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Seasonal Change of the Skin Morphology of Muddy Loach, *Misgurnus anguillicaudatus* (Cobitidae) from Korea

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ABSTRACT The histological morphology on the skin of *Misgurnus anguillicaudatus* was described in the three regions such as dorsal, lateral, occiput and subsequently morphological variations of the skin were monthly observed for a year. The skin consisted of epidermis having epithelial cell, club cell and mucus cell, and dermis of mainly connective tissue fiber, embedded scale and blood capillary. Unicellular mucus cells situated at the epidermis underwent seasonal change in its size, as well as number and amounts of mucus-secreting materials, which they greatly increased in winter, but did not in summer. As it is getting cold, the mucus cells' shape changed from initial spherical to oval or elongated form. Such considerable changes in the mucus cell were particularly most evident in the occiput during winter. Moreover, the dermis largely thickened about 2～3 times in winter than in summer. Based on these results, we discussed function for the mucus on what it mainly acts in nature and information on whether mucus cells' seasonal variations affect on hibernation and cutaneous respiration.

Key words : *Misgurnus anguillicaudatus*, epidermis, dermis, mucus cell, cutaneous respiration

INTRODUCTION

The muddy loach, *Misgurnus anguillicaudatus* is a widely distributed cobitid fish in Korea, Japan and south-eastern China and its habitat is characterized mainly by benthic sand-muddy bottoms with natural growing plants at the edge of the water (Kim and Park, 2002; Hubbs and Lagler, 2004; Kim *et al*., 2005).

Many of the freshwater fishes have a scale on the surface of epidermis, but the scale of *M. anguillicaudatus* was deeply embedded in dermis, not extended to epidermis (Kim, 1997; Kim and Park, 2002; Kim *et al*., 2005). A number of species which the scales are buried or embedded in dermis, as if they look like scaleless fishes, are directly faced with more critical environments for their living when it compared with scaled fishes in epidermis (Fishelson, 1996). In this status, the fishes may have developed mucus gland in the epidermis which serve as chemical and physical protection, disease resistance, offence against the toxic and soluble components, etc. (Fletcher and White, 1973; Shephard, 1994).

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In most cases, these regulatory functional mechanisms are considerably variable at moments, so it is necessary to examine what it has a significant functions in natural habitat and in all seasons (Sadovy *et al*., 2005). That is, the structure of epidermis as a functional unit may be changed in relation to their dynamic environments. However, previous literatures concerning Genus *Misgurnus* were focused on the morphology of its skin (Tsai, 1996; Park and Kim, 1999; Park *et al*., 2001), and moreover, the seasonal changes of its skin have not been touched yet. On the other hand, seasonal variations in number of rodlet cell known as a kind of mucus cell have been reported for the brown trout (Rocha *et al*., 1994) and freshwater bream (Koponen and Myers, 2000), but it was interested in inner organs of fishes, not a skin.

Therefore, we investigated a morphological variation of skin including epidermis and dermis by seasonal change, and discussed the function of mucus cell, particularly regarding ecological views in this species.

MATERIALS AND METHODS

Three to Four specimens of the muddy loaches rang-

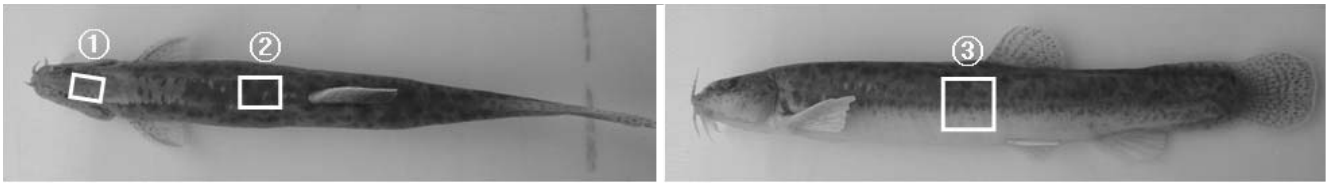

Fig. 1. Tissue-sampling regions (rectangle boxes) of *M. anguillicadatus*: ① Occiput region, ② Dorsal region, ③ Lateral region.

