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## MYCOBACTERIOSIS IN MOUNTAIN WHITEFISH (*Prosopium williamsoni*) FROM THE YAKIMA RIVER,<sup>1</sup> WASHINGTON

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**Abstract:** Mycobacteriosis was found in mountain whitefish (*Prosopium williamsoni*) taken from the Yakima River near Richland, Washington in 1975 and 1976. The disease appeared to affect about 8% of the population sampled. Gross lesions were present in most visceral organs, but were most common in the kidney, liver and pyloric caeca. Microscopically, the lesions consisted of large numbers of macrophages containing numerous intracellular bacilli. An organism was isolated and has been tentatively classified as *Mycobacterium* sp., Runyon group III.

### INTRODUCTION

Piscine mycobacteriosis of fish is world-wide in distribution and has been reported in 151 species of both natural and cultured fish populations.<sup>1</sup> This disease was reported in salmon (*Oncorhynchus tshawytscha*) and trout (*Salmo gairdneri*) in the Columbia River watershed in 1952 and was a problem in hatchery-reared salmon for a decade.<sup>2,3</sup> The disease was perpetuated by the practice of feeding unpasteurized salmon carcasses and viscera to young fry. When the practice was discontinued, the disease was virtually eliminated.<sup>2</sup> The purpose of this paper is to report the occurrence of mycobacteriosis in mountain whitefish (*Prosopium williamsoni*) taken from the Yakima River near Richland, Washington. The sample area was 16 km upstream from the confluence of the Yakima and Columbia Rivers.

### MATERIALS AND METHODS

A sample of approximately 280 mountain whitefish was collected from the Yakima River in February, 1975, and

transported to the Battelle, Pacific Northwest Laboratories. The fish were held in a 1900 l fiberglass circular tank supplied by 6 gpm of Columbia River water at 10 C during the 4 month period of observation. Mortalities not attributable to collection were initially observed in early April when 20 fish died. Gross lesions were present in all fish and consisted of soft white nodules (1-5 mm in diameter) in the following organs (listed in order of frequency): kidney, liver, pyloric caeca, spleen, gills, heart, gonads. Microscopically, the nodular lesions consisted of aggregates of large, round macrophages which were filled with large numbers of acid-fast bacteria. The bacteria were rod-shaped, approximately  $0.5 \times 3 \mu\text{m}$  in tissue sections.

A total of 131 whitefish were collected in the same area on 19 February 1976. Eighteen fish injured during electrofishing and transport were killed and examined. Two had gross lesions similar to those observed in 1975, consisting of accumulations of macrophages and numerous acid-fast organisms. Seventeen days after collection, 25 randomly-

<sup>1</sup> This work was performed for the U.S. Department of Energy, formerly the Energy Research and Development Administration, under Contract EY-76-C-06-1830.

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selected fish were removed from the tank and examined for gross and microscopic lesions. None had gross lesions indicative of mycobacteriosis; however, in one fish, acid-fast organisms were observed in tissue sections of the gill and kidney. Mortalities began to occur in the holding tank on 9 April, 50 days after collection. On 19 April, 5 lethargic and 5 normal fish were removed; two of the former had numerous white nodules on the visceral organs. External signs of infection consisted mainly of loss of condition and lethargy. No color change was observed. Of the 27 fish that died up to 130 days after capture, three had gross visceral lesions typical of mycobacteriosis. On 29 June (131st day), the 51 remaining whitefish were killed; two had very small nodules on gills and kidneys which, on microscopic examination, contained acid-fast organisms. The sex, length and weight were recorded and scale samples taken from all of the fish collected in 1976 except the 16 normal fish of the 18 that were injured during collection. The age of the fish was estimated by examination of annuli on scales.

## RESULTS

There appeared to be no sex difference in the prevalence of mycobacteriosis; but the severity of the infection appeared to be related to age (Table 1). Lesions were small, few in number, and not widespread in age groups III and IV. Lesions were large and widely distributed in age group V and these fish had external signs of infection (loss of condition and lethargy).

