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MFR PAPER 1341

Susceptibility Studies of Various Salmonids to Whirling Disease: Histological Staining and Spore Concentration Procedures

JOSEPH O'GRODNICK

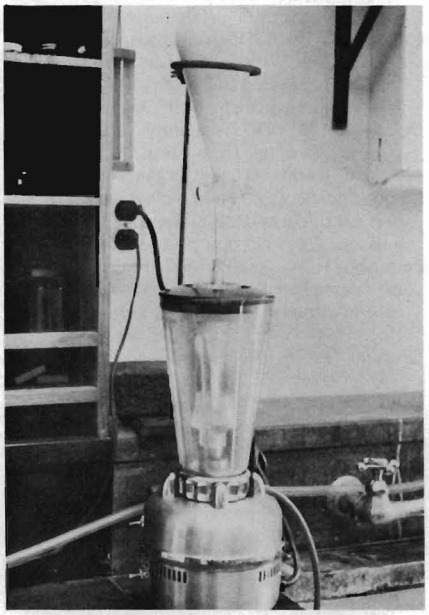

Figure 1.—The plankton centrifuge. The centrifuge removes water through the centrifugal force created by the revolving drum. The excess water is carried away through a rubber tube situated below the drum. Solid particles are deposited on the wall of the revolving drum.

Since 1968, research on the transmission, life history, and control of whirling disease in trout has been conducted by the Pennsylvania Fish Commission. Whirling disease is caused by a myxosporidian parasite, *Myxosoma cerebralis*, which invades and destroys cartilage of susceptible salmonid species. The parasite was introduced into the United States from Europe and has been established as a persistent hatchery disease in several areas.

Affected fish exhibit rapid, tail chasing behavior from the disintegration of the cartilaginous support skeleton of the organs of equilibrium. Also, severe crippling may result from destruction of the spinal skeleton if fish are exposed as fry. "Blacktail," which is caused by pressure on the nerves that control the caudal pigment cells, may be seen in some fish.

In this research, two methods were used to monitor the development of the whirling disease parasite in various salmonid species: 1) examination of histological sections, and 2) quantitative estimates of spores determined by the use of the plankton centrifuge procedure (Fig. 1). (This method involves homogenization of head skeletons, screening out tissue shreds, and concentrating with a continuous plankton centrifuge.

The life cycle of *Myxosoma cerebralis* was monitored in rainbow trout (highly susceptible species) and brown trout (highly resistant species) by the examination of histological sections. Tissues were prepared from infected fish at given intervals, averaging 3 days from initial exposure to the development of spores at 120 days. The following staining methods were used in the study: 1) Hematoxylin and Eosin, 2) Mallory Heidenhain (Casson-Modification), 3) Wright's stain, and 4) Ziehl-Neelson (acid fast staining for spores).

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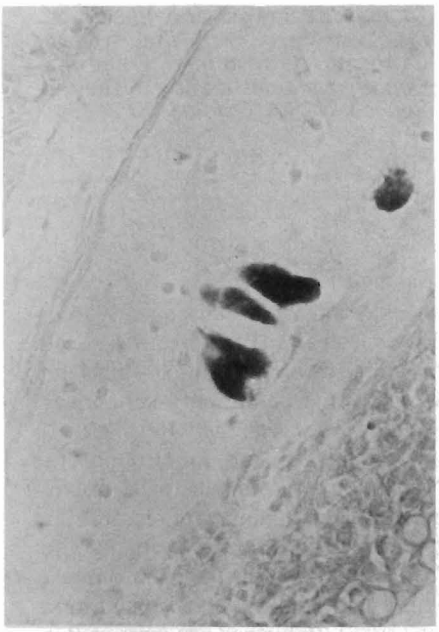
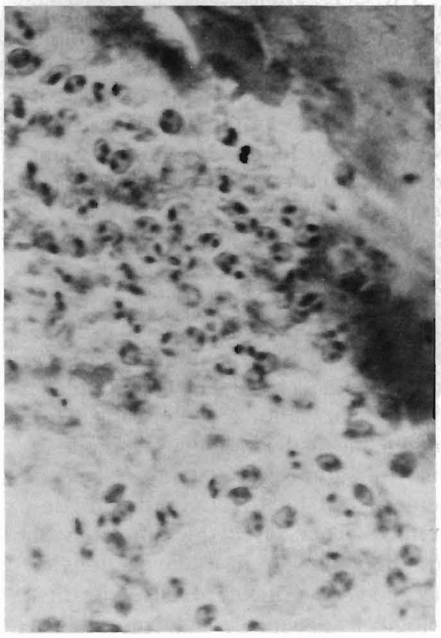


