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The Relationship between Monogenean Skin Parasite *Entobdella hippoglossi* and Mucous Cell Distribution of its Host the Atlantic Halibut *Hippoglossus hippoglossus*

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This study investigated the relationship between attachment site preference of the skin parasite, *Entobdella hippoglossi* and mucous cell density, moucus cell size and epidermis thickness on the surface of the Atlantic halibut *Hippoglossus hippoglossus*.

Parasites occupying the ventral surface of their host were significantly longer and wider than those found on the other zones of the fish ($P<0.05$). The mean size of the mucous cells on the front region was significantly greater than the other regions on the dorsal and ventral surface ($P<0.05$). The average numbers of mucous cells and the epidermal thickness in the skin of the halibut were shown that the front region had significantly higher numbers of mucous cells and thicker layer than the rear region on the dorsal and ventral surface of the halibut, respectively ($P<0.05$).

Key words : Monogenea, skin parasite, halibut, mucous cell

Introduction

Fish skin has many functions such as protection from physical and chemical aggressions, osmoregulation, facilitating locomotion, disease resistance, and social relations (Noakes, 1973; Burton *et al.*, 1984; Hara *et al.*, 1984; Pottinger *et al.*, 1984). Kearn (1976) suggested that the body surface of fishes as a habitat for parasites was especially important. Fish respond to a variety of environmental and pathogenic agents by mucous secretions and fish mucous contains a variety of chemical substances, including nucleic acids, free proteins, glycoproteins and mucopolysaccharides (Askawa, 1970).

There are contrasting reports for the parasitic effect on the mucous cell density of the host skin epidermis. Some researchers (Rogers and Gaines, 1975; Ahmed, 1976) have reported that mucous cell increases during ectoparasitic infections. Other researchers (Logan and Odense, 1974; Paperna, 1980; Roubal *et al.*, 1987) have reported that mucous cell numbers were decreased by parasite infection.

The present study investigated that the attachment pattern of *Entobdella hippoglossi* on the Atlantic halibut *Hippglossus hippoglossus* and relationship between site preference of the parasite and mucous cell density, mucous cell size and epidermis thickness on the skin surface of Atlantic halibut.

Materials and Methods

Parasites were removed from the external surface the Atlantic halibut held in 8 m diameter tanks with running water systems at 34~35 ppt salinity. For this study, a total of 6 female broodstock halibut, infected with the ectoparasitic monogenean, *E. hippoglossi*, were sampled (Average 100 cm in body length and 15 kg in body weight).

All fish were in spawning condition and records showed that there had been no treatments for parasites within the previous 6 months.

Individual parasites at the four principal body regions were counted and measured the total length and the body width using a stereo microscope ($\times 1-4$) with an eyepiece graticule. The total length was taken from the most anterior portion of the adhesive pad to the most posterior edge of the opisthaptor. The width was taken across the center of the testes, which represented the widest part of the body.

The non-parametric Tukey test was used for comparing parasite burden from four different sites (Head dorsal, middle dorsal, tail dorsal and the whole ventral).

The fish skins were obtained from 6 mature Atlantic halibut, and fixed in the paraffin and processed for 1% Alcian blue with pH 2.5/PAS staining method. The number of neutrally stained mucous cells were counted within a 2.5 mm^2 (50 μm × 50 μm) square box. The square box was made under a drawing tube on a Leitz Wetzlar SH Lux compound microscope. Then the slide and square box were overlapped under magnification at ×4.

Counting areas were randomly selected at each side of the fish (10 replicates). Each side had 3 slides each and therefore the slides were also randomly selected. The area of mucous cells and the thickness of the epidermal layer were measured using a KS 300 Imaging System (Kontron Elektronik).

The Non-parametric Dunn's test was used for comparing mucous cell size, epidermal thickness and mucous cell number for the 6 sites: front dorsal; middle dorsal; rear dorsal; front ventral; middle ventral; rear ventral.

