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SHORT COMMUNICATIONS

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Mycotic Gastritis of Juvenile Ayu (*Plecoglossus altivelis*) Caused by *Saprolegnia diclina* Type 1

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ABSTRACT: Histopathological and mycological studies were made on mycotic gastritis in juvenile ayu, *Plecoglossus altivelis*. The disease occurred 5 days after the transportation of fish from a hatchery pond to two rearing ponds of Tochigi Prefectural Fisheries Experiment Station, Tochigi, Japan. The pyloric region of the stomach was red. Numerous fungal hyphae penetrated into the stomach wall. Seven fungal isolates were obtained from the lesions. Three isolates were identified as *Saprolegnia diclina* Type 1. Based on histology, hyphae were most numerous in the pyloric stomach, but also penetrated abdominal adipose tissue, the pancreas, spleen, kidney, swim bladder, gonad, and liver. In some cases, the hyphae penetrated trunk muscles.

Key words: Mycotic gastritis, ayu, *Saprolegnia diclina* Type 1, *Plecoglossus altivelis*.

Visceral mycoses in fry or fingerling of salmonids due to *Saprolegnia* have been reported from the USA (Agersborg, 1933; Davis and Lazer, 1940) and Japan (Tashiro et al., 1977; Hatai and Egusa, 1977; Miyazaki et al., 1977). However, there have been no reports of visceral mycosis in salmonids larger than fingerlings or non-salmonid fish. In 1991, a mycotic gastritis occurred in juvenile ayu, *Plecoglossus altivelis*, reared in ponds at Tochigi Prefectural Fisheries Experiment Station, Tochigi, Japan (36°33′N, 139°52′E). We describe histopathology of the mycotic gastritis in juvenile ayu and isolation of *Saprolegnia diclina* Type 1 from the lesions of infected fish.

Fish averaging approximately 3 g in body weight were transported from an indoor pond of the hatchery of Tochigi Prefectural Federation of Fisheries Cooperative Association to two outdoor ponds of Tochigi Prefectural Fisheries Experiment Station. Morality began 5 days after transportation, resulting in a 1.1% loss of the initial populations in both ponds between 28 February and 2 March 1991. The hatchery pond was supplied with spring water of 16 C. The Experiment Station used the same water source as the hatchery. However, the outdoor ponds of the Station reused the water from some other outdoor ponds. Thus, the water temperature of the outdoor ponds of the station was approximately 12 C during the research period. The fish were fasted for several days before they were transferred. After transportation, the fish were fed immediately with commercial formula food (Ayu deluxe, Nihon Nosan Kogyo K. K., Yokohama, Japan). The initial populations of the two ponds were 37,700 and 36,700 fish, respectively.

Slightly affected fish did not show apparent signs, with the exception of anorexia. Moribund fish floated inactively near the surface of the ponds, submerged to the bottom, and died. Nine moribund fish each about 3 g in body weight were collected for histopathological and mycological examinations at the end of February 1991.

Fungi were isolated by inoculating a small piece of stomach wall of the fish on glucose-yeast extract (GY) agar. The GY agar consisted of 10 g glucose, 2.5 g yeast extract and 15 g agar in 1,000 ml distilled

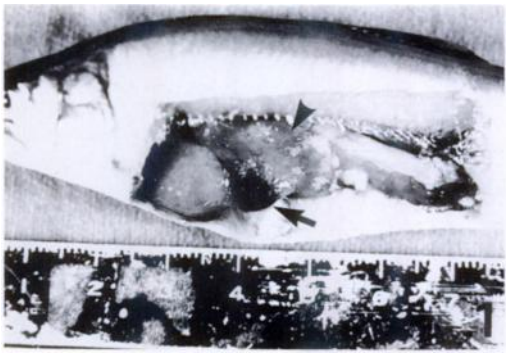

FIGURE 1. Necropsy of an affected fish. Abdominal adipose tissue (arrow head) and pyloric region (arrow) have apparent redness.

