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Seasonal Change of Skin Mucus Cells of *Misgurnus mizolepis* (Cobitidae) Dwelling in a Natural Stream in Korea

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ABSTRACT **Histological observation on the seasonal variation of mucus cells of the mud loach *Misgurnus mizolepis* inhabiting a natural stream was carried out on three skin regions (dorsal, lateral and occiput) from March 2008 to February 2009. Our results showed no differences in general morphology by season, but the mucus cells of the epidermis showed significant seasonal change in their size and number as the water temperature changed. The ratio of surface area of the mucus cell layer and mucus cells, and the number of mucus cells in surface area of the epidermis were the greatest in the cold winter and the least in the hot summer in all regions of the epidermis. In particular, the occiput seemed to be a very sensitive region in response to environmental change, showing wide fluctuations in the size of mucus cells throughout the year and a great change in between seasons, especially from late autumn to early winter when the temperature decreased. As the temperature became colder, a small and spherical-shaped mucus cell was transformed into a large and elongated columnar form with a lot of secreted mucus material in a superficial layer of the epidermis. From our results, we can safely surmise that cold temperature is an important environmental factor having a close relationship with the modification of mucus cells of *M. mizolepis* in winter.**

Key words : Mucus cell, mud loach, *Misgurnus mizolepis*, natural stream, cold temperature, environmental factor

INTRODUCTION

The muddy loach *Misgurnus mizolepis* belonging to the Cobitidae is distributed in Korea and China, and they mainly inhabit the muddy bottom of the swamps, streams, irrigation canals and ricefields (Kim *et al*., 2005). The ricefield undergoes various environmental fluctuation such as an irrigation for cultivating in spring and drainage in autumn, injection of agricultural chemicals that can cause damage to an aquatic organism. And this habitat is also subjected to extraordinary high temperature of the water in summer, drainage and evaporation (drought) for harvest in autumn. These unstable situations are repeated in the following years with similar conditions. Especially, due to the lack of water in winter, *M. mizolepis* dwelling in the ricefield begin to dig into mud bottom to protect their body from a loss of water over the skin. To overcome these difficulties, *M. mizolepis* had bear any changes in mucus cells distributed over the skin, showing a drastic seasonal variation in its size and number according to the fluctuation of the temperature (Oh and Park, 2009).

In general, fishes are primarily gill breather, but in some fishes, they can use secondary respiratory organ as follows; skin (Anguillidae, Cobitidae, Gobiidae, Synbranchidae), intestine (Cobitidae, Trichomycteridae), branchial chamber (Anabantidae, Channidae) and swim bladder (Lebiasinidae) (Liem, 1967; Mittal and Munshi, 1971; Graham *et al*., 1977; Le Moigne *et al*., 1986; Park and Kim, 1999, 2001; Park *et al*., 2000, 2001, 2003, 2005; Park, 2002; Randle and Chapman, 2005; Sayer, 2005). In particular, the fishes doing cutaneous respiration characterized by a thicker epidermis due to a large glandular cell (Park and Kim, 1999), and it undoubtedly correlated with burrowing and amphibious habits (Liem, 1967). Furthermore, cutaneous modification is to resist excessive water loss during the state of aerial exposure (Sayer,

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2005). As the *M. mizolepis* dwelling in the ricefield is directly exposed to the air through the burrow, the size of mucus cell is simultaneously drastically increased in winter (Oh and Park, 2009). Although *M. mizolepis* prefer ricefileld, it is also true that they inhabit natural streams. Therefore, some question still remained whether such changes on mucus cell occurring in the ricefield really appeared in natural streams, not ricefield areas. So we investigated any seasonal variations of mucus cells of *M. mizolepis* living in the natural stream where is not extremely exposed to air, unlike the ricefields, and compared with those of ricefield-dwelling muddy loach.

