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Saprolegniosis in salmonids and their eggs in Japan

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ABSTRACT: An epizootic of the fungal infection saprolegniosis that occurred in freshwater-cultured salmons and their eggs at some hatcheries in Hokkaido (Japan) was investigated. In almost all cases, the initial clinical sign was characterized by the growth of cotton-like mycelia on the fishs' body surface, especially the head, adipose fin, and caudal fin, but the mycelia were not visible to the naked eye in the internal organs. Thirty-three strains isolated from lesions were classified in the genus *Saprolegnia* according to their morphological and biological characteristics on hemp seed cultures at various temperatures. Fifteen of the strains were identified as *Saprolegnia parasitica,* 16 were identified as *S. salmonis,* and two were identified as *S. australis.*

Key words: Epizootic, *Oncorhynchus* spp., salmonids, *Saprolegnia* spp., saprolegniosis.

Saprolegniasis is the most problematic fungal infection of cultured salmonid fish in freshwater ponds in Japan (Hatai, 1980), but it is rarely found in brackish water (Post, 1987). Saprolegniasis occurs most often in mature salmonids and their eggs (Neish and Hughes, 1980; Alderman, 1982). Mass mortality due to saprolegniasis, however, was reported in 20 to 60 g freshwater-cultured coho salmon (*Oncorhynchus kisutch*) in Miayagi Perfecture (Japan), and the pathogenic fungus was identified as *Saprolegnia parasitica* (Hatai and Hoshiai 1992a, b). Since 1992, a similar fungal disease has been found to occur annually in immature salmonids including masu salmon, (*Oncorhynchus masou*) sockeye salmon (*O. nerka*) and chum salmon (*O. keta*) and their eggs at some hatcheries in Hokkaido. We started to investigate the disease in 1997. As a result, a new species of the genus *Saprolegnia* was isolated from lesions in several immature sockeye salmon with saprolegniasis in Hokkaido, and named *S. salmonis* (Hussein and Hatai, 1999). During the sampling in 1998, 33 other isolates, all of the genus *Saprolegnia,* were newly obtained from *O. nerka* and other salmonids and their eggs with saprolegniasis in Hokkaido. The goal of the present study was to identify these thirty-three isolates. All isolates used in this study were collected from two hatcheries of the National Salmon Resources Center (Fisheries Agency, Hokkaido, Japan) during October and November 1998.

The fungi were isolated by inoculating infected eggs and small (approximately 2 mm in diameter) samples of infected muscle taken from different parts of the body, the head, adipose, and caudal fins onto glucose-yeast extract agar (GY) agar (1% glucose from Wako Pure Chemical Industries, Osaka, Japan; 0.25% yeast extract from Difco Laboratoies, Detroit, Michigan, USA) and 1.5% agar (Eiken Chemical, Co., Ltd., Tokyo, Japan) (Hatai and Egusa, 1979). To prevent the growth of most bacteria, traces of ampicillin (Sigma, Chemical Co., St. Louis, Missouri, USA) and streptomycin (Meiji Seika, Kaisha, Ltd., Tokyo, Japan) were added to the medium. The plates were incubated at 15 C for hyphal growth and then purified according to Willoughby (1994). The purified fungal isolates were maintained at 15 C on GY agar, and transferred to fresh GY agar monthly.

Fungal characteristics and identifications were made on hemp seed cultures in sterilized tap water (Coker, 1923; Johnson, 1956). Hemp seed cultures for observation of asexual and sexual organs were done at 5, 15 and 20 C, and observed for 6 wk. The germination type of zoospores was classified according to Yuasa et al. (1997). *Saprolegnia australis* were identified according to Seymour (1970), *S. parasitica*

was identified according to Seymour (1970), Neish (1977) and Willoughby et al. (1983), and *S. salmonis* was identified according to Hussein and Hatai (1999).

Mortality of sockeye salmon fry due to saprolegniasis was first noticed in Hokkaido in 1992. Since that time, epizootics of the disease have occurred in November and April annually. The water temperature when the infections occurred was about 18 C. The fish in the present study, conducted in October and November 1998, showed the typical clinical sign of cotton-like mycelia on the body surface, especially around the head, dorsal and caudal fins, but not in the internal organs. Many aseptate hyphae and zoosporangia were observed in various lesions in a preliminary microscopic examination. Thirty-three fungi isolated from the lesions were divided into four groups (A, B, C, and D) based on their morphological and biological characteristics: Group A (two isolates) isolated from eggs of chum salmon; group B (16 isolates) isolated from body surface of chum and masu salmon; group C (seven isolates) isolated from body surface of sockeye salmon and group D (eight isolates) isolated from body surface of chum salmon.