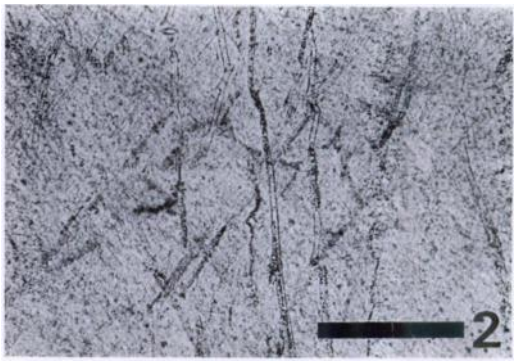

FIGURE 2. Numerous fungal hyphae growing in gastric musculature. The hyphae were aseptate and thick, approximately 15 μm in diameter, and had no zoosporangium-like structures. Unstained. Bar = 200 μm.

water (Hatai and Egusa, 1979). To inhibit bacterial growth, 500 μg/ml each of penicillin G (Meiji Seika Kaisha, Ltd., Tokyo, Japan) and streptomycin sulfate (Meiji Seika Kaisha, Ltd.) were added to the medium. Fungal colonies were subcultured on GY agar to obtain pure cultures. The identification of the fungus was made on hemp seed cultures in sterile tap water at 15 C. Using light microscopy, the hemp seed cultures were examined for secondary zoospore cyst ornamentation, the mode of cyst germination (Willoughby, 1985), and oogonia.

After the removal of the portion of the ventrolateral abdominal body wall, the examined fish were routinely necropsied. All organs were fixed in 10% phosphate buffered (pH 7.0) formalin solution. The fixed tissues were embedded in paraffin and 4 to 5 μm sections were stained with hematoxylin and eosin (H&E). Some sections also were stained with methenamine silver-nitrate, Grocott's variation (Grocott, 1955).

The abdominal adipose tissue and pyloric region had redness (Fig. 1), and the visceral organs often adhered to the abdominal wall. On microscopical examination of the gastric lesion, we observed many aseptate hyphae in the wall of the stomach (Fig. 2). The hyphae were thick, and approximately 15 μm in diameter.

There were numerous fungal hyphae in the pyloric region of the stomach of all specimens (Fig. 3). Hyphae were irregular in width, and were aseptate in the tissue. In mild cases, many hyphae penetrated from the mucous membrane into wall of pyloric stomach, and massive accumulations of hyphae were found in internal body cavity. In severe cases, hyphae penetrated from the pyloric lesion into the surrounding tissues, such as abdominal adipose tissue, pancreas, spleen, liver, kidney, swim bladder, gonad, and trunk muscles; they did not reach the integument, heart, gill, and central nervous system. Hyphae were found in both parenchyma and vascular system of these visceral organs. Thrombi often were observed in the blood vessels due to penetrated hyphae and caused severe congestion. In abdominal adipose tissue and the pyloric region of stomach, a number of the blood vessels were destroyed by penetrating hyphae; severe hemorrhage resulted. One of nine fish examined had hyphae penetration of mucous membrane of cardiac region of stomach reaching the esophagus, but not to the lower regions of the alimentary tract or other visceral organs.

Visceral organs affected with hyphae were severely necrotized and destroyed. Although large quantities of erythrocyte debris and few infiltrating macrophages were observed around the necrotic areas,

