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Histopathological changes in fingerlings of Japanese flounder, *Paralichthys olivaceus*, with severe scuticociliatosis

Nam-Sil LEE, Jeong-Hee PARK, Kyu-Sik HAN and Min-Do HUH

Department of Fish Pathology, College of Fisheries Sciences, National Fisheries University of Pusan, Pusan 608-737, Korea

In order to elucidate the patterns of tissue damage evoked by the scuticociliatids, eighteen fingerlings of Japanese flounder, *Paralichthys olivaceus*, heavily infected with an unidentifed scuticociliatid were histopathologically examined. Skin layer with the underlying musculature were severely necrotized due to the infestation of the ciliates. However in the early lesions, both dermis and myofibres remained relatively intact compared with other surrounding loose connective tissues. Mild damages were found in more dense tissues. One or more scuticociliatids were recognized in the blood and lymph vessels of the loose connective tissues with or without destructive changes. Many of nerve trunks or ganglia were also parasitized with less marked histological damage in the parenchyma. Dura and its adjacent tissues in the spinal cord were severely necrotized with massive accumulation of the ciliates in subdural space. The parasitic invasion in the central nervous system was usually confined to the cortical region. In the gill, variable degenerative changes were occurred due to the invasion of the ciliates recognized in the blood vessels of branchial arches or primary filaments. From these results, it was strongly suggested that the scuticociliatids are very actively penetrated into the deep tissues mainly through the severe destruction of the loose connective tissue components and that the vascular system could play a role in the rapid distribution of the ciliates to the remote tissues or organs.

Key Words : Scuticociliatids, Japanese flounder, Loose connective tissue, Blood and lymphatic vessels

The outbreaks of scuticociliatosis have been explosively increasing in Japanese flounder during recent years. This parasitic disease in the fingerlings often resulted in massive death and considerable economic loss. Scuticociliatid, a protozoan ciliate is not only parasitized in the superficial tissues but also invaded into more deep tissues or organs such as viscera or even central nervous system (Ototake and Matsusato, 1986). The ciliates in the exposed lesions or farming water have been known to be well disinfected with the ozonization or the application of some drugs to the farm water (Mizuno, 1993). However, the procedures as

above appears not to be useful in the case of the parasite located deeply. In order to develop more effective control measures, accumulated informations on this parasitic disease should be needed. However, few studies on the present parasitic disease especially including histopathology have been accomplished.

In this study, we carried out a through histopathlogical observation using heavily infected juvenile Japanese flounders to examine the pattern of tissue damage and to discuss the possible route of invasion into the deep tissue.

Materials and methods

Japanese flounders, *Paralichthys olivaceus* which were clinically diagnosed as scuticociliatosis were sampled from several Korean flounder farms at Pohang and Jeju from February to April, 1993. Among these flounders, eighteen fingerlings (2 - 3 cm in length), which were considered as clinically severe, were selected and all supplied for the histological examination.

Fingerlings were fixed in 10% neutral formalin or Bouin's solution, after dividing the whole body into three parts, head, body and tail regions. All parts of the fish body were dehydrated in ethanol series and embedded in paraffin wax. Hard bony structures were decalcified using a trichloroacetic acid solution. Thin sections (4-5 μm in thickness) were obtained by a microtome (Rotary type) and stained with hematoxylin and eosin.

Results

Only two of eighteen fingerlings were excluded from the present results because they were diagnosed as coinfected with *Edwarsiella tarda*.

Skin and its underlying musculature : Both epidermal and dermal layers of the skin were severely necrotized, along with the invasion of the ciliates and the infiltration of macrophages (Fig. 1). The ciliates phagocytized erythrocytes, variable sizes of melanin granule and even macrophages. The scale-pockets involved were markedly dilated within which often contained one or more ciliates (Fig. 2). Dermal layer was preserved relatively intact in the early lesions. But other loose connective tissue components including adipose tissue were severely necrotized with the invasion of a large number of parasites (Fig. 3). In the trunk musculature with overlying necrotic skin lesions, the muscle fibres showed variable degrees of degeneration and necrosis. It was characterized that the surrounding loose connective tissue was always more prominently disorganized by the invasion of the ciliates than the myofibres themselves (Fig. 4). The scuticociliatids were evidently recognized in the lumina of blood and lymphatic vessels of the connective tissue with (Fig. 5a) or without destructive changes (Fig. 5b and 5c).

