Environmental and molecular aspects of salmonid whirling disease
by Stacie Marie Clark

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Veterinary Molecular Biology
Montana State University

Abstract:
Whirling disease, caused by the metazoan parasite Myxobolus cerebralis, is a significant international problem for feral and hatchery-raised salmonids. Whirling disease continues to have devastating impacts on local economies and recreational activities in certain regions of Russia, British Columbia, Czechoslovakia, Ireland, Europe, New Zealand, Scotland, Denmark and in the United States. In the U. S., whirling disease has been found in 22 states. In the upper Madison River of Montana, whirling disease has been found responsible for a 90% decline in young rainbow trout. The life cycle of M. cerebralis is complex involving several developmental stages in 2 hosts, an oligochaete intermediate host and a salmonid definitive host. To date, there are still no effective means of preventing or managing the disease. One of the major tasks currently underway in Montana and other regions of the U. S. is to develop a rapid and sensitive test that can be used easily for detecting whirling disease in both oligochaete and fish hosts. In this study, a specific DNA-based polymerase chain reaction (PCR) assay was used to detect M. cerebralis DNA in both salmonid and oligochaete hosts. The assay involved isolating and purifying parasite DNA from fish and oligochaetes, using M. cerebralis specific primers, and amplifying a region of parasite rDNA sequence using a nested-PCR approach. Two rounds of PCR were used on each test sample to amplify a known region of parasite 18 S ribosomal DNA sequence. . The amplified parasite product was detected by gel electrophoresis and visualized at approximately 410 bp. Also during this study, an improved method for processing parasite DNA in a background of oligochaete or salmonid DNA was developed. The improved method involved digesting host and parasite tissue with a detergent lysis buffer and testing the resulting material,' without further purification of parasite DNA, directly in the PCR reaction,

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APPROVAL

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Stacie Marie Clark

This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

Whirling disease, caused by the metazoan parasite *Myxobolus cerebralis*, is a significant international problem for feral and hatchery-raised salmonids. Whirling disease continues to have devastating impacts on local economies and recreational activities in certain regions of Russia, British Columbia, Czechoslovakia, Ireland, Europe, New Zealand, Scotland, Denmark and in the United States. In the U. S., whirling disease has been found in 22 states. In the upper Madison River of Montana, whirling disease has been found responsible for a 90% decline in young rainbow trout. The life cycle of *M. cerebralis* is complex involving several developmental stages in 2 hosts, an oligochaete intermediate host and a salmonid definitive host. To date, there are still no effective means of preventing or managing the disease. One of the major tasks currently underway in Montana and other regions of the U. S. is to develop a rapid and sensitive test that can be used easily for detecting whirling disease in both oligochaete and fish hosts. In this study, a specific DNA-based polymerase chain reaction (PCR) assay was used to detect *M. cerebralis* DNA in both salmonid and oligochaete hosts. The assay involved isolating and purifying parasite DNA from fish and oligochaetes, using *M. cerebralis* specific primers, and amplifying a region of parasite rDNA sequence using a nested-PCR approach. Two rounds of PCR were used on each test sample to amplify a known region of parasite 18 S ribosomal DNA sequence. The amplified parasite product was detected by gel electrophoresis and visualized at approximately 410 bp. Also during this study, an improved method for processing parasite DNA in a background of oligochaete or salmonid DNA was developed. The improved method involved digesting host and parasite tissue with a detergent lysis buffer and testing the resulting material, without further purification of parasite DNA, directly in the PCR reaction.