MATERIALS AND METHODS

Three specimens of *Misgurnus mizolepis* were monthly collected from the stream in Jangsu-gun, Jeonbuk-do, Korea from March 2008 to February 2009. The specimens were fixed in 10% formalin solution, and skin tissues containing mucus cell within epidermis were taken from three regions of dorsum, lateral and occiput (Fig. 1). The skin samples were dehydrated by a standard ethanol series, cleared in xylene, and embedded in paraffin. The tissues were sectioned in 5 μm thickness, deparaffinized and stained with Hematoxyin-Eosin (Presnell and Schreibman, 1997). Analysis for seasonal change of mucus cell was conducted using an Axio imager A1 microscope (Carl Zeiss, Germany) and Axio Vision (ver. 4.5, Germany) by a following methods: ① the ratio of surface area of mucus cells layer (m.c.l.) and ② mucus cells (m.c.) in surface area of the epidermis layer per 1 mm length, ③ the number of mucus cells in epidermis layer per 1 mm length (Oh and Park, 2009). One-way analysis of variance (ANOVA) and Duncan's multiple range test ($P = 0.05$) was conducted on the seasonal change of mucus cell (ver. spss 12.0).

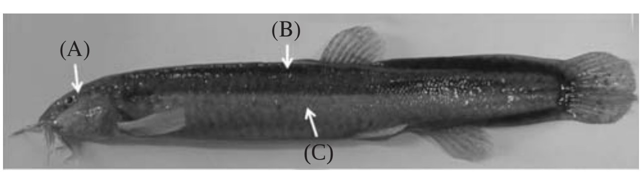

Fig. 1. Tissue sampling regions of a skin of *Misgurnus mizolepis*. (A) occipital region, (B) dorsal region, (C) lateral region.

RESULTS

1. General structure of skin

Structure of skin of *Misgurnus mizolepis* consisted of epidermis and dermis in the dorsal, lateral and occiput regions (Fig. 2).

The epidermis can be divided into three layers of outermost layer, middle layer and stratum germinativum. The outermost layer was composed of cuboidal epithelial cells arranged in 1 to 3 rows, and mucus cell originated from the middle layer expanded outward which its opening connected with superficial layer of the skin. The middle layer had mucus cells and club cells surrounded with epithelial cells. The mucus cell showed Hematoxylin positive (basophilic), but the club cell was rather acidophilic. The stratum germinativum, a structural base of the epidermis consisted of 1 or 2 rows of epithelial cells upon the basal membrane (Fig. 2).

The dermis consisted of stratum laxum and stratum compactum. In the dorsal and lateral regions, the stratum laxum in which the scales were lodged showed somewhat irregularly arrangement of the composing units, so-called a loose connective tissue. This layer is composed of many fibroblast cells that makes a collagen fiber, scales, and blood capillaries. Sometimes, a melanophores were seen in this layer. The upper bony layer of the scale was basophilic, Hematoxylin positive, and the lower fibrillary plate was acidophilic, Eosin positive (Fig. 3A, B). In the occipital region, however, the stratum laxum is absent due to the lack of scales (Fig. 3C). The stratum compac-