Results

On the ventral surface, parasites could not be collected from individual zones because of the potential damage that may be inflicted by inverting the fish, especially to the eyes, if the fish were to flap. Only the dorsal surface could be mapped as turning over the halibut could only be carried out following anaesthetizing and, as a result of this, many of the parasites on the ventral surface detached. These were, therefore, treated as a single group. Though more parasites were found within the head regions on the dorsal surface, this observation was not significantly different from other regions on the same surface (dorsal surface). However, there was a significant difference statistically between parasite numbers from the ventral surface and the tail dorsal region ($P < 0.05$) (Table 1).

Table 1. The number of *E. hippoglossi* collected from the principal zones of their halibut host

Location	Head dorsal (n=6)	Middle dorsal (n=6)	Head dorsal (n=6)	Ventral total (n=6)
Total number	704	334	218	1254
Mean intensity	117.3±77.8 (12~231)	55.7±93.1 (0~236)	36.3±50.9 (0~112)	209±159.9 (17~493)

The mean length and width of the parasites collected from the four principal zones are summarized in Table 2. Parasites occupying the ventral surface of their host were significantly longer and wider than those found on the other zones of the fish ($P < 0.05$). Further, the total length and the body width of the parasites decreased gradually from head to tail dorsal regions.

The mean size of the mucous cells on the head dorsal region was significantly greater than that from the other regions on the dorsal surface ($P < 0.05$). The average mucous cell size on the antero-dorsal region was 189. 52±59.21 μm^2 while those on the mid-dorsal region and the postero-dorsal region were 147.1±59.35 μm^2 and 154.64 ±65.03 μm^2, respectively, from the host.

On the ventral side of the halibut, the mucous cell size pattern was the same as that on the dorsal surface. The average mucous cell size from the antero-ventral region was 186.01±62.69 μm^2, which was significantly bigger than that of the mucous cells on the mid-ventral region (146.62±62.04 μm^2) ($P < 0.05$). However, there was no significant difference between the mu-

Table 2. The mean length and width of *E. hippoglossi* collected from the principal zones of their halibut host

Location	Head dorsal		Middle dorsal		Tail dorsal		Ventral total	
	Length	Width	Length	Width	Length	Width	Length	Width
Mean (mm)	8.09±3.33 (1.46~16.51)	3.68±3.33 (0.49~8.25)	5.73±3.33 (2.14~13.11)	2.41±3.33 (0.78~6.41)	4.86±3.33 (1.55~10.68)	2.11±3.33 (0.58~5.63)	9.67±3.33 (0.97~16.99)	4.48±3.33 (0.29~9.71)

