FINAL REPORT TO SMURFIT-STONE CONTAINER CORPORATION

SURVEY OF UPPER RATTLESNAKE CREEK, A TROUT-SPAWNING TRIBUTARY OF THE CLARK FORK RIVER (MT), FOR *TUBIFEX TUBIFEX*

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Introduction

Rattlesnake Creek is a tributary of the Clark Fork River that flows through parts of western Montana and through Missoula. Currently, an effort is underway to increase spawning habitat for bull and westslope cutthroat trout in the Clark Fork drainage. One proposal in this effort includes the modification or removal of the Mountain Water Company dam on lower Rattlesnake Creek that currently blocks fish passage. The removal or modification of this dam would open miles of prime spawning habitat. However, the Clark Fork River is known to be contaminated with *Myxobolus cerebralis*, the causative agent of salmonid whirling disease. The presence of this parasite in the Clark Fork River has lead to concerns that removal/modification of the dam would allow the parasite to spread into the upper reaches of Rattlesnake Creek, endangering the wild trout populations already established there. Therefore, the objective of this study was to survey the upper portions of Rattlesnake Creek for the presence of the aquatic oligochaete, *Tubifex tubifex*. *T. tubifex* is an obligatory host for *M. cerebralis* and the parasite cannot be transmitted to trout in its absence. An earlier, cursory study by another investigator did not detect *T. tubifex* in the Rattlesnake, so we conducted a more comprehensive survey of the creek for this oligochaete. Further, these worms were screened for the presence of *M. cerebralis* triactinomyxons (TAMs; stage of parasite that infects trout).

Materials and Methods

Oligochaete samples were collected from six sites on Rattlesnake Creek (Figure 1). Four of the sites were along a 9.5-mile stretch above the dam (measured from the U.S. Forest Service's gate at the north end of the parking lot of the Rattlesnake National Recreation Area), the fifth site was about 20 meters above the dam, while the sixth site was located approximately 400 meters below the dam. For all sites, areas that looked like prime *T. tubifex* habitat were intentionally selected.

Oligochaetes were collected from all sites on both October 8, 1999 and again on June 16, 2000, using a modified kick net method (Gilbert and Granath, in press). The worms were placed in 2-liter plastic containers and kept in a cooler during transport back to the laboratory. The oligochaetes were then separated, one per chamber, into 24 well tissue culture dishes, with each chamber containing 1 ml of well water and were kept in an incubator at 15°C with a photoperiod of 14 hr light: 10 hr dark. Water from each well was then periodically examined, during a three-day period, to determine if any of the worms were actively shedding TAMs. After the three-day observation period each worm was fixed in 10% neutral buffered formalin for 24 hours, followed by a secondary fixation in 70% ethanol. The worms were then mounted on microscope slides and identified based on chaetae, sexual organs (when present) and other morphological characteristics (Kathman and Brinkhurst, 1998) to the greatest resolution possible. Further, the identification of these worms was confirmed by an expert in such matters (Dr. R.D. Kathman, Aquatic Resources Center, College Grove, TN). Additionally, standard water quality parameters were measured at each site when the worms were collected (i.e. temperature, pH, dissolved oxygen, conductivity, total dissolved solids).

Results

Although quantitative sampling procedures were not performed, the overall density of oligochaetes was low at all sites. Further, both collection dates resulted in worms that were mostly immature. Unfortunately, sexually mature specimens are required for the positive identification of *T. tubifex*. Despite this, it appears that *T. tubifex* is not abundant in Rattlesnake Creek. Results of the oligochaete collections are presented in Table I. Further, none of these worms released *M. cerebralis* TAMs.

Table 1. Oligochaetes present at six sample sites along Rattlesnake Creek.

Site #	Date	Oligochaete Families Present (Genera and Species, if known, are in parentheses)	Comments
1 (9.5 mile)	10/99	Tubificidae (n=16)	Tubificids were immature but had hair and pectinate chaetae, possibly *T. tubifex.*
		Tubificidae (n=1)	Immature; bifid chaetae; not likely *T. tubifex*.
		Lumbriculidae (*Rhynchelmis sp*.) (n=3)	Immature.
	6/00	Tubificidae (*Rhyacodrilus coccineus*) (n=3)	
		Lumbriculidae (*Rhynchelmis sp*.) (n=4)	Immature.
2 (6.15 mile)	10/99	No oligochaetes recovered	
	6/00	Tubificidae (*Rhyacodrilus coccineus*) (n=9)	
3 (3.75 mile)	10/99	Tubificidae (n=1)	Tubificid was immature but had hair and pectinate chaetae, possibly *T. tubifex.*
		Lumbriculidae (*Rhynchelmis sp*., n=10; probable *Eclipidrilus sp*., n= 4)	Lumbriculids were immature.
		Naididae (*Slavina appendiculata*) (n=2)	
	6/00	No oligochaetes recovered	
4 (0.1 mile)	10/99	Tubificidae (*Rhyacodrilus coccineus*) (n=4)	
		Lumbriculidae (*Rhynchelmis sp*., n=6; probable *Eclipidrilus sp*. n=1)	Lumbriculids were immature.
	6/00	Lumbriculidae (*Rhynchelmis sp*.) (n=2)	Immature.
5 (20 m above dam)	10/99	Tubificidae (n=6)	Immature; bifid chaetae; not likely *T. tubifex*
		Tubificidae (*Rhyacodrilus coccineus*, n= 4; *Limnodrilus profundicola*, n=2; *Limnodrilus hoffmeisteri*, n=4, *Telmatodrilus vejdovskyi*, n=1)	Immature.
		Lumbriculidae (*Rhynchelmis sp*.) (n=8)	Immature.
	6/00	Tubificidae (*Rhyacodrilus coccineus*) (n= 4)	
6 (400 m below dam)	10/99	Tubificidae (n=3)	Immature; bifid chaetae; not likely *T. tubifex*
		Tubificidae (*Limnodrilus profundicola*, n=1; *Rhyacodrilus coccineus*, n=2)	Immature.
		Lumbriculidae (*Rhynchelmis sp*.) (n=12)	Immature
	6/00	Tubificidae (n=3)	Tubificids were immature but had hair and pectinate chaetae, possibly *T. tubifex*
		Tubificidae (*Rhyacodrilus coccineus*) (n=4)	

