Whirling Disease/Habitat Interactions

Federal Aid Project F-427-R3

Kevin G. Thompson
Principal Investigator

Bruce McCloskey, Director

Federal Aid in Fish and Wildlife Restoration

Job Progress Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

June 2006

STATE OF COLORADO

Bill Owens, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Russell George, Executive Director

COLORADO DIVISION OF WILDLIFE

Bruce McCloskey, Director

WILDLIFE COMMISSION

Jeffrey A. Crawford, Chair	Tom Burke, Vice Chair
Claire M. O'Neal, Secretary	Robert Bray
Brad Coors	Rick Enstrom
Roy McAnally	Richard Ray
Ken Torres	Russell George

Don Ament, Dept. of Ag.

AQUATIC RESEARCH STAFF

Mark S. Jones, General Professional VI, Aquatic Wildlife Research Leader
Richard Anderson, General Professional IV, F-289 Riverine Fish/Flow Investigations
Arturo Avalos, Technician III, Research Hatchery
Stephen Brinkman, General Professional IV, F-243, Water Pollution Studies
Patrick Martinez, General Professional V, F-242, Coldwater Reservoir Ecology & GOCO - Westslope Warmwater
R. Barry Nehring, General Professional V, F-237, Stream Fisheries Investigations
Phil Schler, Hatchery Technician V, Research Hatchery
George Schisler, General Professional IV, F-394, Salmonid Disease Investigations
Kevin Rogers, General Professional IV, GOCO - Boreal Toad and Colorado Cutthroat Studies
Kevin Thompson, General Professional IV, F-427, Whirling Disease Habitat Interactions
Rod Van Velson, General Professional IV, F-161, Stream Habitat Investigations and Assistance
Harry Vermillion, Scientific Programmer/Analyst, F-239, Aquatic Data Analysis
Nicole Vieira, Physical Scientist III, Water Quality Issues
Rosemary Black, Program Assistant I

Paula Nichols, Federal Aid Coordinator
Jackie Boss, Librarian

Prepared by: _____
Kevin G. Thompson, GP IV, Aquatic Wildlife Researcher

Approved by: _____
Mark S. Jones, Aquatic Wildlife Research Leader

Date: _____

The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or the Wildlife Commission.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Project Title: Whirling Disease / Habitat Interactions

Project No.: F-427-R

Project Objective: To investigate the influence of aquatic habitat factors on the severity of *Myxobolus cerebralis* infections in free-ranging trout populations in selected stream ecosystems in Colorado, and whether aquatic habitat factors can be managed to reduce the impacts of the parasite.

Job Title: Identification and reduction of *Tubifex tubifex* habitat in streams. Job No. 1

Job Objective: Develop and test strategies to reduce or eliminate *T. tubifex* habitat from areas of streams known to be foci of infectivity in order to reduce the production of actinospores of *Myxobolus cerebralis*.

Period Covered: July 1, 2005 to June 30, 2006

INTRODUCTION

In the early 1990s major declines in numbers of wild rainbow trout *Oncorhynchus mykiss* were observed in certain rivers in Colorado. In most streams in Colorado where rainbow trout numbers declined significantly the effects persist to the present day. Research indicates that these declines are the result of whirling disease (Walker and Nehring 1995; Nehring 1996; Nehring and Walker 1996; Nehring et al. 1998; Nehring 1998; Nehring 1999), caused by the parasite *Myxobolus cerebralis*.

Sentinel fish studies in the Colorado River and *M. cerebralis* actinospore filtration studies in numerous drainages suggest that some areas within streams act as foci of infection for the parasite (Thompson et al. 2002, Nehring and Thompson 2001; Thompson and Nehring 2000). Reservoirs may act as such foci. Stocking Spring Creek Reservoir with catchable trout infected with *Myxobolus cerebralis* resulted in elevated infectivity in Spring Creek below the reservoir (Nehring et al. 2001), as measured by actinospore densities in the water column and myxospore concentrations in samples of brown trout. Additionally, some sites of high infectivity that are not reservoir-related have been detected by actinospore filtration. Examples include some irrigation diversions and beaver ponds or pond complexes.

Infectivity below reservoirs has been addressed by taking steps to insure that fish stocked in them are uninfected with the parasite. Capital improvements to enhance hatchery water supply security, changes in hatchery management, and changes in stocking policy have all played significant roles. The benefits to downstream fisheries from these management actions become more apparent as time passes.

Nevertheless, certain areas of infectivity remain that are not reservoir related but appear to harbor *M. cerebralis* persistently. The objectives of this study are to determine whether it is possible

to remove or greatly reduce these areas of infection by physical habitat manipulation and stream habitat improvement techniques, and to determine if such manipulations result in reduced prevalence and intensity of infection among resident trout downstream of modified sites.

Segment Objectives:

1. Continue collecting post-manipulation triactinomyxon and fish data at study sites modified in previous segments.
2. Continue collecting triactinomyxon and fish data at control study sites.
3. Assist USGS personnel with post-manipulation survey work at the Poudre River study site.
4. Collect post-manipulation oligochaete data at the Poudre River study site.
5. Conduct electrofishing at standard stations on study streams.

METHODS and MATERIALS

Information at each study site was collected to describe the prevalence of infection in the fish, the oligochaete population, and the actinospore production dynamic.

Fish sampling

Samples of age 1+ brown trout were obtained at each location and analyzed for *M. cerebralis* spore concentrations in individual heads by the pepsin-trypsin digest method (PTD, Markiw and Wolf 1974). In some locations young-of-the-year (YOY) trout were collected; they were examined by the polymerase chain reaction (PCR) technique described by Schisler et al. (2001) or a subsequent PCR technique using the HSP-70 gene to determine whether *M. cerebralis* was present. The resulting bands observed on agarose gels were graded independently by two reviewers and reported on a five-point scale ranging from '0' (negative, no band) to '4' (an intense band indicating a severe parasite infection), hence the results are qualitative but more informative than simple presence or absence.

Oligochaete sampling

Oligochaete populations were characterized by sampling what was subjectively judged to be the best oligochaete habitat at each study site on two to four separate occasions. During this segment nine replicate samples were obtained on each occasion by a kicknet technique. A 0.5 m^2 area was selected by surrounding with a frame made of copper water pipe, and a 53.5 cm^2 core sample was removed at the center of the area selected. Depth of the core samples was 10 cm unless the substrate prevented this depth of penetration; all core depths were measured and recorded. The core samples were collected by USGS personnel in order to examine organic content and particle size distributions and determine whether relationships existed between these variables and *T. tubifex* density or lineage composition. Following removal of the core sample the total area was thoroughly disturbed with the sampler's feet for 60 seconds while holding a 250-µm mesh kicknet just downstream in the current to capture the organisms dislodged from the substrate. Each sample

was placed in a 4-L pail and covered with water, labeled, and allowed to sit overnight. The following day, the overlying water was filtered through 20-µm Pecap® screen to concentrate any actinospores present, and the actinospore density was estimated using techniques described previously (Thompson and Nehring 2000, Nehring et al. 2001). All samples were also tested by PCR to confirm the identity of actinospores observed as those of *M. cerebralis*. Following this procedure two samples of 50 haired oligochaete worms were selected from each of the replicate substrate samples. The worm samples were tested by real-time quantitative PCR (qPCR) to estimate the percentage of DNA present from each *T. tubifex* lineage. We also kept track of haired versus non-haired worms during the sample selection process in order to obtain an estimate of the percentage of the oligochaete population that was *T. tubifex*. The remainder of each kicknet sample was preserved for later analysis if needed.

Actinospore sampling

The protocol for collecting water monitoring samples for the purpose of quantifying the waterborne actinospores of *M. cerebralis* was changed in July 2004. Late in the previous segment a vacuum-driven packed-bed sampling device was built, similar to the one used by Lukins (2004). This device was compared in repeated trials with our standard 1900-L bucket method used previously, and a 120-L bucket method that used the same amount of water as the packed-bed system. Results showed conclusively that both of the 120-L methods were superior to the 1900-L method. Apparently as more water is poured through the flat-screen filters used with the bucket method, more actinospores are damaged or washed through the screen and a lower density estimate results. There was no difference in point estimates between the 120-L packed-bed and bucket flat-screen methods. However, the packed bed method was potentially more sensitive and exhibited smaller confidence intervals on individual samples because the filtrates were smaller as a result of a centrifugation step. Only the 120-L screen method was used during this segment. We continued to collect two replicate samples at each monitoring site.

RESULTS

Beaver Creek (South Fork Rio Grande drainage)

Habitat modifications were accomplished at this site in October 2001. Monitoring below this modified site for actinospores ceased after the 2004-05 segment, and indicates low actinospore densities since the habitat manipulation was completed (Figure 1.01). No actinospores have been detected since June 2003, and just three filtrates from June 2002 through May 2005 (n=29) yielded a positive PCR test. Monitoring of surface waters for triactinomyxons ceased after May 2005.

Results of myxospore monitoring suggest that despite the reduction of *M. cerebralis* actinospores in Beaver Creek, prevalence and mean myxospore concentrations have not been significantly reduced among the wild brown trout inhabiting the stream. Infection prevalence reached an apparent low point in 2002 (Figure 1.02), the year after habitat modification. However, the sample in 2003 would have been the first sample that was exposed to *M. cerebralis*

as newly hatched fry under the new habitat conditions. Overall, the mean myxospore concentration in age 1+ brown trout has been higher after habitat modifications than before (38,600 after vs. 23,000 before), indicating that in this stream the lowered detections of triactinomyxons did not result in lower myxospore concentrations. Interpretation of these results is slightly complicated by the use of two labs and two techniques to evaluate the samples. A private lab in Maine was used in 1999 and the analysis was conducted using plankton centrifuge rather than the standard pepsin-trypsin digest. The same private lab was used again in 2004, albeit with pepsin-trypsin digest, but using this technique the private lab tends to have a higher probability of detection than the state lab due to generally lower volumes of PTD product. This affects prevalence more than it does mean concentration as the additional detections occur in fish exhibiting low spore concentrations, but may help explain the highest recorded prevalence noted in 2004.

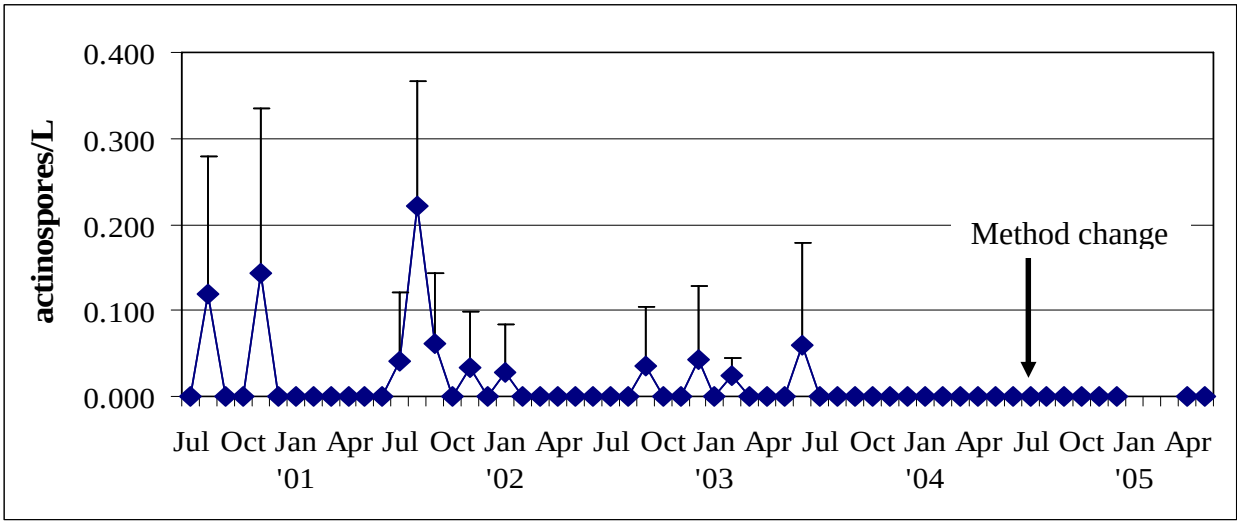

Figure 1.01. Density of actinospores of *M. cerebralis* in Beaver Creek below the side channel containing beaver ponds from July 2000 through May 2005, when monitoring ceased. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the estimate of mean concentration in the stream.

One promising trend at this site is the reappearance of wild age 1+ rainbow trout during the last few years (Table 1.01). During the last three segments the estimated numbers of age 1+ rainbow trout have been the highest in eight years of monitoring. While gains in the wild rainbow trout population have been modest, the timing of these gains does appear to coincide with the habitat modifications implemented in 2001. The average PCR scores are not stable from year to year, but overall are lower since the habitat modifications were made (Table 1.02). Despite these seemingly moderate conditions, the rainbow trout population is not growing. It is possible that fish older than age 1 are creeled by anglers as quickly as they grow to 9 or 10 inches, or they may be emigrating to the South Fork or mainstem of the Rio Grande.