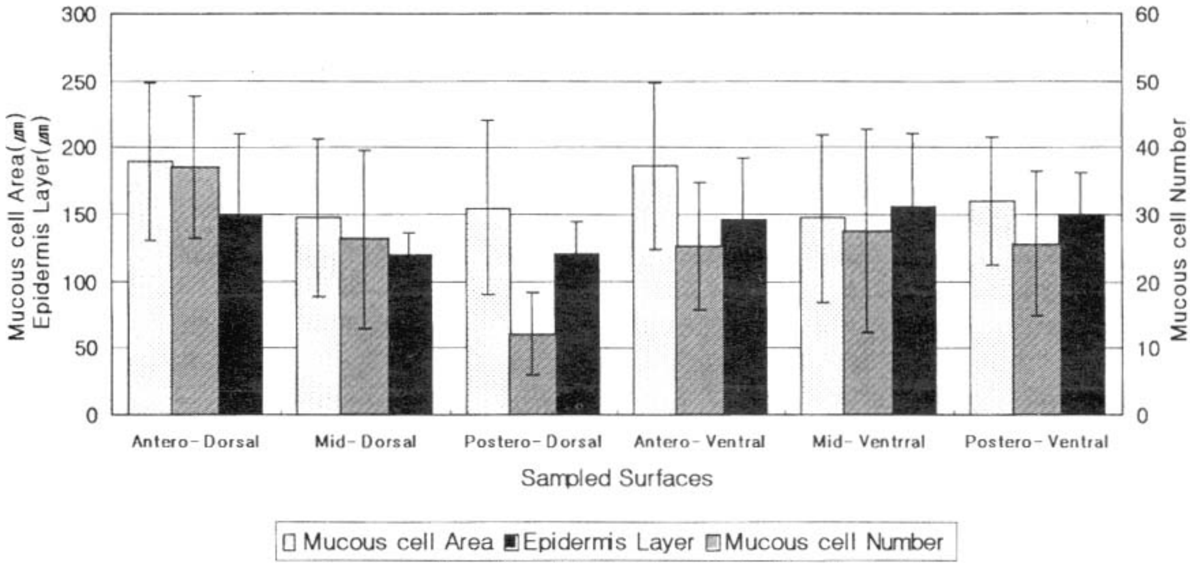

Fig. 1. Mucous cell size, mucous cell number and epidermal thickness of the six different regions of the Atlantic halibut *Hippoglossus hippoglossus*.

cous cell size on the head region and that on the tail region (159.68±47.84 μm²) on the ventral surface of the halibut.

The average numbers of mucous cells in the skin at 6 regions of the halibut skin are shown that the antero- dorsal region (37.05±10.74) and the mid-region (26.3±13.30) had significantly higher numbers of mucous cells than the tail dorsal region (12.05±6.30) ($P<0.05$). The mean number of mucous cells from the different areas of the ventral surface was almost the same on the host (25.25±9.43, 27.45±15.21 and 25.6±10.88, front, middle and rear regions, respectively).

Generally the epidermal layer on the front region was thicker than that on the middle and rear regions from both dorsal and ventral surfaces in this study. The antero-dorsal region (148.54±62.41 μm) was significantly thicker than the other regions (119.51±17.12 μm and 120.64±23.65 μm, the mid-dorsal region and the postero-dorsal region, respectively). On the ventral surface, however, the epidermal layer on the middle region was thicker than the other regions, although there was no significant difference between the head (145.10±47.02 μm), the middle (156.04±54.28 μm) and the tail (149.60±31.09 μm) regions (Fig. 1).

Discussion

In the present study, the numbers of parasites from the dorsal and ventral surfaces were almost the same. On the dorsal surface where their precise location could be studied, however, the parasites were not distributed randomly. The parasites were mainly found in the front region on the dorsal surface of the halibut. The spatial distribution of the oncomiracidia may hold the key to the infection dynamics. Kearn (1963) also observed a larger number of *E. soleae* on the lower surface while smaller parasites were found on the upper surface of heavily infected sole, *Solea solea*. He found that the free-swimming oncomiracidia invaded the anterior part of the upper surface of the fish, which is the only part exposed when the fish is buried in the sand on the bottom of the sea. After a short period of development, the parasites on the upper surface migrated to the lower surface of the host. In contrast to *E. soleae* from the sole, oncomiracidia of *E. hippoglossi* seem to invade the posterior dorsal part of the halibut. Then they migrate from the posterior to anterior part of the dorsal surface and finally they orientate towards the ventral surface of the host.

Mostly, site specificity studies have been concerned with gill parasites. Hanek and Fernando (1978a, b) found that there was a significant preference for the anterior medial section of the hemibranchs by the monogeneans of pumpkinseed sunfish *Lepomis gibbosus* and rock bass *Amblоplites rupestris* Rafinesque.

Kearn (1984) found that most adult and large immature specimens of *E. soleae* from the common sole, Solea solea, were settled on the lower surfaces of their host. He observed that the parasites migrated forward from the tail on the top surface then moved to the lower surface of the host. Kearn (1984) suggested that the advantages of migration from the upper surface to the lower surface were increasing opportunity for exchanging spermatophores, having a better position for attach the eggs to sand grains and avoiding predators. It is assumed that *E. hippoglossi* migrated for the same reasons as *E. soleae* in the present study. However more precise studies are needed to confirm this in *E. hippoglossi*.