Bone and its adjacent tissues : A number of parasites were found between bony spicules of immature skeletons, accompanying with the necrosis of adjacent connective tissues (Fig. 6a). However the bone tissues and dense connective tissue between the vertebrae were not histologically damaged (Fig. 6b).

Nervous system : A large number of ciliates were found in the loose connective tissue around and within the nerve trunks of ganglia in the various parts of the body. Despite of this, there were no marked histological abnormalities in the nervous parenchyma (Fig. 7). Subdural space of spinal cord and brain was always filled with uncountable number of parasites, accompanying with severe necrotic changes of dural connective tissue (Fig. 8). Relatively mild histological damages occurred in the spinal cord in which the ciliates were mainly located in cortical region. Also in the brain, the ciliates were noted mainly in the cortex, and resulting in the liquefactive necrosis occasionally. (Fig. 8, inset).

Gill : Medial branchial epithelium toward the oral cavity generally showed mild histological changes compared with the subepithelial loose connective tissue parasitized severely (Fig. 9). The ciliates in the gill were found sparcely in loose connective tissue or in groups around the branchial cartilages. The striated muscle of branchial septum was invaded by the parasites with a variety of degenerative histological findings (Fig. 10a). The scuticociliatids were also observed in the vasculatures in the branchial arches and the primary gill filaments (Fig. 10b).

Histologically no remarkable changes were found in other tissues or organs except a slight inflammatory reaction in the liver, in which no scuticociliatids were recognized. Only from one fingerling, the scuticociliatid infection was suspected in the eye (Not presented here).

Discussion

This yet unidentified ciliate infesting in Japanese flounder, *Paralichthys olivaceus* has been only classified as a holotrichid protozoa belonging to Order scuticociliata (Ototake, M. and Matsusato, T., 1986). It is also one of freeliving ciliate in the marine environment which has pear-shape and measures 30 - 40 μm in length (Yoshimizu *et al.*, 1993). Other marine two scuticociliatids, *Miamiensis avidus* (Moewus, 1962) and *Uronema marium* (Cheung *et al.*, 1980; Roberts, 1989) have been already reported. It has been known that these ciliates becomes occsionally parasitic to the marine fish under an unfavorable environmental condition. Also a morphologically quite similar scuticociliatid, *Orchitophyra stellarum*, has been reported as parasitizing in asteroid gonads (Michel, J., 1987; Leighton *et al.*, 1991). At present, exact relationships in taxonomy between the scuticociliatid in the flounder and the other identified ciliates still remained to be solved.

In our results, most of lesions evoked by the scuticociliatids generally showed variable degrees of degeneration. The accumulation of macrophages in the lesions was considered only as a response of protection. Characteristically in the early lesions, more severe degenerative changes occurred in the loose connective tissue than the parenchymatous components, dense connective tissue or its specialized forms such as cartilages or bones. Most of pathological lesions in the fingerling flounders were very similar in the aspect of histopathology to those by the other identified scuticociliatids (Cheung

et al., 1989] and a freshwater species, *Tetrahymena* sp. [Hoffmam, 1975]. However there are no reports that dealt with the comparative damage patterns in terms of various tissue components. Our results strongly suggest that the loose connective tissue is particularly vulnerable to the destruction by the ciliates compared with the other tissue components. The connective tissue is widely distributed in various tissues or organs for the nutritive and supportive functions and also carries a number of blood and lymph vessels. Therefore, the scuticociliatids might be rapidly accessible to the deep tissue through the destruction of the connective tissue components, especially of the loose one. Furthermore, it seems very likely that the ciliates could easily enter into the circulation, which consequently is responsible for the transportation of the parasites into the remote tissue or organs during a short period of time. In our results, the scuticociliatids were frequently recognized in the blood and/or lymphatic vessels of the loose connective tissue that was not always damaged. Also most of *Tetrahymena* sp. have been reported as easily recognizable in the circulatory system [Hoffman, 1975; Gratzek, 1993].