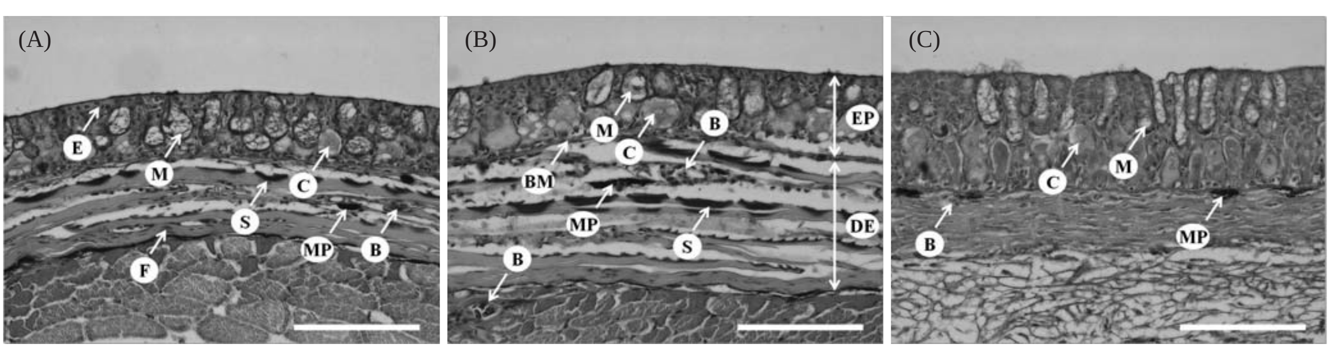

Fig. 2. General structure of skin of *M. mizolepis* in the dorsal, lateral and occiput region. (A) dorsal region, July 2008, (B) lateral region, July 2008, (C) occipital region, July 2008. B, blood capillary; BM, basement membrane; C, club cell; DE, dermis; E, epithelial cell; EP, epidermis; F, fibroblast cell; M, mucus cell; MP, melanophore; S, scale. H-E staining. Bars indicate 100 μm.

tum, a dense connective tissue was characterized by regularly stratified collagen fiber bundles and it also exhibited acidophilic. This laminar propria located under the stratum laxum and provided function of muscle adhesion region (Fig. 2).

2. Seasonal variation of mucus cell

The mucus cells observed from the three skin's regions showed seasonal changes as the water temperature changes. The surface area of mucus cell layer and mucus cell, and the number of mucus cell in epidermis layer per 1 mm length were the greatest in cold winter and the least in hot summer.

In the dorsal region, the ratio of surface area of mucus cells layer was the greatest in December (79.2±3.7), and the least in August (40.6±4.1) (Fig. 3A). There was also a similar tendency in the ratio of surface area of mucus cells that is the greatest in December (62.9±6.4), and the least in August (9.5±2.5) (Fig. 3B). The number of mucus cells was also abundant in January (52.0±3.7), and few in August (23.0±2.6) (Fig. 3C).

In the lateral region, the changing rate of mucus cell showed somewhat moderate aspect than those of other

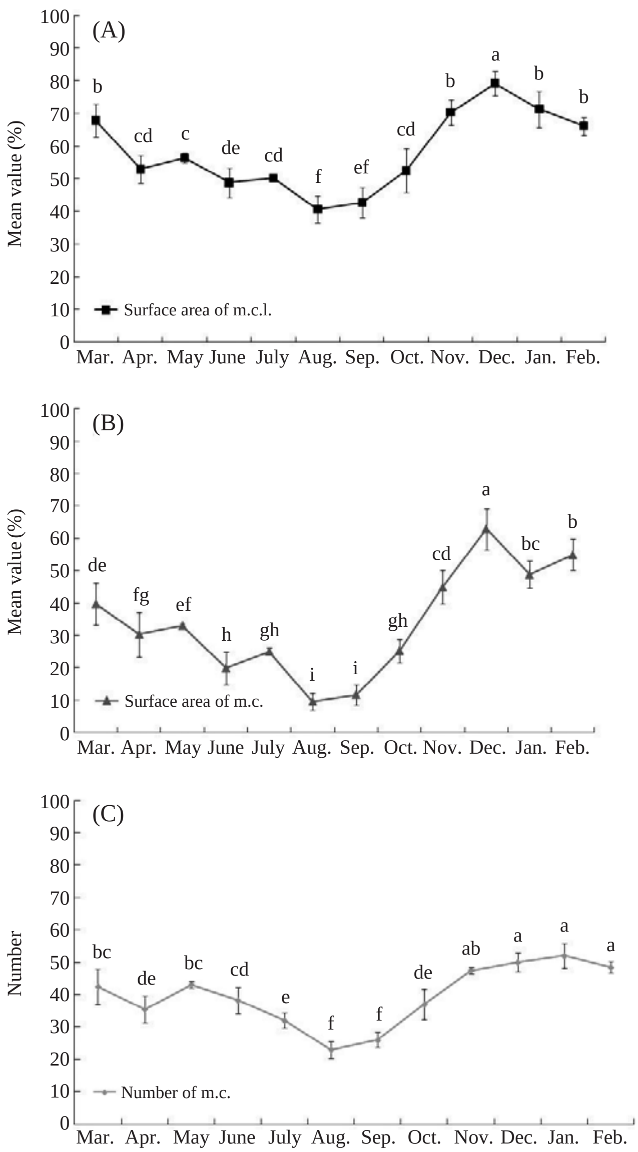

Fig. 3. Seasonal changes of the mucus cell over dorsal region from March 2008 to February 2009, *M. mizolepis*. Same letters above the values mean no significant differences ($P > 0.05$).