Results of taking standard water quality measurements at the time of oligochaete collections are presented in Table 2.

Table 2. Water quality measurements taken at each collection site along Rattlesnake Creek.

Site #	Date	Temperature (ºC)	pH	Dissolved oxygen (%)	Conductivity (ųS/cm)	Total dissolved solids
1 (9.55 mile)	10/99	6.8	7.60	79.2	ND*	ND*
	6/00	5.0	7.74	87.0	19.2	9.5
2 (6.15 mile)	10/99	6.9	7.31	89.5	ND*	ND*
	6/00	5.6	7.18	88.5	19.4	9.7
3 (3.75 mile)	10/99	8.4	6.89	63.3	ND*	ND*
	6/00	6.5	7.44	87.6	19.7	9.7
4 (0.1 mile)	10/99	8.3	7.20	74.2	ND*	ND*
	6/00	7.2	7.34	89.4	18.5	9.2
5 (20 m above dam)	10/99	8.1	8.90	89.9	ND*	ND*
	6/00	7.5	7.39	89.2	22.2	11.1
6 (400 m be-low dam)	10/99	ND**	ND**	ND**	ND*	ND*
	6/00	7.8	7.31	68.5	49.7	25.1

*ND = Not Done (equipment not available).
**Not Done because samples were taken from mud and there was not enough standing water to make accurate measurements.

Discussion and Conclusions

The major objective of this study was to survey the upper Rattlesnake Creek drainage for *T. tubifex*. Therefore, collection sites were specifically chosen in areas containing prime oligochaete habitat. Despite this, the overall abundance of oligochaetes appeared low, although quantitative sampling was not performed (i.e. random, quantitative samples would have yielded even fewer oligochaetes then we detected). Unfortunately, *T. tubifex* could not be positively identified from any of the collection sites due to the fact that probable specimens (tubificids with hair and pectinate chaetae) were sexually immature. Even so, such worms were found at few sites and in relatively low abundance (Table 1). Further, efforts to grow these worms to sexual maturity in the laboratory were not successful.

The overall water quality of Rattlesnake Creek, based on the measurements taken in this study, appears to be excellent (Table 2). Moreover, the water temperatures at the sites were quite cold. This could be important to the establishment and transmission of *M. cerebralis* since *T. tubifex* release significantly fewer TAMs below 10ºC (El-Matbouli et al., 1999). Unfortunately, long-term seasonal temperature data for Rattlesnake Creek is not available.

In comparison to other studies we have conducted (e.g. Gilbert and Granath, in press) and continue to pursue, it is our opinion that upper Rattlesnake Creek, in its present condition, would not be threatened by whirling disease should fish passage around the dam occur. A combination of low water temperature, pristine habitat, and relatively low oligochaete densities would seem to make the establishment of *M. cerebralis* difficult here. Having worked extensively in other areas endemic for whirling disease, Rattlesnake Creek does not appear to meet the conditions for successful parasite transmission. However, we do realize that our non-exhaustive study had limitations. For example, *T. tubifex* may be more abundant then our results indicate, but we feel that this is a remote possibility. Also, the mere presence of possible *T. tubifex* at a few of our collection sites should not be used to infer that whirling disease will establish itself here. For example, since this simple survey was not quantitative, no data on whether there are sufficient numbers of *T. tubifex* to maintain the life cycle of *M. cerebralis* was generated. Although, given that we intentionally searched for *T. tubifex* habitat and found few oligochaetes, we feel that, if present, *T. tubifex* abundance is low. In addition, research by my laboratory and others have shown that there are genetic variants of *T. tubifex* that vary in their ability to support development and release TAMs. That is, such studies have indicated that some geographic variants of *T. tubifex* are very effective at transmitting whirling disease whereas populations of these worms in other locations are unable to transmit the parasite. Since our study did not examine the susceptibility of the presumptive *T. tubifex* that we collected, no data on their potential to transmit whirling disease was assessed. However, none of these worms were infected with *M. cerebralis*.

Literature Cited

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Acknowledgements

Smurfit-Stone Container Corporation, Missoula, MT, supplied financial support for this project. We also thank Ladd Knotek (Montana Fish, Wildlife and Parks) for valuable discussions concerning Rattlesnake Creek and for arranging permission to access the study sites via motorized vehicle.


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