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Temporal Variations of Skin Mucus Cells of *Misgurnus mizolepis* (Cobitidae) by a Change of Water Temperature

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ABSTRACT We studied any variations of skin mucus cells of *Misgurnus mizolepis* caused by inducing a great change of water temperature including high temperature- and low temperature-adapted groups and then compared them with a control group of normal water condition. The high temperature-adapted group showed no significant different in size and number of the mucus cell ($P>0.01$), whereas in the low temperature-adapted group, the surface area of mucus cell layer and shape of its mucus cell, and the number of mucus cell remarkably increased in all the skin regions of dorsum, lateral region and occiput ($P<0.01$). Returned to the same condition as the control group, the low temperature-adapted groups showed the same features that appear in the normal water condition ($P<0.01$). Based on these results, the skin mucus cells of *M. mizolepis* seems to be very sensitive to cold water temperature and therefore they may play a key role in assessment of its environmental conditions.

Key words : Mucus cell, *Misgurnus mizolepis*, high temperature-adapted group, low temperature-adapted group, environmental conditions

INTRODUCTION

A muddy loach *Misgurnus mizolepis* (Cobitidae) is distributed in the East Asia (Korea and China), and they live in muddy bottom of swamps, irrigation canals, and rice fields (Kim *et al.*, 2005). The mucus cell covering their skin is considered to be a sensitive organ responding to variable environmental conditions, a water temperature and an aerial exposure of the skin (Oh and Park, 2009, 2010, 2011). In the field study, the size and the number of the mucus cell were predominantly increased in cases of that the loach is directly exposed to the air, regardless of temperature surrounding them (Oh and Park, 2009, 2011) and even in the winter season getting cold (Oh and Park, 2010). This similar phenomenon was already revealed in a closely related species *M. anguillicaudatus* (Oh and Park, 2008).

In many species, it was suggested that the function of the mucus cell is closely related to chemical and physical protection, disease resistance and cell renewal, cutaneous respiration for a bimodal respiration system (Liem, 1967; Mittal and Munshi, 1971; Shephard, 1994; Fishelson, 1996; Tsai, 1996; Park and Kim, 1999; Park *et al.*, 2001; Sadovy *et al.*, 2005; Sayer, 2005; Oh and Park, 2009). With various functions, the mucus cell is certain to represent well its characteristics adapted to the surrounding environment. Therefore, the skin mucus cell of *M. mizolepis* could used for environmental indicator that can detect any change degree of aquatic circumstances, especially water temperature. Meanwhile, it still need more information to support relation between water temperature and indicator in the field. Therefore, we conducted artificially a water temperature-changing experiment to know about the change degree that the mucus cell is likely affected by water temperature.

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MATERIALS AND METHODS

Specimens of *Misgurnus mizolepis* reaching 98 ~ 105 mm in total length were collected from a rice field in Jangsu-gun, Jeollabuk-do, Korea in August 2009. They were transferred to a small tank (47 × 29 × 28 cm) and acclimated to the normal water temperature (18°C) for three months. The experiment for radical change of water temperature consisted of two experimental groups, a high temperature-adapted group and a low temperature-adapted group, and finally two control groups. The control groups were just fishes acclimated in 18°C water temperature, pre-experiment (C1) and post-experiment (C2). The two experimental groups were taken from the control group of pre-experiment (C1).

For the high temperature-adapted group, the water temperature reached 18°C to 32°C for 7 days, rising 2°C per a day and then maintained 32°C for 3 days (H1). Once again, the water temperature adjusted to primary water temperature (18°C) for 7 days, falling 2°C per a day, and kept up with the temperature for 3 days (H2). In the same manner, the low temperature-adapted group was induced by dropping down 4°C from normal 18°C for 7 days and acclimated for 3 days (L1), and inversely, it's water temperature rose to 18°C through 7 days and finally was adapted for 3 days (L2). Setting up the water temperature in both of two experiment groups were based on the seasonal variations of water temperature in Korea.