In conclusion, it was strongly suggested in our histopathological study that the scuticociliatids parasitizing in the fingerlings of Japanese flounder have a suprising power for the destruction of loose connective tissue which is responsible for the rapid propagation throughout the fish body, particularly with the aid of circulatory paths.

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Legends for figures

Figure 1. A lesion in the skin. Scuticociliates (s) are massively invaded in severely necrotized dermal layer with the loss of epidermis. Many erythrocytes phagocytised by the ciliates are seen (inset, × 400). A number of macrophages (arrow) are accumulted. H & E, × 200.

Figure 2. The ciliates (s) are found in a dilated scale-pocket with the necrotized epidermis. Subdermal adipose tissue (at) is also invaded by the ciliates (s). H & E, × 200.

Figure 3. An early skin lesion with relatively mild damage of dermis (d). A number of ciliates (s) are seen between dermis (d) and its underlying musculature. H & E, × 200.

Figure 4. The ciliates (s) are found in the destructive connective tissue among the muscle fibres (m) with less marked degenrative changes. H & E, × 200.

Figure 5. The ciliates (s) are seen in blood vessels (bv) and lympahtic spaces (c, ls) in the connective tissue with (5a, × 400), or without the degeneration (5b and 5c, × 200). H & E.

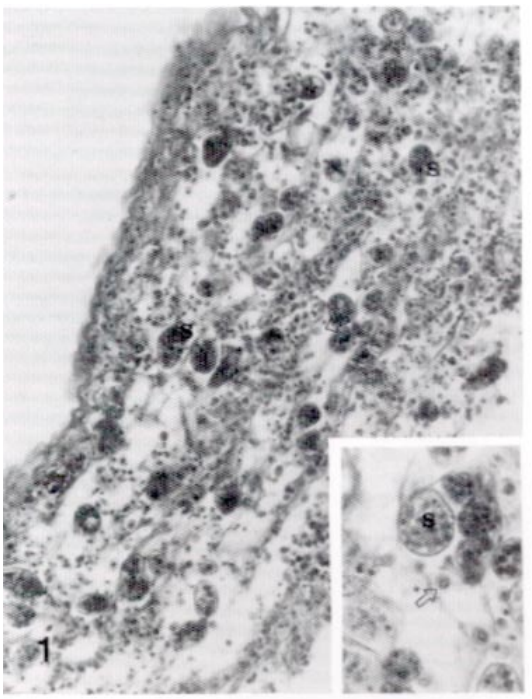
Figure 6. The spaces betwen around the vertebrae (6a) and the bony spicules (6b) are invaded by a large number of the ciliates (s). H & E, × 400.

Figure 7. A number of the ciliates (s) are seen within or aorund a nerve trunk (nt) and a ganglion (gn). But no marked degenerative changes are recognized in the nerve parenchymatous area. H & E, × 200.

Figure 8. A great number of the ciliates (s) were accumulated in the subdural space (ss) of spinal cord (sp). H & E, × 200. Inset; the ciliates (s) are located maily in the cortical regions of spinal cord and brain, × 400).

Figure 9. The ciliates (s) are invaded in the subepithelial loose connective tissue of the medial side of a branchial arch (9a) with marked infiltration of macrophages and in the branchial septal muscle. H & E, × 200.