No worm collections were made at this site, because plans were in progress to remedy the site before the baseline data collection scheme was fully formulated, and because it appeared possible to completely seal off the side channel containing the senescent beaver pond.

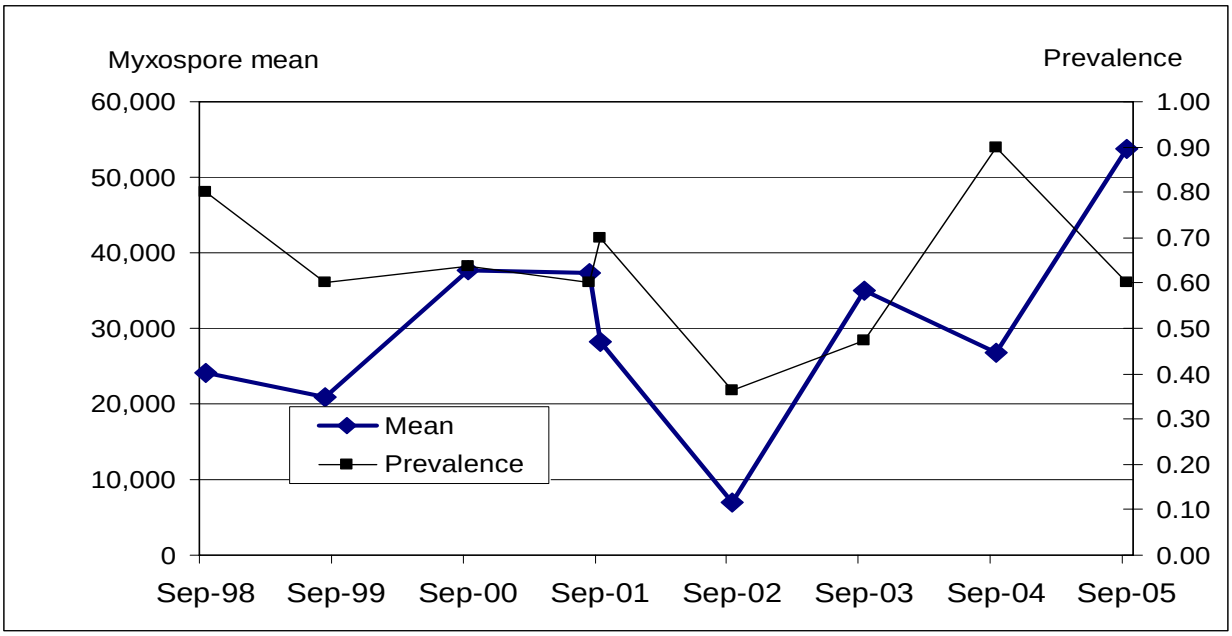

Figure 1.02. Mean myxospore concentration and prevalence of infection in samples of age 1+ brown trout (n = 11 – 20) collected from Beaver Creek below the habitat manipulation site.

Table 1.01. Trout population biostatistics (fish ≥ 15 cm) for Beaver Creek 1 km below Beaver Creek Reservoir from the fall (September and October) of 1998 through 2005.

	Brown Trout					Rainbow Trout				
Year	N	95% CI	Kg/ha	N/ha ≥ 15 cm	N/ha Age 1+	N	95% CI	Kg/ha	N/ha ≥ 15 cm	N/ha Age 1+
1998	103	± 3	190.2	1,704	1,014	2	± 0	2.3	33	0
1999	100	± 3	140.3	1,282	1,505	5	± 0	7.4	77	0
2000	232	± 6	344.5	2,828	891	5	± 0	6.7	61	24
2001	155	± 5	196.1	1,908	948	3	± 0	3.7	37	77
2002	152	± 4	244.1	1,852	811	4	± 0	5.3	49	49
2003	136	± 5	199.0	1,664	671	8	± 0	6.0	98	184
2004	138	± 7	185.0	1,695	974	10	± 1	9.0	123	89
2005	129	± 3	190.0	1,577	891	4	± 0	6.0	49	86

Table 1.02. Results of PCR tests on young-of-the-year brown and rainbow trout from Beaver Creek below Beaver Creek Reservoir from 2000 to 2005.

Date	Sample size (N)	Positive fish	Mean PCR score	Sample size (N)	Positive fish	Mean PCR score
	Brown trout			Rainbow trout		
09/22/00	11	9	2.55	2	2	3.50
09/26/01	10	10	3.50	10	10	2.60
09/13/02	13	8	2.71	22	21	3.68
09/23/03	20	15	1.80	15	10	1.87
09/19/04	20	18	2.40	11	8	2.82
09/22/05	15	13	3.20	15	12	1.80

Cache la Poudre River

The Cache la Poudre River was added to the work schedule during the 2002-03 segment. Significant strides have been made in reducing *M. cerebralis* actinospores emanating from the Poudre Rearing Unit (PRU) (see Job 2 of Nehring and Thompson 2003, Schisler 2003), so additional attention was focused on in-stream habitats near the PRU. Allen (1999) found that the main channel of the river in the low-gradient reach above PRU contained few oligochaetes, but that they were often numerous in side-pockets, alcoves, and side channels. While not detailed in Allen's thesis, one such site identified was at Kinikinik. In the area there are two significant backwater areas that appear to be excellent habitat for *T. tubifex*.

Berms designed to isolate both of the backwater areas at Kinikinik were constructed in September and October 2004 and described in Thompson (2005). The berms were designed to preclude 90% or more of all average daily flows in this reach from entering the backwater areas, based on historic data from a discontinued gage near Rustic. The hypothesis is that TAMs produced in the backwater areas will not reach the river since they are more likely to be present during non-runoff periods.

Water samples have been collected above and below the Kinikinik site since January 2003 (Figure 1.03). Density estimates were low on all occasions when actinospores were actually observed. It has been two years since actinospores were observed at the downstream end of the Kinikinik study area, but they were observed on several occasions during this segment at the top of the study reach. Actinospores were also observed from both of the isolated backwater areas during the fall of 2005.

Baseline oligochaete sampling was completed in 2003 and 2004 (Table 1.03), and two post-construction samples were collected in 2005. Lineage V, having few if any susceptible individuals, has not been represented in the oligochaete samples collected from this area to date. Lineage III is presently believed to be the *T. tubifex* most susceptible to *M. cerebralis* infection (Beauchamp et al. 2002, DuBey et al. 2005), and predominated at this site in early baseline sampling. However, the proportion of lineage III DNA in the worm samples tested by qPCR showed a significant downward trend over the 13 month time span of the baseline sampling (ANOVA, $P = 0.0002$), with a concomitant rise in the percentage DNA of the less-susceptible lineages I and VI.

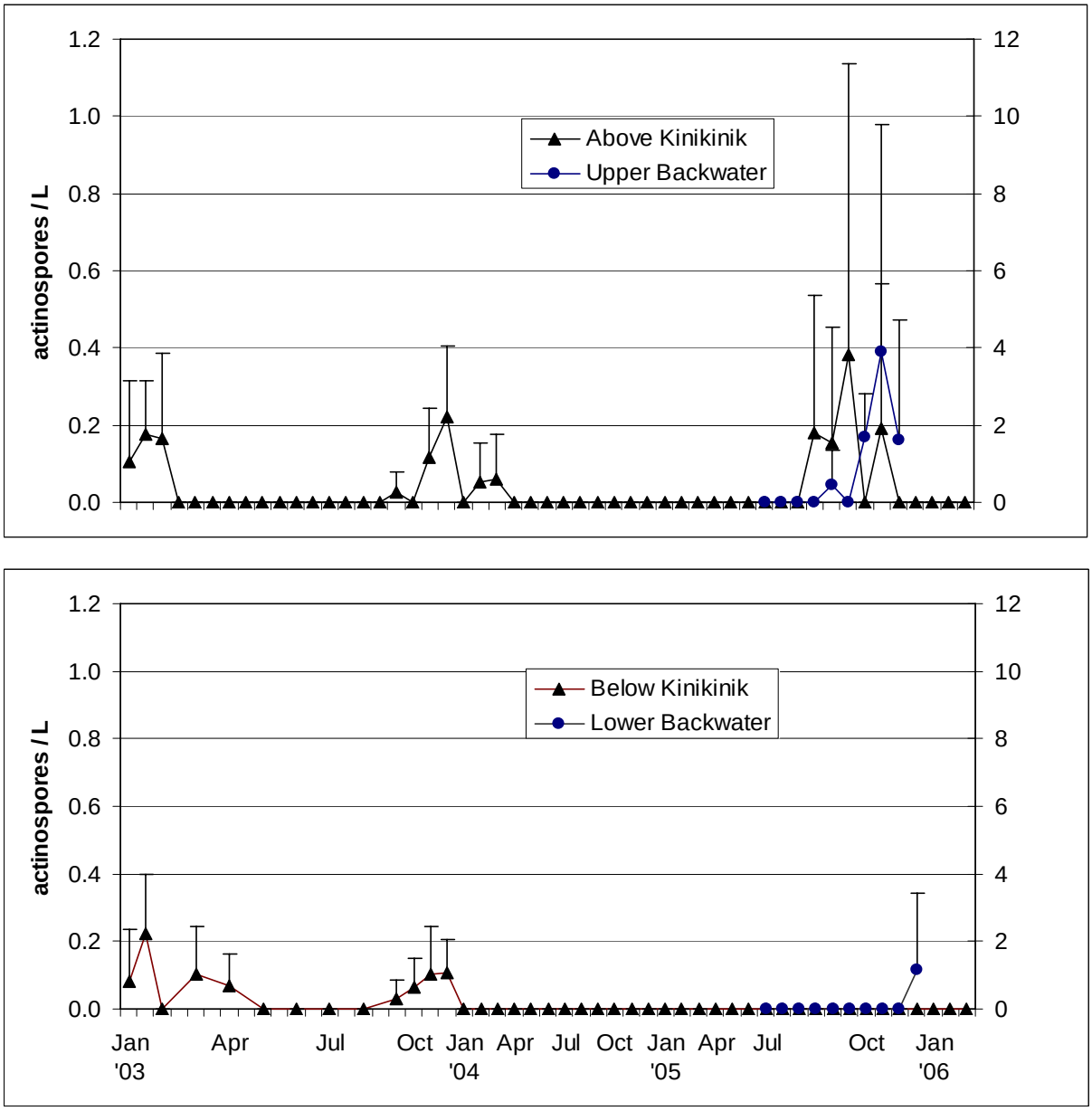

Figure 1.03. Estimates of actinospores/L in the Poudre River at above and below Kinikinik from January 2003 through March 2006. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream. Sampling frequency was twice per month early in the monitoring and during late summer and fall 2005, hence the uneven x-axis. Backwater values are referenced to the 2nd y-axis.

This observed change in lineage DNA was not as apparent in post construction samples. Instead, lineage III percentages seemed to hold at about the same level as the late baseline data. It is also noted that fewer of the nine samples contained *T. tubifex* in the post construction samples compared to those collected before construction. Lineage I DNA has been steadily rising relative to

lineage III and VI.

Table 1.03. Estimates of the proportion of each *Tubifex tubifex* lineage DNA found in oligochaete samples at the Kinikinik site. N refers to the number of the nine kicknet samples collected on each occasion that contained *T. tubifex*. The values in parentheses in the percent DNA composition columns are 95% confidence intervals.

Date	N	Approximate percent DNA composition by *M. cerebralis* lineage			
		I	III	V	VI
		Pre-modification			
8/25/03	9	2.8 (1.7)	73.9 (11.2)	0.0 (0.0)	23.3 (10.2)
10/01/03	8	4.8 (2.6)	67.5 (10.8)	0.0 (0.0)	27.7 (9.9)
06/22/04	9	5.6 (6.1)	55.5 (25.1)	0.0 (0.0)	38.9 (23.3)
09/13/04	8	13.7 (6.6)	37.3 (21.8)	0.0 (0.0)	48.9 (20.1)
		Post-modification			
07/18/05	4	22.5 (54.6)	58.9 (62.7)	0.0 (0.0)	18.6 (22.3)
10/24/05	6	40.6 (18.7)	37.7 (19.3)	0.0 (0.0)	21.7 (9.7)

The results of myxospore analyses from samples of age 1+ brown trout collected over the last several years are presented in Table 1.04. On average the data suggest that a somewhat higher proportion of wild brown trout are infected with *M. cerebralis* below the Kinikinik site than above it. Mean concentrations have been fairly low at both sites.

Table 1.04. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from the Poudre River.

