Whirling Disease Habitat Interactions

Federal Aid Project F-427-R1

Kevin G. Thompson
Principal Investigator

Bruce McCloskey, Director

Federal Aid in Fish and Wildlife Restoration

Job Progress Report

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Fish Research Section

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STATE OF COLORADO

Bill Owens, Governor

COLORADO DEPARTMENT OF NATURAL RESOURCES

Russell George, Executive Director

COLORADO DIVISION OF WILDLIFE

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Richard Anderson, General Professional IV, F-289 Riverine Fish/Flow Investigations
Arturo Avalos, Technician III, Research Hatchery
Stephen Brinkman, Laboratory Technician II, F-243, Water Pollution Studies
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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
Rosemary Black, Program Assistant I

Paula Nichols, Federal Aid Coordinator
Jackie Boss, Librarian

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

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

Date: 7/28/04

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.

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Project Title: Whirling Disease / Habitat Interactions

Project No.: F-427-R1

Project Objective: To investigate the influence of aquatic habitat factors on the severity of *Myxobolus cerebralis (Mc)* 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 No 1: Identification and Reduction of *Tubifex tubifex (T. tubifex)* Habitat in Streams.

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 *Mc.*

Period Covered: July 1, 2003 to June 30, 2004

INTRODUCTION

Since 1994 major declines in numbers of wild rainbow trout *Oncorhynchus mykiss* have been observed in certain rivers in Colorado. Research suggested that these declines resulted from whirling disease (Walker and Nehring 1995; Nehring 1996; Nehring and Walker 1996; Nehring et al. 1998; Nehring 1998; Nehring 1999), caused by the Mc parasite. Sentinel fish studies in the Colorado River and *Mc* actinospore filtration studies in numerous drainages suggested that there are areas within streams that act as foci of infection for the parasite (Thompson et al. 2002, Nehring and Thompson 2001; Thompson and Nehring 2000a). Reservoirs may also act as such foci. Stocking Spring Creek Reservoir with catchable trout infected with *Mc* 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. However, some sites of high infectivity that were not reservoir-related were detected by actinospore filtration. Examples included some irrigation diversions and beaver ponds or pond complexes.

The problem of infectivity below reservoirs was initially addressed by taking steps to insure that fish stocked in them were uninfected with the parasite. Included were intense capital efforts to enhance hatchery water supply security, changes in hatchery management, and changes in stocking policy. The benefits to downstream fisheries from these management actions became more apparent as time passed. Nevertheless, certain areas of infectivity remained that were not reservoir related but appeared to harbor *Mc* persistently. The objectives of this study were to determine whether it is possible to remove or greatly reduce such foci of infection by physical habitat manipulation and stream habitat improvement techniques, and to determine if such manipulations resulted in reduced prevalence and intensity of infection among resident trout within the stream reach presumably affected by the focus of infection.

Segment Objectives were to:

1. Implement habitat modifications at one study site (Willow Creek below Willow Creek Reservoir),

2. Collect baseline oligochaete and triactinomyxon data at the Cache la Poudre River study site above the Poudre Rearing Unit,

3. Assist USGS personnel with survey work at the Cache la Poudre River study site,

4. Finish collecting post-manipulation data at study sites modified during previous segment,

5. Design habitat modification structures to reduce *T. tubifex* habitat for Poudre River study sites, and

6. Begin the Corps of Engineers permitting process for in-stream work.

METHODS and MATERIALS

Site Selection

Sites previously under study in project F-237 were initially considered. Those that showed consistent infectivity in the past and appeared to be linked to discrete natural or anthropogenic features of the stream were considered the best candidates as study sites for this job. These sites had been monitored by water filtration according to the protocol described in Thompson and Nehring (2000a) and Nehring et al. (2001). Other sites not previously monitored by water filtration were evaluated based on visual inspection and the detection of infected *T. tubifex* by examining water held overnight over substrate samples and then filtered to concentrate any actinospores present. If infected *T. tubifex* were present; holding them for 16-24 hours in a static condition that allowed the water to warm somewhat stimulated a release of mature actinospores (El-Matbouli et al. 1999). At such sites, monitoring by water filtration was initiated to confirm long-term infectivity and gather baseline data on actinospore density in the water column.

Baseline Information

Baseline information at each study site was collected to describe the prevalence of infection in the fish, the oligochaete population, and the actinospore production dynamic. At some study sites data were also available or collected on the fish population structure. At these sites standard electrofishing stations encompassing or just downstream of each study site were established and a fish population estimate obtained by removal estimator (Seber and LeCren 1967). At one study site, the population data were obtained by the Lincoln-Peterson mark and recapture estimator. Samples of age 1+ brown trout were obtained at each location and analyzed for *Mc* spore concentrations in individual heads by the pepsin-trypsin digest (PTD) method (Markiw and Wolf 1974). For some

locations, such samples were available from several years. In instances where young-of-the-year (YOY) trout were collected they were examined using 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 *Mc* was present. Results were graded subjectively by two reviewers and reported on a five-point scale ranging from '0' (negative) to '4' (an intense band indicating a severe parasite infection).

Oligochaete populations were characterized by sampling what was judged to be the best oligochaete habitat at each study site on two or three 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 dropping a frame made of copper tubing, and a 53.5 cm^2, 10-cm deep core sample was removed at the center of the area selected. The core samples were collected by USGS personnel to examine organic content and particle size distributions and to 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. The actinospore density was estimated using techniques described previously (Thompson and Nehring 2000a, Nehring et al. 2001). All samples were also tested by PCR to confirm the identity of actinospores observed as those of *Mc.* 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 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.

Water samples consisting of 1900 L filtered through 20-μm Pecap screen were collected each month from each site, with some exceptions. These concentrated samples were examined for actinospores. Density of the organism was estimated with protocols established in earlier research (Thompson and Nehring 2000a, Nehring and Thompson 2001). The samples were also typically tested by PCR to confirm the identity of the actinospores observed and to test those samples in which no actinospores were observed.

RESULTS

Beaver Creek (South Fork Rio Grande drainage)

The identified focus of infection in Beaver Creek below Beaver Creek Reservoir was a small senescent beaver pond located in a small side channel (Nehring and Thompson 2003). The inlet to the side channel was sealed off by the San Luis Valley chapter of Trout Unlimited in October 2001.

Monitoring of the site for actinospores continued through the current segment, and indicated low actinospore densities since the habitat manipulation was completed (Table 1.01, Figure 1.01). Actinospores were observed on a similar number of occasions in the 20 months following manipulation compared to the 20 months preceding manipulation. However, in 2002 and 2003 there have been fewer occasions when actinospores were detected during the months of June through September, the period of maximum vulnerability for YOY trout. No actinospores were detected during this segment (Figure 1.01). None of the filtrates yielded a positive PCR test.

Table 1.01. Results of water filtration to quantify *Mc* actinospores (number/L) in 1900-L samples of water from Beaver Creek below a small side channel containing a beaver pond, July 1, 1998 to May 30, 2004. Numbers in parentheses are the half-width of 95% confidence intervals.