Figure 10. The ciliates (s) are found in the blood vessels of a branchial arch (10a) and a primary gill filament (10b). H & E, × 200.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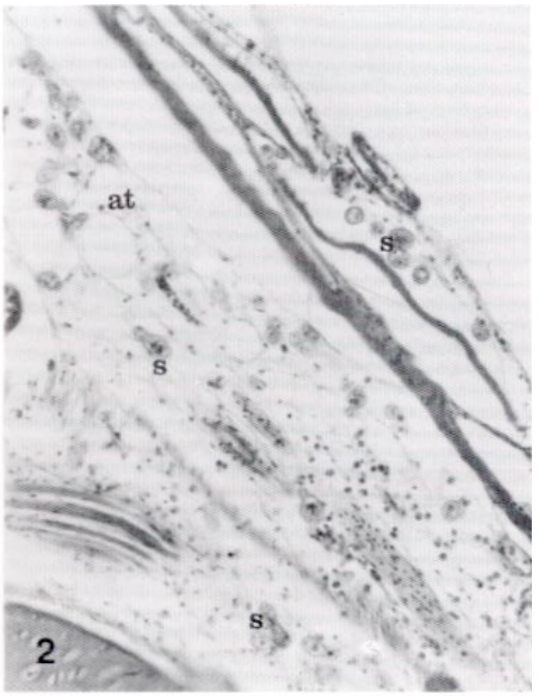
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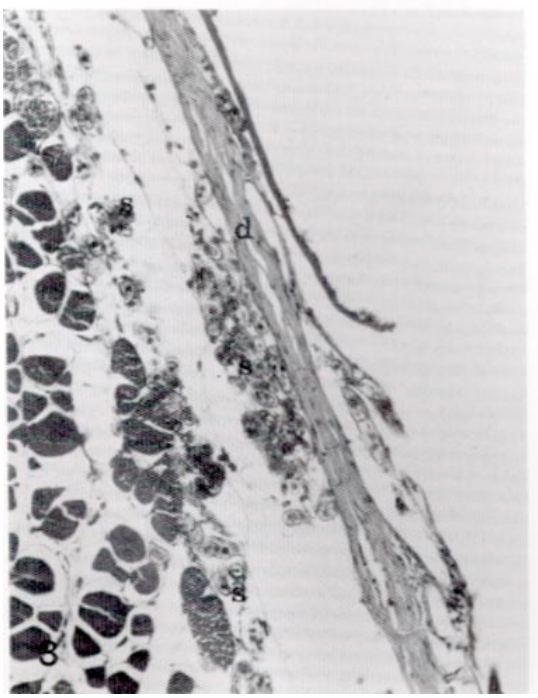
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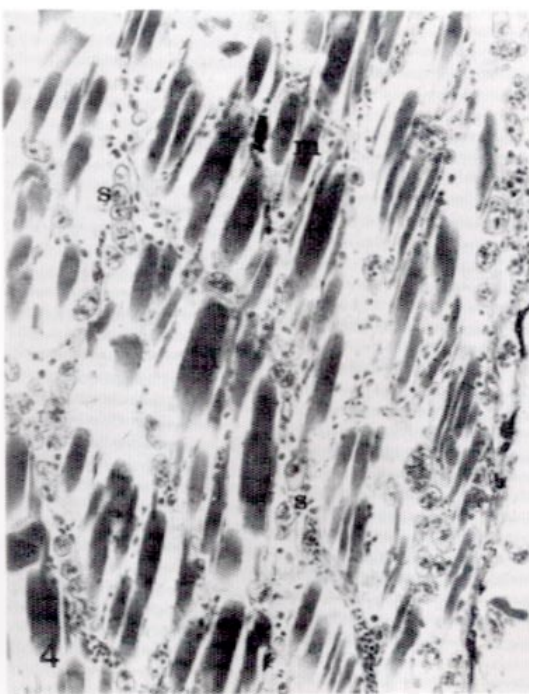