Date mm/dd/yy	N	Prevalence	Overall Mean Concentration	Positive Fish	
				Mean	Range
Bliss State Wildlife Area – above Kinikinik					
09/30/02	10	10.0%	2,800	28,100	28,100
10/22/03	20	40.0%	4,400	11,000	2,300 – 31,600
10/28/04	10	20.0%	2,600	13,000	9,200 – 16,700
11/02/05	17	70.6%	2,000	2,800	560 – 13,300
Big Bend – below Kinikinik					
09/19/00	10	50.0%	6,300	12,600	990 – 37,600
10/22/03	12	41.7%	3,900	9,400	920 – 16,000
10/28/04	15	40.0%	17,100	42,900	5,600 – 92,300
11/02/05	15	60.0%	3,600	6,000	560 – 27,200

Colorado River

No habitat or other manipulations have occurred in this stream segment. All stocked rainbow trout are the Colorado River strain and most are 7.5 – 12.5 cm in length at stocking. Small numbers of larger fish have been stocked in this section. Monitoring in the Colorado River at the Kemp/Breeze Wildlife Area continued during this segment for triactinomyxon and myxospore information.

Samples of juvenile brown trout obtained since 1999 for analysis of cranial myxospore concentrations by PTD (Markiw and Wolf 1974) indicate that prevalence of infection is routinely 60% or greater (Table 1.05). Samples were collected in 2005 at the Kemp/Breeze Wildlife Area as well as at the Hitching Post Bridge downstream of Windy Gap Reservoir. The year-to-year variations in prevalence and myxospore concentration are not really different than those being observed in treated stream sections. This suggests that the habitat manipulations implemented elsewhere may be no more responsible for changes in these metrics than the random processes occurring in all the streams.

Table 1.05. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from the Colorado River during the fall in 1999-2004.

Date	N	Prevalence	Overall Mean Concentration	Positive Fish	
				Mean	Range
Hitching Post Bridge 1.9 km below Windy Gap Reservoir					
09/29/99	10	80.0%	6,330	7,920	1,110 – 15,550
10/12/00	10	100.0%	58,700	58,700	8,700 – 208,700
09/13/01	20	75.0%	20,300	27,500	4,000 – 96,000
09/27/02	10	60.0%	12,300	20,400	3,500 – 73,800
09/29/03	16	68.8%	11,700	17,000	2,500 – 43,700
09/27/04	22	95.5%	19,700	20,700	560 – 96,700
10/17/05	15	60.0%	4,900	8,200	560 – 24,400
Kemp/Breeze Wildlife Area 26 km below Windy Gap Reservoir					
09/29/99	10	60.0%	2,330	3,890	2,220 – 6,670
09/18/01	19	36.8%	13,800	37,300	1,900 – 160,600
10/08/02	13	84.6%	19,900	23,600	3,300 – 68,100
09/17/03	15	93.3%	14,400	15,400	3,300 – 70,100
09/30/04	21	76.2%	7,900	10,400	1,100 – 50,000
10/17/05	14	78.6%	9,800	12,500	560 – 25,600

Actinospore densities were monitored at the Breeze Bridge once each month during the last segment. *Myxobolus cerebralis* actinospores have been observed only infrequently during the last two segments (Figure 1.04).

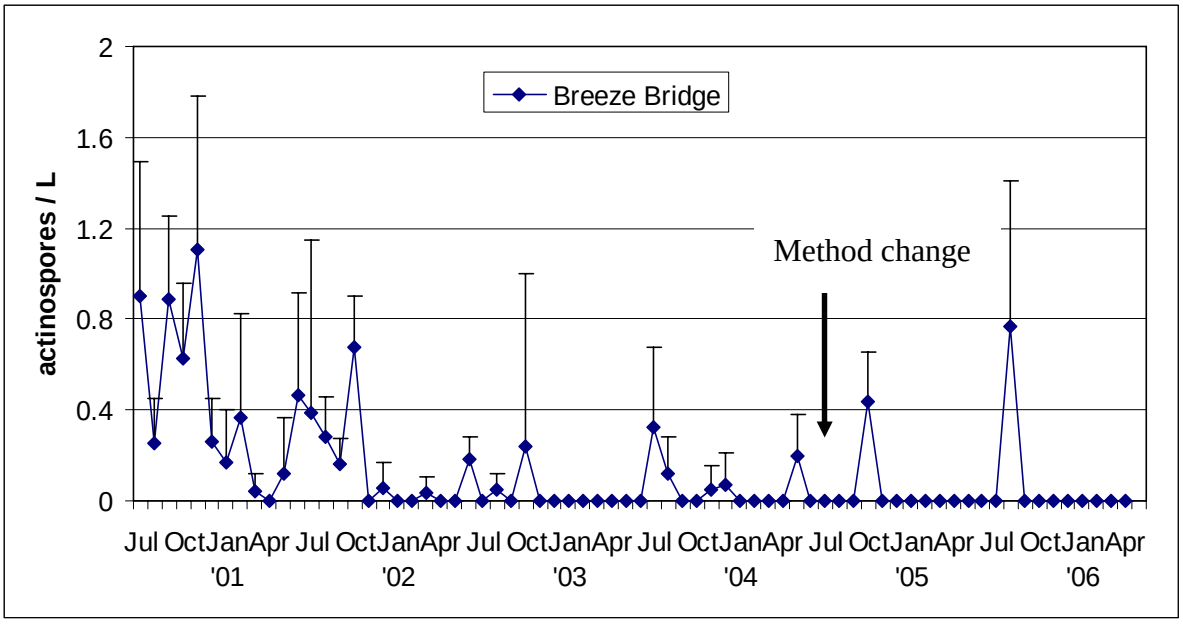

Figure 1.04. Results of water filtration to estimate ambient density of *M. cerebralis* actinospores (N/L) in the Colorado River at Breeze Bridge July 1, 2000 to May 31, 2006. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Spring Creek (Taylor River drainage)

Habitat modifications occurred on this stream in 2002. Monitoring continued in this segment at both the study site and at upstream and downstream control sites to collect data on ambient actinospore density and on prevalence of infection and myxospore concentration in brown trout. The brown trout population remains stable in this stream; the rainbow trout population is sparse and consists largely of stocked catchable trout (Table 1.06).

Table 1.06. Trout population biostatistics for three sites upstream from, downstream from, and at Salsbury Gulch on Spring Creek, from fall electrofishing efforts.

	Brown Trout					Rainbow Trout				
Year	N	95% CI	Kg/ha	N/ha ≥ 15 cm	N/ha Age 1+	N	95% CI	Kg/ha	N/ha ≥ 15 cm	N/ha Age 1+
	Spring Creek below Spring Creek Reservoir									
2002	265	± 2	506	5,725	4,571	0	---	0	0	0
2003	246	± 5	261	3,173	4,345	0	---	0	0	0
2004	231	± 1	258	2,967	2,323	0	---	0	0	0
2005	199	± 4	178	2,564	3,193	2	± 0	3	26	0
	Above Spring Creek Campground (control site)									
2002	175	± 5	207	2,105	1,814	207 [a]	± 1	427	2,435	24
2003	157	± 8	180	1,653	1,664	52 [a]	± 2	102	554	21
2004	146	± 5	124	1,538	1,245	71 [a]	± 4	124	748	0
2005	160	± 9	181	1,687	1,725	34 [a]	± 0	67	359	11
	At Salsbury Gulch (treatment site)									
2002	393	± 1	329	2,861	1,182	0	---	0	0	0
2003	309	± 8	288	2,803	1,240	7	± 1	10	63	0
2004	347	± 2	315	3,143	1,875	72 [a]	± 8	99	649	205
2005	308	± 5	282	2,789	1,283	50 [a]	± 3	88	451	0

a: The vast majority of the rainbow trout comprising this population were stocked catchables.

The samples of age 1+ brown trout collected during this segment and the last are "post-treatment" samples, having been exposed at hatch to the new conditions in Spring Creek. Results indicate that there is no difference in prevalence or infection intensity between the fish collected at the treatment site versus those collected at upstream and downstream control sites (Table 1.07). Also, there is no evidence at the treatment site that myxospore concentration or parasite prevalence in brown trout decreased after treatment compared to pre-treatment.

Samples of young-of-the-year (YOY) brown trout were collected at the same three sites in September of the last four years. The YOY samples collected in 2003 were the first post-manipulation data. The heads were analyzed by the PCR technique and indicate that there is a high prevalence of infection among YOY brown trout at all three sites for all years (Table 1.08). Prevalence was 100% in the samples from all three sites over the last two years, and there is little difference in the average PCR score among the three sites within any given year. In 2005 rainbow trout fry were observed at both upper and lower control stations in September for the first time in

several years. Nearly all rainbow trout fry PCR results were scored as '4', indicating they likely will not survive to age 1. Their presence during the electrofishing in this segment may well be a product of a higher than average water year, resulting in dilution of TAMs. In recent years, rainbow trout fry have been observed at the lower control station in August, but they have all been dead by September when electrofishing occurs.

Table 1.07. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from Spring Creek.

Date mm/dd/yy	N	Prevalence	Overall Mean Concentration	Positive Fish	
				Mean	Range
0.8 km downstream of Spring Creek Reservoir					
05/18/01	20	45%	6,500	14,400	1,400 – 56,000
08/01/01	20	80%	21,200	26,500	4,200 – 82,300
09/17/02	19	79%	43,900	55,700	2,000 – 195,000
09/22/03	23	78%	63,300	80,900	4,100 – 316,000
09/07/04	26	92%	50,700	54,900	4,400 – 56,700
09/21/05	20	65%	59,700	91,800	2,800 – 590,700
5 km downstream of Spring Creek Reservoir at Salsbury Gulch (treatment)					
05/18/01	20	90%	87,900	97,600	1,800 – 590,200
08/01/01	20	85%	67,300	79,200	3,900 – 401,000
09/17/02	20	85%	24,600	28,900	2,200 – 158,000
09/22/03	20	80%	39,600	49,600	2,700 – 151,600
09/07/04	20	100%	41,000	41,000	560 – 191,100
09/21/05	21	86%	64,900	76,900	7,100 – 422,500
19 km downstream of Spring Creek Reservoir (control)					
05/18/01	20	95%	57,000	60,000	15,200 – 173,200
08/01/01	20	90%	76,400	84,900	6,600 – 225,300
09/17/02	20	95%	13,200	13,900	1,300 – 30,300
09/23/03	20	90%	40,900	45,400	7,700 – 153,100
09/07/04	20	95%	53,300	56,100	4,400 – 212,200
09/21/05	20	90%	46,600	51,800	3,300 – 208,400

Water samples taken during the segment continued to indicate that habitat manipulation at this site did not result in reduced actinospore densities following construction. To the contrary, post-construction monitoring has resulted in a greater frequency of actinospore detection compared to pre-construction sampling (Figure 1.05) at both the treatment and control sites. In conjunction with the myxospore and PCR data this suggests that infectivity in this stream remains high, despite the decrease in oligochaete worm biomass originally observed within the

treatment section following habitat modifications (Thompson 2005).

Table 1.08. Results of polymerase chain reaction (PCR) tests of samples of young-of-the-year brown trout collected from Spring Creek. Mean PCR score is based on assigning numerical values to the qualitative score given to indicate strength of signal as follows: negative = 0, weak positive = 1, positive = 2, strong positive = 3, and very strong positive = 4.

Date	Sample size (N)	Positive fish	Mean PCR score
0.8 km downstream of Spring Creek Reservoir			
09/26/01	10	10	3.4
09/17/02	18	14	1.6
09/22/03	20	20	2.8
09/07/04	25	25	3.8
09/19/05	16	16	3.9
5 km downstream of Spring Creek Reservoir at Treatment site			
09/26/01	not sampled		
09/18/02	21	18	1.9
08/22/03	20	20	3.1
09/08/04	20	20	3.4
09/21/05	15	15	3.8
19 km downstream of Spring Creek Reservoir at Control site			
09/26/01	10	10	3.8
09/18/02	10	10	2.3
09/23/03	20	20	2.7
09/08/04	20	20	3.9
09/22/05	15	15	3.9

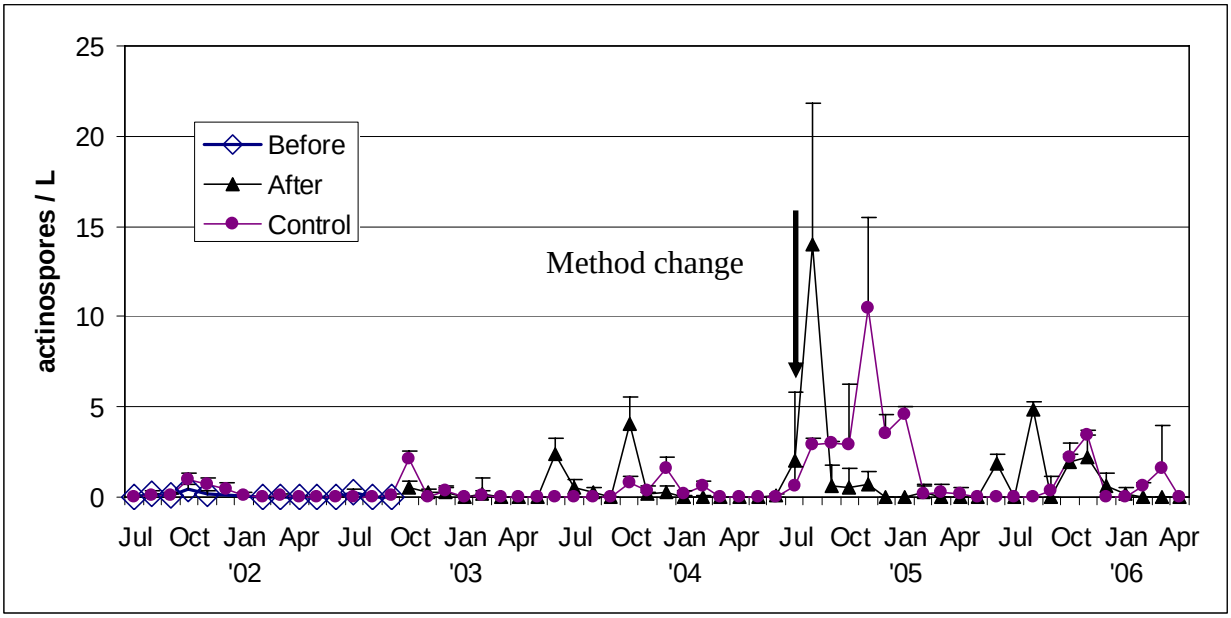

Figure 1.05. Density of actinospores observed in surface water samples collected at the Spring Creek treatment and lower control sites. “Before” designates the 15 months preceding construction.