The postspawning condition of the sexual organs of infected fish was comparable to that of a noninfected fish. No atrophied eggs were present in ovarian tissues of infected females, in contrast to a previous description of ovarian development in infected chinook salmon.<sup>4</sup>

Attempts to culture the organism on Lowenstein's agar,<sup>5</sup> after sodium hydroxide digestion of tissues, were not successful. The surface of the kidney of one affected fish was heat-sterilized and material obtained from a granuloma with a culture loop was spread directly on Lowenstein's agar. Culture tubes were incubated both at room temperature (22

TABLE 1. Mountain whitefish with gross or microscopic evidence of mycobacterial infection collected in the Yakima River in 1976. Total number of fish observed shown in parentheses.

	Age Group					TOTAL
	III	IV	V	VI	VII	
Male	0(3)	1(9)	1(11)	0(5)	0(1)	2(29)
Female	1(15)	2(36)	3(24)	2(10)	0(1)	8(86)
% Infected	5.6	6.7	11.4	13.3	0.0	7.6 <sup>a</sup>
Average grade of infection <sup>b</sup>	1	2	4	3.5	0	

<sup>a</sup>Total % infected includes 16 noninfected fish for which age and sex were not recorded.

<sup>b</sup>Lesions rated 1 to 4; 1 = slight, 4 = severe

<sup>5</sup> DIFCO, Detroit, Michigan

C) and in an incubator at 37 C. Small colonies of acid-fast organisms were observed on the agar slants incubated at room temperature after 3 to 6 weeks. The agar slants incubated at 37 C had no visible colony formation after 3 months, and were discarded. The organism has tentatively been classified as belonging in Runyon group III <sup>□</sup> but has not been further characterized at this time.

### DISCUSSION

The method of collection, the season, and the sample site were selective for the adult spawning population. The prevalence of mycobacteriosis in whitefish may be nearly 8%, if the sample is representative of the wild population. The stress of collection and maintenance of whitefish under artificial conditions may have enhanced the development of the disease to some degree. However, the increased severity of infection with age suggests that holding the stock for several weeks probably did not affect the prevalence of mycobacteriosis as much as it would affect the prevalence of a disease with a shorter incubation period.

Neither the source of the acid-fast organism nor the mode of transmission are known. An adequate number of other species of fish from the river have not been examined to determine the extent of the disease. The identity of the organism has not been definitely established. The slow growth at 20-22 C, lack of growth at 37 C, and the pleomorphic morphology and stain reactions are similar to those of other piscine mycobacterial isolates. Attempts to further characterize the bacterial isolates are continuing.

Media other than Lowenstein's are now being used to aid in characterization; thus far, all cultures have grown very slowly. Cultures from the kidneys of chinook salmon, incubated at 20 C for 5 days on BBL Dubos agar, have been

reported successful for the isolation of *Mycobacterium* sp.<sup>4</sup>

Although the gross lesions of mycobacteriosis in the whitefish examined were similar to those reported in other fish,<sup>1</sup> some microscopic features of the lesions were different. The disease in salmon is characterized by a lack of an inflammatory response to the organism;<sup>5</sup> in contrast, our findings in whitefish indicated a generous mononuclear cell response. The lesions in whitefish (Fig. 1) were not encapsulated by fibrous connective tissue as has been reported in tropical fish.<sup>1</sup>

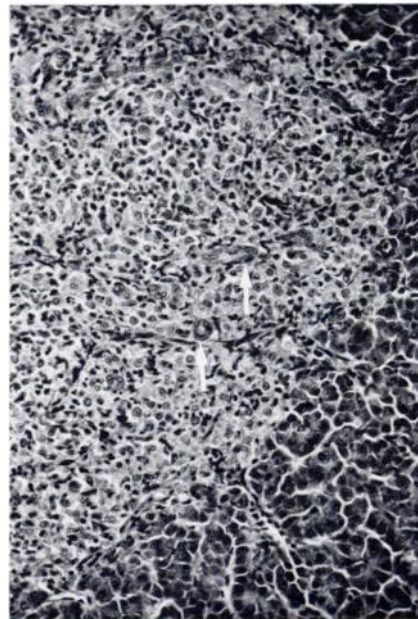


FIGURE 1. A mycobacterial lesion in the liver of a whitefish. Most of the cells are macrophages. Structures resembling multinucleated giant cells (arrows) are regenerating bile ducts. Note the lack of fibrosis at the periphery of the lesion. Hematoxylin and eosin. 125X.

<sup>□</sup> U.S. Department of Agriculture, Animal and Plant Health Inspection Service, Ames, Iowa 50010, USA.

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