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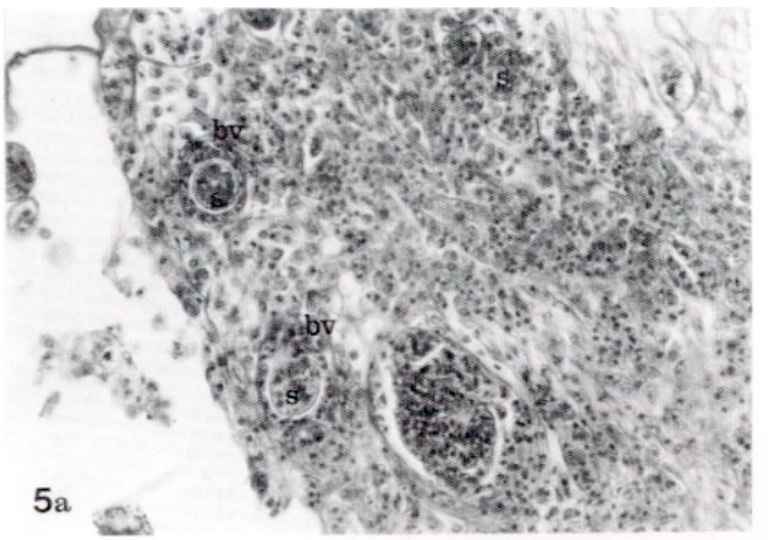
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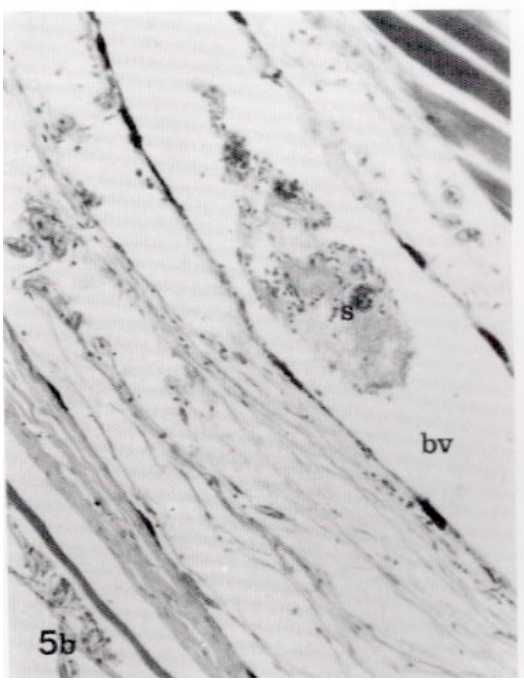
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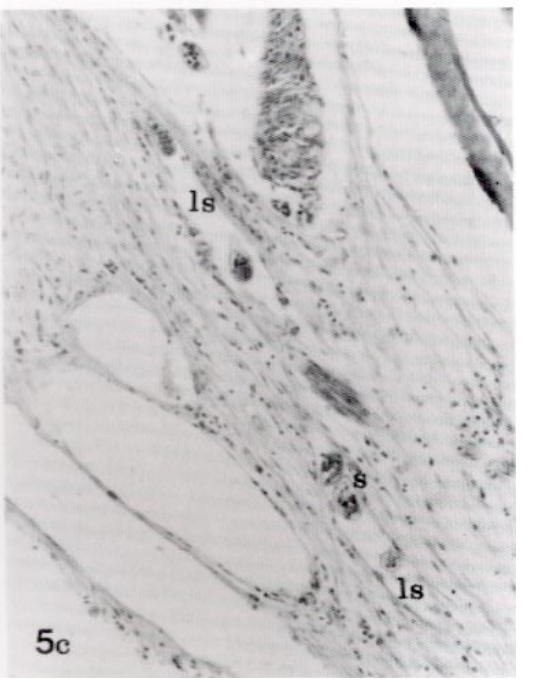
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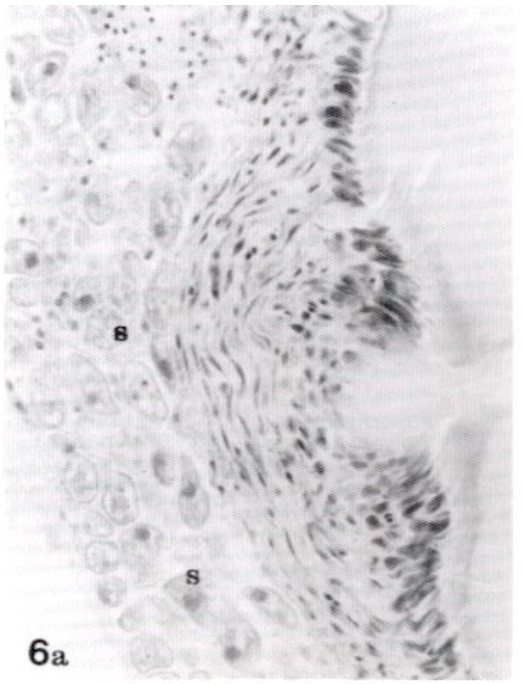