Three specimens from every control groups (C1, C2) and experimental groups (H1, H2, L1, L2) were anesthetized with MS222, fixed in 10% of neutralized formalin solution, and the skin tissues were taken from three regions of dorsum, lateral and occiput to investigate any change of the mucus cell between the control group and experimental group (Fig. 1). All tissue was dehydrated in ethanol series, cleared in xylene, and embedded in wax. The preparations were sectioned with 5 μm thickness, deparaffined in xylene and stained with Hematoxylin-Eosin (Presnell and Schreibman, 1997).

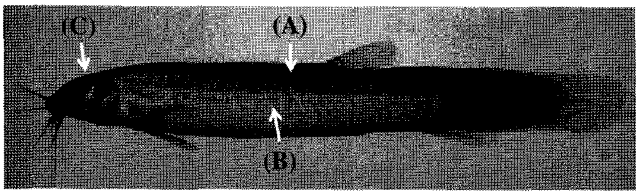

Fig. 1. Tissue sampling regions of skin of *Misgurnus mizolepis*. (A) dorsum, (B) lateral and (C) occiput.

Analysis of the skin mucus cell was conducted with an Axio imager A1 microscope (Carl Zeiss, Germany) and an Axio Vision (ver. 4.5, Germany), and the methods were as follows: The ratio of surface area of mucus cells layer (mcl) and mucus cells (mc) in surface area of the epidermis layer per 1 mm length; the number of mucus cells in epidermis layer per 1 mm length (Oh and Park, 2009, 2010, 2011). One-way analysis of variance (ANOVA) followed by Duncan's multiple range test ($P=0.01$) were conducted using SPSS ver. 12.0.

RESULTS AND DISCUSSION

1. General structure of the skin

The skin of *Misgurnus mizolepis* consisted of epidermis and dermis in every regions of dorsum, lateral and occiput. The epidermis is occupied by three major cells-epithelial cell, club cell, and mucus cell secreting mucus outside. The mucus cell was positive for Hematoxylin, whereas the club cell was stained weakly with Eosin. The dermis is located underneath the basement membrane belonging to stratum germinativum of the epidermis (Fig. 2). They have more various components including scales, melanophores, blood capillaries, fibroblast cells and connective tissue. This basic structure was nearly similar with *M. mizolepis* dwelling in a natural stream (Oh and Park, 2010) and *M. anguillicaudatus* (Oh and Park, 2009).

2. Variations of the mucus cells

1) Control groups

The proportions of the skin mucus cell of *M. mizolepis* in two control groups of pre- and post-experiment were analyzed by a given method, and the values were compared each other to verify whether it is adequate to control group. As a result, there were not any differences between two control groups in all skin region ($P>0.01$).