Month	1998-99	1999-2000	2000-01	2001-02	2002-03	2003-04
Jul	2.150 (0.76)	0.217 (0.16)	0.0 (---)	0.041 (0.08)	0.0 (---)	0.0 (---)
Aug	1.681 (0.90)	1.445 (0.79)	0.119 (0.16)	0.222 (0.15)	0.0 (---)	0.0 (---)
Sep	2.101 (0.38)	0.333 (0.30)	0.0 (---)	0.061 (0.08)	0.035 (0.07)	0.0 (---)
Oct	0.606 (0.41)	0.093 (0.18)	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)
Nov	0.040 (0.08)	0.099 (0.11)	0.143 (0.19)	0.033 (0.07)	0.0 (---)	0.0 (---)
Dec	0.097 (0.01)	0.0 (---)	0.0 (---)	0.0 (---)	0.044 (0.09)	0.0 (---)
Jan	0.0 (---)	0.0 (---)	0.0 (---)	0.028 (0.06)	0.0 (---)	no sample
Feb	0.030 (0.04)	0.0 (---)	0.0 (---)	0.0 (---)	0.024 (0.02)	no sample
Mar	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	no sample
Apr	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)
May	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)
Jun	0.0 (---)	0.0 (---)	0.0 (---)	0.0 (---)	0.060 (0.12)	

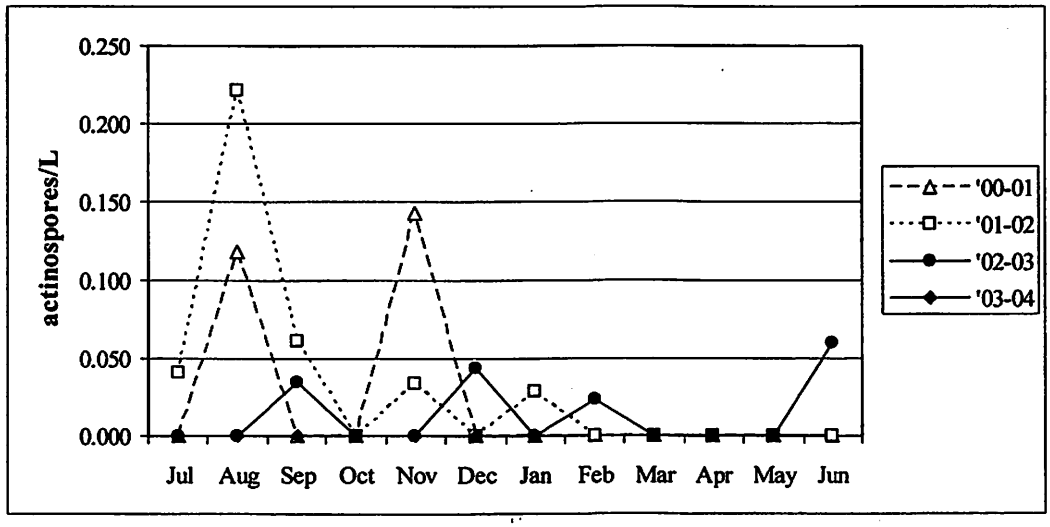

Figure 1.01. Density of actinospores of *Mc* in Beaver Creek below the side channel containing beaver ponds from July 2000 through May 2004.

Samples of juvenile brown trout taken since 1998 established the trout population in this section of stream was substantially infected by the whirling disease parasite. The average prevalence of infection in age 1 brown trout inhabiting Beaver Creek below Beaver Creek Reservoir was 70.5% during 1998 - 2001. The average concentration consistently exceeded 20,000 myxospores (Figure 1.02).

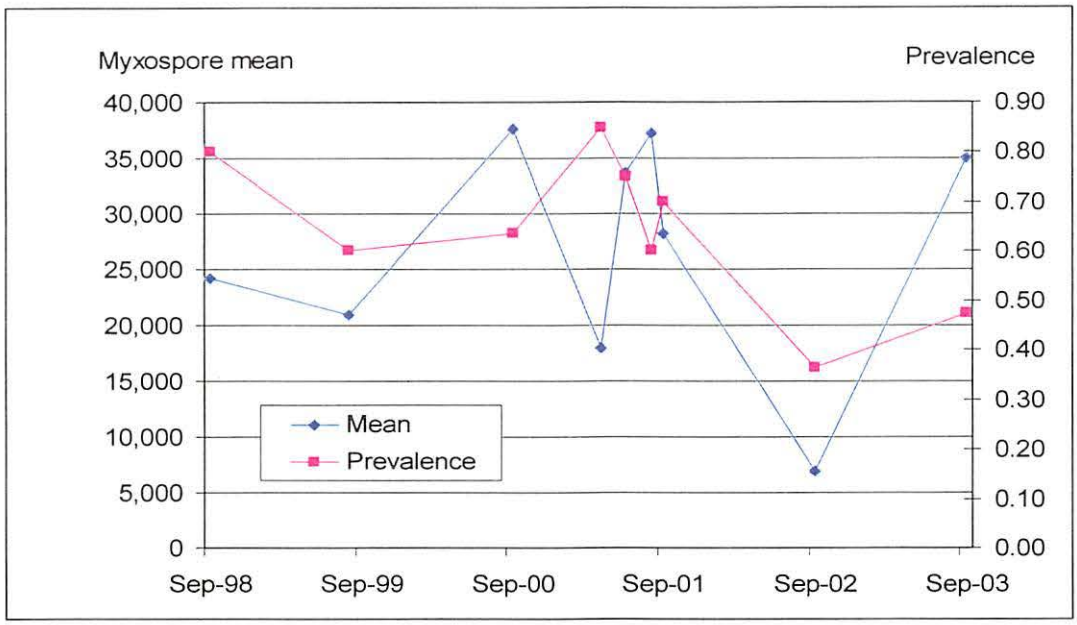

Figure 1.02. Mean myxospore concentration and prevalence of infection in samples of age 1+ brown trout (n = 11 – 20) collected from Beaver Creek below the habitat manipulation site.

Results of myxospore evaluations in brown trout suggested that infection prevalence was lower during the last two years than before the habitat modification (Figure 1.02). Compared to a mean prevalence of 70.5% in the years prior to manipulation, only 36.4% of age 1+ trout collected in the Fall 2002 and 47.4% collected in 2003 tested positive. While these values do not represent statistically significant decreases compared to the 70% prevalence observed in 2001 (close to the mean pre-treatment value), the pattern was promising, even though the mean myxospore concentration was high in the Fall 2003 sample. The high myxospore mean was greatly influenced by two high values. The mean without the highest value was 17,340 myxospores, without the two highest values the mean was 9,519.

Perhaps an even more promising trend at this site was the reappearance of wild age 1+ rainbow trout during the last few years (Table 1.02). These rainbow trout first were detected again in 2000, a year after *Mc*-positive catchable rainbow trout were last observed in Beaver Creek Reservoir in samples of fish collected from fishermen during the Fall. During the last segment the highest number of rainbow trout in six years was observed. Also, the average PCR score obtained from Y-O-Y rainbow trout decreased from 3.7 in 2002 (n = 22) to 1.9 in 2003 (n = 15).

Table 1.02.Trout population biostatistics (fish ≥ 15 cm) for Beaver Creek 1 km below Beaver Creek Reservoir from the fall (September and October) 1998 through 2002.

	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

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.

Cache la Poudre River

The Cache la Poudre River was added to the work schedule during the 2002-03 segment. Previously, great strides were made in reducing *Mc* actinospores emanating from the Poudre Rearing Unit (PRU) (see Job 2 of Nehring and Thompson 2003, Schisler 2003). So it seemed the time was right to focus additional attention 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 during his work was at Kinikinik. At the site there are two significant backwater areas that appear to be excellent habitat for *T. tubifex*.

Water samples were collected above and below the Kinikinik site since January 2003 (Figure 1.03). Additional samples were collected at the Bliss State Wildlife Area, which was about 1.5 km upstream of the upper Kinikinik site. To date the site below Kinikinik yielded actinospores on 44% of filtering occasions, compared to 32% of occasions above Kinikinik and 18% of occasions at the Bliss SWA site. Density estimates were low on all occasions.