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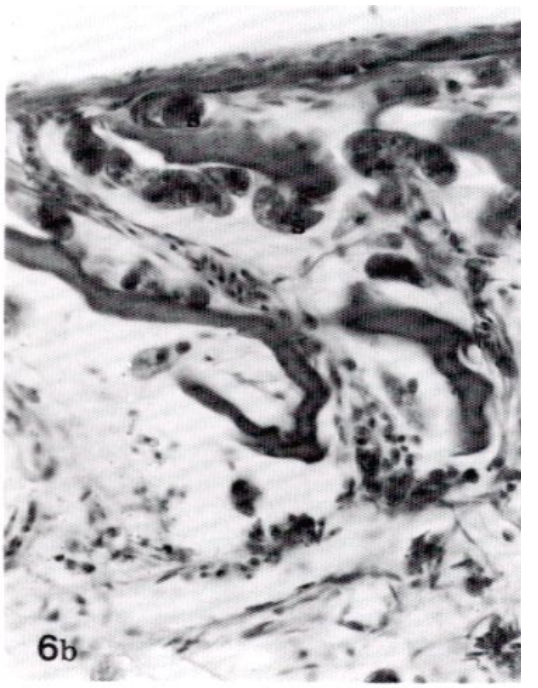

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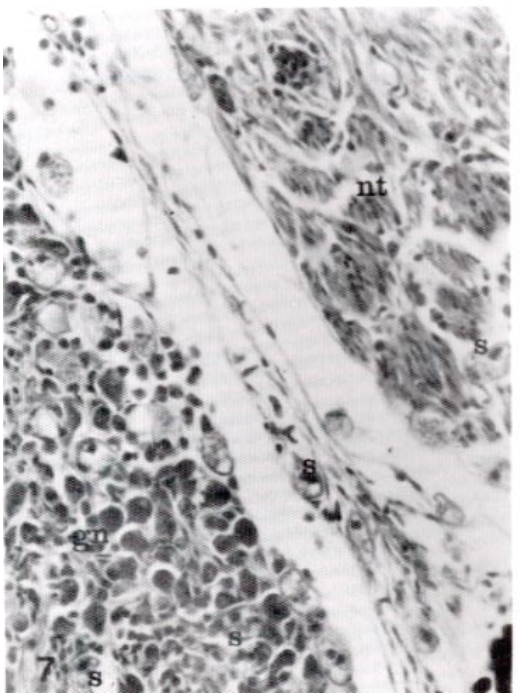