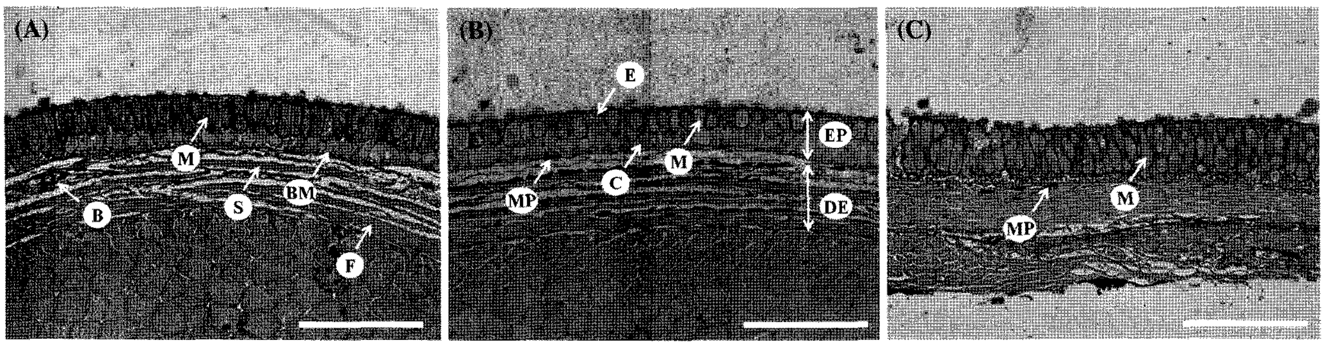

Fig. 2. General structure of *Misgurnus mizolepis* in the normal water temperature corresponding to two control groups (C1 and C2) in present study. (A) dorsum, (B) lateral and (C) occiput. B, blood capillary; BM, basement membrane; C, club cell; DE, dermis; E, epithelial cell; EP, epidermis; F, fibroblast cell; M, mucus cell; MP, melanophore; S, scale. Bars indicate 200 μm. H-E staining.

Table 1. Variations of the skin mucus cell in three regions of dorsum, lateral region and occiput in *Misgurnus mizolepis*

	Dorsum				Lateral				Occiput			
	C1	H1	H2	C2	C1	H1	H2	C2	C1	H1	H2	C2
mcl	66.1±0.3	58.6±5.3	55.6±4.2	65.8±7.3	52±8.2	40.3±6.7	48.2±3.8	51.3±8.2	83.8±3.4	75.6±4.8	80±3.7	81.8±5.8
mc	51.7±0.9	48.6±7.7	39.3±4.2	51.2±8.4	37.8±11.1	29.6±5.6	35.8±5.6	38.7±9.5	65.2±0.8	70.5±5.8	68.4±2.2	68.2±7.2
N.	45±1.2	46±2.3	42±1.7	42±2.1	42±4.2	39±3.4	40±0.5	41±3.8	55±1.2	56±3.7	53±3.1	52±3.7

C1: control group of pre-experiment, C2: control group of post-experiment, H1: high temperature-adapted group (32°C), H2: high temperature-adapted group (H1→18°C), mcl: surface area of mucus cells layer, mc: surface area of mucus cells, N: the number of mucus cell.

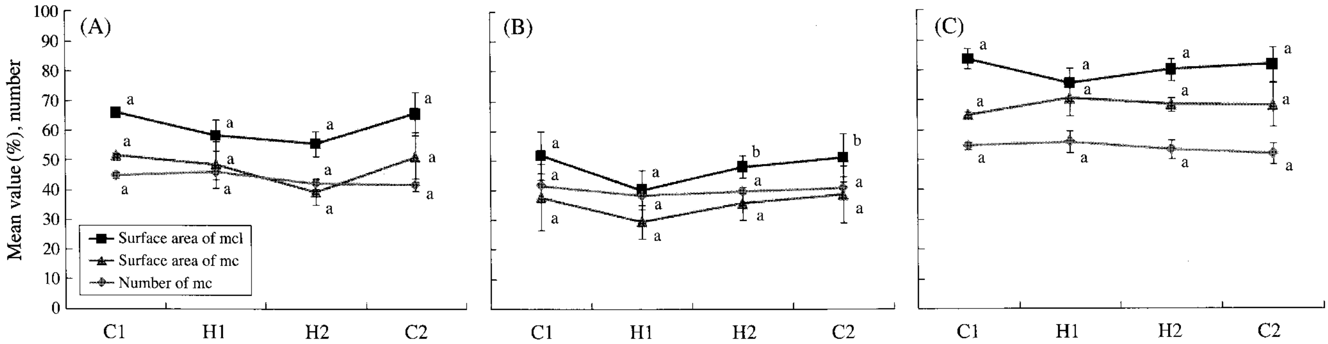

Fig. 3. Change of the skin mucus cell from the high temperature-adapted group of *Misgurnus mizolepis* in dorsum (A), lateral (B) and occiput (C). C1: control group of pre-experiment, C2: control group of post-experiment, H1: high temperature-adapted group (32°C), H2: high temperature-adapted group (H1→18°C). Same letters on the values are no significant differences each other ($P>0.01$).