Oligochaete samples were collected on two pre-construction occasions thus far, with a third occasion scheduled in June 2004. The first two sampling occasions indicated that *T. tubifex* lineage III predominated in this area (Table 1.03). Lineage III was shown in the past to be the strain of *T. tubifex* most susceptible to *Mc* infection (Beauchamp et al. 2002). Lineage V, considered to contain few if any susceptible individuals, was not represented in the oligochaete samples collected from this area to date.

The available results of myxospore analyses from samples of age 1+ brown trout collected over the last several years are presented in Table 1.04.

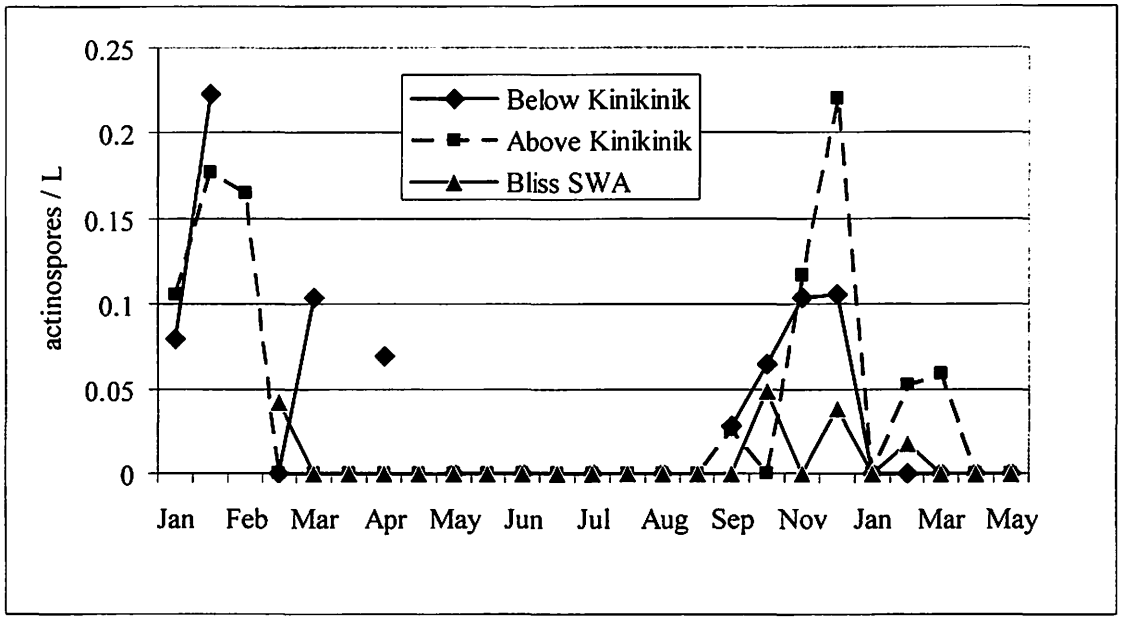

Figure 1.03. Estimates of actinospores/L in the Poudre River at three sites near Kinikinik from January 2003 through May 2004. Bliss State Wildlife Area (SWA) was upstream of the Kinikinik sites.

Table 1.03. Estimates of the proportion of each *T. tubifex* lineage comprising the pre-modification samples at the Kinikinik site. N refers to the number of the nine 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 *Mc* lineage			
		I	III	V	VI
8/25/03	9	2.8 (1.8)	73.9 (10.6)	0.0 (0.0)	23.3 (11.0)
10/01/03	8	4.8 (2.9)	67.5 (11.8)	0.0 (0.0)	27.7 (10.8)

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

Date mm/dd/yy	Sample Size		Overall Mean Concentration	Positive Fish	
	N	prevalence		Mean	Range
Bliss State Wildlife Area – above Kinikinik					
09/30/02	10	10%	2.800	28.100	28.100
10/22/03	Results not available				
Big Bend – below Kinikinik					
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

A design to modify the river channel to isolate the two backwater habitats at flows less than 800 cfs with native material berms was produced in association with a hydrologist from USGS and DOW engineers. These berms precluded about 90% of all average daily flows in this area from entering the backwater areas, according to data from a discontinued gage near Rustic. Permits to execute the work were obtained from the U. S. Army Corps of Engineers and the U. S. Forest Service (Wild and Scenic River authority). The work was scheduled to be completed during the next segment of this project.

Colorado River

A long history of trout population evaluation on the 3.2-km reach of the Colorado River that encompassed the Kemp/Breeze Wildlife Area clearly indicated that what was once a prodigious self-sustaining rainbow trout fishery all but collapsed, beginning in the early 1990's (Figure 1.04, Table 1.05). Previous intensive study in the area pointed to the whirling disease epizootic as the primary factor in the failure of rainbow trout recruitment over the past decade and the concomitant population crash (Nehring and Thompson 2001). The brown trout population in this same reach of river has not experienced a population crash, but instead appeared to have benefited from reduced competition. The trend in brown trout numbers and biomass reflected substantial increases over the values observed in the 1980's (Table 1.04).

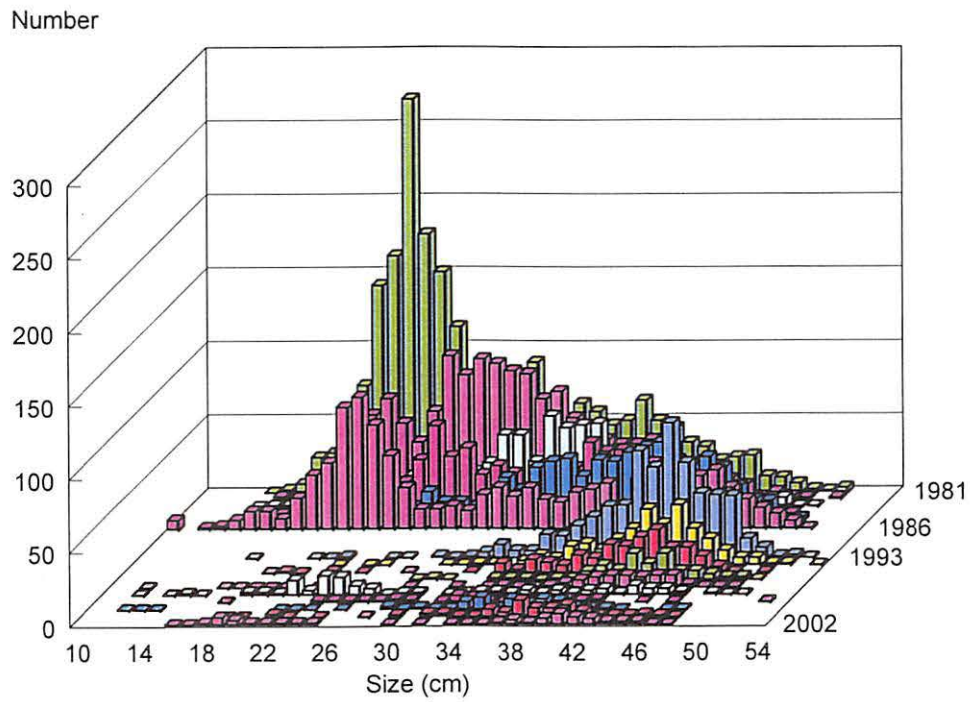

Figure 1.04. Total numbers of unmarked rainbow trout caught during mark and recapture population estimates on the Kemp/Breeze Wildlife Area of the Colorado River in 1981 – 1986 (pre whirling disease years) and 1993 – 2002 (post whirling disease years).