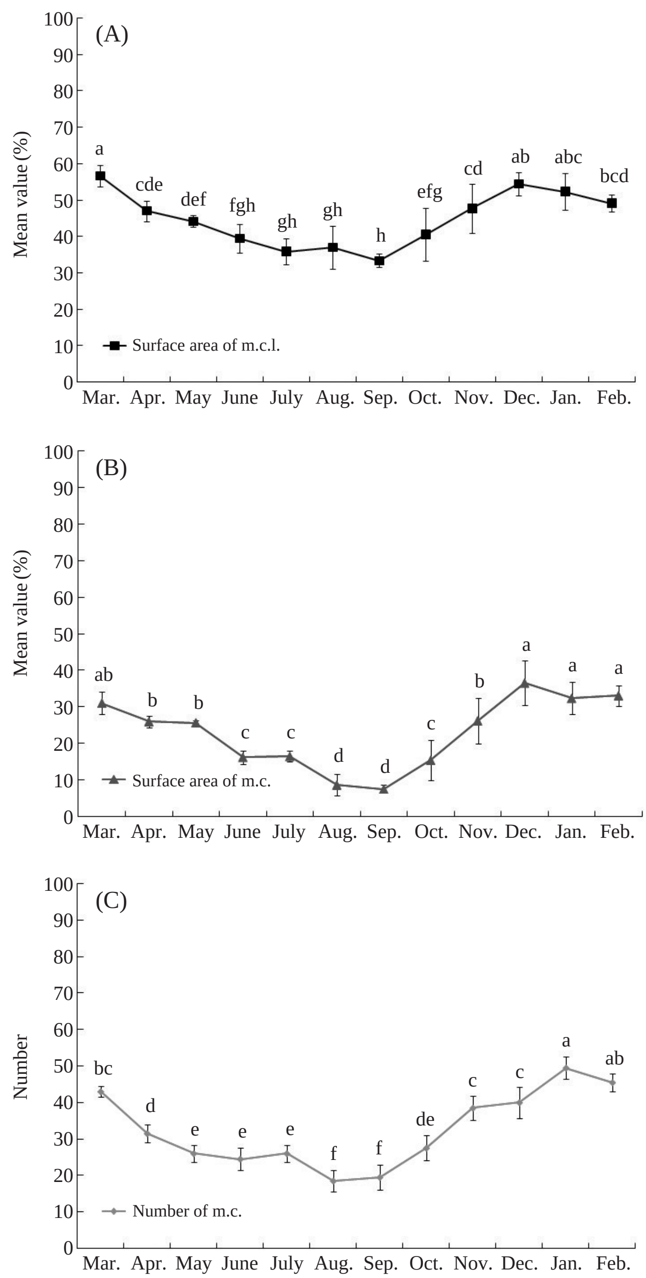

Fig. 4. Seasonal changes of the mucus cell of *M. mizolepis* in lateral region from March 2008 to February 2009. Same letters above the values mean no significant differences ($P > 0.05$).