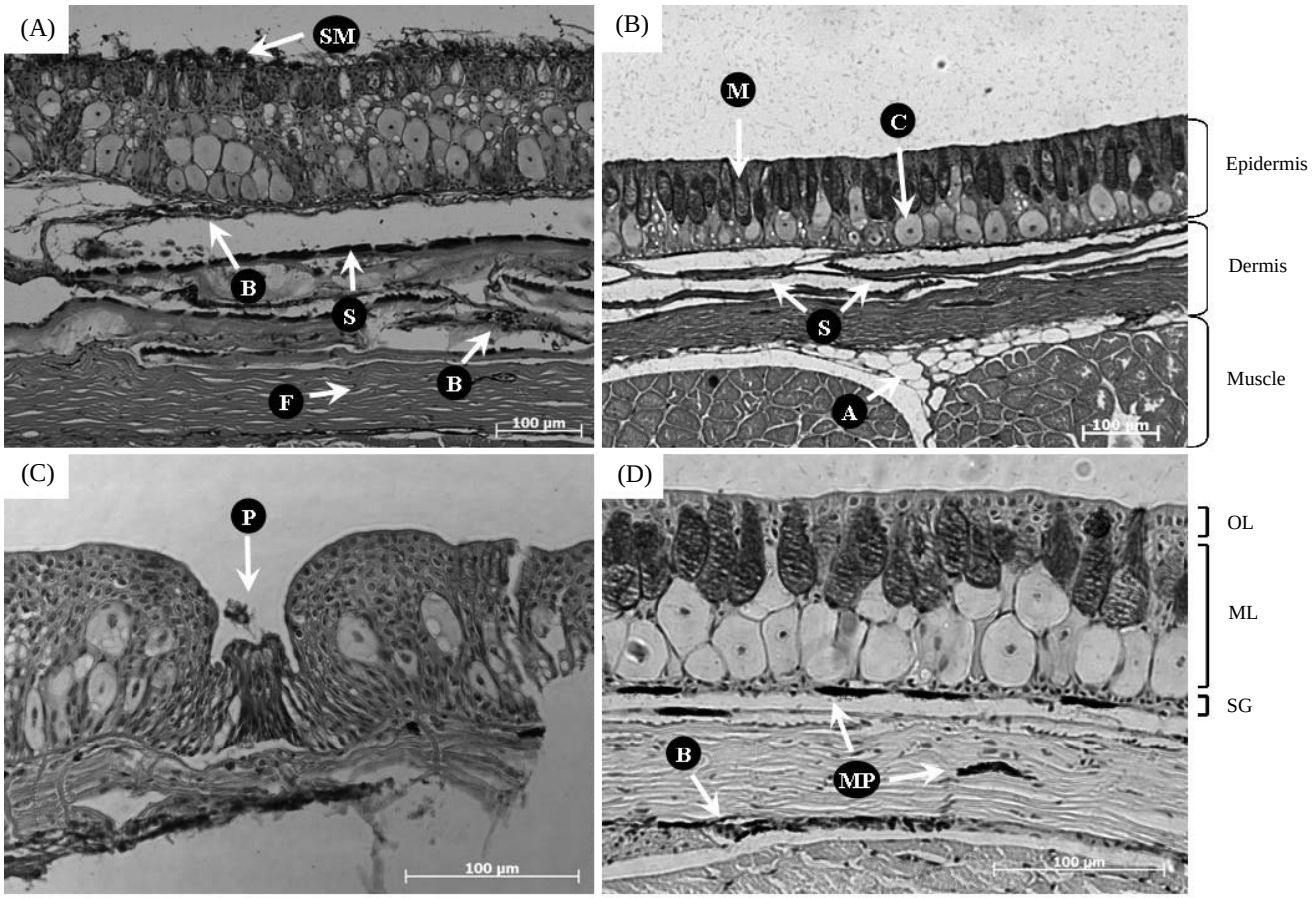

Fig. 2. General morphology of *M. anguillicaudatus* in occiput, lateral and dorsal region with hematoxylin-eosin. (A) Lateral region, Jan. 2008; (B) Dorsal region, April 2007; (C) Occiput region, June 2007; (D) Dorsal region, March 2007. A, adipose cell; B, blood capillary; C, club cell; F, fibroblast; M, mucus cell; ML, middle layer; MP, melanophore; OL, outermost layer; P, pit organ; S, scale; SG, stratum germinativum; SM, secreted mucous material.