Williams Fork River (Colorado River drainage)

Work on the Williams Fork River during this segment was limited to monitoring actinospore densities in surface water below the habitat modification site, collecting fish population information at two sites, and collecting age 1+ brown trout samples at two sites for myxospore information.

Trout population data have been collected from the Williams Fork River for the past four years (Table 1.09). The rainbow trout population remains sparse. Biomass and overall density of rainbow trout remain consistently higher just below Williams Fork Dam versus below the habitat modification site. This circumstance supports the hypothesis that the majority of present-day infectivity comes from within the river rather than the reservoir.

Table 1.09. Trout population biostatistics for two sites on the Williams Fork River below Williams Fork Reservoir.

	Brown Trout					Rainbow Trout				
Year	N	95% CI	Kg/ha	N/ha ≥ 15 cm	N/ha Age 1+	N	95% CI	Kg/ha	N/ha ≥ 15 cm	N/ha Age 1+
	0.3 km below Williams Fork Reservoir									
2002	269	± 6	279	1559	522	30	± 1	56	174	93
2003	999	± 9	816	5779	1003	24	± 3	45	138	74
2004	430	± 4	455	2490	213	33	± 2	70	188	54
2005	523	± 13	383	3028	666	24	± 5	55	137	71
	1.6 km below Williams Fork Reservoir, below Kemp/Breeze Wildlife Area irrigation									
2002 [a]	593	± 15	651	2952	1600	25	± 1	56.8	125	55
2003 [b]	711	± 7	360	1811	1172	32	± 2	21	80	42
2004 [b]	472	± 8	373	1202	1336	21	± 2	21	54	3
2005 [b]	403	± 24	214	1026	796	33	± 7	13	83	79

a: Station length 385 feet
b: Station length 813 feet.

Construction at the Williams Fork River site occurred during the first week of June 2002. Details of the habitat modifications and initial actinospore and oligochaete monitoring were presented previously (Nehring and Thompson 2003). The high actinospore density observed 12 months post-construction still appears to be an aberration, although low densities of actinospores have been observed on three other occasions since (Figure 1.06). Overall, we continue to detect actinospores less frequently than was the case before habitat modification. Although three of the seven highest actinospore densities observed have occurred after habitat modification, two of the samples were collected with the newer, more sensitive technique.

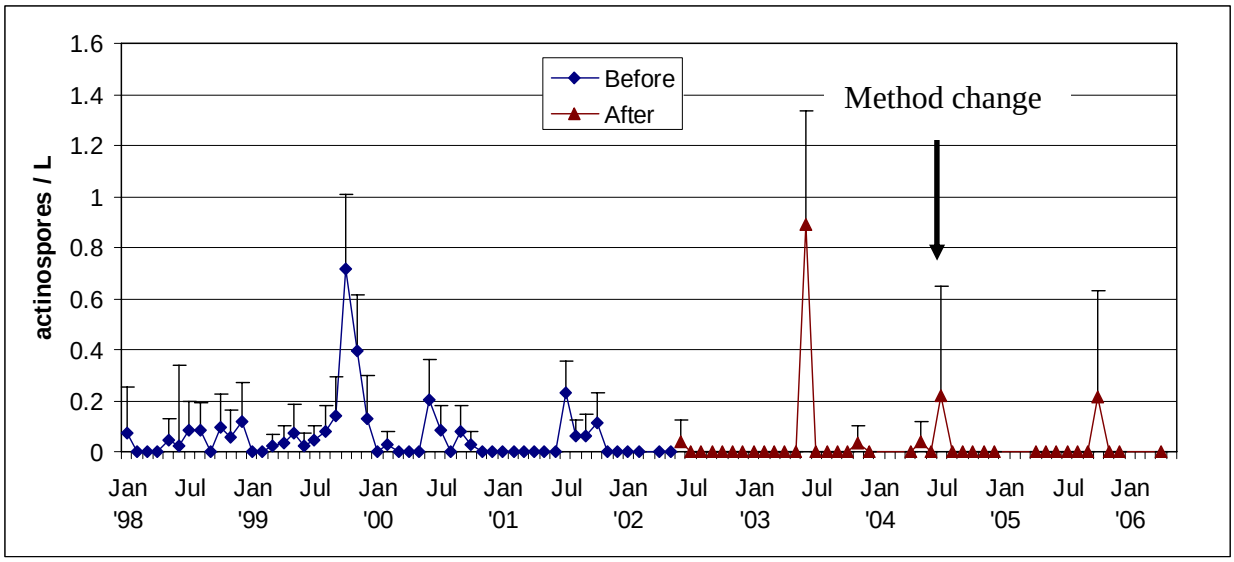

Figure 1.06. Density of actinospores observed in concentrates of surface water samples collected at the Williams Fork treatment site from January 1998 through April 2005. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream. "Before" designates samples collected prior to construction and "After" the samples collected following construction.

The brown trout collected in the fall of 2004 and 2005 represent the first true post-manipulation samples. Until 2005 prevalence of infection and average myxospore concentration have been higher at the downstream sites compared to just below the Williams Fork Dam (Table 1.10). In the most recent samples the average spore count was higher just below the Dam, however the difference is not significant. The average spore count observed at the treatment site was the lowest observed in the last five years.

Table 1.10. Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from three sites in the Williams Fork River.

Date mm/dd/yy	N	Prevalence	Overall Mean Concentration	Positive Fish	
				Mean	Range
0.3 km below Williams Fork Reservoir, above Treatment site					
09/13/01	15	13%	970	7,300	6,400 – 8,100
11/18/02	10	60%	6,900	10,400	2,000 – 42,400
11/18/03	20	35%	10,500	30,000	4,900 – 141,300
11/16/04	21	43%	710	1,700	560 – 4,400
11/15/05	20	55%	5,100	9,300	560 – 32,200

Table 1.10 (continued). Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from three sites in the Williams Fork River.

Date mm/dd/yy	N	Prevalence	Overall Mean Concentration	Positive Fish	
				Mean	Range
1.6 km below Williams Fork Reservoir, immediately below Treatment site					
08/06/01	20	45%	12,600	28,000	5,600 – 57,900
11/18/02	15	53%	26,900	50,500	1,900 – 342,700
11/18/03	20	80%	18,800	23,500	2,100 – 99,200
11/16/04	21	76%	12,200	16,100	560 – 66,700
11/15/05	20	55%	1,900	3,500	560 – 15,000
2.6 km below Williams Fork Reservoir					
09/12/01	20	55%	21,600	39,200	4,300 – 113,700
11/18/02	15	53%	3,600	6,700	1,600 – 13,600
11/18/03	20	90%	14,300	15,800	2,900 – 61,500
11/16/04	20	60%	31,500	52,500	5,600 – 240,000
Not collected					

Willow Creek (Colorado River drainage)

Willow Creek was added to the work schedule in the 2003-04 segment to take advantage of extensive baseline work accomplished by the U. S. Geological Survey (USGS) and the Colorado Cooperative Fish and Wildlife Research Unit. Further baseline oligochaete sampling occurred during the summer of 2003 (Table 1.11). Habitat modifications occurred during fall 2003. Both before and after the habitat changes lineages III and VI were the only ones detected. Lineage III is considered to contain the highest proportion of susceptible individuals (Beauchamp et al. 2002), whereas recent research indicates lineage VI is not susceptible (DuBey et al 2005). The average proportion of lineage III DNA in the qPCR samples was less in the samples collected in 2004 after habitat modification than in those collected before (Kruskal-Wallis non-parametric ranks test, $p = 0.0745$, Table 1.11). However, if that change may be attributed to habitat modification, it was a short-lived effect as lineage III DNA increased dramatically in the 2005 samples. Using all the data, the same non-parametric test indicates no difference overall in before versus after proportions of lineage III DNA ($p = 0.3975$).

Collections of brown trout collected above and below the site indicate a high prevalence of *M. cerebralis* infection in the area of the backwater both by PTD from age 1+ fish (Table 1.12) and PCR from YOY fish (Table 1.13). The 2005 PTD samples are considered post-modification samples because of the timing of exposure. The fish collected in 2005 exhibited lower myxospore concentrations than the baseline samples, but prevalence was not reduced. The fish samples were

much more difficult to collect in 2004 and 2005 than in 2003. Beaver activity through the project area resulted in extensive ponds that reduced electrofishing efficiency. The entire treatment area was inundated for all of 2005 to such an extent that the habitat structures and the berm are completely under water. In 2005, only one YOY brown trout was encountered in the entire study area.

Table 1.11. Estimates of the proportion of each *Tubifex tubifex* lineage comprising the pre-modification samples at Willow Creek. N refers to the number of the nine substrate samples collected on each occasion that contained *T. tubifex*. The values in parentheses in the percent DNA composition columns are 95% confidence intervals.

Date	N	Approximate percent DNA composition by *M. cerebralis* lineage			
		I	III	V	VI
		Prior to habitat modification			
6/23/03	8	0.0 (0.0)	22.1 (21.0)	0.0 (0.0)	77.9 (21.0)
8/18/03	9	0.0 (0.0)	19.2 (14.2)	0.0 (0.0)	80.8 (14.2)
		After habitat modification			
5/18/04	9	0.0 (0.0)	5.3 (4.7)	0.0 (0.0)	94.7 (4.7)
8/16/04	9	0.0 (0.0)	9.3 (9.7)	0.0 (0.0)	90.7 (9.7)
6/01/05	6	0.0 (0.0)	16.9 (14.3)	0.0 (0.0)	83.1 (14.3)
8/22/05	9	0.0 (0.0)	29.2 (16.5)	0.0 (0.0)	70.8 (16.5)

Table 1.12. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from Willow Creek.

Date mm/dd/yy	Age	N	Prevalence	Overall Mean Concentration	Positive Fish	
					Mean	Range
Above Willow Creek Gage						
09/30/03	1+	20	70%	21,400	30,600	2,600 – 194,700
09/29/04	1+	15	40%	8,100	20,100	5,000 – 64,500
10/17/05	1+	15	73%	3,800	5,200	560 – 12,200
Downstream of backwater site						
09/30/03	1+	20	60%	10,700	17,900	2,000 – 41,200
09/29/04	1+	10	30%	29,200	97,400	57,700 – 128,800
10/17/05	1+	13	38.5%	1,300	3,400	560 – 7,800

Table 1.13. Results of polymerase chain reaction (PCR) tests of samples of young-of-the-year brown trout collected from Willow Creek. Mean PCR score is based on assigning numerical values to the qualitative score given to indicate strength of signal as follows: negative = 0, weak positive = 1, positive = 2, strong positive = 3, and very strong positive = 4.

Date	Sample size (N)	Positive fish	Mean PCR score
Above Willow Creek Gage			
09/30/03	10	10	2.6
09/29/04	13	11	2.4
10/17/05	none encountered		
Downstream of backwater site			
09/30/03	11	7	1.7
09/29/04	20	16	2.9
10/17/05	1	0	0.0

DISCUSSION

The final project designed to isolate or remove discrete areas of good *T. tubifex* habitat from streams was constructed in the autumn of 2004 on the Poudre River near Kinikinik. While evaluation will continue for two more segments on most of the projects, early indications are that the habitat modification strategy is unlikely to result in dramatic improvement of conditions for fish populations. Some indications have been positive, such as reduced actinospore detection in the Williams Fork River, reductions in the apparent amount of lineage III *T. tubifex* in Willow Creek and the Poudre River (the latter occurring before any habitat modifications were made), and the lower biomass of oligochaetes within the Spring Creek study site following habitat improvements. The ultimate goal is evidence of reduced prevalence and severity of infection in the trout populations downstream of the project sites, and to date that goal does not appear to be realized. This is evidenced not only by year-to-year comparisons in the study streams, but also in the fact that un-manipulated control sections exhibit the same sort of year-to-year variability in prevalence and concentration seen in the treatment sections. If improvement in the fish population could be asserted to have occurred on any study stream, it would be Beaver Creek, where somewhat higher age 1 rainbow trout densities have been observed the last couple of years compared to prior years.

