvenile Chinese longsnout catfish *Leiocassis longirostris* (Günther), were evaluated. The barbles consisted of an epidermis, a dermis, and a central rod. The epidermis harbored taste buds, granular cells and epidermal cells. The taste buds were basophilic and situated along the distal portion of the epidermis. The dermis was composed of loose connective tissue containing blood vessels pigment cells. The innermost central region was cartilage enclosed within layers of muscle layers. After 30 days, the regenerated barbel length measured 0.92 ± 0.404 mm at 15°C (regenerated growth curve: $y = 0.5085x + 4.0678, r^2 = 0.9654$, where $y$ is regenerated length and $x$ is experimental period in days), 1.88 ± 0.521 mm at 20°C ($y = 0.1806x + 4.808, r^2 = 0.9822$), and 6.44 ± 0.751 mm at 25°C ($y = 0.0914x + 4.9918, r^2 = 0.9944$). Fifteen days after amputation, the regenerated length was significantly longer at 25°C than at 15 or 20°C ($P < 0.05$). The barbels of the Chinese longsnout catfish was the tender and flexible type, and our experimental findings provide evidence of temperature-dependent regeneration. Additional investigation of the behavior and physiology of the Chinese longsnout catfish is needed, particularly histological studies of regenerated barbels and the measurements of the numbers of taste buds per barbel under various environmental conditions.

Key words: *Leiocassis longirostris*, Chinese longsnout catfish Barbel, Histology, Regeneration

Introduction
A wide variety of marine and freshwater fish possess externally situated appendages, which are referred to as the barbels. Barbels are accessory feeding structures, that harbor sensory organs and perform functions important to the daily activities of fish (Kapoor and Bhargava, 1967; Park et al., 2005, 2006). The number, length, and position of barbels vary markedly (Park et al., 2005), and their histological structures have been compared among several fish species (Satô and Kapoor, 1957; Nagar and Mathur, 1958; Srivastava and Sinha, 1961; Agarwal and Rajbanshi, 1965; Rajbanshi, 1966; Kapoor and Bhargava, 1967; Singh and Kapoor, 1967; Satô, 1977; Kim et al., 2001; Park and Kim, 2005; Park et al., 2005, 2006).

Barbels also have the ability to regenerate after amputation, similar to the tails of salamanders. Nerves in the barbel are likely related to the regenerative ability. In a study of the effects of nerves on the maintenance of taste buds, Olmstead (1920) reported that injured barbels did not regenerate completely when denervated.

The Chinese longsnout catfish *Leiocassis longirostris* (Günther) is a benthic freshwater fish that inhabits the sandy bottoms of lakes throughout China and Korea (Kim and Park, 2002). It was not found in Korea prior to 2007 because

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*Corresponding Author
E-mail: ispark@hhu.ac.kr
of pollution and development (Kim and Park, 2002), but the Chinese longsnout catfish was recently re-introduced to Korea from Chinese stocks. In 2007, artificial breeding succeeded, suggesting that aquaculture of this species would soon be possible. The Chinese longsnout catfish harbors four pairs of barbels. According to Kapoor and Bhargava (1967), its barbel is a tender and flexible type. Similar to the barbels of the striped eel catfish, *Plotosus lineatus*, and the amblycipitidae *Liobagrus andersoni*, both of the Siluridae family, the barbels of the Chinese longsnout catfish offer gustatory sensibility and function in locating prey (Park et al., 2005, 2006). However, barbel structure and regeneration in the Chinese longsnout catfish have yet to be elucidated. The objective of the present work was to determine the structure and regeneration properties of the barbel of the Chinese longsnout catfish a member of the Bagridae family.

**Materials and Methods**

In the present study, we used juvenile specimens of the Chinese longsnout catfish *Leiocassis longirostris*, which were acquired from the Central Inland Fisheries Research Institute, National Fisheries Research and Development Institute (NFRDI) in Korea. The mean standard length of the study fish was 15.6 ± 2.11 cm (*n* = 35).

**Histological observations**

Eight specimens were used for histological observations. Specimens were fixed in 10% neutral-buffered formaldehyde. Barbels were dehydrated via a standard ethanol series to 100%, cleared in xylene, and then embedded in paraffin. Sections of 6-μm thickness were deparaffinized and stained using Mayer’s hematoxylin and eosin. Observations and evaluations were conducted under a light microscope (Serien-Nr: 3109000586 Axiostar Plus; Carl Zeiss, Oberkochen, Germany).