ing from 90 to 120 mm in total length were monthly collected from sand-muddy bottoms in the tributary of Mangyeong River (Sam-Cheon stream), Korea from March 2006 to February 2007 using 5 mm × 5 mm scooping net. The specimens were fixed in 10% neutral formalin solution and the skin fragments were taken out from the following three regions: occiput, dorsal and lateral region (Fig. 1, Hubbs and Lagler, 2004). Skin fragments were subsequently prepared for general paraffin method and routine hematoxylin-eosin staining preparation (Presnell and Schreibman, 1997). The Axio imager.A1 (Carl Zeiss, Germany) and the Axio Vision (Ver. 4.5, Germany) was used for image capture and measurement. Analysis was conducted by the following methods: surface area of mucus cells layer, surface area and number of mucus cell in surface area of the epidermis per 1 mm length, respectively.

RESULTS

1. General morphology of the skin

The skin of *Misgurnus anguillicaudatus* is composed of epidermis and dermis. The epidermis could be divid-

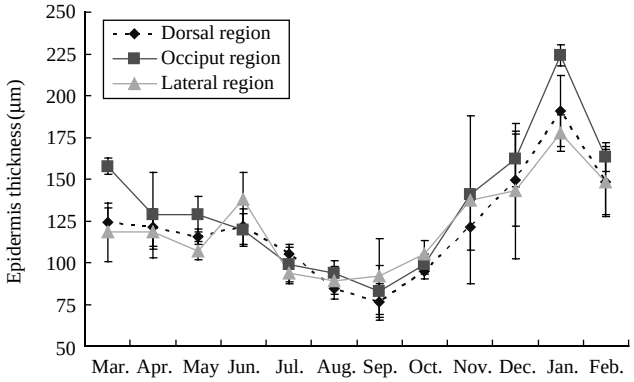

Fig. 3. Seasonal variations of epidermal thickness of *M. anguillicaudatus* from March 2006 to February 2007.

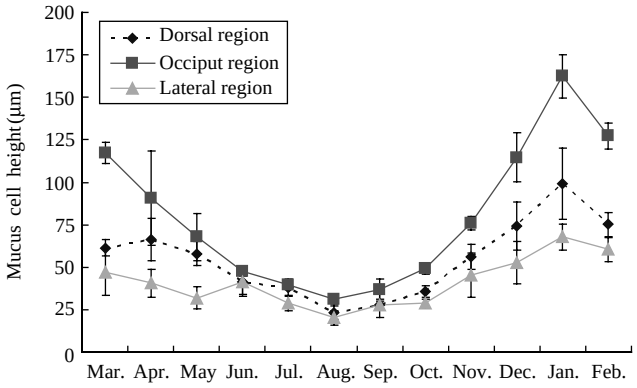

Fig. 4. Seasonal variations of mucus cell's height of *M. anguillicaudatus* from March 2006 to February 2007.

ed into three layers including outermost layer, middle layer and stratum germinativum (Fig. 2). The outermost layer is composed of stratified squamous cells in 1～3 rows and secretory openings of mucus cell, a mouth-part where the secretory materials are discharged outside the skin. The middle layer has two types of glandular cells, oval or spherical-shaped mucus cell and large club cell. Sometimes, the mucus cells in occiput were mostly transformed into large elongated unicellular form. They are stained dark-blue with haematoxylin, and club cell is stained pink with eosin. The stratum germinativum composed of a layer of cuboidal cells and a pear-shaped pit organ is found in overall three regions sunk into the basement membrane.

