Field and Laboratory Evaluation of Whirling Disease Using a Novel Polymerase Chain-Reaction Diagnostic Assay, and Assessing the Risk of Whirling Disease Becoming Established in Wisconsin

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ABSTRACT

Since its introduction into North America around 1956 *Myxobolus cerebralis* (Mc) has caused extensive damage to wild trout populations in the intermountain west (Montana, Colorado, and Utah). The parasite has worked its way out of the initial infestation areas (Pennsylvania and Nevada), and is spreading across the country threatening unaffected areas. As of this time, Mc has not been reported in Minnesota and Wisconsin. To date, this project has emphasized examining local streams which are rated highly for their brook, brown, and rainbow trout populations. Trout from these streams were examined for Mc, and cohabiting fish were examined for closely related species of myxosporeans. Local streams were also examined for the presence of *Tubifex tubifex*, the only known oligochaete intermediate host for Mc, and for the presence of other potential intermediate hosts. Other genera of oligocheates that have been collected have been placed into pure cultures to later evaluate their abilities to serve as intermediate hosts for Mc. To date, no myxosporeans have been recovered from any of the trout sampled, but cohabiting white suckers routinely harbor *Myxobolus bibullatum* in the gills, and slimy sculpins harbored *Thelohanellus* sp in muscle tissue. To obtain additional myxosporeans for testing the specificity of the polymerase chain reaction diagnostic assay developed by Hedrick, fish were also examined from the Mississippi River. Several *Myxobolus* spp were obtained from the gills of shorthead, silver and golden redhorse. *Myxobolus neurophila* has been recovered from cranial areas of bluegill. *Henneguya exilis* is found on gills of channel catfish. In all, more than 206 fish in 27 species have been examined for myxosporeans. *Tubifex tubifex* has been recovered from Coulee Region trout streams, indicating that whirling disease may be able to establish itself in this area.

INTRODUCTION

Myxobolus cerebralis (Mc) is an exotic species of myxosporean parasite that is thought to have been introduced into North America in 1956 (Markiw 1992). Mc is the cause of salmonid whirling disease (WD) and is suspected to be the cause of major declines in wild trout populations in three intermountian western states. WD can be devastating to trout populations. For example, the Colorado River has lost four consecutive

year classes (generations) of rainbow trout since the invasion of Mc into the river (Walker and Nehring 1995). Also, WD is thought to have caused the rainbow trout fishery in the Madison River (in Montana) to decline by 91% in the three years between 1991 and 1994 (McCoffery and Saile 1995).

Wisconsin, Minnesota and a few other states are the only states with major self-sustaining trout populations that have not been affected by WD (Mathews 1995). These states are at risk of having Mc invade their trout populations. Introduction of WD into Wisconsin and Minnesota would place a multi billion dollar a year Great Lakes sport fishery and a multi-million dollar a year inland trout fishery at risk.

MATERIALS AND METHODS

Collection of parasitic samples: Sample sites included Coulee Region trout streams, cool water creeks, the Mississippi River main channel, and Coulee Region marshes. Fish were collected from these areas using back pack electrofishers, boat electrofishers, dip nets, and seines. These fish were necropsied, and examined for myxosporean parasites using dissecting microscopes. Subsamples of myxosporeans recovered were fixed in 70% EtOH and later identified using standard spore morphometric parameters. Additional subsamples of the same myxosporeans were frozen at -70° C and later thawed for analysis with the polymerase chain reaction assay developed by Hedrick (Andre et al. 1997). Trout heads also were examined for *Myxobolus cerebralis* using the modified plankton centrifuge method of O'Grodnik (1975).

Collection of oligochaete samples: Benthos samples were also collected from trout streams using Surber samplers, kick nets and Ekman dredges and preserved in 20% formalin. Preserved benthos samples were screened for tubificid oligochaetes. Subsamples of tubificids were mounted on slides and examined for the presence of *Tubifex tubifex*, the only known intermediate host for *Myxobolus cerebralis.* Oligochaetes were identified using the taxonomic keys of Brinkhurst (1986).