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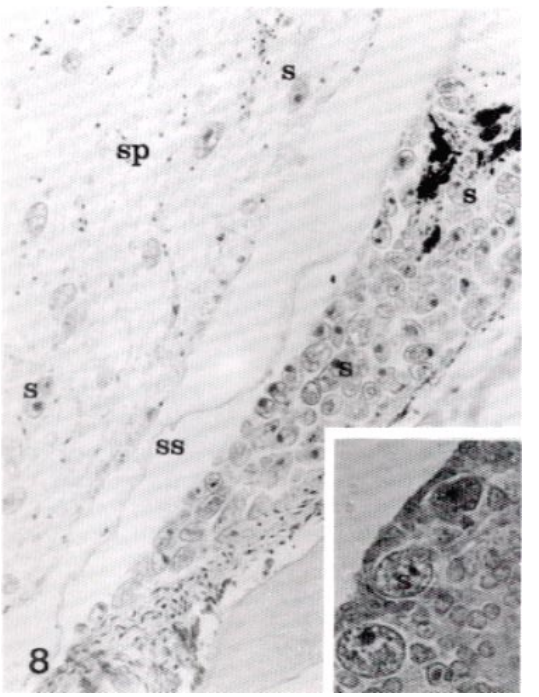

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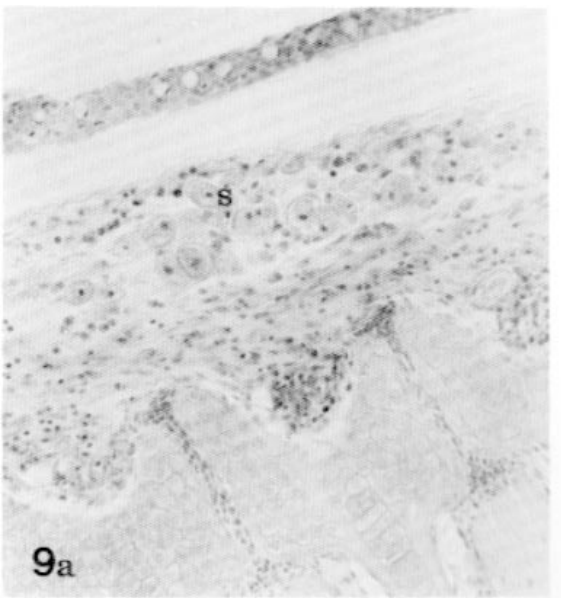

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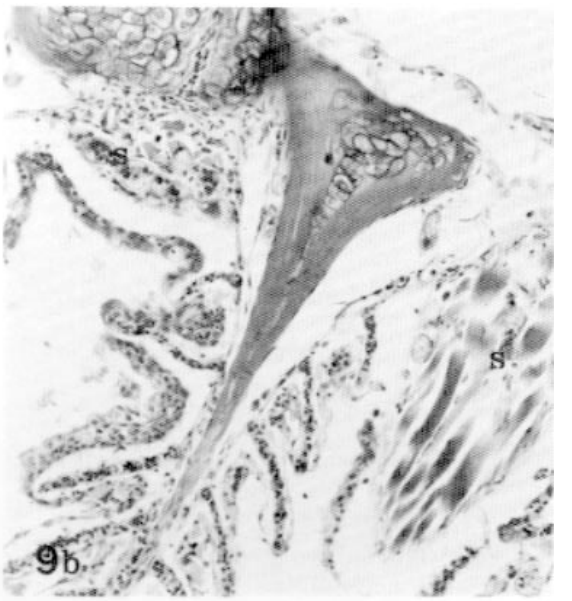

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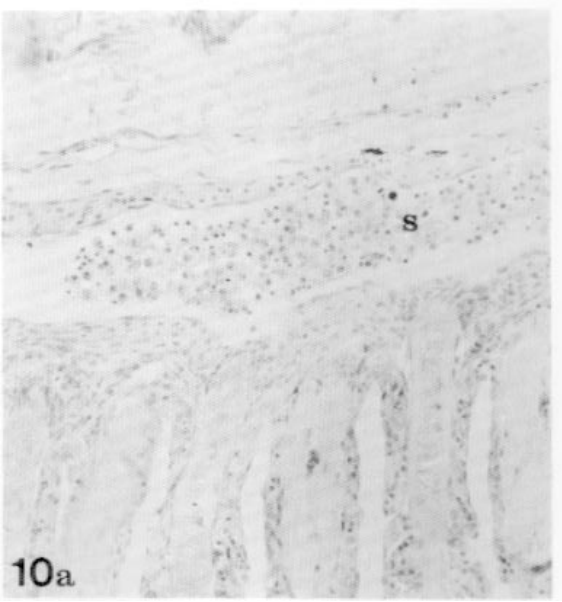

