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Authors: Hatai, K., and Hoshiai, G.

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MASS MORTALITY IN CULTURED COHO SALMON (*ONCORHYNCHUS KISUTCH*) DUE TO *SAPROLEGNIA PARASITICA* COKER

K. Hatai[1] **and G. Hoshiai**[2]

[1] Division of Fish Diseases, Nippon Veterinary and Animal Science University, 1-7-1 Kyonan Cho, Musashino, Tokyo 180, Japan
[2] Miyagi Prefectural Fisheries Experimental Station, Japan

ABSTRACT: Epizootics of saprolegniasis occurred in 20 to 60 g freshwater-cultured coho salmon (*Oncorhynchus kisutch*) in Miyagi Prefecture, Japan. Cotton-like mycelia occurred on the body surface of infected fish, especially around the head, the adipose fin and the caudal fin, and aseptate hyphae occurred in the lesions. The hyphae also penetrated into the muscle and blood vessels. The isolated fungus was identified by asexual morphological characteristics as *Saprolegnia parasitica* (syn. *S. diclina* Type 1), a known salmonid fish pathogen.

Key words: Saprolegniasis, coho salmon, *Oncorhynchus kisutch, Saprolegnia parasitica,* case report.

INTRODUCTION

Coho salmon (*Oncorhynchus kisutch*) have been cultured in the northern coastal waters of Japan, usually in floating net pens. Based on information provided by the Miyagi Prefectural Fisheries Experimental Station, there has been a steady increase in the numbers of cultured fish each year since 1979; in 1988, it reached 14,000 metric tons. The fish are cultured in freshwater until about 100 g in body weight and then transferred to seawater.

Mass mortality due to saprolegniasis, a fungal disease caused by *Saprolegnia* spp., was first noticed at a farm in Miyagi Prefecture, Japan (38°18′N, 140°64′E) in 1985. Since that time epizootics of the disease occurred annually on several farms. Saprolegniasis is very common in cold-freshwater fishes, especially salmonids (Neish and Hughes, 1980). The disease occurs most often in mature salmonids regardless of their location (Neish and Hughes, 1980). To date, mass mortality in immature fish due to this agent has not been documented in Japan (Hatai et al., 1977a).

In Japan, *Saprolegnia diclina* was reported from the viscera of salmonid fish fry (*Oncorhynchus rhodurus*) (Hatai and Egusa, 1977), *S. australis* from the saprolegniasis of rainbow trout fingerling (*Oncorhynchus mykiss*) (Hatai et al., 1977a) and *S. shikotsuensis* from the saprolegniasis of kokanee salmon (*O. nerka* var. *adonis*) (Hatai et al. 1977b).

We report the first known mass mortality in coho salmon in Japan due to saprolegniasis caused by *Saprolegnia parasitica*.

MATERIALS AND METHODS

Several fish ranging from 20 g to 40 g in body weight, dying from saprolegniasis, were collected from the infected pond on 9 July 1986. Tissues from the lesions were fixed in 10% phosphate-buffered formalin solution. The fixed tissues were embedded in paraffin and cut at 3 to 5 μm. Sections were stained with haematoxylin and eosin (H&E), Grocott's stain, or Periodic-Acid Schiff stain (PAS) (Lennette et al., 1985).

The fungi were isolated by inoculating samples of infected muscle taken from different parts of the body, approximately 2 mm in diameter, from a moribund coho salmon onto 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 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.) was added to the medium. The isolated fungi were maintained at 15 C on GY agar, and transferred to fresh GY agar monthly.

About 20 fungi strains were isolated from the diseased fish. After a preliminary check of the characteristics, two were chosen for further study. These were the most common isolate, NJM 8604 (=H2), and a much rarer isolate, NJM

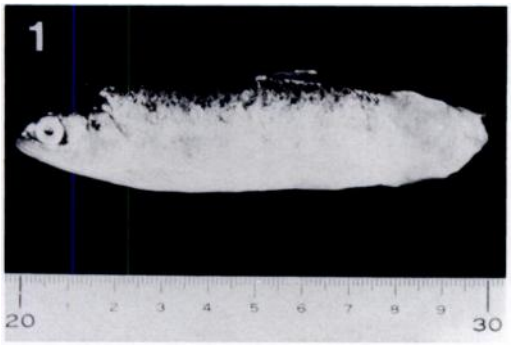

FIGURE 1. Gross lesions of coho salmon infected with *Saprolegnia parasitica*. Note cotton-like mycelia of the caudal peduncle.