The dermis consisted of distinguishable two layers, outer loose connective tissue and inner dense connective tissue. The scales are embedded in loose layer, except for the occiput where no scales and loose layer exist. Many blood capillaries are distributed just above loose layer contacting with basement membrane. The inner dense connective tissues are characterized by regu-

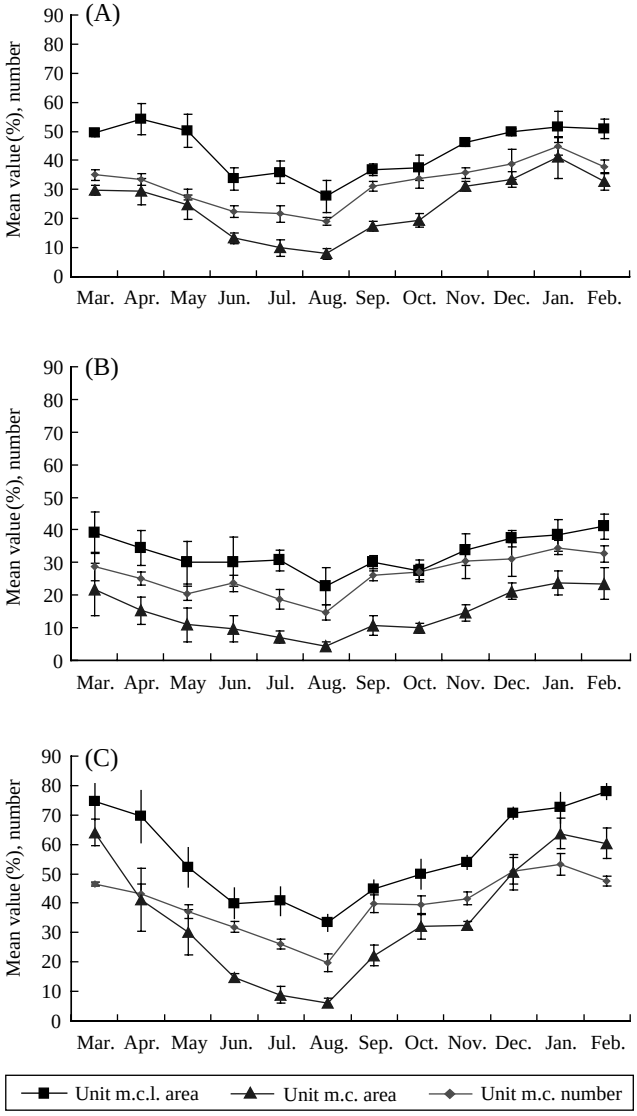

Fig. 5. The proportional mean value in surface area of the epidermis per 1 mm length of *M. anguillicaudatus* from March 2006 to February 2007. (A) Dorsal region, (B) Lateral region, (C) Occiput region. Abbreviations are as follows: m.c. (mucus cell); m.c.l. (mucus cells layer).

lar predominant collagen fiber with numerous fibroblast cells. Adipose cells are located in boundary between dense connective tissue and muscle part. Sometimes the melanophores existed in stratum germinativum and overall dermis region.

2. Seasonal change of the mucus cell

1) The thickness of the epidermis and the height of the mucus cell

The thickness of epidermis was considerably varied by season, particularly in both summer and winter in all three regions: 82.5±15.5 μm and 224.2±6.3 μm in the