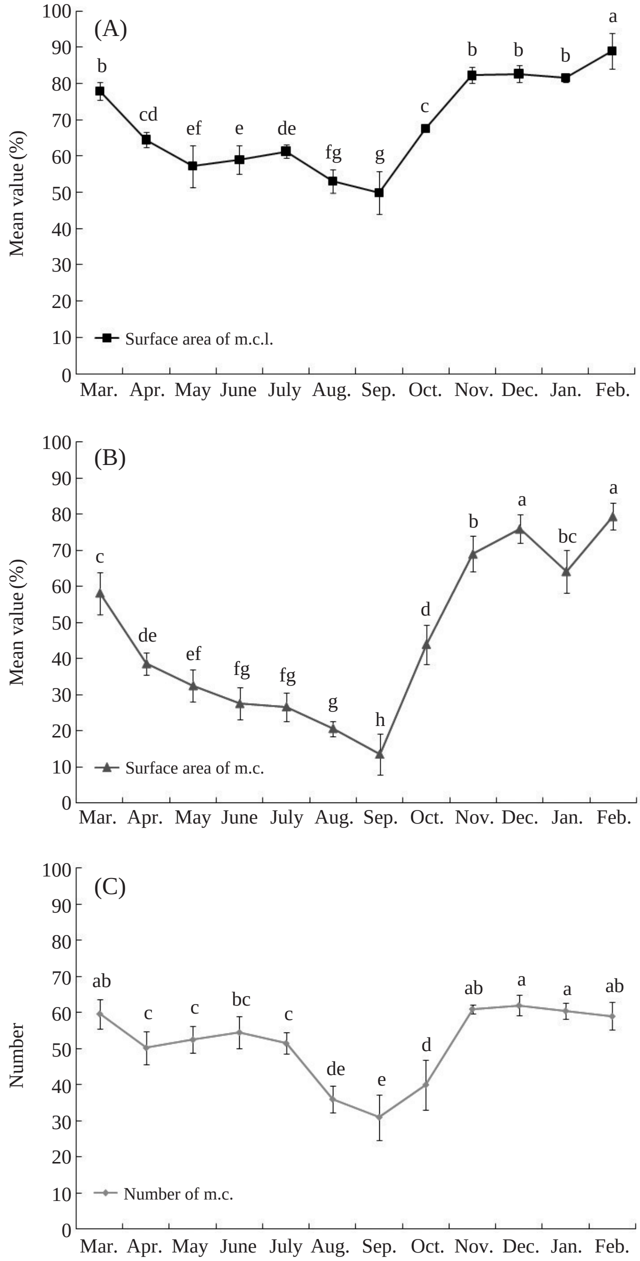

Fig. 5. Seasonal change of the mucus cell of *M. mizolepis* in occipital region from March 2008 to February 2009. Same letters above the values mean no significant differences ($P > 0.05$).

regional skins. The surface area of mucus cell layer showed the maximum value in March (56.7±3.1), and the minimum in September (33.4±3.1) (Fig. 4A). The number and the size of the mucus cell were remarkably reduced around the summer (19～20 and 7.5～8.6%, respectively), but in the winter season, it was the greatest from December to January (40～50 and 32.3～36.5%, respectively) (Fig. 4B, 4C).

In the occipital region, the surface area of mucus cell layer was the greatest in February (88.9±5.0) and the least in September (49.8±5.9), which the lowermost value was even higher than those of the other regions (Fig. 5A). From the analysis of the surface area of mucus cell, the values were the greatest in February (79.5±3.7) and the least in September (13.5±5.8). Since September, the value highly rose by December (Fig. 5B). Therefore, such tendency on the changing rate make the difference between the lowest and highest value greater among the three skin regions. The number of mucus cells also showed a similar pattern like its size (Fig. 5C).

3. Shape of mucus cell

The mucus cell was also changed in shape as the water temperature fluctuate (Fig. 6). In summer in moderate water temperatures ranging from 21～25°C, the cells were generally small and spherical, and sometime it seemed to be somewhat thin-and-rod type in the occipital region. These mucus cells were mostly located near to the superficial layer of the epidermis. In winter (5～11°C), however, the mucus cells became enlarged and finally showed a columnar form. Moreover, its number was predominantly increased and then the surface of the skin was covered with massive mucous materials secreted by mucus cell.