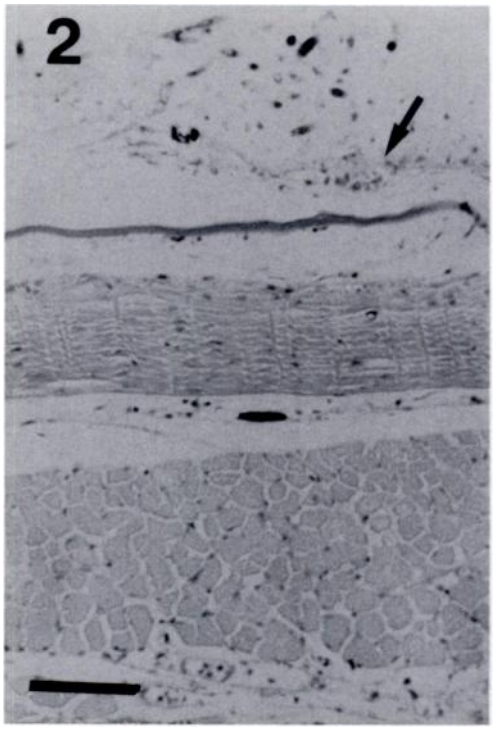

FIGURE 2. Cross section of skin of coho salmon with saprolegniasis. Note the destruction and disappearance of the epidermis (arrow). H&E. Bar = 50 μm.

0005 (=H3), which was the only one to produce oogonia. For purposes of comparison, three other strains, NJM 0007 (=H9), NJM 8751 (=H5) and NJM 8761 (=H15) also were used for some experiments.

Fungal characteristics and identifications were made on hemp seed cultures in sterile tap water (Coker, 1923; Johnson, 1956); after inoculation, hemp seed cultures were incubated at 10 and 20 C. Using 400× negative phase-contrast light microscopy, the hemp seed cultures were examined for secondary zoospore cyst ornamentation, mode of cyst germination (Willoughby, 1985), and oogonia. Finally, the isolated strains were identified according to the criteria of Coker (1923), Seymour (1970), Willoughby (1978) and Pickering et al. (1979).

To determine the growth rates of each fungal isolate, two plastic 83 mm diameter petri dishes, each containing 20 ml GY agar, were inoculated with centrally placed circular 6 mm diameter blocks of GY agar cut from the margins of actively growing colonies.

The growth rates were measured at intervals of 18, 25, 42, 50, 66, 73, 92, 116 and 140 hr after inoculation. Colony diameters were measured with vernier calipers along two radii at right angles to each other. Mean radii were calculated for each fungal strain based on two petri dishes.

RESULTS

Approximately 130,000 and 110,000 coho salmon were reared in two ponds at fish farms A and B, respectively. Between 1 and 30 June 1987, 52,910 fish died at farm A; 43,120 fish died at farm B between 6 and 30 June 1987. Moribund fish had serious infections, usually in the head and caudal peduncle (Fig. 1). Cotton-like mycelia were present on the head, adipose fin, and caudal fin. In most cases, there were no internal gross signs. Many aseptate hyphae were observed in the various lesions.

Severe destruction of the epidermis occurred (Fig. 2). Fungal hyphae also were present in the dermis and musculature (Fig. 3). However, no bacteria or endoparasites were observed in these lesions. In some fish, hyphae penetrated into blood vessels. No inflammatory reactions were observed at the site of the lesions.

All isolates were identified as fungi belonging to the genus *Saprolegnia*, based on the mode of zoospores release from zoosporangia. Isolate H3 grown on hemp seed produced oogonia both at 10 and 20 C. Antheridial branches almost always were present and diclinous. Oogonia usually were abundant and spherical or pyriform.

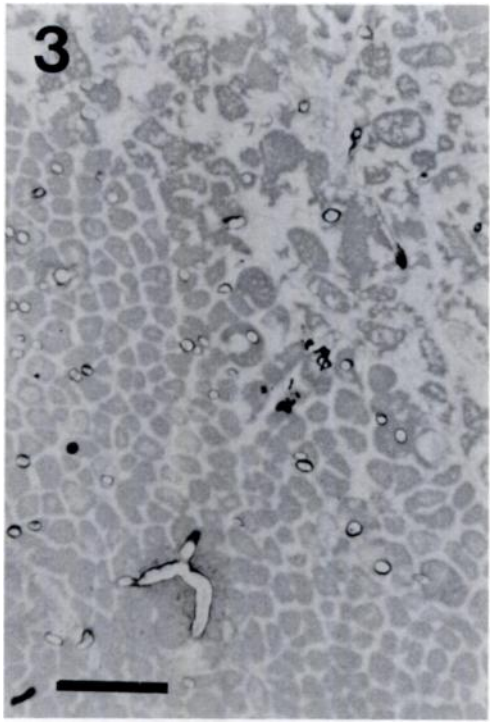

FIGURE 3. Cross section of skin of coho salmon with saprolegniasis. Note hyphae in the musculature, and absence of necrosis or inflammatory response. Grocott's stain. Bar = 50 μm.

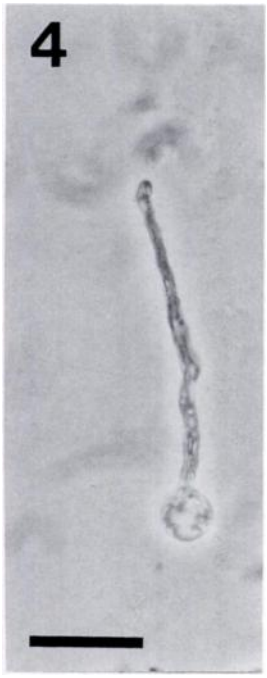

FIGURE 4. Isolate H3. Direct germination of *Saprolegnia diclina* from encysted spore. Bar = 25 μm.