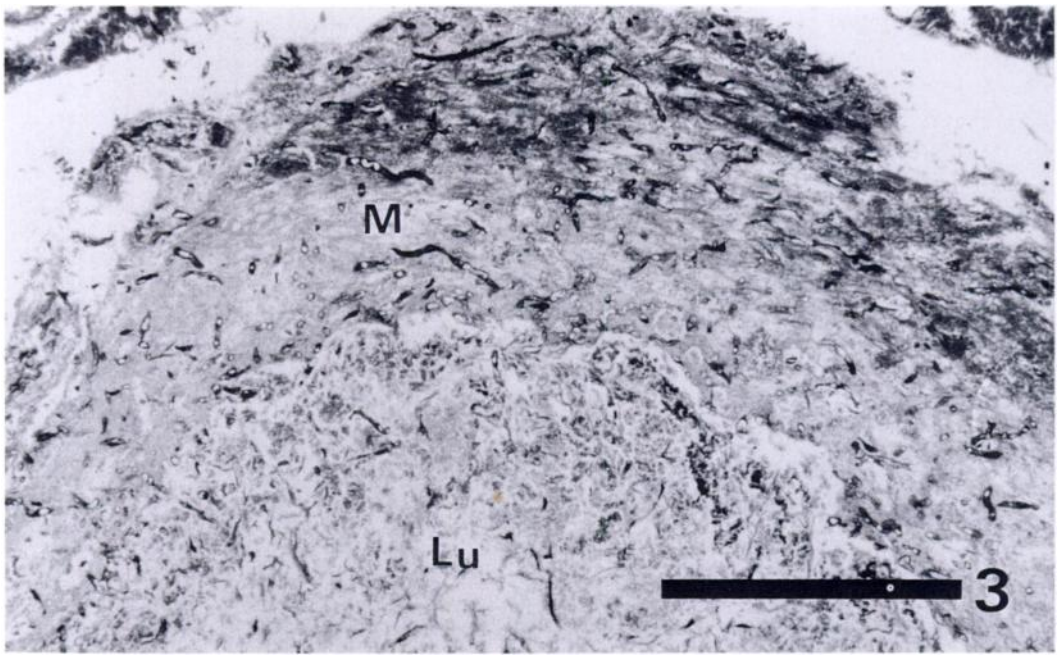

FIGURE 3. A number of hyphae penetrating from the digestive lumen (Lu) into the muscular layer (M) of pyloric region. Grocott's variation. Bar = 500 μm.

there were no granulomas around the hyphae.

Seven isolates from the pyloric lesions were identified as fungi belonging to the genus *Saprolegnia* based on the mode of zoospore release from zoosporangia. A more detailed examination was conducted on three isolates (NJM 9101, NJM 9102, and NJM 9103). They did not produce oogonia at 15 C. The presence of long hooked-hair ornaments on the secondary zoospore cysts in hemp seed cultures was observed in all of them. Secondary zoospores from the three isolates showed indirect germination in hemp seed culture. Based on Willoughby (1978, 1985), we identified isolates NJM 9101, NJM 9102 and NJM 9103 as *Saprolegnia diclina* Type 1.

Based on histology, the primary infectious lesions were established at the pyloric region, and the fungus invaded from the mucous membrane of the pyloric region into the visceral organs of moribund fish. However, other fungal lesions were found which had been established at the cardiac region. Thus, we propose that the primary fungal lesions could be established over a wide area of the mucous membrane of the stomach. The fish were diagnosed as mycotic gastritis due to *Saprolegnia diclina* Type 1.

Saprolegniasis in salmonids may follow inhibition of host defense mechanisms (Neish, 1977; Pickering, 1977). Pickering et al. (1982) reported that handling stress could increase the susceptibility of brown trout (*Salmo trutta*) to saprolegniasis. It seemed likely that the sudden change of the water temperature and handling at the time of transferring the fish might have induced stress and disturbance in defense mechanisms and induced mycotic gastritis in ayu of the present study.

Agersborg (1933) reported a visceral mycosis in brook trout (*Salivelinus fontinalis*) in which numerous fungal hyphae were observed in the intestine and formation of zoosporangia occurred at the tip of hyphae. In the present case, however, hyphae were not observed in the intestine and had no zoosporangium-like structures. On the other hand, visceral mycosis re-

ported in rainbow trout, *Oncorhynchus mykiss* (Davis and Lazer, 1940), and in amago salmon, *O. rhodurus* (Tashiro et al., 1977; Hatai and Egusa, 1977; Miyazaki et al., 1977) were characterized by fungal hyphae which invaded the stomach and penetrated into the body cavity. The causative agent of amago salmon case was *S. diclina* Type 3 (Hatai and Egusa, 1977); most of its histopathological findings (Miyazaki et al., 1977) were similar to those of ayu of the present study except that hyphae were absent in the integument and heart in ayu. Furthermore, Miyazaki et al. (1977) considered that the primary lesion due to *S. diclina* Type 3 in amago salmon might have been established at the pyloric region. The earlier workers described amago salmon cases as a visceral mycosis (Tashiro et al., 1977; Hatai and Egusa, 1977; Miyazaki et al., 1977) because the hyphae penetrated into many visceral organs. However, the pathogenesis of *S. diclina* Type 3 found in amago salmon case resembled that of ayu of the present study. Re-evaluation of visceral mycosis of amago salmon caused by *S. diclina* Type 3 is needed to establish its primary infection site.

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