**Regeneration of barbels**

The Chinese longsnout catfish harbors four pairs of barbels (Fig. 1), but regeneration was observed only for the longest barbel, which was proximal to the mandibular snout (a in Fig. 1B). Twenty-seven fish were acclimated in nine 40-L tanks, each of which held three fish. The fish were provided food pellets daily. Barbel regeneration was observed at three different temperatures (15, 20 and 25°C), with three replicate tanks for each temperature. The mean mandibular snout barbel length was 13.1 ± 0.32 mm prior to amputation. Amputation was accomplished by clipping 5 mm from the upper side of the basal barbel. Barbel length was then measured every 3 days over a 30-day period, using digital vernier calipers (CD-20CP; Mitutoyo, Kawasaki, Japan).

**Statistical analysis**

One-way ANOVA and Duncan’s multiple range test (Duncan, 1955) were used to assess the significance of differences among the mean lengths at the three temperatures. Statistical analyses were performed with SPSS version 9.0 (SPSS Inc., Chicago, IL, USA).

**Results**

We noted no structural differences among the barbels of the Chinese longsnout catfish *Leiocassis longirostris*. All barbels consisted of three principal layers: an epidermis, a dermis, and a central axis of cartilage (axial rod) (Fig. 2A). As shown in Fig. 2, the epidermal layer was composed of stratified epidermal cells having a spherical or ovoid shape in the surface layer and a columnar in shape in the basal layer.

In the surface of the epidermis, a number of taste buds were scattered among the epidermal cells (Fig. 2B and 2C). The taste buds varied in size and were flush with or even crossed the periphery, lying closer to the distal portion than the proximal portion of the barbel. The taste buds were basophilic. Also
among the epidermal cells, we noted granular cells with deeply stained nuclei and small quantities of faintly stained cytoplasm. The dermis was separated from the epidermal layer by a basement membrane and was composed of loose connective fibers that harbored abundant blood vessels and pigment cells (Fig. 2D-2F). The pigment cells were scattered throughout the superficial dermis (Fig. 2D).

An axial rod consisting of cartilaginous tissue was located at the innermost region of the barbel (Fig. 2B and 2E) and formed a “supporting rod” for the barbel. The cartilage cells had irregular shapes with a nucleus in the center (Fig. 2E), and the cartilaginous tissue contained a small amount of ground substance containing dispersed fibers. The axial rod was ensheathed by an inner circular layer of muscle and several bundles of longitudinal muscle, which were in turn surrounded by a circular layer of smooth muscles (Fig. 2E and 2F).

Fig. 2. Structure of barbel of Chinese longnout catfish Leiocassis longirostris. (A) Cross section of the mandibular barbel showing the structure of the axial rod of cartilage, dermis, and epidermis. (B) Longitudinal section of the maxillary barbell. (C) Taste bud and basement membrane. (D) Dermis and pigment cell. (E) Axial rod of cartilage, loose connective tissue, and smooth muscle layer. (F) Blood vessel. Ar, axial rod of cartilage; bm, basement membrane; bv, blood vessel; ct, loose connective tissue; d, dermis; ep, epidermis; ml, smooth muscle layer; pc, pigment cell; tb, taste bud. Scale bars: A, B = 40 μm, C-F = 10 μm.
After the 30-day experimental period, the length of the barbel regenerated at 15°C was 0.92 ± 0.404 mm (regenerated growth curve: \( y = 0.5085x + 4.0678 \), \( r^2 = 0.9654 \), where \( y \) is the regenerated length, and \( x \) is the experimental period in days) (Fig. 3). The regenerated barbel length was 1.88 ± 0.521 mm at 20°C (regenerated growth curve: \( y = 0.1806x + 4.808 \), \( r^2 = 0.9822 \)) and 6.44 ± 0.751 mm (regenerated growth curve: \( y = 0.0914x + 4.9918 \), \( r^2 = 0.9944 \)) (Fig. 3). Fifteen days after amputation, the regenerated length was significantly longer at 25°C than at 15 or 20°C (\( P < 0.05 \)).

**Discussion**

The barbels of fish are fleshy and elongated structures that harbor tactile and chemosensory receptors, and some species-specific differences have been observed (Bond, 1996). In particular, the movable barbels of some catfishes appear to serve an important function in food localization (Singh and Kapoor, 1967). The substantial gustatory sensitivity of the barbels extends the usefulness of these chemoreceptors a reasonable distance from the fish, which presumably facilitates the location of food in murky water (Moyle and Cech, 2000).