Samples of juvenile brown trout obtained since 1999 for analysis of cranial myxospore concentrations by the PTD method (Markiw and Wolf 1974) indicated prevalence of infection was routinely 60% or greater (Table 1.06). The sample collected during the last segment contained a higher proportion of infected individuals than any previous sample.

Baseline oligochaete sampling in the Kemp/Breeze Wildlife Area was completed in 2001 (Table 1.07). Wingdam structures downstream of the Breeze Bridge that were suspected as prime areas of sediment deposition and therefore potential areas of infection did not prove to be so. Biomass of likely *T. tubifex* in the wingdam area was low compared to the values observed upstream in the area of the Breeze Bridge. In the bridge vicinity higher biomass values were observed in subsamples from near the bridge, and lower biomass values were observed in subsamples taken from the head of an irrigation out-take pool just downstream of the bridge.

Table 1.05. Trout population biostatistics for the 3.2 km reach of the Colorado River on the present-day Kemp/Breeze Wildlife Area, fall 1981-1986 (pre-whirling disease) versus Fall 1993-2002 (post-whirling disease).

	Brown Trout					Rainbow Trout				
Year	N	95% CI	Kg/ha	N/ha	N/ha ≥35 cm	N	95% CI	Kg/ha	N/ha	N/ha ≥35 cm
1981	3,415	± 1335	82	294	36	10,300	±1,635	231	889	185
1982	2,031	± 588	48	175	53	4,756	±739	124	410	173
1983	1,476	±710	42	127	25	2,341	±452	81	202	86
1984	1,735	±408	35	150	11	2,410	±410	78	210	78
1985	1,651	±613	55	142	34	1,976	±329	94	170	115
1986	1,230	±389	44	106	33	3,214	±538	109	277	111
1993	3,280	±1,244	91	283	51	1,881	±396	128	162	154
1994	4,965	±1,817	119	428	49	774	±232	53.1	67	65
1995	9,707	±2,084	224	837	138	610	±219	41.2	53	50
1996	5,857	±1,274	190	505	164	288	±126	19.9	25	24
1997	4,330	±926	162	373	149	293	±199	17.0	25	23
1998	7,333	±1,300	224	632	142	359	±168	15.3	31	13
1999	9,761	±3,329	224	842	199	527	±748	19.7	45	18
2000	8,113	±2,115	232	699	248	265	±208	11.5	23	17
2001	8,358	±1,637	235	721	211	362	±289	17.6	31	25
2002	19,515	±3,678	419	1682	291	1,364[a]	±1,966	58	118	73
2003	22,615	±4,790	414	1970	150	276	±350	10	24	12

a: A total of 72 rainbow trout were captured on the mark and recapture occasions. No individual rainbow trout were recaptured, resulting in an infinite population estimate by the Lincoln-Petersen estimator. The reported estimate was based on the hypothetical recapture of one fish, but clearly did not satisfy the assumptions of the model for the rainbow trout component of the population.

Table 1.06. Cranial *Mc* myxospore concentrations in brown trout sampled from the Colorado River at the Kemp/Breeze Wildlife Area in 2001-2002

Date Mo/Da/Yr	Age	Sample Size		Overall Mean Myxospore Concentration	Myxospores in Positive Fish	
		N	N+		Mean	Range
09/29/99	1+	10	6	2,330	3,890	2,220 – 6,670
05/31/00	1	10	8	6,720	8,400	830 – 25,000
04/05/01	1	20	15	14,200	18,000	1,100 – 132,200
08/06/01	1+	21	16	40,900	53,700	2,900 – 337,500
09/18/01	1+	19	7	13,800	37,300	1,900 – 160,600
10/08/02	1+	13	11	19,900	23,600	3,300 – 68,100
09/17/03	1+	15	14	14,400	15,400	3,300 – 70,100

Table 1.07. Oligochaete sampling results from the Colorado River. Biomass values are expressed in grams. Spores observed refers to the number of oligochaete samples from which actinospores were observed by microscopy over the total number of samples examined, after holding the worms overnight in the lab. PCR refers to the number of filtered water samples taken from the worm samples that tested positive for the DNA of the parasite over the total number of samples examined.

Date	Mean Total Biomass	SE Mean	% likely *T. tubifex*	*T. tubifex* Mean Biomass	SE Mean	Spores observed	PCR
			Breeze Bridge Area				
06/27/01	1.763	0.5569	49.9	0.911	0.3308	3/6	---
11/26/01	3.923	2.2097	31.3	3.073	1.9326	3/6	5/6
			Wingdams 0.5 km below Breeze Bridge				
11/06/01	2.676	2.5214	3.9	0.006	0.004	0/6	3/6

Actinospore densities were monitored at the Breeze Bridge once each month during the last segment. *Mc* actinospores were observed on five of eleven occasions so far in this segment (Table 1.08). This represented a higher detection rate than during the previous segment, but was still lower than the earlier years of actinospore monitoring at this site.

Table 1.08. Results of water filtration to quantify *Mc* actinospores (Tams/L) in 1900-L (500-gallon) samples of water drawn from the Colorado River at Breeze Bridge July 1, 1999 to June 30, 2003. Numbers in parentheses are the half-width of 95% confidence intervals.

Month	1999-2000	2000-01	2001-02	2002-03	2003-04
July	0.357 (0.21)	0.903 (0.59)	0.386 (0.76)	0.000	0.324 (0.35)
August	0.299 (0.23)	0.251 (0.20)	0.285 (0.17)	0.052 (0.07)	0.120 (0.16)
September	0.103 (0.91)	0.887 (0.37)	0.162 (0.11)	0.000 (---)	0.000 (---)
October	0.487 (0.24)	0.627 (0.33)	0.674 (0.23)	0.237 (0.76)	0.000 (---)
November	0.565 (0.41)	1.109 (0.67)	0.000 (---)	0.000 (---)	0.052 (0.10)
December	0.068 (0.09)	0.263 (0.19)	0.058 (0.11)	0.000 (---)	0.070 (0.14)
January	0.178 (0.14)	0.169 (0.23)	0.000 (---)	0.000 (---)	0.000 (---)
February	0.000 (---)	0.363 (0.46)	0.000 (---)	0.000 (---)	0.000 (---)
March	0.000 (---)	0.040 (0.08)	0.038 (0.07)	0.000 (---)	0.000 (---)
April	0.178 (0.24)	0.000 (---)	0.000 (---)	0.000 (---)	0.000 (---)
May	0.124 (0.17)	0.123 (0.24)	0.000 (---)	0.000 (---)	0.197 (0.18)
June	2.005 (0.68)	0.466 (0.45)	0.182 (0.10)	0.000 (---)	

The Colorado River site will continue to be monitored as a site that has not been modified to eliminate or reduce oligochaete habitat.

Fryingpan River

The Fryingpan River below Ruedi Dam was another stream with a long history of trout population study to draw from. Previous study indicated *Mc* infection had a noticeable impact on the rainbow trout population in the lower part of the reach. One focus of infectivity was 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 the Fall 2002. The conclusion of the study was that the Cap-K Ranch ponds were a far more consistent source of infectivity than any other site studied.

A potential in-river site existed on the Christine Unit of the Basalt Wildlife Area, about four km above the town of Basalt. The site consisted of a low-volume side channel that contained a series of beaver dams, resulting in an area rich in sediment and organic debris. The adjacent main channel also made a tortuous turn where the channels rejoin, creating a pool with very low velocity and substantial debris accumulation. Study of this site was initiated in 2001 to evaluate whether it contributed significant infectivity to the Fryingpan River.