2) High temperature-adapted group

As the water temperature rose to 32°C (H1), the surface area of mucus cell layer showed a decreasing tendency in three regions of dorsum, lateral and occiput. Similarly, the surface area of mucus cell showed the same aspect in dorsum and lateral regions except the occiput region (Table 1, Fig. 3). These decreasing patterns were somewhat similar to those of hot summer in a rice field (Oh and Park, 2009) and a natural stream (Oh and Park, 2010). With these observation, we can surmised that the water temperature of 32°C treated in the present study might be moderate for fish to live. However, Oh and Park (2009) reported that the mucus cell of *M. mizolepis* dwelling in a rice field suddenly increased around June while at about 28°C. Regarding this case, they assumed that it was for more oxygen uptake, based on the result that because the higher a water temperature was, the more a respiration rate increased in rainbow trout (So *et al.*, 2008). As pointed out by Oh and Park (2009), however, the cause on sudden increase of the mucus cell should be reviewed in various ways due to no significant differences in all skin regions ($P<0.01$) even in this experiment.

When the water temperature returned to 18°C (H2), there was slight increase or decrease in the size and the number of the mucus cell, but it was not a significant different in dorsum, lateral region and occiput region ($P<0.01$).

3) Low temperature-adapted group

When the water temperature fell to 4°C (L1) from 18°C of control group, there were remarkable changes in the dorsum, lateral and occiput. In the dorsal region, the surface area of mucus cell layer increased to 88.4±4.4, compared with 66.1±0.3 of the control group (C1). The surface area of mucus cells also increased from 51.7±0.9 in control group to 77.4±9.4 (Table 2, Fig. 4). Although the change rare in the number of mucus cells was relatively small, its increase was significantly different ($P<0.01$). In the lateral region, the change of mucus cells was only observed in the surface area of mucus cell layer and mucus cells, 72.9±2.4 and 58.8±2.2, respectively ($P<0.01$). The surface area of mucus cells and its number in occiput region also increased from 65.0±0.8 and 55±1.2 to 88.1±1.3 and 63±2.6, respectively ($P<0.01$), but the surface area of mucus cell layer showed no significant increase ($P>0.01$).

After recovering the water temperature from 4°C (C1) to 18°C (C2), the mucus cells also returned to a similar level to those of the control group ($P<0.01$), but the surface area of mucus cell layer and mucus cells in the occiput region were somewhat decreased, meaning not be a significant decline ($P>0.01$).

From the results, an immediate change of the skin mucus cells gives us an important meaning in that is very sensitive to a cold water temperature. It is also possible for making it useful as an indicator for assesment of its aquatic environmental conditions. There are some

Table 2. Variations of the skin mucus cell in three regions of dorsum, lateral and occiput in *Misgurnus mizolepis*

	Dorsum				Lateral				Occiput			
	C1	L1	L2	C2	C1	L1	L2	C2	C1	L1	L2	C2
mcl	66.1±0.3	88.4±4.4	63.6±5.7	65.8±7.3	52±8.2	72.9±2.4	52.2±7.2	51.3±8.2	83.8±3.4	91.1±1.4	89.3±1.3	81.8±5.8
mc	51.7±0.9	77.4±9.4	51.9±6.3	51.2±8.4	38.2±11	58.8±2.2	36.5±8.7	38.7±9.5	65.2±0.8	88.1±1.3	81.4±3.4	68.2±7.2
N.	45±1.2	50±1.6	45±1.9	42±2.1	42±4.2	45±3.8	44±6.7	41±3.8	55±1.2	63±2.6	54±5.4	52±3.7

C1: control group of pre-experiment, C2: control group of post-experiment, L1: low temperature-adapted group (4°C), L2: low temperature-adapted group (L1 → 18°C), mcl: surface area of mucus cells layer, mc: surface area of mucus cells, N: the number of mucus cell.