Figure 2.—Trophozoites of *Myxosoma cerebralis* developing in rainbow trout cartilage 35 days postexposure. Mallory Heidenhain (Casson-Modification); 225×.

Figure 3.—Mature spores of *Myxosoma cerebralis* in rainbow trout cartilage. Wright's stain. 225×.



The study revealed the following sequence of events in rainbow trout tissue. The trophozoite stage (Fig. 2) was first observed at 20 days postexposure. No definite parasitic stage was observed prior to invasion of the cartilage at 20 days. There was rapid proliferation of the multinucleated trophozoites by numerous divisions for 70 days—90 days from initial exposure. At 90 days, the trophozoites began the transformation to the spore stage of the life cycle (pansporoblast stage). A connective tissue network began to surround the developing spores, and mature spores (Fig. 3) were seen at 120 days. The fish in this study were reared at 11°C water temperature.

The brown trout study revealed a similar development of *Myxosoma cerebralis*, but very few parasitic lesions were observed. There appeared to be some disintegration of active trophozoites 60-90 days after initial exposure. There were also many tissue eosinophils present in surrounding connective tissue of infected brown trout. These cells were also observed in control fish tissue sections. Spores developed in smaller pockets in brown trout cartilage and were difficult to find in histological sections.

A study of the relative susceptibility of various salmonids to whirling disease was conducted during 1975 and 1976. In 1975, fingerlings of four different salmonids were exposed to whirling disease for a 60-day period and then reared in uncontaminated water until spores developed at 160 days. Samples were run in five pools of five fish each (25 heads) for the plankton centrifuge technique and five fish (25 slides) for the histologic section technique. Table 1 contains the results of the 1975 study.

In 1976, salmonid fry were exposed for 3 days to whirling disease and then reared in spore-free water until spores developed at 160 days. Samples were run as in 1975. Table 2 contains the results of the 1976 study.

Table 1.—Susceptibility of four species of salmonid fingerlings exposed to whirling disease for 60 days as assessed by histologic and plankton centrifuge techniques.

Species	Histology: no. of positive slides (5 fish/25 slides)	Plankton centrifuge: average no. of spores per head (25 heads)
Rainbow trout	Not done	404,800
Brook trout	3	47,336
Brown trout	Not done	784
Coho salmon	0	0

Table 2.—Susceptibility of seven species of salmonid fry exposed to whirling disease for 3 days as assessed by histologic and plankton centrifuge techniques.

Species	Histology: no. of positive slides (5 fish/25 slides)	Plankton centrifuge: average no. of spores per head (25 heads)
Rainbow trout	100	1,619,156
Brook trout	56	552,150
Brown trout	8	6,075
Lake trout	0	0
Kokanee salmon	100	116,775
Chinook salmon	20	60,775
Coho salmon	0	[1]0

[1]Accidental loss, only one pool run.

The development of *Myxosoma cerebralis* varied extensively in the salmonids studied. Under controlled conditions, brown trout, lake trout, and coho salmon (which exhibited little or no evidence of spores) did not develop characteristic whirling disease signs. Rainbow trout, brook trout, kokanee salmon, and chinook salmon did develop whirling disease, exhibiting clinical signs of disease. Since whirling disease can cause substantial losses in some hatcheries, resistant species may be reared to sustain production in areas where *Myxosoma cerebralis* is endemic.

Recent studies have revealed that species other than salmonids may be carriers of the whirling disease parasite. We have found similar sporozoan parasites in cartilage of suckers and in brain tissue of creek chubs.

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