Samples of age one brown trout were collected in April 2002 and 2003 to begin establishing the baseline cranial myxospore concentration in the brown trout population (Table 1.09). These samples indicated that a high proportion of the brown trout were positive for *Mc*. The prevalence and intensity of infection, however, was not significantly different than what was detected further upstream near the Taylor Creek confluence.

Table 1.09. Cranial *Mc* myxospore concentrations in brown trout sampled from the Fryingpan River at the Christine Unit of the Basalt Wildlife Area and near the Taylor Creek confluence

Date Mo/Da/Yr	Age	Sample Size		Overall Mean Myxospore Concentration	Myxospores in Positive Fish	
		N	N+		Mean	Range
Christine Unit of Basalt State Wildlife Area						
04/05/02	1	20	18	18,400	20,400	500 – 73,800
04/25/03	1	18	15	23,200	27,800	1,500 – 113,300
Taylor Creek Confluence						
05/30/00	1	10	7	6,740	9,600	430 – 31,200
10/31/00	1+	9	5	37,700	67,800	9,300 – 181,900
10/30/01	1+	11	7	35,900	56,400	2,528 – 147,500
10/29/03	1+	20	16	29,600	37,000	1,500 – 189,900

Oligochaete samples were obtained on three occasions at the Christine Wildlife area (Table 1.10). Total biomass figures for this study site were somewhat lower than those observed in the Colorado River, but the proportion of the worms that were likely to be *T. tubifex* was quite high. *Mc* actinospores were observed in the majority of filtrates taken from the worm samples. In all but one instance, PCR confirmed the identifications. There were two other subsamples in which no actinospores were observed yet tested positive by PCR.

Table 1.10. Oligochaete sampling results from the Fryingpan River. Biomass values are expressed in grams. Spores observed refers to the number of oligochaete samples from which actinospores were observed by microscopy over the total number of samples examined, after holding the worms overnight in the lab. PCR refers to the number of filtered water samples taken from the worm samples that tested positive for the DNA of the parasite over the total number of samples examined.

Date	Mean Total Biomass	SE Mean	% likely *T. tubifex*	*T. tubifex* Mean Biomass	SE Mean	Spores observed	PCR
Christine Unit of Basalt State Wildlife Area							
10/31/01	0.725	0.1544	72.0	0.346	0.1103	5/6	---
11/19/01	0.474	0.0678	78.6	0.298	0.0676	4/6	5/6
05/02/02	1.180[a]	0.8578	77.8	1.074	0.8495	3/6	3/6

a: Biomass figures are based on n = 4. Subsample 3 was mistakenly washed in a 500 um mesh sieve at the lab. Subsample 6 was intentionally taken in the recently re-wetted side channel to determine whether *T. tubifex* would had recolonized. Few worms (no tubificids) were observed.

Actinospores were observed sporadically at this site (Table 1.11). The side channel also dried up during low winter flows. The filtration site in the side channel was moved to the pool in the river immediately below the side channel in April 2002. This pool also contained good oligochaete habitat, as evidenced by the oligochaete samples taken in May 2002, when five of the six subsamples were collected.

Table 1.11. Results of water filtration to quantify *Mc* actinospores (N/L) in 1900-L (500-gallon) samples of water drawn from the Fryingpan River at the Christine Unit of Basalt Wildlife Area November 1, 2001 to September 30, 2003.

Month	2001-2002	2002-2003	2003-2004	2001-2002	2002-2003
	Below side channel			Above side channel	
Jul	a	0.0 (---)	0.0 (---)	a	0.0 (---)
Aug	a	0.0 (---)	0.0 (---)	a	0.0 (---)
Sep	a	0.023 (0.05)	0.0 (---)	a	0.0 (---)
Oct	a	0.025 (0.05)	c	a	c
Nov	0.050 (0.10)	0.0 (---)		0.0 (---)	
Dec	b	0.086 (0.08)		0.0 (---)	
Jan	b	0.0 (---)		0.0 (---)	
Feb	b	0.0 (---)		0.0 (---)	
Mar	b	0.0 (---)		0.0 (---)	
Apr	0.0 (---)	0.0 (---)		0.0 (---)	
May	0.030 (0.06)	0.059 (0.12)		0.0 (---)	
Jun	0.0 (---)	0.0 (---)		0.0 (---)	

a: Sampling not yet initiated
b: Sample not collected because side channel was dry.
c: Sampling ceased after September 2002 (above side channel) and 2003 (below side channel).

This site may be a point source of infectivity. If so infectivity most likely originated in the pool below the side channel. The side channel itself, although it could harbor oligochaetes, did not seem to be a significant threat to the river since it most likely dried up annually through the winter months. Overall, the area did not appear to elevate prevalence and intensity of infection over what was observed at the upstream Taylor Creek confluence. Consequently, we ceased monitoring this site, but continued to monitor the Taylor Creek confluence site. Actinospore monitoring results are presented in Table 1.12 for the Taylor Creek site.

Table 1.12. Results of water filtration to quantify *Mc* actinospores (N/L) in 1900-L (500-gallon) samples of water drawn from the Fryingpan River near the Taylor Creek confluence November 1, 2001 to May 31, 2004.

Month	2001-2002	2002-2003	2003-2004
Jul	a	0.0 (---)	0.0 (---)
Aug	a	0.0 (---)	0.0 (---)
Sep	a	0.0 (---)	0.0 (---)
Oct	a	0.0 (---)	0.0 (---)
Nov	0.029 (0.06)	0.017 (0.03)	0.0 (---)
Dec	0.036 (0.07)	0.0 (---)	0.0 (---)
Jan	0.014 (0.03)	0.0 (---)	0.0 (---)
Feb	0.0 (---)	0.0 (---)	0.0 (---)
Mar	0.0 (---)	0.0 (---)	0.0 (---)
Apr	0.0 (---)	0.0 (---)	0.0 (---)
May	0.0 (---)	0.0 (---)	0.0 (---)
Jun	0.0 (---)	0.0 (---)	

a: Sampling not yet initiated

Spring Creek (Taylor River drainage)

Spring Creek below Spring Creek Reservoir was a fairly high gradient, low sediment-supply stream for about two km. It then flowed through a reach where gradient lessened and several small tributary streams contributed to the sediment load. Log-drop structures were placed in this moderate gradient reach years ago to enhance trout habitat. These structures were viewed with suspicion as potential foci of *Mc* infection because they tended to pool water upstream of the logs. Preliminary investigations through this reach revealed that a more recognizable focus of infection existed at the confluence of Salsbury Gulch, just below the Doctor Gulch road ford (FS road 744.2c). The channel in this area was undergoing adjustment, which resulted in some bank erosion and the accumulation of sediment and organic debris.

Spring Creek supported a substantial trout population predominated by brown trout (Table 1.13). Biomass tended to be higher above the study site at Salsbury Gulch than below it. The rainbow trout component of the population was sparse, but Spring Creek was occasionally stocked with catchable rainbow trout. Natural reproduction of rainbow trout occurred sporadically. YOY rainbow trout were captured at the station four km above Salsbury Gulch (0.8 km below Spring Creek Reservoir) in 1999, 2000 and 2003, but not in 1998, 2001 or 2002. YOY rainbow trout were captured at the control station 14 km below Salsbury Gulch in 1999, 2001, 2002 and 2003.