DISCUSSION

Functions for fish mucus were well known for the respiratory gas exchange, ion and water regulation, disease resistance, chemical and physical protection, and so on (Shephard, 1994). These features are very important for survival in bimodal breathing species like *Misgurnus*, *Monopterus*, *Anguilla* species concerning to cutaneous respiration (Liem, 1967; Shephard, 1994, Park and Kim, 1999; Park *et al*., 2001; Sayer, 2005; Oh and Park, 2008, 2009). In particular, *Monopterus albus*, an absolutely bimodal respirator operated their cutaneous system across the mucous coat of the epithelium even in water, and this event took place more intense in air just about twice in quantity of oxygen consumption (Liem, 1967). But its osmoregulatory ability could be fail if the mucous coat removed from the epidermis, which it was supported by osmotic and ionic regulation experiments from *Anguilla anguilla* (Shephard, 1994). Remarkablely, the cutaneous respiration is most effective at the low temperature, that is, not above 15°C in *Anguilla*, at 10°C in *Misgurnus*, and at a relatively high temperature (23°C) in *Monopterus* (Liem, 1967). These results coincided with our result that the mucus cell of the skin of *M. mizolepis* was predominantly increased in cold winter season, which the range of the water temperature recorded 5～11°C and Dissolved Oxygen (DO) was 12～14 mg/L from November 2008 to March 2009. Moreover, superficial layer of the skin was covered with a lot of secreted mucous mate-