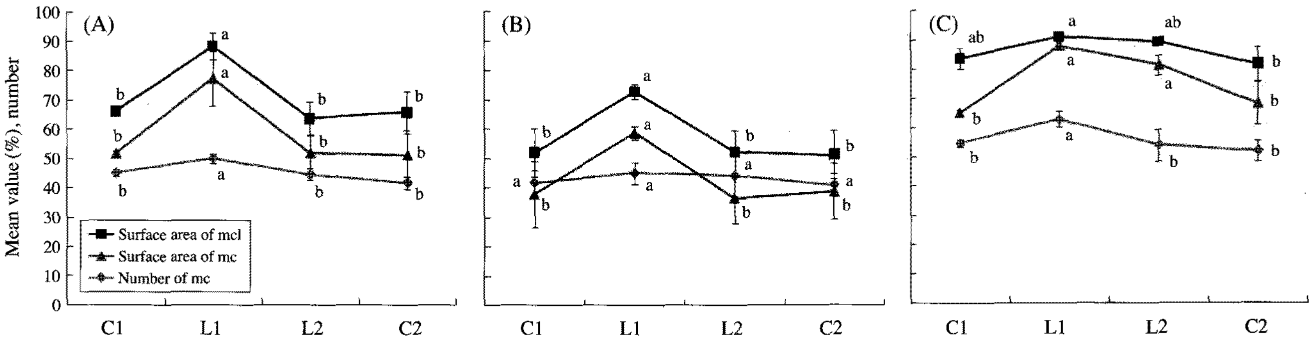

Fig. 4. Change of the skin mucus cell from the low temperature-adapted group of *Misgurnus mizolepis* in dorsum (A), lateral (B) and occiput (C). C1: control group of pre-experiment, C2: control group of post-experiment, L1: low temperature-adapted group (4°C), L2: low temperature-adapted group (L1 → 18°C). Same letters on the values are no significant differences each other ($P > 0.01$).

reports that an abundant mucus coat of *Callionymus risso* (Callionymidae) might serve as protection from the sand or mud in which it buries itself (Sadovy *et al.*, 2005) and an extraordinarily large mucus glands is undoubtedly correlated with burrowing and amphibious habits (Liem, 1967). Also a moray eel (Muraenidae) thicken their skin to protect against abrasion and stress by the substrate (Fishelson, 1996). In the present study, the skin mucus cells of *M. mizolepis* is directly contacted with its environment, and its change causing from the cold water temperature seemed to be an adaptation to dynamic environment. However, as there were various complicated environmental factors, further details are required in the future study.

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수온변화에 의한 미꾸라지, *Misgurnus mizolepis* 표피 점액세포의 일시적 변화

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요 약 : 인위적 수온변화에 의한 미꾸라지 피부의 점액세포 반응을 확인하기 위해 고온(32°C)과 저온(4°C)에 적응시킨 실험군의 표피조직을 실온(18°C)의 대조군 집단과 비교분석하였다. 그 결과, 고온적응 실험군에서는 대조군과 유의한 차이를 보이지 않았으나, 저온적응 실험군에서는 등, 체측, 후두부의 점액세포 수와 크기가 현저하게 증가하는 경향을 보였다($P<0.01$). 또한 저온적응 실험군의 수온을 증가시켜 상온에서 재적응시킨 결과에서도 피부 점액세포의 수와 크기가 현저히 감소하는 경향을 보였다($P<0.01$). 따라서 미꾸라지 피부의 점액세포가 저온에 매우 민감하게 반응하는 양상은 어류의 조직학적 환경지표로서 유용할 것으로 여겨진다.

찾아보기 낱말 : 점액세포, 미꾸라지, 고온적응 실험군, 저온적응 실험군, 환경지표