Two strains were isolated from some eggs of chum salmon, group A. One of these strains (NJM 9852) was observed in detail. The isolate produced oogonia within 15 days at 5 C and 10 days at 15 and 20 C. They were abundant, spherical, laterally and/or terminally represented. Oogonial walls were conspicuously pitted and the origins of the antheridial branches were mainly diclinous. Oospores were spherical, 22 to 24 μm in diam with an average number of 6 to 12. The internal structures of the oospores were subcentric. The zoospore germination type was direct. Gemmae formation took at least three weeks at 5, 15 and 20 C and they were abundant, spherical, single, and frequently in chains. From these characteristics, the fungi in this group were identified as *Saprolegnia australis.*

Twelve strains were isolated from masu salmon and four strains were isolated from chum salmon, group B. One of these isolates, NJM 9858, was observed in detail. Characteristically, oogonia formation took from 20 days to four weeks at 5 C. The oogonia were elongate (40%), spherical (35%) and pyriform (25%). The oogonial wall was pitted or not pitted. The origins of antheridial branches were mainly diclinous. Some of the oogonia, both spherical and pyriform, were often lacking antheridial branches. Oospores were spherical, 20 μm in diam. Spherical and pyriform oogonia had 4 to 12 (8) oospores and elongate oogonia had 16 to 20 (18) oospores. The internal structures of the oospores were mostly subcentric but some were centric. Zoospore germination type was direct or indirect. Gemmae were not observed at 5, 15, and 20 C. The isolate NJM 9858 was identified as *Saprolegnia salmonis.*

One strain was isolated from chum salmon and seven strains were isolated from sockeye salmon, group C. One of these isolates (NJM 9868) was observed in detail. Only gemmae formed at 5, 15, and 20 C after more than four weeks. They were abundant and usually in chains. No oogonia were observed. The zoospore germination type was indirect. The isolate NJM 9868 was identified as *Saprolegnia parasitica.*

Two isolates were isolated from masu salmon and chum salmon, group D. One of these isolates (NJM 9873) was observed in detail. This isolate produced neither oogonia nor gemmae at 5, 15, and 20 C. However, the mode of their zoospore germination was indirect, which according to Hatai and Hoshiai (1992b) and Yuasa et al. (1997) is a characteristic of *Saprolegnia parasitica.* Thus, the isolate NJM 9873 was identified as *Saprolegnia parasitica.* The characteristics of these four groups we have summarized in Table 1.

It has been established that fungi belonging to the genus *Saprolegnia* are principally associated with fungal infections of

TABLE 1. Morphological and biological characteristics of *Saprolegnia* spp. isolated from salmon and eggs with saprolegniosis in Hokkaido (Japan).

	Group A NJM9852	Group B NJM9858	Group C NJM9868	Group D NJM9873
Oogonial formation at				
5 C	+	++[a]	−	−
15	+++	−[b]	−	−
20	+++	−	−	−
Oogonial shape	spherical	spherical, pyriform, or elongate	NO[c]	NO
Oogonial wall	conspicuously pitted	pitted or not pitted		
Oospores	subcentric	centric or subcentric	NO	NO
Antheridial branches	diclinous	diclinous	NO	NO
Zoospores germination	direct	direct or indirect	indirect	indirect
Gemmae formation at				
5 C	++	−	+++	−
15	+++	−	+++	−
20	++	−	++	−
Identification	*S. australis*	*S. salmonis*	*S. parasitica*	*S. parasitica*

[a] Slight formation = +, good formation = ++, excellent formation = +++.
[b] Indicates no formation.
[c] Not observed.

freshwater fishes, fish eggs, amphibians and reptiles (Willoughby, 1970, 1978, 1994; Hatai and Hoshiai, 1992a, b; Bly et al., 1992; Blausteine et al., 1994; Kitancharoen et al., 1995, 1997). In the genus *Saprolegnia*, *S. parasitica* is the most important fungal parasite of fish (Neish and Hughes, 1980; Beakes et al., 1994) and has consistently been isolated as a pathogen of epizootics and mass mortalities, especially in cultured trout and salmon (Willoughby, 1978; Hatai and Hoshiai 1992a, b). *Saprolegnia salmonis* was first described in 1999 when it was isolated from sockeye salmon in 1998 (Hussein and Hatai, 1999). In this survey, *S. salmonis* was also found in masu salmon and chum salmon. *Saprolegnia salmonis* has never been found in Honshu (Japan) but is commonly found in the rivers of Hokkaido.

Hatai et al. (1977) reported the isolation of *S. australis* from rainbow trout fingerlings with saprolegniasis. In the present study, *S. australis* was isolated from some eggs of chum salmon with saprolegniasis. This suggests that *S. australis* can cause disease in both fish and eggs either as a primary pathogen or as an opportunistic one.

There is no doubt that ecological differences in different geographical locations play an important role in the species diversity of the fungi that develop on both fish and eggs (Alabi, 1971; Avtalion et al., 1973; Wood and Willoughby, 1986). Although environmental variables were not studied herein, they are known to influence the growth, reproduction, and intensity of aquatic fungal infections. In addition, the occurrence of saprolegniasis may be related to environmental changes or seasonal variations, water quality, temperature as well as physiological changes and the immune response of fish. From our investigation it can be concluded that *S. parasitica*, *S. salmonis*, and *S. australis* are the most common *Saprolegnia* spp. associated with the occurrence of saprolegniosis in salmonids in Hokkaido. Further investigations to evaluate the degree of pathogenicity of such species and the environmental factors and host factors that influence the disease epizootics are needed.

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