Table 1.13. 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									
1998	171	± 1	466	4,876	2,724	5	± 0	18	143	0
1999	187	± 5	441	4,036	3,469	3	± 0	17	65	345
2000	285	± 2	580	6,147	6,577	0	---	0	0	0
2001	243	± 2	474	5,238	3,766	4	± 0	12	86	0
2002	265	± 2	506	5,725	4,571	0	---	0	0	0
2003	246	± 5	261	3,173	4,345	0	---	0	0	0
	Above Spring Creek Campground									
1998	124	± 3	215	1,612	1,366	5	± 0	18	143	0
1999	106	± 9	229	1,244	1,330	3	± 0	17	65	345
2000	172	± 3	264	2,019	2,198	0	---	0	0	0
2001	157	± 5	190	1,961	2,574	4	± 0	12	86	0
2002	175	± 5	207	2,105	1,814	207[a]	± 1	427	2,435	24
2003	157	± 8	180	1,653	1,664	52	± 2	102	554	21
	At Salsbury Gulch									
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

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

Samples of brown trout were collected at three sites during this segment for myxospore analysis (Table 1.14). Over the last couple of years, prevalence of *Mc* infection in fish collected at Salsbury Gulch and at Spring Creek Campground tended slightly higher than in brown trout collected just below Spring Creek Reservoir. However, these values were not significantly different, nor were the average myxospore concentrations. All samples collected at the treatment site were considered baseline data because of the age of fish sampled. The first "post-treatment" samples will not be available until Fall 2004. Samples from the last couple of years suggested that myxospore concentrations may be increasing below the reservoir. A likely explanation for this was increased beaver activity in the first 0.75 km of stream downstream of the reservoir since 2001.

Samples of YOY brown trout were collected at the same three sites in September 2002 and 2003. The heads were analyzed by the PCR technique, which indicated there was high prevalence of infection among YOY brown trout at all three sites for all years (Table 1.14).

Table 1.14. Cranial *Mc* myxospore concentrations in age 1+ brown trout sampled from Spring Creek. The first "post-treatment" samples will not be collected until Fall 2004.

Date mm/dd/yy	Sample Size		Overall Mean Concentration	Positive Fish	
	N	prevalence		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
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
19 km downstream of Spring Creek Reservoir at Control site					
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

Table 1.15. Results of PCR tests of samples of YOY brown trout collected from Spring Creek. The mean PCR score was 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			
09/27/01	10	10	3.4
09/17/02	18	14	1.6
09/22/03	20	20	2.8
5 km downstream of Spring Creek Reservoir at Treatment site			
09/18/02	21	18	1.9
08/22/03	20	20	3.1
19 km downstream of Spring Creek Reservoir at Control site			
09/27/01	10	10	3.8
09/18/02	10	10	2.3
09/23/03	20	20	2.7

Habitat modification was accomplished at the Salsbury Gulch site during early October 2002 (Nehring and Thompson 2003). The post-construction oligochaete sampling was completed during the last segment (Table 1.16). In addition to the Salsbury Gulch site where habitat modifications took place, samples were collected 19 km downstream of the reservoir at Spring

Creek Campground. The campground served as an un-manipulated control site for comparison to the sites on Spring Creek and the Williams Fork River that underwent habitat modifications in 2002. Oligochaete samples taken at the Salsbury Gulch site and the Spring Creek Campground control site consistently yielded actinospores that were confirmed as those of *Mc* by the PCR test.

Table 1.16. Oligochaete sampling results from Spring Creek. Biomass values are expressed in grams. Spores observed refers to the number of oligochaete samples from which actinospores were observed by microscopy over the total number of samples examined, after holding the worms overnight in the lab. PCR refers to the number of filtered water samples taken from worm samples that tested positive for *Mc* DNA over the total number of samples examined. Habitat modifications were completed at the Salsbury Gulch site during the first week of October 2002.

Date	Mean Total Biomass	SE Mean	% likely *T. tubifex*	*T. tubifex* Mean Biomass	SE Mean	Spores observed	PCR
			At Salsbury Gulch				
07/23/01	1.67	0.668	51.7	0.99	0.481	5/6	---
10/17/01	1.06	0.448	33.9	0.43	0.239	5/6	---
11/15/01	2.07	0.615	26.3	0.68	0.262	6/6	5/6
11/07/02	0.15	0.059	26.3	0.02	0.007	2/6	2/6
07/14/03	0.43	0.303	65.2	0.29	0.206	5/6	1/6[a]
09/24/03	0.35	0.124	67.0	0.18	0.070	4/6	0/6[a]
			Upper end of Spring Creek Campground				
04/22/02	1.82	0.929	57.4	0.49	0.297	4/6	3/6
07/15/02	0.62	0.261	48.4	0.26	0.106	5/6	5/6
11/07/02	0.59	0.315	50.8	0.46	0.364	5/6	5/6
07/14/03	0.09	0.046	59.2	0.03	0.012	5/6	3/6[a]
09/24/03	0.63	0.131	53.0	0.32	0.081	6/6	2/6[a]

a: The detection of *Mc* DNA by PCR and the relative scores for these samples were lower than anticipated. The use of ordinary tap water to rinse material from the filter screen may have resulted in the degeneration of much of the DNA by chlorine exposure prior to processing at the lab.

Water filtration samples indicated that habitat manipulation at this site did not result in reduced actinospore densities during the first year following construction. To the contrary, post-construction monitoring resulted in a greater frequency of actinospore detection compared to pre-construction sampling, and several occasions showed higher actinospore densities than those seen during pre-construction sampling (Figure 1.05). A one-tailed paired t-test (Ho: No difference, Ha: actinospore densities lower after treatment; $\alpha = 0.05$) of the 12 months preceding construction versus the 12 months following construction indicated the mean differences were not significant ($p = 0.899$, 95% lower bound for mean difference = -0.753 actinospores/L). In fact, the result of this test indicated post-construction densities were very nearly significantly higher than pre-construction densities over that time period.

In contrast, the substrate sampling indicated that the habitat modification had a substantial effect on reducing the oligochaete population within the treatment area. Post-manipulation monitoring samples collected nearly a year after completion of the project showed oligochaete biomass to still be considerably lower than was observed before construction (Table 1.16). These contrasting results suggested the work was effective in reducing oligochaete habitat in the treated area, but there were other suitable habitat areas nearby harboring infected *T. tubifex*.

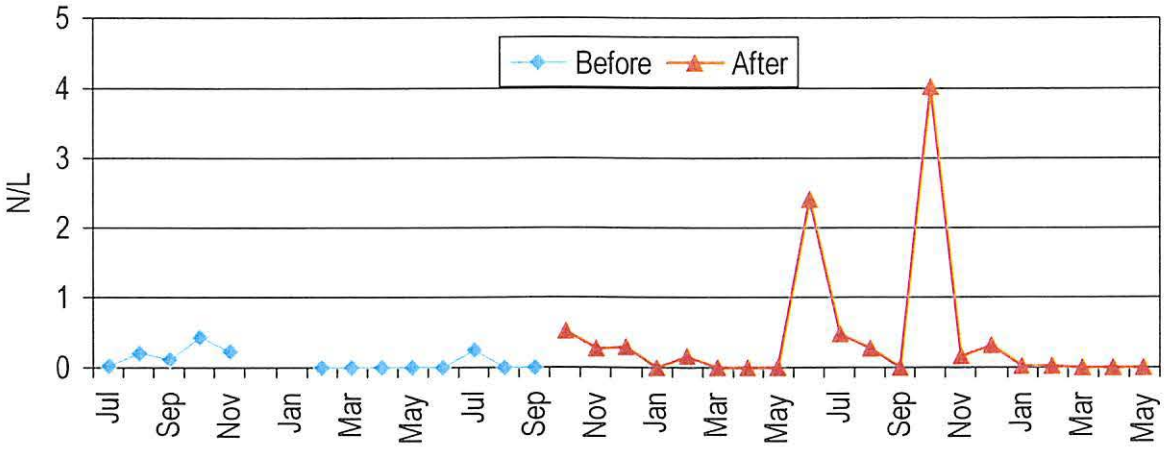

Figure 1.05. Density of actinospores observed in concentrates of 1900-L surface water samples collected at the Spring Creek treatment site. "Before" designates the 15 months (13 samples) immediately preceding construction, and "After" the 20 months following construction.