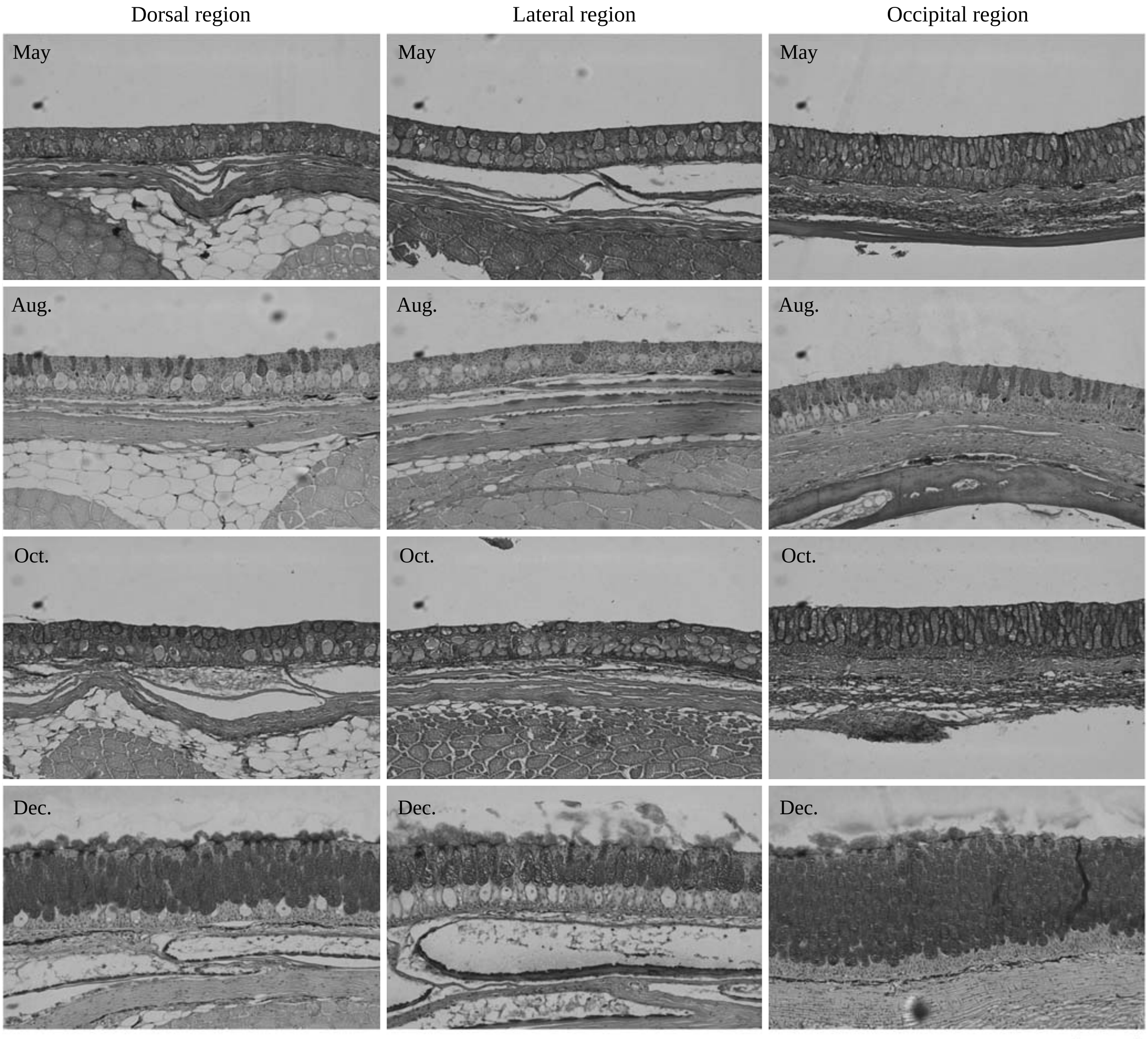

Fig. 6. Seasonal changes of mucus cell of *M. mizolepis* in shape from March 2008 to February 2009. Bar indicates 100 µm. H-E staining.

rial (see Fig. 6), these figures were as same as *M. anguillicaudatus* (Oh and Park, 2008) and *M. mizolepis* (Oh and Park, 2009) in winter season. As the great abundance of extraordinarily large mucous glands is undoubtedly correlated with burrowing and amphibious habits (Liem, 1967), *M. mizolepis* seemed to be adapted to an unstable environment by means of modification of skin structure for survival.

A general structure of epidermis of *M. mizolepis* in present study was very similar to *M. anguillicaudatus* inhabiting in natural stream where the habitat was always full of water (Oh and Park, 2008), and also to *M. mizolepis* in ricefield (Oh and Park, 2009). Furthermore, the seasonal change of the mucus cell was very similar in even different habitat, ricefilelds and streams, indicating that the size and number of the mucus cell was distinctively increased in winter, and vice versa in summer. In spite of such similar changes of the mucus cells, there was a different pattern in the ricefield-dwelling mud loach (Oh and Park, 2009), because there might be environmental fluctuations as followings; an irrigation for cultivating in spring, a drainage for harvesting in autumn, and sudden increase of water temperature in summer. In these conditions, the mucus cell showed an abnormal modification pattern. In natural stream, however, as these unstable environmental factors rarely occur in the natural

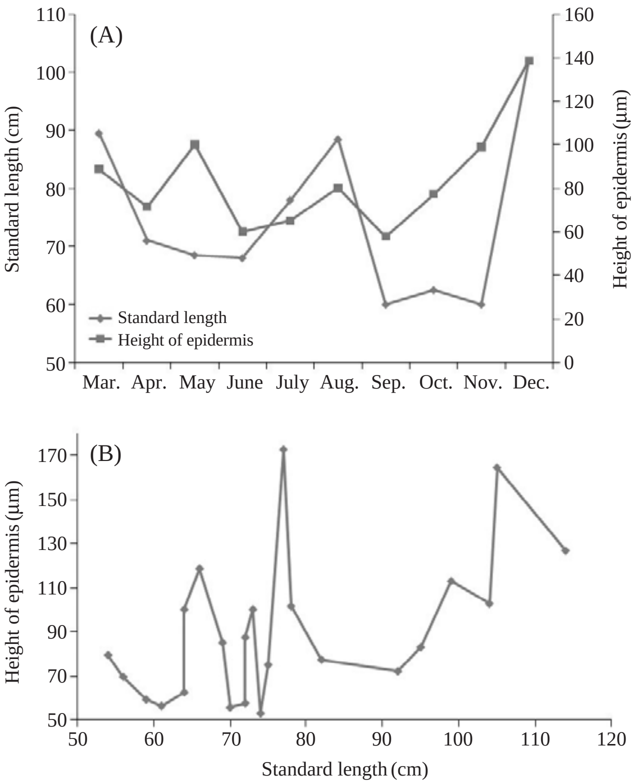

Fig. 7. Relationship between standard length and height of epidermis in *M. mizolepis*. (A) mean values arranged by season, (B) raw data excluding seasonal factor.

stream, the modification pattern of the mucus cell of *M. mizolepis* in present study was somewhat gradual, not drastic, except for the occipital region (Fig. 5B). We think that a drastic change in the occipital region is more likely be very sensitive than other regions.