At the annual Whirling Disease Symposium convened in Denver in February 2005, infectious disease authority and keynote speaker Dr. Paul Ewald (University of Louisville) noted that the two spores involved in the transmission of the parasite from host to host employ differing strategies. The myxospore is thought to be rather immobile once it is deposited, thus the transmission technique is to “sit and wait” for a suitable host to encounter it. Typically, disease agents characterized by this sort of strategy have a high impact on the host (Ewald 1994). In contrast, the actinospore is waterborne and disease agents characterized by this method of transmission generally have a lesser impact on the host than do “sit and wait” disease agents. Dr. Ewald asserted a focus on resistance to the parasite in the hosts would be the most productive avenue of research. For the trout host, this would suggest continued research into a resistant

rainbow trout as a primary component of many important sport fisheries throughout North America.

An avenue of host resistance largely unexploited to date lies in the oligochaete hosts. Only recently has it become apparent that differences in susceptibility of *T. tubifex* to the parasite are lineage-related (Beauchamp et al. 2002). This evidence, coupled with the knowledge that we have a number of places where to date only the susceptible lineage III has been documented (Thompson 2005, Nehring 2005), leads to the conclusion that research into taking advantage of worm host resistance may be productive. While a resistant rainbow trout may be a suitable answer to the whirling disease problem in many waters, they would not be an acceptable solution in native cutthroat habitat. In such places it would be more desirable to displace susceptible worm hosts with non-susceptible ones.

Job Title: Actinospore Hot Spot Abatement Studies. Job No. 2

Job Objective: Develop and test strategies to reduce, control, or eliminate the production of triactinomyxon actinospores of *Myxobolus cerebralis* from man-made ponds and settling ponds known to be focuses of infectivity.

Period Covered: July 1, 2005 to June 30, 2006

INTRODUCTION

Whirling disease is a serious malady of some salmonid fishes that can result from exposure of susceptible salmonid fry or fingerlings to the waterborne actinospore of the myxosporean parasite *Myxobolus cerebralis* (Wolf and Markiw 1984; Markiw 1991). Phagocytic vegetative stages of the parasite feed on cartilage in young trout. A granulomatous inflammatory response usually develops in peripheral tissues adjacent to sites of infection. Destruction of the cartilage by the parasite interferes with normal bone development and can result in skeletal and cranial deformities. Young fish that are infected may display an erratic swimming behavior known as "whirling", hence the name whirling disease. Rose et al. (2000) suggested that the cause of the erratic swimming pattern is inflammatory response to parasite activity in the cranial and anterior spinal region, resulting in multiple compressions of the spinal cord.

Once considered an aggravating nuisance for salmonid aquaculture, it is now recognized that this disease can significantly impact wild trout populations (Walker 1997; Hedrick 1998). Nehring and Thompson (2001) found no substantive evidence that any environmental perturbation or stressor other than *M. cerebralis* adequately explained the recurring losses of young wild rainbow trout observed on nearly 600 km of Colorado's premier trout streams. In some instances in Colorado off-channel sources of infectivity have apparently influenced the rate and intensity of infection in trout. In the Fryingpan River, abundance of age 1 wild rainbow trout in the 15-km reach upstream from its confluence with the Roaring Fork River declined 90% between 1994 and 1998 (Nehring 1999). That trend continued in 1999, 2000, and 2001. A localized area of *Myxobolus cerebralis* infectivity emanating from a series of off-channel ponds was documented (Nehring et al. 2000). The most severe reduction in abundance of age 1 wild rainbow trout has occurred downstream of this focus of infection, suggesting that whirling disease induced the decline.

Fish rearing facilities may also contribute infectivity to waters receiving settling pond effluent. The number of State-owned rearing units experiencing parasite infestations peaked in 1998 at 11 facilities. Currently the number stands at just four that actually stock fish; two of those are working toward *M. cerebralis*-free status. However, in some cases rearing units are free of the parasite but the settling ponds are not. In other cases there is no expectation of ever succeeding in freeing the rearing unit of the parasite.

The objective of this job is to document the changes in *M. cerebralis* infectivity that may occur in response to management actions on such off-channel sites, and to help develop best management practices for such sites.

Segment Objectives:

1. Continue to monitor triactinomyxon densities at established study sites.
2. Monitor triactinomyxon densities in the inflow to and effluent of the sand filter wetland on the Cap K Ranch in the Fryingpan River drainage.
3. De-populate the Roaring Judy effluent ditch between the end of the concrete raceways and the new kokanee trap.
4. Remove brook trout from the upper two ponds on the Cap K Ranch.
5. Obtain estimates of rainbow trout remaining in Roaring Judy ponds after the kokanee spawn-take. Remove all stocked rainbow trout and submit samples for analysis of myxospore concentration by PTD.
6. Collect samples of age 1+ brown trout above and below off-channel sources of *Mc* infectivity on Quartz Creek, East River, and Fryingpan River.

METHODS and MATERIALS

Field Filtration and Sample Collection

The technique for collecting field filtration samples was changed at the beginning of the 2003-04 segment based on experiments conducted late in the previous segment (see Job 1, Materials and Methods: Actinospore sampling). Rather than sampling a single 1900-L volume of water at each site, we sampled duplicate 120-L samples.

Actinospores of *M. cerebralis* were identified on the basis of general appearance, overall conformation, size and shape according to descriptive criteria in El-Matbouli and Hoffmann (1998). However, size was considered to a lesser degree than conformation because recent evidence shows that there may considerably more variability in the size of *M. cerebralis* triactinomyxons than previously thought (Hallett et al. 2004)

A single 1.6-mL sample (equal to the volume examined from 20 aliquots) of filtrate from some field samples was subjected to the polymerase chain reaction (PCR) test. Since April 2001, we have used a PCR test developed by Pisces Molecular, Inc., that amplifies a segment from a heat shock protein gene of *M. cerebralis* designated as hsp70. Each sample tested by PCR was preserved in 70% ethyl alcohol in a 15-mL centrifuge tube, and was identified only by alphanumeric code when sent to the laboratory.

Fish Removal

Electrofishing equipment was used to accomplish fish removal from the Cap K Ranch ponds. Pitkin Rearing Unit personnel have accomplished fish removal in the Pitkin settling pond using gill-nets.

RESULTS and DISCUSSION

Cap K Ranch Ponds (Fryingpan River drainage)

The Fryingpan River between Ruedi Dam and the Roaring Fork River confluence is a stream that has a long history of trout population study to draw from. Previous study indicates that *Myxobolus cerebralis* infection has had a noticeable impact on the rainbow trout population in the lower part of the reach. One focus of infectivity has been identified as the effluent of the Cap K Ranch ponds (Nehring 1999, Nehring et al. 2000). A search for others throughout the drainage below Ruedi Dam was completed in fall 2002. The conclusion was that the Cap K Ranch ponds were a far more significant and consistent source of infectivity than any other site studied.

The Cap K Ranch ponds are in a series designated by numbers 1 – 6, with pond 1 at the top and pond 6 at the terminus of the series. The effluent from pond 6 returns to the Fryingpan River, although the capability exists to divert pond water back to the Fryingpan River before it enters Pond 5. Filtration data obtained since July 2000 from ponds 1 and 2 indicates that pond 2 continues to be a consistent producer of actinospores (Figure 2.01).

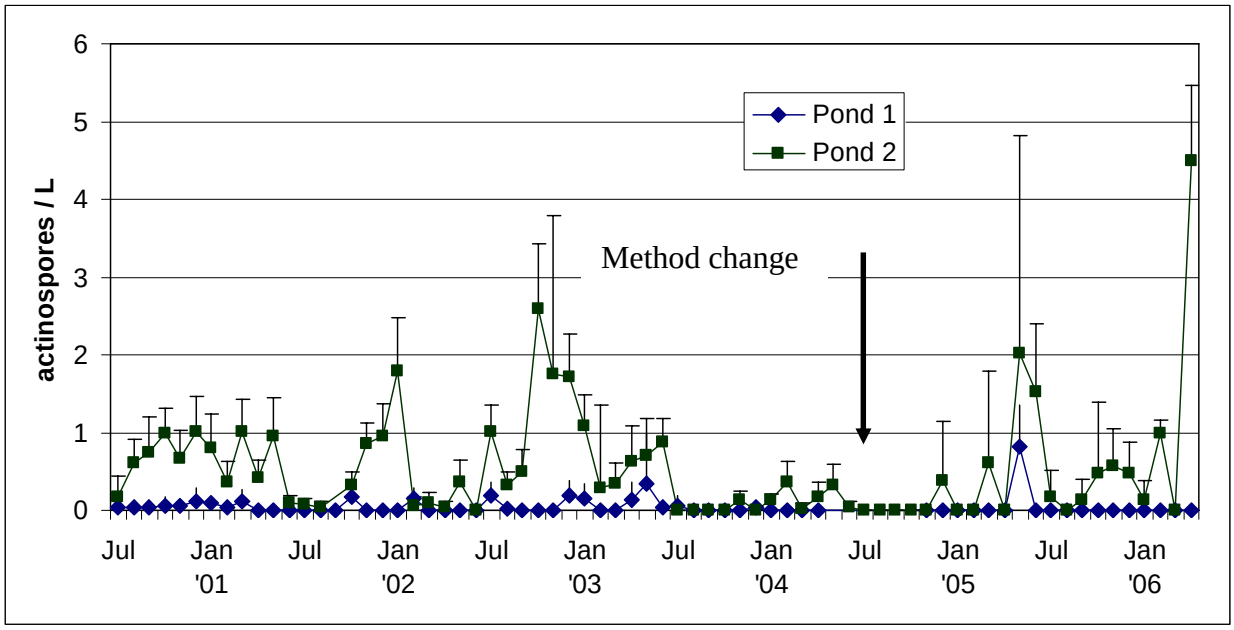

Figure 2.01. Estimates of *M. cerebralis* actinospore density in samples of water in the effluents of Cap K Ranch ponds 1 and 2. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

During March through May 2006 pond 2 was electrofished on three occasions to remove brook trout fry. In this fifth year of brook trout fry removal, over 2500 fry were taken out of pond 2. By reducing the population of this susceptible species it is hoped that infectivity in the system will also be reduced, however in the last year there have been numerous occasions when actinospores were detected at higher levels than were seen in 2004 (Figure 2.01).

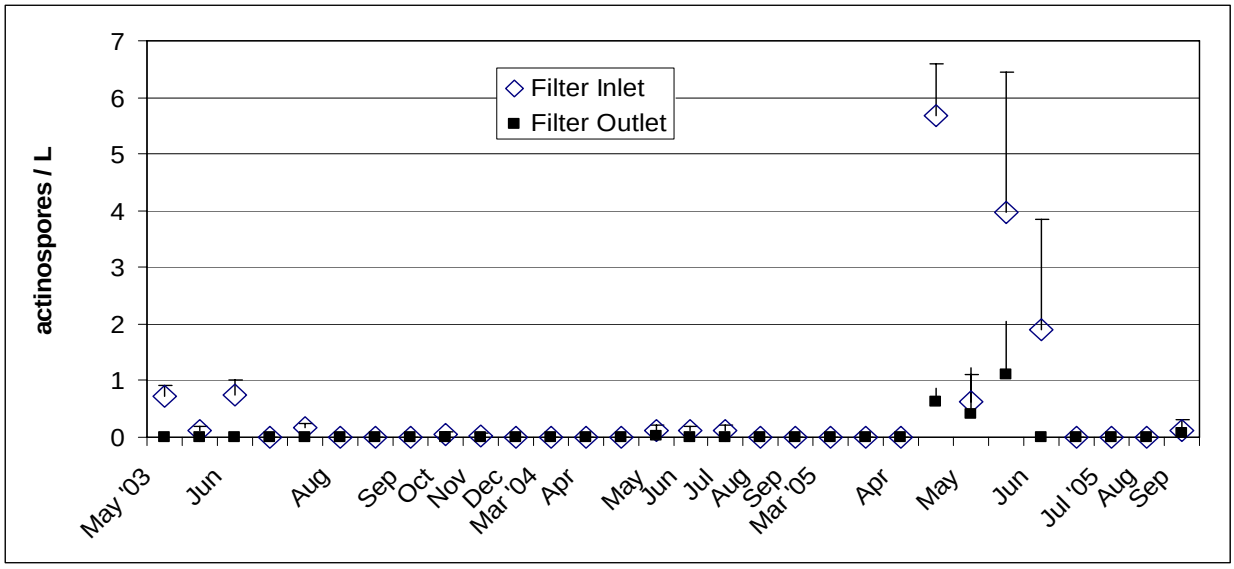

Figure 2.02. Paired water filtrations from the inlet and outlet of the wetland filter installed in Pond 6 on the Cap K Ranch.