The oogonial wall was unpitted and smooth. Oospores were centric. Long hooked hairs on secondary zoospore cysts from hemp seed culture were not detected. Isolate H3 was identified as *Saprolegnia diclina*. None of the other isolates (H2, H5, H9 and H15) produced oogonia. Long hooked hairs on secondary zoospore cysts were detected for these latter four isolates.

Secondary zoospores had direct germination in hemp seed dish water for isolate H3, while the other four isolates had indirect germination (Figs. 4, 5). Based on the criteria of Willoughby (1978, 1985), isolates H2, H5, H9 and H15 from coho salmon were identified as *Saprolegnia parasitica* (syn. *S. diclina* Type 1). Although the growth rates of the isolates identified as *S. parasitica* were variable at 30 C, isolate H5 had the fastest growth rate of those isolates. After 92 hr, the growth rates were 26 mm for H2, 0 mm for H3, 40 mm for H5, 15 mm for H9 and 22 mm for H15. There was a striking difference between isolate H3, identified as *S. diclina* Humphery (syn. *S. diclina* Type 3), which did not grow at all, and the other isolates.

DISCUSSION

Based on preliminary tests, the same growth rate occurred for all strains at temperatures below 30 C. Growth rates at 30 C however were clearly different and as a result all experiments were conducted at this temperature.

Two kinds of fungi belonging to the genus *Saprolegnia* were isolated from the lesions of coho salmon; *S. parasitica* was common, while *S. diclina* was rare.

This is the first report in Japan of saprolegniasis in coho salmon (*O. kisutch*) caused by *S. parasitica*. Further, there are no previous reports of high mortality in cultured salmonids in Japan due to saprolegniasis. Although it is generally assumed that saprolegniasis occurs frequent-

ly in Japanese salmonids during spawning, there are no published reports of losses in Japan during spawning.

Based on histopathology, the main site of infection was the skin and underlying musculature. The infection can apparently cause rapid destruction of the epidermis; hence, the direct cause of death probably is related to impaired osmoregulation (Gardner, 1974; Hargens and Perez, 1975). We believe that *S. parasitica* was the primary pathogen in this case because there was no evidence of prior bacterial or protozoal infection. Rapid growth of the fungi made it difficult to see inflammatory reactions in the affected musculature. Based on histology, we were uncertain whether the tissue damage was due to *S. parasitica* or *S. diclina*.

According to Willoughby (1978), *S. parasitica* is regarded as *S. diclina* Type 1, a common pathogen of salmonid fish. It often is called "salmonid *Saprolegnia*." The oogonia of this species are either completely absent or are formed only after a prolonged period of time. Hence, *Saprolegnia* which do not produce oogonia and are pathogenic to salmonids have been identified as *S. parasitica*.

Hatai and Egusa (1977) reported visceral mycosis in salmonid fish fry (*Oncorhynchus rhodurus*) caused by *S. diclina*. The fungus isolated from the lesion of fish with the disease produced oogonia easily. The fungi reported in the present paper had abundant oogonia and were regarded as *S. diclina* Type 3. In this case *S. diclina* was regarded as a secondary parasite. It is unclear why *S. diclina*, a saprophyte, was involved.

Based on these data, we propose that *S. parasitica* also is pathogenic for coho salmon. Isolate H2 was very pathogenic for coho salmon when the fish were exposed to a suspension of zoospores from the isolate (Hatai and Hoshiai, unpubl.).

Comparative growth rates of both species at 30 C were diagnostic. Specifically, isolate H3 of *S. diclina* showed no growth at 30 C. Future studies will be made to see if growth rates at 30 C can be used for rapid identification of fungi belonging to the genus *Saprolegnia* isolated from coho salmon.

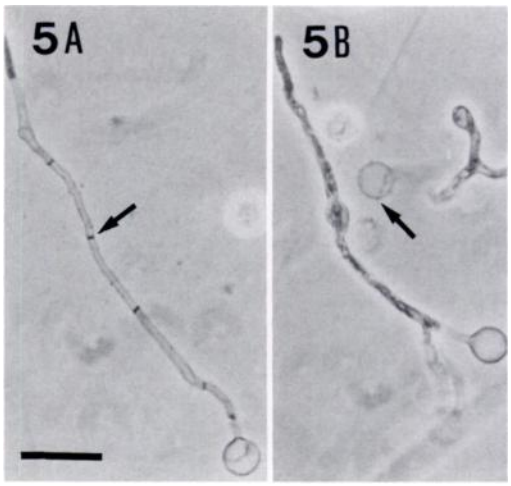

FIGURE 5. Isolate H2. Indirect germination of *Saprolegnia parasitica* from encysted spore. Bar = 25 μm. A. Note the empty long germ tube with several septa (arrow). B. Note the long hooked-hairs (arrow) on the empty spore.

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