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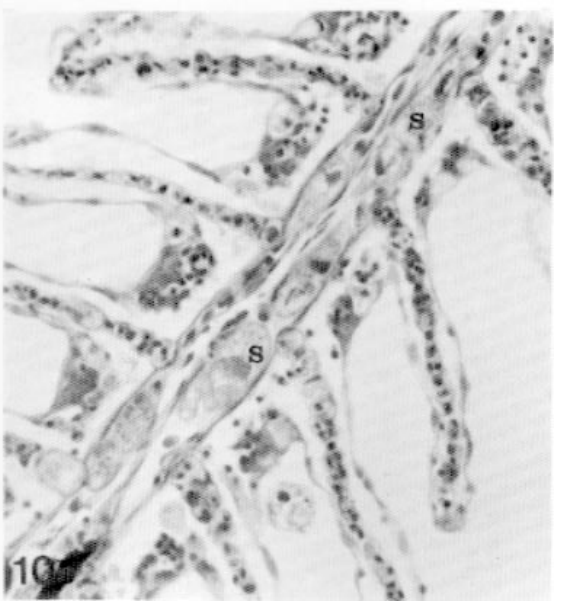

10b

스쿠지카섬모충에 중감염된 넙치 (*Paralichthys olivaceus*) 치어에 대한 병리조직학적 관찰

이남실 · 박정희 · 한규식 · 허민도

부산수산대학교 어병학과

스쿠지카섬모충 (Scuticociliatid) 감염에 따르는 어체조직의 조직학적 손상유형과 심부조직으로의 이행경로를 규명하기 위하여 중감염된 빈사상태의 넙치 (*Paralichthys olivaceus*) 치어 18 마리를 대상으로 전 장기 및 조직에 대한 병리조직학적 검사를 실시하였다. 피부 및 하부의 골격근조직은 다수의 스쿠지카충의 침입에 기인하여 심한 변성 또는 괴사소견과 함께 대식구의 침윤이 현저하였다. 비교적 초기병변에서는 치밀결합조직인 진피나 골격근섬유의 변성보다 이들을 지지하는 소성결합조직성분이 더욱 심한 변성소견을 보였다. 이들 병변부내 또는 병변부와 격리된 소성결합조직내의 혈관 또는 임파공간내에 수개의 충체가 확인되었다. 신경다발과 신경절내 또는 주위 소성결합조직내에 다수의 충체침입이 확인되었으나 실질의 조직학적 이상은 비교적 경미하였다. 뇌 및 척수의 경막하강에 다수의 충체밀집과 함께 신경실질을 포함한 인접조직은 경도 내지 심한 괴사소견을 보였으며 충체의 침입부위는 피질역에 주로 한정되어 있었다. 각종 아가미관련조직에서 섬모충의 기생이 확인되었으며 특히 소성결합조직은 다수의 충체침입으로 심한 변성소견을 수반하였으며, 특히 새궁 및 일차새변의 혈관내에서 충체가 인정되었다. 본 병리학적 검사결과에서 넙치치어에서의 스쿠지카섬모충은 어체내 침입 후 실질조직보다 소성결합조직을 우선적으로 파괴하는 동시에 결합조직내의 혈관 또는 임파관으로 쉽게 이행하여 단시간내에 심부조직으로 확산되는 것으로 사료되었다.

Key Words : Scuticociliatids, Japanese flounder, Loose connective tissue, Blood and lymphatic vessels