CHAPTER 1

INTRODUCTION

Historical Perspective

Whirling disease, caused by the metazoan parasite *Myxobolus cerebralis*, is considered a major disease of the fishes in the family Salmonidae (Markiw, 1992). Since whirling disease was first described in Germany by Hofer (1903), it has spread to over 28 countries on 5 continents (Halliday, 1976). Whirling disease was first diagnosed in the U. S. in 1958 at the Benner Spring Fish Research Station in Pennsylvania. It is speculated that *M. cerebralis* contaminated frozen trout were imported to the station from Europe (Hoffman, 1962; Margolis, 1981; Horsch, 1987; Yasutake, 1970). Subsequently, the disease spread to Nevada (1958), Connecticut (1961), Virginia (1965), California (1966), and Massachusetts (1966) (Hoffman, 1990). Since then, whirling disease has spread to 22 states, including Montana.

In December, 1994, *M. cerebralis* was first discovered in Montana's upper Madison River. There has been additional confirmation of 52 positive *M. cerebralis* Montana waters since that time (anonymous, 1997) (Table 1). Major Montana drainages contaminated with *M. cerebralis* include the Beaverhead, Madison, Jefferson and Yellowstone.

Table 1. Summary of 52 Montana waters which have tested positive for *M. cerebralis* from December 20, 1994 through July 29, 1997. (Montana Fish Wildlife and Parks, 1997).

LOCATION	DRAINAGE	SPECIES
Alder Gulch Creek	Beaverhead	BNT
Beaverhead River	Beaverhead	BNT
Big Hole River	Big Hole	RBT
Big Sheep Creek	Beaverhead	BNT
Birch Creek Reservoir	Beaverhead	RBT
Blacktail Deer Creek	Beaverhead	RBT, BKT
Blaine Spring Creek	Madison	RBT
Boulder River	Jefferson	RBT, BNT, BKT
Canyon Pond	Beaverhead	RBT, BNT
Cherry Creek	Madison	RBT
Clark Canyon res.	Beaverhead	RBT
Clark Fork River	Clark Fork	RBT
Cottonwood Creek	Blackfoot	RBT
Culver Pond	Beaverhead	BKT
E. Fork Rock Creek	Clark Fork	RBT, BKT, WCT
Flint Creek	Clark Fork	RBT, BNT, BKT
Flint Creek, N. Fork	Clark Fork	RBT, BKT
Georgetown Lake	Clark Fork	KOK
Grasshopper Cr. (upper)	Beaverhead	RBT, BNT
Hells Canyon Creed	Jefferson	RBT, BNT
Horse Creek	Madison	RBT, BNT
Horse Prairie Creek	Beaverhead	BKT
Hound Creek	Smith	BNT
Jack Creek	Madison	BNT

Table 1 Cont.

Jefferson River	Jefferson	RBT, BNT
Little Prickly Pear Cr.	Missouri	RBT
Lost Creek	Clark Fork	BNT
Madison River	Madison	RBT
Missouri River	Missouri	RBT
Moore Creek	Madison	BNT
Moose Creek	Madison	BNT
O'dell Creek	Madison	RBT, BNT
Papoose Creek	Madison	RBT
Poindexter Slough	Beaverhead	BNT
Racetrack Creek	Clark Fork	BNT, BKT
Red Rock R. (Springs)	Beaverhead	RBT, BNT
Red Rock R. Creek	Beaverhead	BKT, RBX
Rock Creek	Clark Fork	RBT, BNT, BKT, RBX
Ruby River	Beaverhead	BNT
Ruby Reservoir	Beaverhead	RBT
Soap Creek	Madison	RBT
South Boulder River	Jefferson	RBT
Squaw Creek	Madison	unknown
Stuart Mill Creek	Clark Fork	BKT
Swan River	Flathead	RBT
Warm Spring Creek	Clark Fork	BKT
West Fork Madison River	Madison	RBT, BNT
Whitehall Creek	Jefferson	BNT
Willow Creek (above Res.)	Jefferson	RBT
Willow Springs Creek	Jefferson	BNT
Wolf Creek	Madison	RBT
Yellowstone River	Yellowstone	RBT

Species Codes: **RBT**- Rainbow Trout
BNT- Brown Trout **KOK**- Kokanee
BKT- Brook Trout **RBX**- RBT x Cutthroat hybrid
WCT- Westslope Cutthroat