Exploratory sampling during July 2003 confirmed other, smaller oligochaete habitats in close proximity to the study site, both upstream and downstream (Figure 1.06). Worm samples obtained from such sites yielded actinospores after holding the samples overnight in the lab. In addition, testing of these oligochaete samples for the DNA of four lineages of *T. tubifex* suggested the entire length of Spring Creek between Spring Creek Reservoir and Spring Creek Campground 19 km downstream was heavily dominated by the very susceptible lineage III. Fourteen samples representing seven different sites yielded 100% lineage III DNA.

Monitoring of *Mc* actinospores at the control site on Spring Creek began in December 1997 and continued unabated (Figure 1.07). A one-tailed paired t-test (Ho: No difference, Ha: densities lower after treatment, alpha = 0.05) of the 12 months preceding construction at the treatment site versus the 12 months following construction indicated the mean differences were not significant ($p = 0.561$, 95% lower bound for mean difference = -0.228 actinospores/L). Moreover, plotting the long-term average actinospore density by month compared to the point estimates by month following construction at the treatment site indicated the baseline data were closely mirrored by the data collected at the control site after construction at the treatment site.

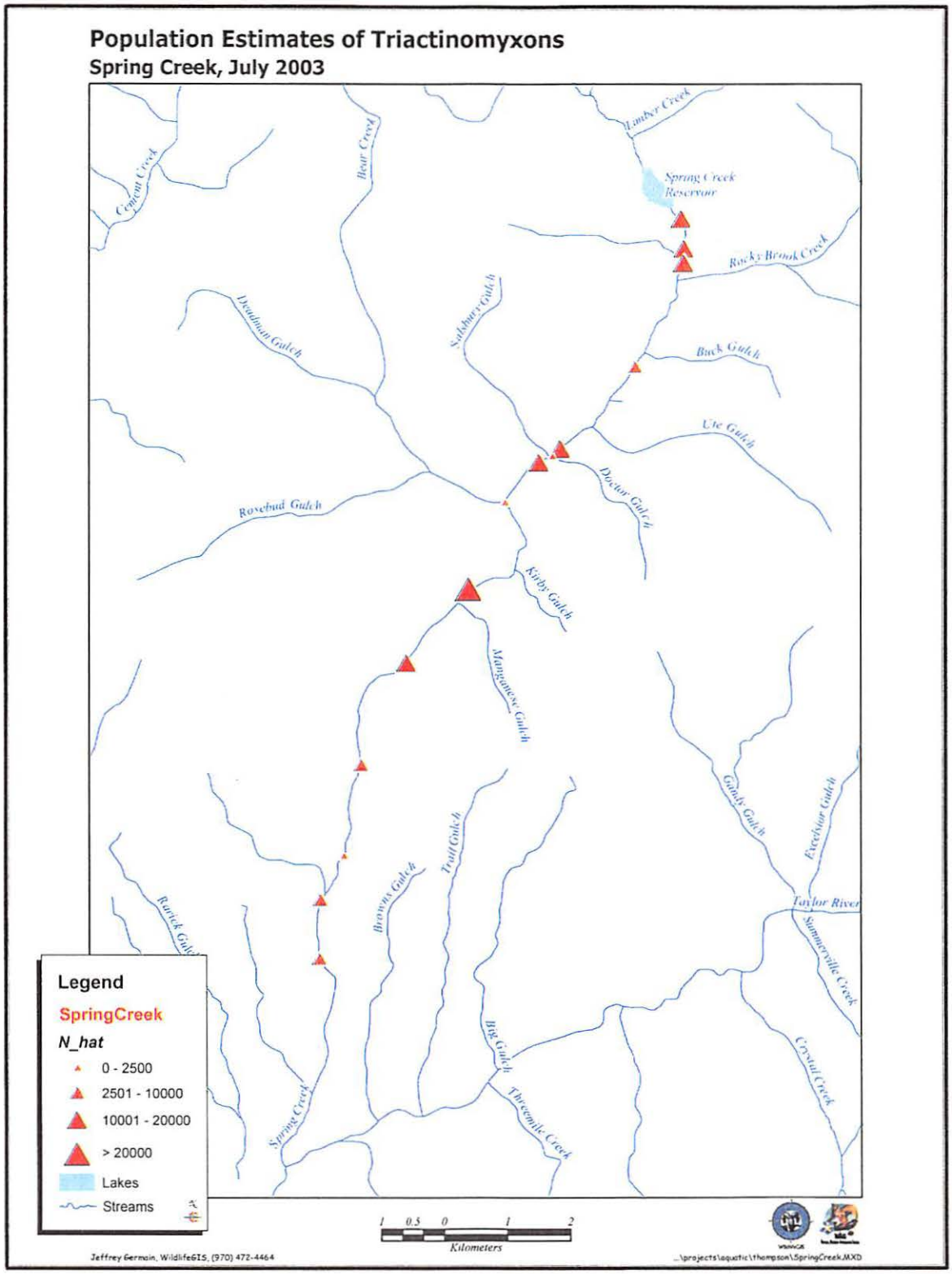

Figure 1.06. Estimates of the number of actinospores produced overnight from 14 60-second kicknet substrate samples collected from Spring Creek and containing infected *T. tubifex*.

Two substrate samples were collected by kicknet at the Spring Creek control site during 2002 as baseline data for the control site. Following construction at the treatment site, the treatment and control sites were sampled on the same days. As with the treatment sites on both the Williams Fork River and Spring Creek, oligochaete biomass estimates were highly variable, with no apparent trend evident. The particularly low biomass values obtained in July 2003 may be partly attributed to a natural loss of suitable oligochaete habitat at the control site caused by the loss of some instream woody debris that was previously encouraging sedimentation.

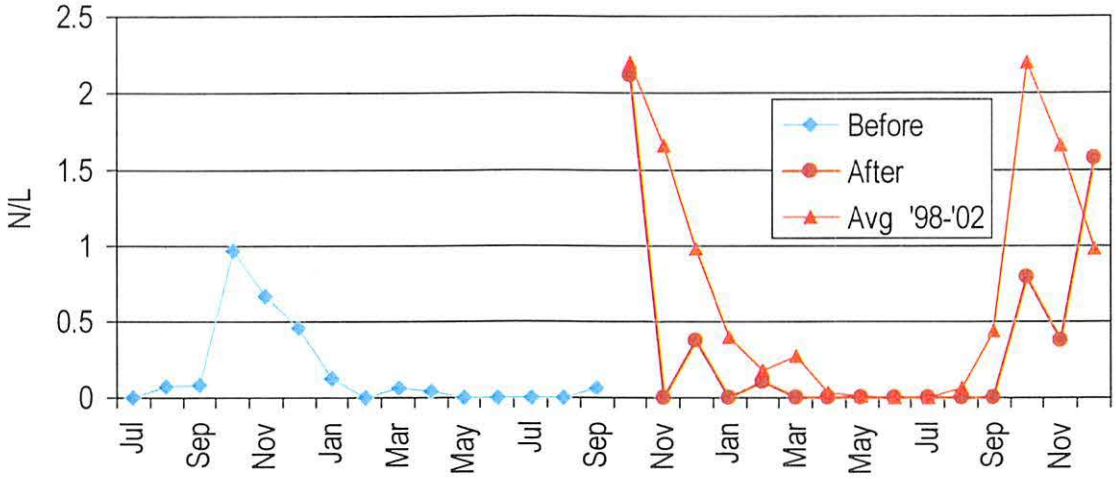

Figure 1.07. Density of actinospores observed in concentrates of 1900-L surface water samples collected at the Spring Creek control site. "Before" designates the 15 months immediately preceding construction at the treatment site, and "After" the 14 months following construction. The "Avg '98-'02" series shows the monthly average density of actinospores from 1998 – 2002 over the same 15 month period (i.e. the first and last three data points in that series are the same).