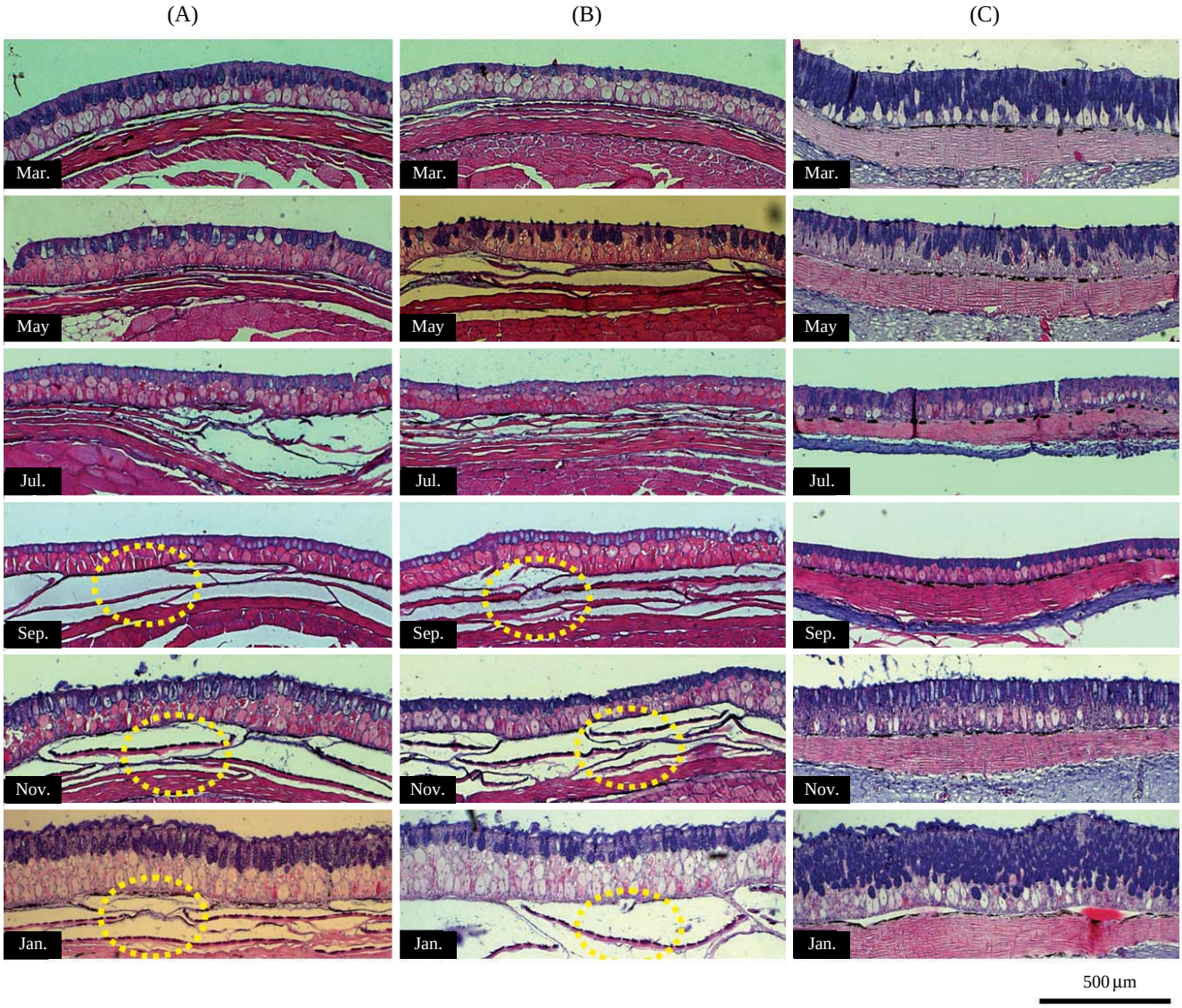

Fig. 6. Seasonal variations of epidermal three regions showing the evidence of morphological change of *M. anguillicaudatus* from March 2006 to February 2007. The yellow-dotted circles indicate voluminous dermis layer. Mucus cell stained with bluish haematoxylin are tiny in summer (July and Sep.), but they become elongated or enlarged one in winter (Nov. and Jan.). (A) Dorsal region, (B) Lateral region, (C) Occiput region. The magnification of all figures are × 100.

occiput, 89.3±8.1 μm and 177.5±11.1 μm in the lateral region, and 76.3±10.8 μm and 190.9±21.1 μm in the dorsal region, respectively (Fig. 3). As it is getting warmer, the thickness of epidermis decreased, except for the case of lateral region in June. The height of the mucus cell also showed a similar pattern as that of the epidermis: 31.1±2.7 μm for summer and 162.5±12.8 μm for winter in the occiput, 20.2±4.4 μm and 67.8±7.6 μm in the lateral region, and 23.2±3.8 μm and 99.3±21.0 μm in the dorsal region, respectively (Fig. 4). In particular, those of the occiput region for winter, 162.5±12.8 μm, are approximately 5 times larger than in summer, 31.1±2.7 μm, showing the most dramatic increase. The thickness of the epidermis and the height of the mucus cell increased or decreased coincidently as the season is changing.