Kapoor and Bhargava (1967) classified barbels into two types: 1) tender and yielding barbels, which lack an axial cartilaginous rod and have a dermis harboring a network of blood vessels and 2) stiff barbels which can be motionless or flexible. Motionless stiff barbels have a supporting axis of true bone, whereas flexible stiff barbels contain a cartilaginous axis. The barbels of the Chinese longsnout catfish *Leiocassis longirostris* have an epidermis with taste buds and epidermal cells, a dermis containing plentiful blood vessels, and an axial cartilaginous rod. In accordance with the above classifications, the barbel of the Chinese longsnout catfish is the tender and flexible type.

In catfishes, including the striped eel catfish, tender and flexible barbels have gustatory sensitivity and function in the location of prey (Singh and Kapoor, 1967, Park and Kim 2005). The epidermis of the Chinese longsnout catfish barbel contains taste buds buried among the epidermal cells. Typically, taste buds control gustatory secretions by mucous and club cells. However, the Chinese longsnout catfish did not appear to have mucous cells or club cells. Consequently, the barbels of Chinese longsnout catfish are probably restricted to gustatory functions.

Singh and Kapoor (1967) demonstrated that mucous cells in the barbels secrete mucus for protection and escape from predators. Taste buds and club cells in the skin of the barbel have been described in the marine catfish *Arius thalassinus* (Kapoor and Bhargava, 1967). In addition, taste buds, mucous cells and club cells have been observed in the barbels of *Callichrous bimaculatus*, *Heteropneustes fossilis*, *Clarias batrachus* and *Rita rita* (Satô and Kapoor, 1957; Srivastava and Sinha, 1961; Singh and Kapoor, 1967). Taste buds were also detected in the barbels of *Bagarius bagarius* and *Blepsias cirrohosus draciscus* (Nagar and Mathur, 1958; Satô, 1977). Taste buds, mucous cells, and club cells were not detected in the barbels of *Mystus vittatus*; thus, the barbels were restricted to tactile, and not gustatory, functions (Agarwal and Rajbanshi, 1965).

The regeneration of fish barbels has been well documented. After amputation catfish barbels regenerate nerves, cartilage, connective tissue, epidermis, and taste buds, all of which are dependent upon the regeneration of nerves (Kamrin and Singer, 1953, 1955). A recent study has shown that the small chin barbell of *Lota lota* can regenerate several millimeters in length within 3 months following transection at the base (Cochran, 1987). Whitear (1990) demonstrated that within 2 days following the amputation of the barbell in *H. fossilis*, the stump was covered by epidermis; by the third day, regenerated tissues were detected. After 5 days, 1-1.5 mm of regenerated tissue were detected; after 7-8 days, 2.4-3.5 mm were observed, corresponding to approximately 0.5 mm of growth per day (Whitear, 1990). The axis harbored blood vessels, regenerated nerves with Schwann cells, fibroblasts, and collagen, but no cartilaginous skeleton. Tissue remodeling included simultaneous phagocytosis and the synthesis of collagen by fibroblasts (Whitear, 1990). Although the regenerated region in the present study was not examined histologically its regenerative capacity was similar to that of other species, considering that the Chinese longsnout catfish was a juvenile.

May (1925) demonstrated that after nerve section, the regeneration of taste buds begins only after the regenerated barbel nerve reaches the area of the degenerated taste buds; a nerve factor was thought to have influenced the growth and integrity of the taste buds. This was also demonstrated by Kamrin and Singer (1955) for the regeneration and maintenance
of the barbel of catfish. Kamrin and Singer (1955) used normal regeneration of the barbel as a control for comparison with regeneration of denervated and amputated barbels and denervated but not amputated barbels. Their results confirmed the regenerative abilities of barbels and the dependence of regeneration upon nerve supply. Moreover, barbels that were denervated but not amputated regressed in the absence of nerves (Kamrin and Singer, 1953).

These previous results were verified by Olivo (1928) and Torrey (1934, 1936), who demonstrated that low temperature inhibits nerve regeneration and hence the reappearance of the taste buds. The optimal temperature for this process was determined to be 20°C, suggesting that the causative factor may be an enzyme. Temperature-dependent barbel regeneration also occurred in the present study; low temperature inhibited nerve regeneration in the barbels, and barbel regeneration at 25°C was attributed to active metabolism, most notably enzymatic activity.

Based on the present study, the barbels of the Chinese longsnout catfish are the tender and flexible type, and exhibit temperature-dependent regeneration. However, these results do not fully elucidate the physiology of barbels in the Chinese longsnout catfish, and future work should include histological studies of regenerated barbels and measurements of the numbers of taste buds per barbel under various environmental conditions.

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