RESULTS

Results of the myxosporean collections are summarized in Tables 1, 2. To date, no myxosporeans have been recovered from any of the trout sampled, but cohabiting white suckers routinely harbor *Myxobolus bibullatum* in the gills, and slimy sculpins harbored *Thelohanellus* in muscle tissue. The PCR results indicate that the nested PCR protocol is specific for Mc. There was one non specific cross reaction with the first primers for a myxosporean that inhabits the meninges of bluegill (*M. neurophila*).

Tubifex tubifex and *Limnodrilus hoffmeisteri* are the two predominant tubifid oligochaetes found in Coulee Region trout streams.

DISCUSSION

To date no Mc have been identified from brook and brown trout in Coulee Region trout streams. The absence of the parasite in Coulee Region trout streams was expected, and confirms the theory that the disease has not yet infected area trout populations.

The oligochaete host *Tubifex tubifex* has been identified in benthos samples taken from the areas trout streams. The presence of the intermediate host suggests that if Mc is introduced into Coulee Region trout streams that the life cycle will be able to establish,

and the disease may have a great impact on the rainbow trout in the area. There is also a risk to steelhead trout populations that inhabit the Great Lakes. The introduction of this parasite into either the Great Lakes or Wisconsin trout streams would have a great impact on the numbers of rainbow and steelhead trout, the Great Lakes sport fishery, and the local fishery environment.

CONCLUSION

Whirling disease has not yet been introduced into Coulee Region trout streams. Analysis of all of myxosporeans collected did not show cross reaction when tested with the Hedrick WD specific PCR assay, except that *M. neurophila* non specifically cross reacted with the outer primers. *Tubifex tubifex* does inhabit many of the Coulee Region trout streams; therefore, if *M. cerebralis* does become introduced into Wisconsin, and the *T. tubifex* resident in Wisconsin is a subspecies susceptible to Mc, then WD should be able to establish.

LITERATURE CITED

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Table 1. Prevalence of myxosporean infections in all fish speices necropsied during summer. 1997 from all habitats (trout streams, cool water creeks, Mississippi River main channel, and marshes).

Species	Number of Fish Sampled	Prevalence of Myxosporean Infections
White Sucker	35	25.7%
Spotted Sucker	3	66.0%
Slimy Sculpin	9	44.9%
Central Mudminnow	13	15.4%
Brook Stickleback	20	00.0%
Blacknose Dace	1	00.0%
Fantail Darter	1	00.0%
Banded Darter	5	00.0%
Johnny Darter	4	00.0%
Channel Catfish	3	00.0%
Freshwater Drum	3	00.0%
Bowfin	1	00.0%
Creek Chub	21	09.5%
Carp	5	00.0%
Bluegill	1	00.0%
Black Crappie	1	00.0%
Sauger	2	00.0%
Walleye	3	00.0%
Silver Redhorse	4	50.0%
Golden Redhorse	4	50.0%
Shorthead Redhorse	3	33.3%
Smallmouth Bass	3	00.0%
Largemouth Bass	2	00.0%
Rock Bass	2	00.0%
White Bass	3	00.0%
Brook Trout	25	00.0%
Brown Trout	29	00.0%

Table 2. Prevalence of myxosporean infection in various Coulee Region trout streams.

Stream	Species	Number of Fish Sampled	Prevalence of Myxosporean Infection
Timber Coulee	Brown Trout	8	0.0%
	White Sucker	3	100.0%
	Slimy Sculpin	9	44.4%
Spring Coulee	Brown Trout	13	0.0%
	White Sucker	8	25.0%
Burns Creek	Brown Trout	25	0.0%
Dutch Creek	Brown Trout	8	0.0%
Bohemian Coulee	White Sucker	4	25.0%
	Brown Trout	17	0.0%
North Fork Chipmunk Creek	White Sucker	11	27.3%