2) Surface area of the mucus cells layer, surface area and number of mucus cell

In surface area of the epidermis per 1 mm length, the proportional mean values including surface area of mucus cells layer, surface area and number of mucus cell showed similar tendencies to the thickness of epidermis and the height of the mucus cell. It was lowest in summer and highest in winter: In the dorsal region, surface area of the mucus cells layer, surface area and

number of mucus cell were lowest in summer, 27.6±5.4 μm, 8.0±1.8 μm, 19.0±1.0 μm, and highest in winter, 54.2±5.4 μm, 41.0±7.1 μm, 45.0±3.0 μm, respectively (Fig. 5A). In the lateral region, also, 22.8±5.7 μm, 4.5±1.3 μm, 15±2.0 μm in summer and 41.0±3.9 μm, 23.9±3.7 μm, 35.0±2.0 μm in winter (Fig. 5B). In the occiput region, 33.4±2.8 μm, 6.1±1.5 μm, 20.0±3.0 μm in summer and 78.0±2.6 μm, 64.0±4.5 μm, 53.0±4.0 μm in winter, respectively (Fig. 5C). In the case of surface area of mucus cell in occiput region, especially, the difference between summer and winter is the most about 10 times in mean value.

3) Morphological change of the mucus cell

As it is gradually getting warmer, spring to summer, the mucus cell still keeps its spherical-shaped form in all three regions, which is mostly located at the outermost region with obviously downsized form. Otherwise, as it is getting colder, small and spherical mucus cells are transformed into elongated or enlarged one, which are generally arranged in 2～3 rows, particularly 3～5 rows in the occiput region (Fig. 6).

3. Seasonal change of dermis

Seasonal variations occurs in a definite area between the basement membrane and scale, forming some free space which scales are embedded in the loose connective tissue of the epidermis. These areas are filled with eosinophilic materials. Around summer season, the area is close toward the basement membrane. While getting cold, the area is accumulated with voluminous materials and then become more large. As a result, the dermis obviously thicken about 2 or 3 times than usual, summer season (Fig. 6A, B-Nov., Jan.).

DISCUSSION

3 Mud loaches, *Misgurnus anguillicaudaus*, *M. mizolepis* and *M. fossilis*, are often subjected to extremely bad conditions such as hypoxia in the water. To overcome such a unstable respiratory system, they are using skin for air-uptake, so-called cutaneous respiratory fishes (Jakubowski, 1958; Park and Kim, 1999; Park *et al*., 2001). Unfortunately, however, there are no studies dealing with seasonal change of epidermis related to the uptake of air in its natural habitat.

For *M. anguillicaudatus*, studying on seasonal change of mucus cell including size, shape, and density as well as the thickness of the epidermis and dermis throughout a year, we could confirm that there were morphological variations related to its environmental conditions such as temperature (T), dissolved oxygen (DO), pH, etc.. The habitat of *M. anguillicaudatus* had various environmental conditions by season and in particular showed a drastic change between summer and winter. According to annual records, T_{WATER}, DO and pH showed 4˚C, 14.0 mg/L, 8.6 in winter and 27˚C, 7.2 mg/L, 6.9 in summer, respectively. In both distinct seasons, summer and winter, unicellular mucus cells situated at the epidermis underwent seasonal changes in its size, as well as the number and amounts of mucus-secreting materials, affecting the thickness of the skin. All of them greatly increased in winter but did not in summer. Of course, some fishes were reported that rodlet cells, resembling mucus cell, were vary in number from fish to fish and several factors such as crowding, the season, ionic concentration of the water are closely related (Leino, 1974). However, these reports are not described in the natural habit throughout a year but in the short period-based artificial experiments. Moreover, the monthly change of epidermal structure had still little touched.