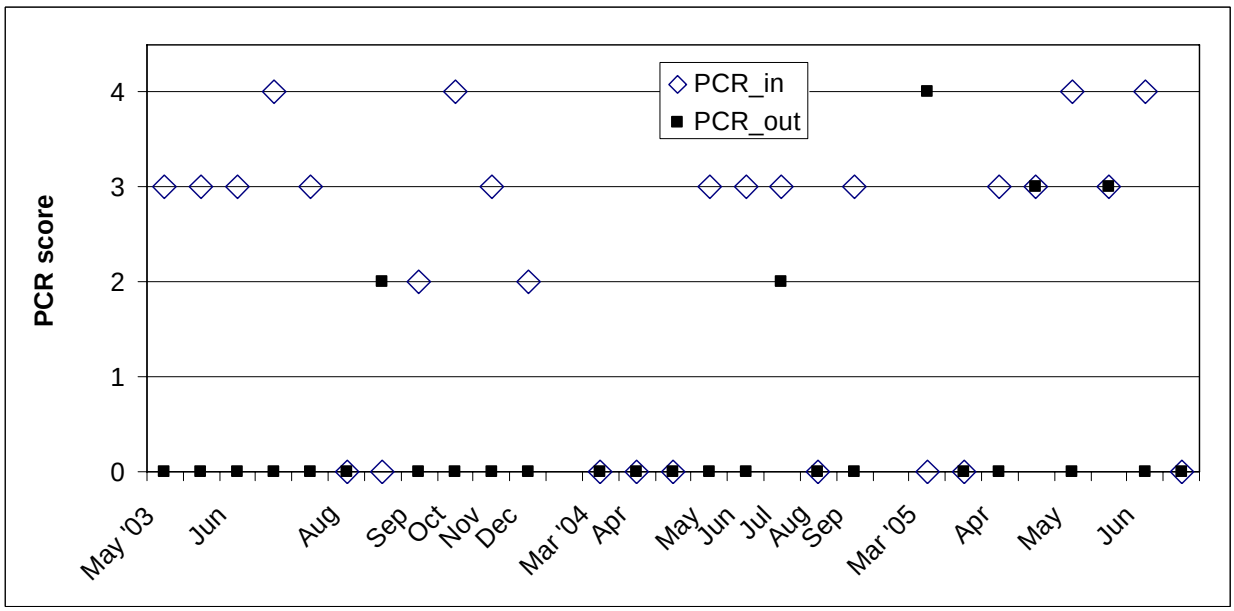

Figure 2.03. Comparison of PCR score for filtrates from the inlet and outlet of the wetland filter. PCR score is based on a scale from '0' (no signal) to '4' (a very strong signal). The first positive test was likely caused by improperly set controls that allowed unfiltered water to exit the filter.

Pond 6 has historically been a source of *M. cerebralis* actinospores to the Fryingpan River (Thompson 2004). This pond was modified during February and March of 2003. A description of the filter installed was previously provided (Nehring and Thompson 2003). Monitoring of the inlet and outlet of the filter concluded during this segment. Actinospores were documented in the filter inlet on 14 occasions (two points in Figure 2.02 are not clearly evident because of the compressed scale). A single actinospore was observed in the effluent on May 5, 2004. In late April through June 2005 multiple actinospores were observed in the filter effluent,

indicating that the filter clearly has exceeded its useful lifespan. This was further seen in the increasing proportion of positive PCR samples observed in the filter effluent samples (Figure 2.03).

The Fryingpan River has been sampled each month at four or more sites since August 1998. Water samples drawn from the Fryingpan River immediately downstream from Ruedi Dam were collected on 81 sampling occasions from October 1998 through July 2005. Actinospores were detected three times, in April 2001, November 2004 and June 2005. One water sample tested weakly positive by PCR for *M. cerebralis* in August 2004 and the June 2005 sample tested positive. The sites 1.9 km above Cap K Ranch and Taylor Creek confluence serve as upstream and downstream evaluation sites for the manipulations occurring on the Cap K Ranch (Figure 2.04).

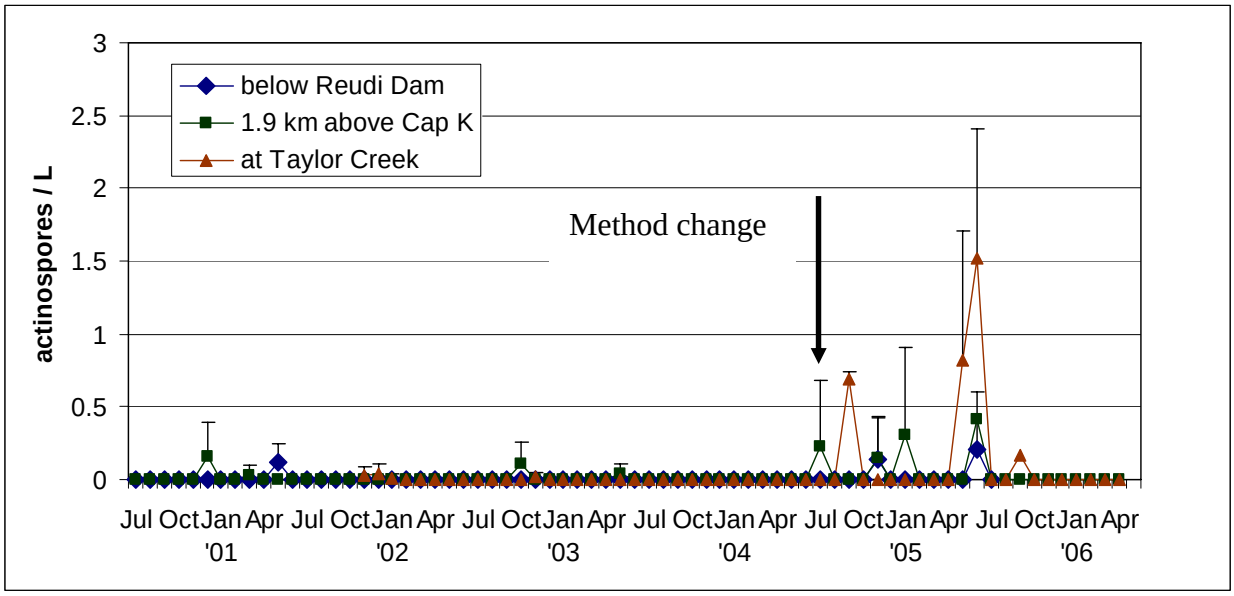

Figure 2.04. Results of water filtration to estimate ambient density of *M. cerebralis* actinospores (N/L) at three sites in the Fryingpan River from July 1, 2001 to May 31, 2005. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Samples of age 1+ brown trout were obtained during this segment from sites in the Fryingpan River above and below the Cap K Ranch (Table 2.01). The samples acquired in 2005 are the second year-class of brown trout hatched in the river after the construction of the filter in Pond 6. These samples suggest that prevalence of parasite infection remains high downstream of the Cap K Ranch, but the decreasing trend in average spore concentration continues. It is doubtful the lower concentration may be regarded as a result of the filter preventing actinospores from entering the Fryingpan River. The average concentration was lower in the upstream sample also, in an area unaffected by the Cap K Ranch effluent. Moreover, during the last two summers the filter was handling only a small fraction of the water from pond 6, so even if it had remained effective there were still actinospores being introduced to the Fryingpan River through the overflow channel.

Table 2.01. Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from locations in the Fryingpan River above and below Cap K Ranch.

Date	Age	N	Prevalence	Overall Mean Concentration	Positive Fish Mean	Positive Fish Range
			1 Km below Ruedi Dam			
10/28/00	1+	10	0%	---	---	---
10/30/01	1+	11	36.4%	7,800	21,500	2,780 – 35,800
10/29/03	1+	20	45.0%	38,500	85,600	4,880 – 541,300
10/26/04	1+	23	82.6%	26,500	33,200	560 – 254,400
11/02/05	1+	15	53.3%	3,700	6,900	560 – 20,600
			1.6 Km above Cap K Ranch			
10/28/00	1+	10	10.0%	26,900	269,300	269,300
10/30/01	1+	10	60.0%	15,700	26,100	2,670 – 71,000
10/29/03	1+	20	55.0%	21,800	39,600	4,560 – 112,300
10/26/04	1+	21	85.7%	46,900	55,200	1,670 – 197,800
11/02/05	1+	20	90.0%	18,100	20,100	1,100 – 173,300
			Taylor Creek 4.8 km below Cap K Ranch			
10/31/00	1+	9	55.6%	37,700	67,800	9,300 – 181,900
10/30/01	1+	11	63.6%	35,900	56,400	2,500 – 147,500
10/29/03	1+	20	80.0%	29,600	37,000	1,500 – 189,900
10/27/04	1+	20	85.0%	16,000	18,800	1,100 – 60,000
11/02/05	1+	20	85.0%	10,600	12,400	1,100 – 82,200

Pitkin Rearing Unit

Trout reared at the Pitkin Rearing unit first tested positive for *M. cerebralis* in March 1997. The unit was taken out of production in 2001 and extensive renovation, modernization and securing of springs and well-water supplies was accomplished. The use of Quartz Creek surface water for rearing fish was discontinued upon re-start of the unit. If future tests result in no *M. cerebralis* detection this unit should be certified as negative for the parasite in January 2007.

Monitoring of actinospore densities began at the Pitkin Rearing Unit in November 2001. Actinospores of *M. cerebralis* were routinely observed in the effluent of the settling pond, including a large "pulse" during November and December 2002. No actinospores were detected in the effluent or in Quartz Creek during this segment (Figure 2.05). These results indicate that the hatchery effluent appears no more infected than Quartz Creek upstream of the effluent.

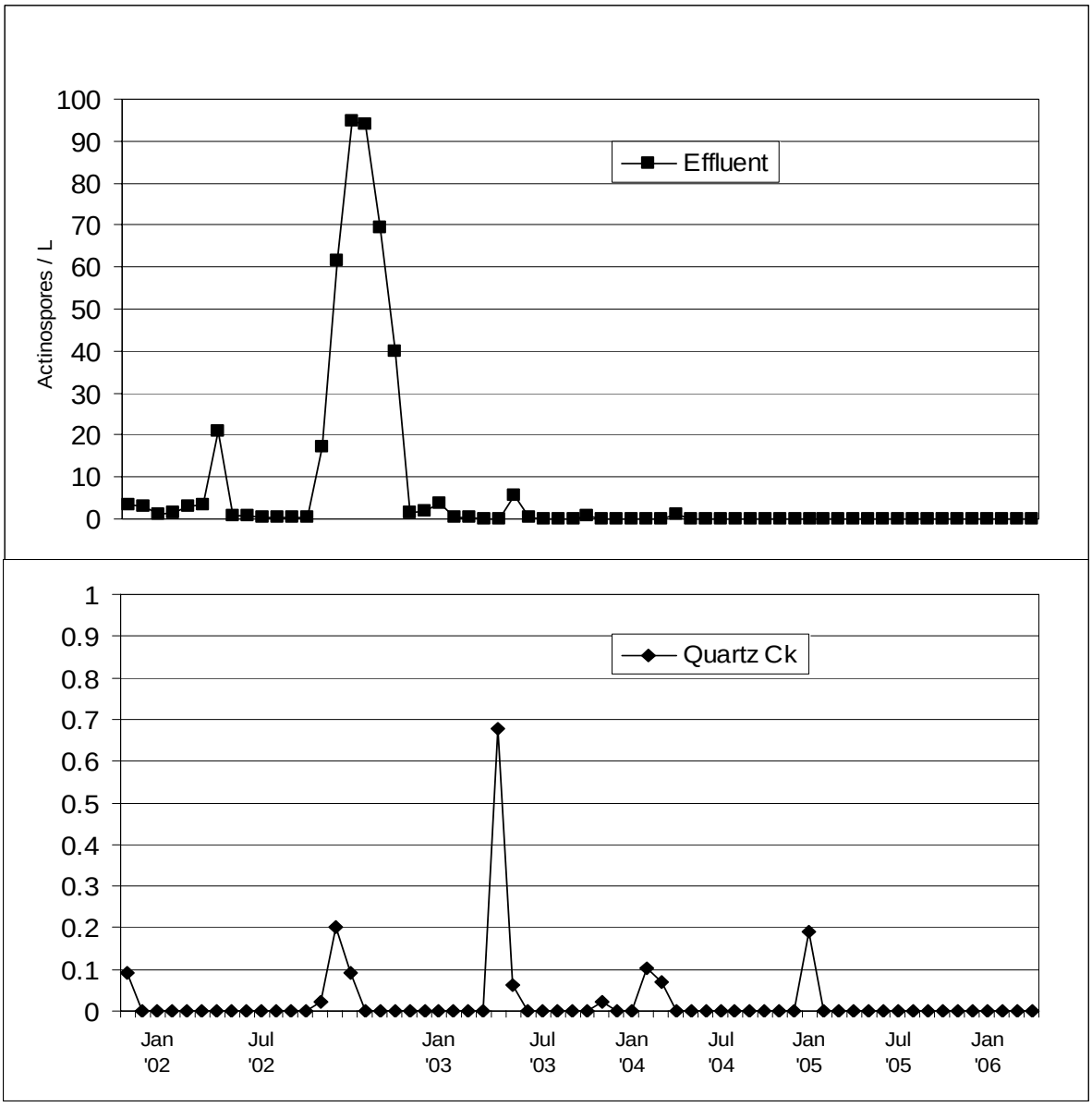

Figure 2.05. Results of water filtration to quantify actinospores of *M. cerebralis* in samples of water at Pitkin Hatchery, August 2002 through April 2005. PCR results are based on a scale of '0' (negative) to '4' (very strongly positive).