Economic Impacts

Whirling disease threatens some of Montana's best wild trout fisheries, and is responsible for tremendous economic and recreational losses. The economic value of sport fishing in Montana has been estimated to be $500,000,000 with sport fishing on the Madison River alone generating approximately $50,000,000 (Table 2) (R.Vincent, pers. commun., 1997). According to R. Vincent, whirling disease research coordinator for Montana Fish Wildlife and Parks, the number of angler days spent on southwest Montana streams in 1996 declined 10 to 15%. Although poor weather conditions may have been a contributing factor, Vincent (pers. commun., 1997) believes this decline can be partly attributed to whirling disease.

Table 2. Estimated economic value of selected Montana streams in 1993 (Brooks, pers. commun., 1996).

RIVER	*TOTAL ANGLER DAYS*	*TOTAL USE VALUE*
Madison	146,039	$ 47,756,238
Beaverhead	20,736	$ 4,619,394
Big Hole	63,247	$ 13,744,591
Gallatin	71,129	$ 19,944,504
Jefferson	16,865	NOT AVAILABLE
Upper Yellowstone	79,718	$ 27,728,476

Host Susceptibility

Whirling disease is a chronic disease of salmonids which may cause high mortality rates, especially in rainbow (*Oncorhynchus mykiss*) and brook (*Salvelinus fontinalis*) trout under one year of age (Hoffman, 1974; Markiw, 1991, 1992; Putz and Hoffman, 1966). Rainbow trout, cutthroat trout (*Oncorhynchus clarki*), brook trout, kokanee salmon, and chinook salmon have been shown to exhibit clinical signs of whirling disease. In contrast, brown trout (*Salmo truttta*) , lake trout (*Salvelinus namaycush*) and coho salmon appear to be relatively resistant (O' Grodnick, 1979). Other salmonids susceptible to whirling disease include golden trout (*Oncorhynchus aguabonita*) (Yasutake and Wolf, 1970) and Atlantic salmon (Hoffman, 1990).

Pathogenesis

Clinical signs of whirling disease appear at approximately 35 to 80 days after initial exposure to *M. cerebralis* spores depending on the intensity of infection and water temperature (Markiw, 1997). Earlier initial clinical signs may appear at higher water temperatures (up to 17° C) or in the presence of a higher concentration of spores. In a study by Halliday (1973), symptomatic signs of whirling disease (whirling and black tail) appeared approximately 70 days post exposure at 12° C. In contrast, fry exposed to approximately the same number of *M. cerebralis* spores at 17° C exhibited clinical signs of disease at 55 days post exposure. Additionally, a greater percentage of trout with whirling disease symptoms was observed at 17° C than at 12 °C.

The caudal peduncle and tail may become dark or even black ("blacktail") due to neural damage from lesions and deterioration of cartilaginous tissue (Fig. 2) (Elson, 1969; Halliday, 1973; Hastein, 1971; Hoffman, 1966; Roberts, 1970). When alarmed or feeding, some infected fish show an abnormal whirling behavior, hence the origin of the disease name. Since *M. cerebralis* has a selective attraction to cartilage it mainly affects young fish in which bone ossification has not progressed to maturity (Halliday, 1973; Lucky, 1970).

Although internal organs appear normal, histological sections of cartilage, particularly gill, skull, and vertebrae show areas of inflammation and deterioration (Fig. 3). Spores from *M. cerebralis* may be visible around cartilage lesions (Fig. 4). The presence of *M. cerebralis* spores in cartilage is considered pathognomonic, but subsequent confirmation by polymerase chain reaction testing is required by the U.S. Fish and Wildlife Service as part of the newly implemented National Wild Fish Health Survey (anonymous, 1997).

Figure 1. Skeletal deformities in a rainbow trout infected with *M. cerebralis*.