A thick epidermis in *M. anguillicaudatus* has been considered as one of characteristics needed for making active cell renewals in its epidermis (Tsai, 1996). According to Fishelson (1996), marin eels have a thick epidermis to compensates for a lack of armour against the abrasion and stress by the substrates during the digging. In contrast, the fishes having a thick epidermis consisted of two kinds of glandular cells (mucus cell and club cells), like *M. anguillicaudatus*, has been known as cutaneous respiratory fishes: Anguillidae, Cobitidae, Gobiidae, Synbranchidae, Channidae (Jakubowski, 1958; Liem, 1967; Mittal and Munshi, 1971; Graham *et al*., 1977; Graham, 1997; Park and Kim, 1999, 2001; Park *et al*., 2000, 2001, 2003, 2005; Park, 2002; Sayer, 2005; Jang, 2007). Park and Kim (1999) suggested that thick epidemis of *M. anguillicaudaus* is one of proof allowing them to uptake air through skin. However, there was no the detailed information on seasonal change of epidermis and overlooked on which season is more effective for air-uptake.

Fish mucus have been well known various functions as the following: cell for respiratory gas exchange, ion and water osmoregulation, chemical and physical protection, disease resistance as immune system, and filter feeding (Liem, 1967; Mittal and Munshi, 1971; Shephard, 1994). In particular, Letterer (1959) and Rogers (1961) have reported that mucus substances had great ability to bind a large amount of water. Hora (1934) and Mittal *et al*. (1980) have shown that mucus has a remarkable power for precipitating mud held in suspension in water, and that the mucus secreted by the skin in air breathing fishes may also be used to keep the skin clear for respiration. Liem (1967) mentioned the feature of integument mucus that the great abundance of extraordinarily large unicellular mucus glands is undoubtedly correlated with burrowing and amphibious habits.

Through the present study, it was confirmed that the size and number of the mucus cell, as well as thickness

of the mucus cells layer and the skin were very well developed as it is getting cold, which reach up to 2 times than summer. Also the amount of mucus secreted by mucus cell became more in winter. On the basis of the above results, we could draw up at least 2 facts. Firstly, *M. anguillicaudatus* known as a cutaneous respiratory fish seems that winter season diminishing in water temperature and dissolved oxygen in natural habitats let them respire through skin more frequently than any other seasons. Secondly, they inhabit under the sand or muddy bottom, mainly in winter and they are obliged to dig down the burrow deeply. To prevent physical wounds or abrasions during the digging burrow, they looks like lubricate their body with mucus secreted by mucus cell. Among the observed 3 body regions, the occiput region showed a great increase in thickness and mucus cell. Because the occiput head sandy-muddy bottom to dig the burrow, the occiput region may be require any ecological features such as more thick skin and lots of mucus than other body regions. In addition, however, other environmental factors may be related to the seasonal change of skin in various ways. To verify the relationship between seasonal chance of the skin and environmental factors, further experiments will be necessary.

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계절변화에 따른 한국산 미꾸리, *Misgurnus anguillicaudatus* 피부의 조직학적 연구

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요 약 : 미꾸리 *M. anguillicaudatus*의 등, 후두부, 체측 부위에서 적출한 피부조직의 형태를 분석하여 계절의 변화에 따른 각 부위 조직의 형태변화를 관찰하였다. 각 피부조직은 점액세포를 포함한 상피세포들로 구성된 표피층과 결합조직의 진피층으로 구성되어 있었으며, 표피와 진피층의 경계에 모세혈관이 분포하고 있었다. 표피의 middle layer에 분포하는 점액세포는 수온이 낮아짐에 따라 그 형태가 원형에서 타원형 또는 장타원형으로 변화하였고, 단위면적당 세포의 개수와 크기 및 분비량이 증가하였는데, 특히 후두부에서 그 변화양상이 가장 크게 나타났다. 또한, 등과 체측 부위의 비늘이 존재하는 진피층에서는 eosin에 염색되는 염기성 물질이 상당량 분비되어 진피층의 두께가 두꺼워지는 경향을 보였다. 계절변화에 따른 이러한 피부조직의 구조적 변화를 미꾸리의 동면, 피부호흡 및 점액의 기능과 관련하여 고찰하였다.

찾아보기 낱말 : 미꾸리, 점액세포, 표피층, 진피층, 피부호흡