Pitkin Unit personnel removed all feral fish from the unit's settling pond during unit renovation in 2001-02. It remains essentially free of fish. To no longer have a myxospore source available to the *T. tubifex* community residing in the settling pond has had a positive impact on the infectivity observed in the effluent.

During this segment brown trout samples were collected from Quartz Creek approximately one mile above and below Pitkin Rearing Unit. Prevalence of *M. cerebralis* infection fell significantly at both locations compared to 2004 (Table 2.02, both $p < 0.005$), but mean spore

concentration remained unchanged at the upstream site while falling back to 2003 levels at the downstream site. The prevalence and average concentration upstream of Pitkin Rearing Unit over the last two years suggests that the parasite may be spreading in Quartz Creek.

Table 2.02. Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from Quartz Creek above and below the Pitkin Fish Rearing Unit.

Date mm/dd/yy	Age	N	Prevalence	Overall Mean Concentration	Positive Fish	
					Mean	Range
	Upstream of Pitkin Rearing Unit					
08/28/03	1+	20	10.0%	2,900	29,400	25,300 – 33,500
08/09/04	1+	20	85.0%	15,400	18,100	1,700 – 50,100
08/17/05	1+	20	40.0%	17,400	43,600	2,500 – 151,500
	Downstream of Pitkin Rearing Unit					
08/28/03	1+	20	45.0%	10,200	22,700	4,900 – 59,400
08/09/04	1+	20	95.0%	67,200	70,700	1,500 – 489,300
08/17/05	1+	20	60.0%	10,400	17,300	2,800 – 68,800

Poudre Rearing Unit

Actinospore monitoring began at numerous sites on the Poudre River in 1997. The data from 1997 through June 2001 clearly indicated that the Poudre State Fish Rearing Unit (PRU) had become a major point source of *M. cerebralis* actinospore production. This resulted in severe infection in brown and rainbow trout downstream from the unit compared to upstream (Nehring et al. 2001; Schisler 2001).

Actinospores of *M. cerebralis* were still encountered frequently at the filtration sites on the PRU during this segment. Estimated densities remained low in the PRU effluent compared to the historic high numbers seen in 1999-2000 when it was common to observe > 10 / L (Figure 2.06). Actinospores are also seen much less frequently over the last two segments compared to previous years when they were detected on a majority of sampling occasions. Water samples from the unit supply pond contained actinospores on 3 of 5 occasions compared to 1 of 11 occasions in the Poudre River inflow to the supply pond. The densities of actinospores observed in the supply pond were higher than those in the Poudre River inflow.

The modification of the supply pipeline system at the Poudre Unit was completed in 2005. The unit now uses water directly from the Poudre River rather than from the supply pond except during a couple of critical months when warmer water from the supply pond is needed to prevent icing problems. Monitoring of the supply pond ceased once the pipeline project was completed.

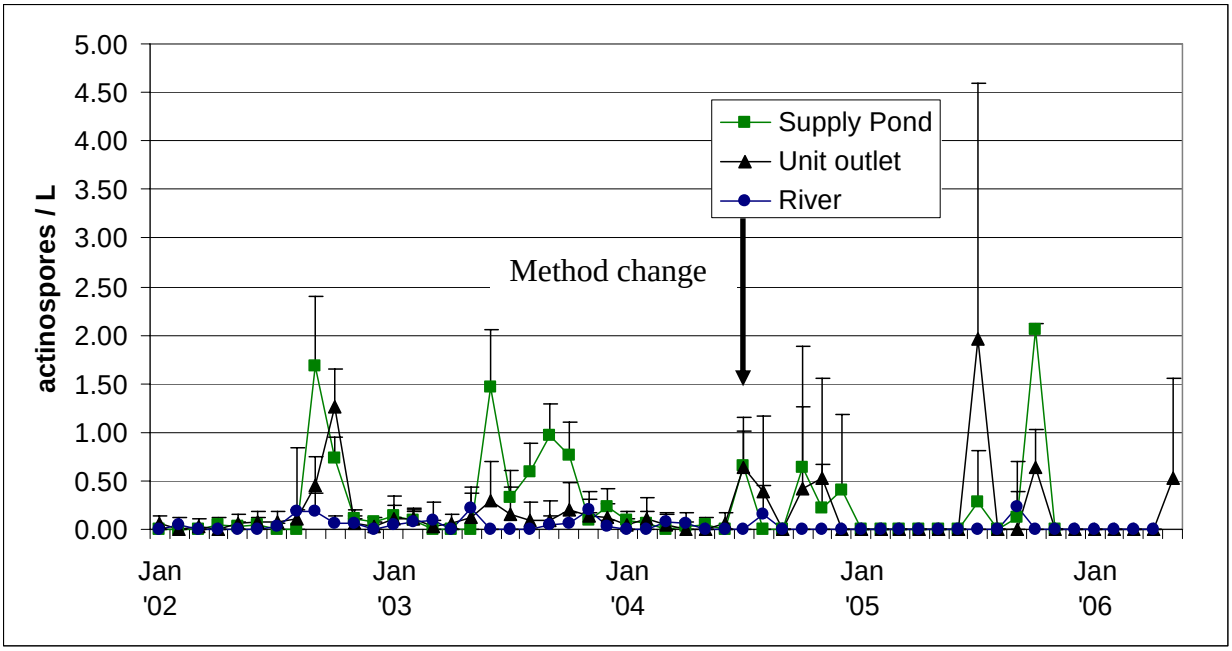

Figure 2.06. Comparison of actinospore densities from the Poudre River, the Supply pond, and the Unit effluent through May 2005. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Table 2.03. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from the Poudre River above and below the Poudre Rearing Unit (PRU).

Date mm/dd/yy	N	Prevalence	Overall Mean Concentration	Positive Fish	
				Mean	Range
Big Bend – above PRU					
09/19/00	10	50%	6,300	12,600	990 – 37,600
10/22/03	12	41.7%	3,900	9,400	920 – 16,000
10/28/04	15	40.0%	17,100	42,900	5,600 – 92,300
11/02/05	15	60.0%	3,600	6,000	560 – 27,200
Pasquinel's cabin – below PRU					
09/19/00	9	22.2%	4,300	21,000	3,900 – 35,100
10/22/03	21	14.3%	1,800	12,600	6,900 – 21,000
10/28/04	6	0%	---	---	---
11/02/05	15	60.0%	3,500	5,900	560 – 27,200

Samples of brown trout obtained above and below PRU suggest that over the last several years the Unit's effluent has had a minimal effect on infectivity in the brown trout population downstream (Table 2.03).

Roaring Judy Rearing Unit

Inspection records at the CDOW Aquatic Animal Health Laboratory show trout from the Roaring Judy State Fish Rearing Unit (ROJ) first tested positive for the presence of *M. cerebralis* in early 1992. Those same records indicate the parasite was detected in free-ranging rainbow trout collected from Meridian Lake in the Slate River drainage, tributary to the East River near Crested Butte, in 1988. Meridian Lake, about 25 km upstream of ROJ, was stocked with rainbow trout by a private aquaculturist whose facility tested positive for the parasite in late 1987.

In the spring of 2005 the Roaring Judy Unit regained certification as a *M. cerebralis*-free facility. However, the effluent channel, new kokanee spawning unit, and settling ponds remain enzootic habitats. Research continues on methods and management strategies to minimize the number of actinospores in the settling pond effluent. Monthly monitoring during this segment resulted in the detection of actinospores in the unit effluent on the fewest occasions since monitoring began, just 3 of 11 months (Figure 2.07).

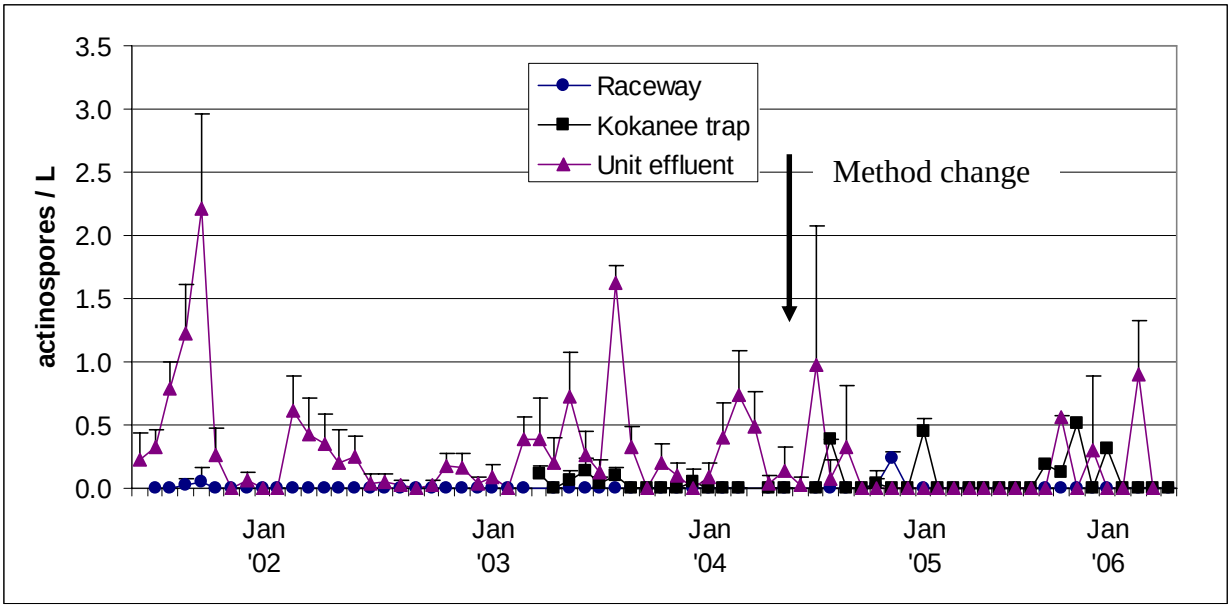

Figure 2.07. Comparison of actinospore densities from the ROJ concrete Raceway tailbox, the kokanee trap (downstream of the concrete raceways), and the Unit effluent through May 2006. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

The removal of kokanee salmon carcasses from the west settling ponds and connecting waterways continued during this segment, accomplished by Division of Wildlife employees. Carcasses were disposed in an upland area. Removal occurred once each week during the spawning season.

Table 2.04. Cranial *Myxobolus cerebralis* myxospore concentrations in trout sampled from the Roaring Judy State Fish Rearing Unit effluent channel.

Date mm/dd/yy	Age (yrs)	Sample Size		Overall Mean Concentration	Positive Fish	
		N	prevalence		Mean	Range
				Brown trout		
05/16/03	1	12	75%	39,900	53,200	2,000 – 177,750
11/25/03	1+	20	70%	29,700	42,400	4,400 – 150,700
05/24/04	1	20	75%	23,500	31,300	300 – 161,900
11/30/04	1+	20	75%	25,700	34,300	1,100 – 193,300
11/22/05	1+	19	68%	37,500	54,700	2,900 – 335,800
				Rainbow trout		
05/16/03	2	21	100%	367,400	367,400	3,700 – 2,242,500
11/25/03	1+	22	50%	57,700	115,300	5,400 – 597,400
05/24/04	1	20	5%	100	1,850	1,850
05/24/04	2	20	80%	458,000	572,200	4,200 – 3,111,800
11/30/04	1+	20	20%	9,600	47,800	5,500 – 157,000
11/30/04	2+	25	12%	3,700	30,600	5,000 – 80,400
11/22/05	2+	25	36%	21,400	59,400	7,400 – 234,600

Trout were not removed from the effluent channel between the concrete raceway outlet and the top of the kokanee spawning facility during this segment as it became apparent that it would not be possible to keep the channel fish population reduced due to escapement from the rearing unit. Samples of the trout removed from the effluent channel show that prevalence and intensity of *M. cerebralis* infection can be substantial (Table 2.04). However, recent samples of older rainbow trout show lower prevalence and intensity of infection after the removal efforts that occurred in 2003 and 2004. This population will continue to be monitored for myxospore concentration and parasite prevalence. If these metrics rise in the future, additional fish removals may be prudent to eliminate heavily infected fish from the channel.