With the change of the mucus cell in its size and number, we investigated relationship between standard length and height of epidermis. As a result, there were not significant difference ($P > 0.05$, Pearson's correlation coefficient; Fig. 7A), whereas the height of epidermis was absolutely influenced by the water temperature like the mucus cell. The height of epidermis at 74 cm standard length (SL) in July was average 53 μm (the lowest value in present study), but that of 77 cm (SL) in February was average 173 μm (the highest one), over three times thicker than that of summer. And at 95 cm (SL) in August it was average 83 μm and 114 cm (SL) in February was average 127 μm (Fig. 7B). In spite of ambiguous relationship between them, however, there was a conspicuous fact that the height of epidermis is greatly increased as soon as the water temperature decrease. This phenomenon might be somewhat related with their ecological habitat. According to Fishelson (1996), when the moray eel (Muraenidae) inhabiting in the rock and sand bottom had a thick epidermis reaching up to 140 μm and consisted of 4~15 rows of the epithelial cells, these features were not only a compensation for a lack of armour on their skin, but also to prevent abrasion and stress by the substrate when they crawl on the bottom and dig between the rocks and sand. And the dotted dragonet *Callionymus risso* has an abundant mucous film that it might serve as protection from the sand or mud in which it buries itself (Sadovy *et al*., 2005). This finding is coincide with the result of present study that *M. mizolepis* is subject to abrasion even in the water throughout a year, which is their ecological nature (Kim, 1997). Seeing Fig. 7A, the height of epidermis gradually increase in the winter, and then during this period, the mud loach does not appear any more in the sand-muddy bottom by digging and hibernating in the burrow till the next spring. According to Oh and Park (2009), in reality, a ricefield-dwelling mud loach have made burrows of about 15~30 cm deep and came out close to spring after hibernation (wintering). Comparing *M. mizolepis* of ricefields and streams, we can surmise that at least the factor of "cold temperature" would be closely related with any change of the mucus cell occurring in the skin in *M. mizolepis*. However, further controlled experiments regarding aerial exposure are required for proving this hypothesis.

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자연하천에 서식하는 미꾸라지 *Misgurnus mizolepis* 피부 점액세포의 계절변화

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요 약 : 자연하천에 서식하는 미꾸라지의 등, 체측, 후두부 피부조직에 대한 점액세포의 계절변화를 조사하였다. 2008년 3월부터 2009년 2월까지 계절변화에 대한 월별 조사에서 피부조직의 일반적인 형태변화는 없었으나, 상피조직 단위면적당 존재하는 점액세포의 크기와 개수는 겨울철에 가장 높게 나타났고, 여름철에 가장 낮게 나타나 계절변동에 따른 점액세포의 유의한 변화가 관찰되었다. 특히 후두부 부위는 점액세포의 계절변동 폭이 크고, 늦가을 환절기의 기온급감에 따른 점액세포의 급격한 변화를 보여 환경변화에 대한 반응이 가장 민감한 부위로 여겨진다. 또한 점액세포는 수온이 감소함에 따라 외부형태가 작은 구형에서 크고 긴 원주세포형으로 변화하였으며, 다량의 점액물질이 분비되었다. 본 연구를 통해 "저온"은 겨울철 미꾸라지 피부의 점액세포를 변화시키는데 밀접한 연관이 있는 중요한 환경요인으로 여겨진다.

찾아보기 낱말 : 점액세포, 미꾸라지, 자연하천, 저온, 환경요인