Results from the present study showed that there was quite a regular pattern of mucous cell distribution and thickness of epidermis in the Atlantic halibut skin. Usually, the anterior region had a greater density of mucous cells compared to other regions.

Pickering (1974) found that the epidermis of both male and female brown trout, *Salmo trutta* undergoes rhythmical changes in thickness during successive spawning cycles, and at male *S. trutta* had a significantly thicker epidermis than the female for most of the year. Wilkins and Jancsar (1979) also reported from many salmonid fish that the thickness of skin was greater in sexually mature than in immature fish and the male skin was thicker than female skin due to the level of sex hormones. Pickering and Macey (1977) reported that repeated handling, which stressed the fish host, the char, *Salvelinus alpinus* (L.), induced an increase in the number of mucous cells but it did not affect the size of the mucous cells and the thickness of the epidermis. They suggested that some changes would occur in the number or size of superficial goblet cells immediately under stress condition. Pickering (1974) reported that the mucous cells in the brown trout, *S. trutta*, and the char, *S. alpinus* gradually increased in size from the region of the basal cells to the surface of the epidermis. Wells and Cone (1990) found that a mixed infection of *Gyrodactylus colemanensis* and *G. salmonis* did not influence rainbow trout, *Oncorhynchus mykiss* fry growth or survival but the parasitic infection reduced mucous cell number of 50% in the epidermis of the fin. They suggested that the reason why the mucous cell number was decreased in infected fish was that surface mucins served as an important food source for these ectoparasites.

Considering the results from this study, there is a clear positive relationship between the population structure of *E. hippoglossi* and mucous cell distribution of halibut. The results may suggested that the adult parasites migrated to the antero-ventral surface. The size distribution and the mean size of the parasites correlated with the mucous cell concentration of the halibut skin. It was evident that *E. hippoglossi*, at least the adult, preferred the halibut skin area which had the greatest mucus concentration. Taking into account the present results, the oncomiracidia of *E. hippoglossi* attach to an area where there is a smaller volume of mucous cells. Alternatively the oncomiracidia may not be selective in their attachment but survival in some areas may be greater than others. For example, if mucus is acting as a defence mechanism, protecting the fish from the parasite then the parasites might survive better in areas where there is less mucus. It is not evident why a thinner epidermis might be an advantage and it may simply be that the epidermal thickness correlates with mucous cell density and size.

After the oncomiracidia have become accustomed to the new environment of the host, they are able to migrate in search of a better environment on the host. This would explain the distribution of the different sizes of *E. hippoglossi* on the halibut skin.

However, more precise studies will be needed, such as the analysis of mucous components and their role in defence against these parasites and cues for migration of parasites to the ventral surface.

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Atlantic halibut, *Hippoglossus hippoglossus*에 기생하는 피부흡충, *Entobdella hippoglossi*의 기생밀도와 숙주 점액 세포와의 관계

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피부흡충인 *Entobdella hippoglossi*가 숙주인 Atlantic halibut의 피부에 기생할 때 기생 부위별 밀도와 숙주의 피부 점액세포 밀도를 조사하여 *E. hippoglossi*의 기생이 피부점액 세포의 밀도와 관계가 있는가를 조사하였다.

숙주인 Atlantic halibut 복측과 배측에 기생하는 *E. hippoglossi*의 밀도는 비슷하였으나 기생충의 크기는 복측이 배측보다 더 컸으며, 배측내에서는 배측의 머리부분에 기생하는 *E. hippoglossi*의 밀도가 배측의 중앙부나 꼬리부분에서보다 높았다. 숙주의 점액세포 크기, 밀도 그리고 표피 점액층의 두께는 대체로 기생충의 밀도와 비례하였고, 숙주내에서 기생충의 선택적 기생은 점액 세포의 밀도와 관계가 있었다.