Table 2.05. Cranial *Myxobolus cerebralis* myxospore concentrations in trout sampled from the Roaring Judy State Fish Rearing Unit settling ponds.

Date mm/dd/yy	Species or Strain	Sample Size		Overall Mean Concentration	Positive Fish	
		N	prevalence		Mean	Range
		Settling Ponds				
11/04/03	Tasmanian[a]	28	28.6%	5,100	17,800	3,300 – 40,000
11/04/03	Bellaire[a]	23	43.5%	17,200	39,600	3,300 –136,500
11/04/03	Rainbow[b]	16	93.8%	365,700	390,100	7,200 – 1,387,400
11/30/04	Brown[c]	20	90%	22,600	25,100	560 – 142,200
11/30/04	Brown[d]	20	85%	11,800	14,100	1,100 – 64,400
11/30/04	Erwin[e]	18	72.2%	35.800	49.500	4.400 – 199.500
11/30/04	Bellaire[e]	30	20%	34.700	173.400	6.300 – 942.200
11/30/04	Rainbow[b]	6	50%	93.800	187.600	44.500 – 370.300
11/08/05	Tasmanian	25	8%	3.100	38.900	3.800 – 74.000
11/08/05	Bellaire	25	4%	315	7,900	7,900

a: Tasmanian strain rainbow trout were from the *M. cerebralis*-negative Crystal Rearing Unit, and the Bellaire strain rainbow trout were from the *M. cerebralis*-negative Rifle Rearing Unit.
b: Unmarked rainbow trout, presumed to be feral inhabitants of the ponds or immigrants from the East River.
c: Captured in upper pond.
d: Captured in lower pond.
e: Erwin strain rainbow trout were from the *M. cerebralis*-negative Rifle Rearing Unit, and the Bellaire strain rainbow trout were from the *M. cerebralis*-negative Durango Rearing Unit.

The two west settling ponds were stocked with 4000 fin-clipped catchable rainbow trout in May and June 2005 with Bellaire strain and Tasmanian strain from the Roaring Judy Rearing Unit. This was an increase from 3000 total catchable trout stocked during the previous two years. Samples of the remaining fish were collected in November during the kokanee spawn, having followed the kokanee into the trap. The samples showed very low prevalence and myxospore concentrations (Table 2.05).

Population estimates on the west settling ponds were again conducted during early December, and indicated that very few of the stocked catchable rainbow trout remained in the ponds (Table 2.06). It would appear that the annual stocking of 3-4000 catchable rainbow trout into the settling ponds for the purpose of providing recreational fishing opportunity will not appreciably influence the density of actinospores in the pond effluent because most catchables are removed by anglers before they develop myxospores. Stocking in the future should continue to be completed prior to July to ensure that most catchable trout are removed from the system each year.

Table 2.06. Trout population estimates from the Roaring Judy Fish Rearing Unit settling ponds for fish 15 cm and greater.

Date	Rainbow trout			Brown trout		
	N	95% CI	Kg/ha	N	95% CI	Kg/ha
	Upper pond					
12/03/03	30	23	8	1135	269	310
11/23/04	12	14	5	1132	249	315
11/22/05	39	---	15	944	169	234
	Lower pond					
12/03/03	8[a]	---	4	924	220	625
11/23/04	10[a]	---	6	1355	296	1098
11/22/05	31	65	53	620	101	459

a: No marked fish were recaptured, resulting in an infinite population estimate. These values represent the total numbers of rainbow trout captured in the lower pond. Biomass estimates were based upon actual and estimated rainbow trout weights on the fish captured.

RECOMMENDATIONS and CONCLUSIONS

Filtration studies at the CDOW's Pitkin, Poudre and Roaring Judy trout rearing units have identified earthen bottom settling ponds as major sources of actinospore production that doubtless contributed to the infection of wild trout stocks in the streams receiving the effluents of these units. Efforts to ameliorate the infectivity emanating from these ponds have been successful, with progress continuing to be made toward bringing effluent actinospore densities at these units into equilibrium with the adjacent streams.

It is recommended that the settling pond at Pitkin continue to be kept as free of fish as possible. Since it appears impractical to depopulate the settling ponds at Roaring Judy at this time, it is further recommended that any catchable rainbow trout stocked into these ponds be stocked no later than the end of June. Such stocked fish should continue to be sampled and monitored following the kokanee spawning season to determine prevalence and intensity of infection of the different strains used.

The removal of kokanee salmon carcasses from the ponds and stream channel during the kokanee spawning period at Roaring Judy Fish Rearing Unit should continue. Encouragement of angling harvest in the effluent channel would result in beneficial use of the trout resources that do occupy that area, and would seem preferable to removing them by electrofishing. Signs were posted in 2005 to encourage angler use; these should remain in place.

The Cap K Ranch sand filter proved to be a disappointment in the loss of water capacity experienced over a short period of use. Now, it is clear that the filter is no longer effectively capturing actinospores. Any further efforts to construct sand filtration systems must include changes to filter design as recommended by the engineering proponent of the previous filter,

namely, that the filter media be graded crushed glass, probably in a thinner layer than was used for the existing filter, and finally, that backwash air lines be laid in a much higher density than was the case with the existing filter. Other strategies for reducing infectivity from the Cap K Ranch ponds and similar habitats appear more appropriate at this time.

Job Title: Technical Assistance. Job No. 3

Job Objective: Provide information on impacts of whirling disease on wild trout populations to the Colorado Division of Wildlife Management and Hatchery Sections and to other interested agencies or publics.

Period Covered: July 1, 2005 to June 30, 2006

During this segment, requests for technical assistance were not limited to whirling disease information. Consultations included the following:

1) Fulfilled a request to complete three metadata questionnaires and submit metadata to a contractor working for the National Partnership for the Management of Wild and Native Coldwater Fisheries.
2) Participated in the review process for the new Whirling Disease brochure published by the Whirling Disease Initiative.
3) Reviewed one paper for the Journal of Aquatic Animal Health.
4) Sent *Tubifex tubifex* worms to researchers at New Mexico State University and at the National Fish Health Laboratory in West Virginia for use in whirling disease research projects.
5) Accommodated a number of internal requests from researchers, hatchery managers, and biologists for actinospore density, temperature, and myxospore concentration data.
6) Reviewed the Brush Hollow Reservoir application for a stocking exemption.
7) Responded to a request to send photographs of trout with clinical signs of whirling disease to a researcher in Vermont.
8) Consulted with California associate fish pathologist regarding sentinel fish exposures in natural waters.
9) Shared information with Montana FWP biologist on the configuration and specifications of the raft-mounted electrofishing gear used by our research team.

LITERATURE CITED

Allen, M. B. 1999. Factors influencing the distribution of *Myxobolus cerebralis*, the causative agent of whirling disease, in the Cache la Poudre River, Colorado. Master of Science Thesis. Department of Fishery and Wildlife Biology, Colorado State University, Fort Collins.

Beauchamp, K. A., M. Gay, G. O. Kelley, M. El-Matbouli, R. D. Kathman, R. B. Nehring, and R. P. Hedrick 2002. "Prevalence and susceptibility of infection to *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex*." Diseases of Aquatic Organisms 51: 113-121.

Dubey, R., C. Caldwell, and W. R. Gould. 2005. Effects of temperature, photoperiod, and *Myxobolus cerebralis* infection on growth, reproduction, and survival of *Tubifex tubifex* lineages. Journal of Aquatic Animal Health 17:338-344.

El-Matbouli, M., and R. W. Hoffmann. 1998. Light and electron microscopic studies on the chronological development of *Myxobolus cerebralis* to the actinosporean stage in *Tubifex tubifex*. International Journal for Parasitology 28:195-217.

Ewald, P. W. 1994. Evolution of infectious diseases. Oxford University Press. New York.

Hallett, S. L., S. D. Atkinson, C. Erseus, and M. El-Matbouli. 2004. Molecular methods clarify morphometric variation in triactinomyxon spores (Myxozoa) released from different oligochaete hosts. Systematic Parasitology 57:1-14.

Hedrick, R. P. 1998. Relationships of the host, pathogen, and environment: implications for diseases of cultured and wild fish populations. Journal of Aquatic Animal Health 10:107-111.

Lukins, H. J. 2004. Dynamics of the waterborne stage of *Myxobolus cerebralis* estimated directly by packed-bed filtration. Master's Thesis, Montana State University, Bozeman.

Markiw, M. E. 1991. Whirling disease: earliest susceptible age of rainbow trout to the triactinomyxon of *Myxobolus cerebralis.* Aquaculture 92:1-6.

Markiw, M. E., and K. Wolf. 1974. *Myxosoma cerebralis:* isolation and concentration from fish skeletal elements - sequential enzymatic digestions and purification by differential centrifugation. Journal of the Fisheries Research Board of Canada. 31:15-20.

Nehring, R. B. 2005. Stream fisheries investigations. Colorado Division of Wildlife Final Report. Federal Aid Project F237-R11. Fort Collins.

Nehring, R. B. 1999. Whirling disease investigations. Colorado Division of Wildlife Job Progress Report. Federal Aid Project F237-R6. Fort Collins.

Nehring, R. B. 1998. Stream fisheries investigations. Colorado Division of Wildlife Final Report. Federal Aid Project F237-R5. Fort Collins.

Nehring, R. B. 1996. Whirling disease in feral trout populations in Colorado. Pages 126-144 *In* E. P. Bergersen and B. A. Knopf, editors. Proceedings: Whirling disease workshop-where do we go from here? Colorado Cooperative Fish and Wildlife Research Unit. Fort Collins, Colorado.

Nehring, R. B., and K.G. Thompson. 2003. Whirling disease investigations. Colorado Division of Wildlife Job Final Report. Federal Aid Project F237-R10. Fort Collins.

Nehring, R. B., and K. G. Thompson. 2001. Impact assessment of some physical and biological factors in the whirling disease epizootic among wild trout in Colorado. Colorado Division of Wildlife Special Report Number 76. Fort Collins.

Nehring, R. B., K. G. Thompson, and S. Hebein. 1998. Impacts of whirling disease on wild trout populations in Colorado. Pages 82 – 94 *In* K. G. Wadsworth, editor. Transactions of the 63rd North American Wildlife and Natural Resources Conference. Wildlife Management Institute. Washington, D.C.

Nehring, R. B., K.G. Thompson, J. Padia, and B. Neuschwanger. 2000. Whirling disease investigations. Colorado Division of Wildlife Job Progress Report. Federal Aid Project F237-R7. Fort Collins.

Nehring, R. B., K.G. Thompson, D. Chacon, J. Padia, and A. Nikirk. 2001. Whirling disease investigations. Colorado Division of Wildlife Job Progress Report. Federal Aid Project F237-R8. Fort Collins.

Nehring, R. B., and P. G. Walker. 1996. Whirling disease in the wild: the new reality in the intermountain west. Fisheries 21(6):28-30.

Rose, J. D., G. S. Marrs, C. Lewis, and G. Schisler. 2000. Whirling disease behavior and its relation to pathology of brain stem and spinal cord in rainbow trout. Journal of Aquatic Animal Health 12:107-118.

Schisler, G. J. 2003. Salmonid Disease Studies. Evaluation of habitat influences. Colorado Division of Wildlife Progress Report. Federal Aid Project F394-R3. Fort Collins.

Schisler, G. J. 2001. Whirling disease investigations. Evaluation of habitat influences. Colorado Division of Wildlife Progress Report. Federal Aid Project F237-R8. Fort Collins.

Schisler, G. J., E. P. Bergersen, and P.G. Walker, J. Wood, and J. K. Epp. 2001. Comparison of single-round polymerase chain reaction (PCR) and pepsin-trypsin digest (PTD) methods for detection of *Myxobolus cerebralis*. Diseases of Aquatic Organisms 45:109-114.

Thompson, K. G. 2005. Whirling Disease Habitat Interactions. Colorado Division of Wildlife Job Progress Report. Federal Aid Project F-427-R2. Fort Collins.

Thompson, K. G. 2004. Whirling Disease Habitat Interactions. Colorado Division of Wildlife Job Progress Report. Federal Aid Project F-427-R1. Fort Collins.

Thompson, K. G., R. B. Nehring, D. C. Bowden, and T. Wygant. 2002. Response of rainbow trout *Oncorhynchus mykiss* to exposure to *Myxobolus cerebralis* above and below a point source of infectivity in the upper Colorado River. Diseases of Aquatic Organisms 49:171-178.

Thompson, K.G., and R. B. Nehring. 2000. A simple technique used to filter and quantify the actinospore of *Myxobolus cerebralis* and determine its seasonal abundance in the Colorado River. Journal of Aquatic Animal Health 12:316-323.

Walker, P. G. 1997. Whirling disease problem reveals need to redirect agency fish health programs. Fisheries 22(8):4.

Walker, P. G., and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife Report. Denver.

Wolf, K., and M. E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science 225:1449-1452.