Williams Fork River (Colorado River drainage)

This 3.2-km reach of river situated between the Williams Fork Reservoir and the Colorado River seemed to hold potential as a refuge from the *Mc* parasite in the upper Colorado River drainage. Water filtration showed that actinospore densities in this reach typically were lower than in the Kemp/Breeze, and much lower than sites further upstream in the Colorado River. Still, rainbow trout recruitment in the Williams Fork over the last eight years or more was negligible. Rainbow trout fry survived longer than in the adjacent Colorado River, but ultimately failed to recruit to the juvenile population. Early evidence in 1997 suggested the irrigation diversion that supplies water to both the Kemp and Breeze Units of the Wildlife Area produced more actinospores than other sites sampled in the Williams Fork (Nehring 1998).

We collected trout population data from the Williams Fork River for the past four years (Table 1.17). The rainbow trout population remained sparse. However, the data also revealed that greater numbers of rainbow trout were present near the dam than further downstream. Although this was partly explained by a lack of fishing pressure on the Denver Water Board property, the presence of more young rainbow trout at the uppermost station suggested whirling disease

infectivity was lower there. Age 1+ rainbow trout abundance density decreased with distance downstream of the dam, which supported the idea that the majority of present-day infectivity came from within the river rather than the reservoir.

Table 1.17. Trout population biostatistics for three sites on the Williams Fork River below Williams Fork Reservoir.

	Brown Trout					Rainbow Trout[a]				
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									
2001[b]	134	± 2	191	810	579	27	± 0	36	164	105
						33	± 0	39	200	143
2001	99	± 2	217	569	243	31	± 2	53	180	94
						34	± 3	57	195	106
2002	269	± 6	279	1559	522	30	± 1	56	174	93
2003	999	± 9	816	5779	4844	24	± 3	45	138	74
	1.6 km below Williams Fork Reservoir at Kemp/Breeze Wildlife Area irrigation diversion									
2000	239	± 2	303	1,144	511	6	± 0	8.4	29	60
						28	± 1	18.9	136	188
2001[b]	207	± 5	165	607	379	22	± 0	36	65	24
						38	± 0	45	111	65
2001	254	± 36	214	744	831	11	± 2	18.9	31	18
2002[c]	593	± 15	651	2952	1600	25	± 1	56.8	125	55
2003[d]	711	± 7	360	1811	1172	32	± 2	21	80	42
	2.6 km below Williams Fork Reservoir									
2000	237	± 5	374	934	515	23	± 0	53	91	8
						39	± 0	66	154	56
2001[b]	185	± 15	230	597	242	14	± 3	23	44	0
						18	± 2	26	58	10
2001	207	± 17	228	631	1,103	13	± 1	22.5	38	0
						16	± 2	29.3	49	6

a: The second line of estimates for rainbow trout, where present, includes stocked fish. No stocked rainbow trout were found in 2001 at the irrigation diversion.
b: Spring estimates conducted May 2001.
c: Station length at this site was truncated at 385 feet compared to 875 feet in 2001 and 500 feet in 2000 because of the numbers of fish caught.
d: 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 very high actinospore density observed

12 months post-construction still appeared to be an aberration, although low densities of actinospores were observed on two other occasions since (Figure 1.08). Monitoring of the site was suspended from January through March since historically we never detected actinospores at this site during those months.

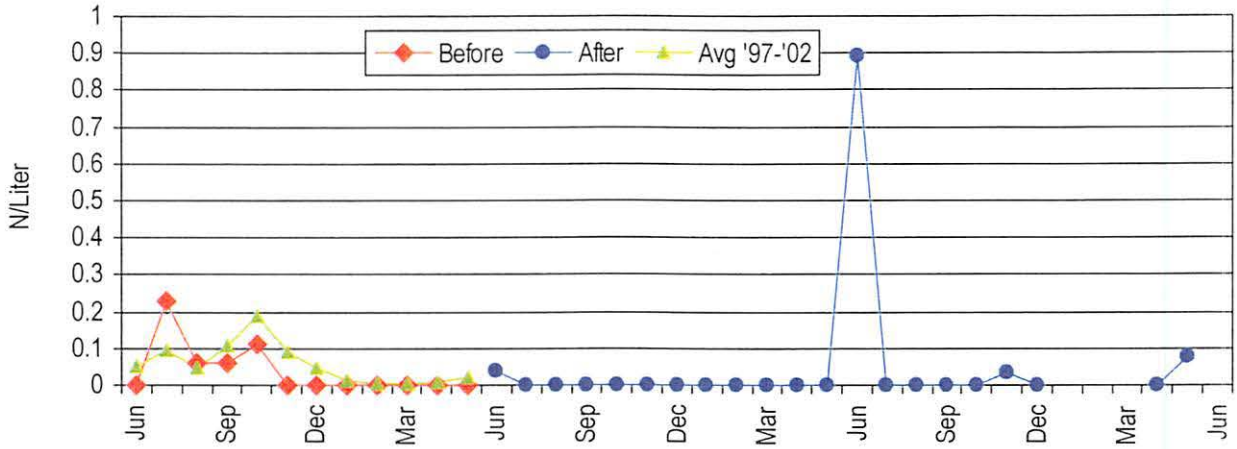

Figure 1.08. Density of actinospores observed in concentrates of 1900-L surface water samples collected at the Williams Fork treatment site. "Before" designates the 12 months immediately preceding construction, "After" the 19 months following construction, and "Avg '97-'02" designates the monthly average values obtained over the five years preceding construction.

During this segment we again observed dense oligochaete colonies at this site, particularly immediately in front of the west bank headgate. Efforts were again directed toward dislodging many of the worms and allowing them to be flushed into the irrigation ditch. In July 2003, we also conducted a search for additional oligochaete concentrations from 1 km above the treatment site to 1.2 km below. While no comparable visible colonies were observed, seven different likely sites were sampled. Six of the worm samples produced actinospores after holding the samples in buckets overnight in the lab (Figure 1.09). Replicate samples of likely T. tubifex from each site indicated that virtually no lineage I worms were present. But, there was a widely varying mix of lineages III, V, and VI among the sample sites. The baseline and post-construction oligochaete monitoring data are presented in Table 1.18.

Baseline data on myxospore prevalence and concentration in age 1+ brown trout revealed a substantial proportion of the juvenile brown trout population was infected with the parasite in this stream prior to habitat modifications (Table 1.19). Mean concentration of myxospores tended to be lower just below the dam, even in samples in which prevalence was high. The sample collected in 2003 was considered as pre-manipulation because the project was not completed until several weeks after most brown trout fry in 2002 emerged. Consequently no meaningful conclusions were drawn from the myxospore sampling.

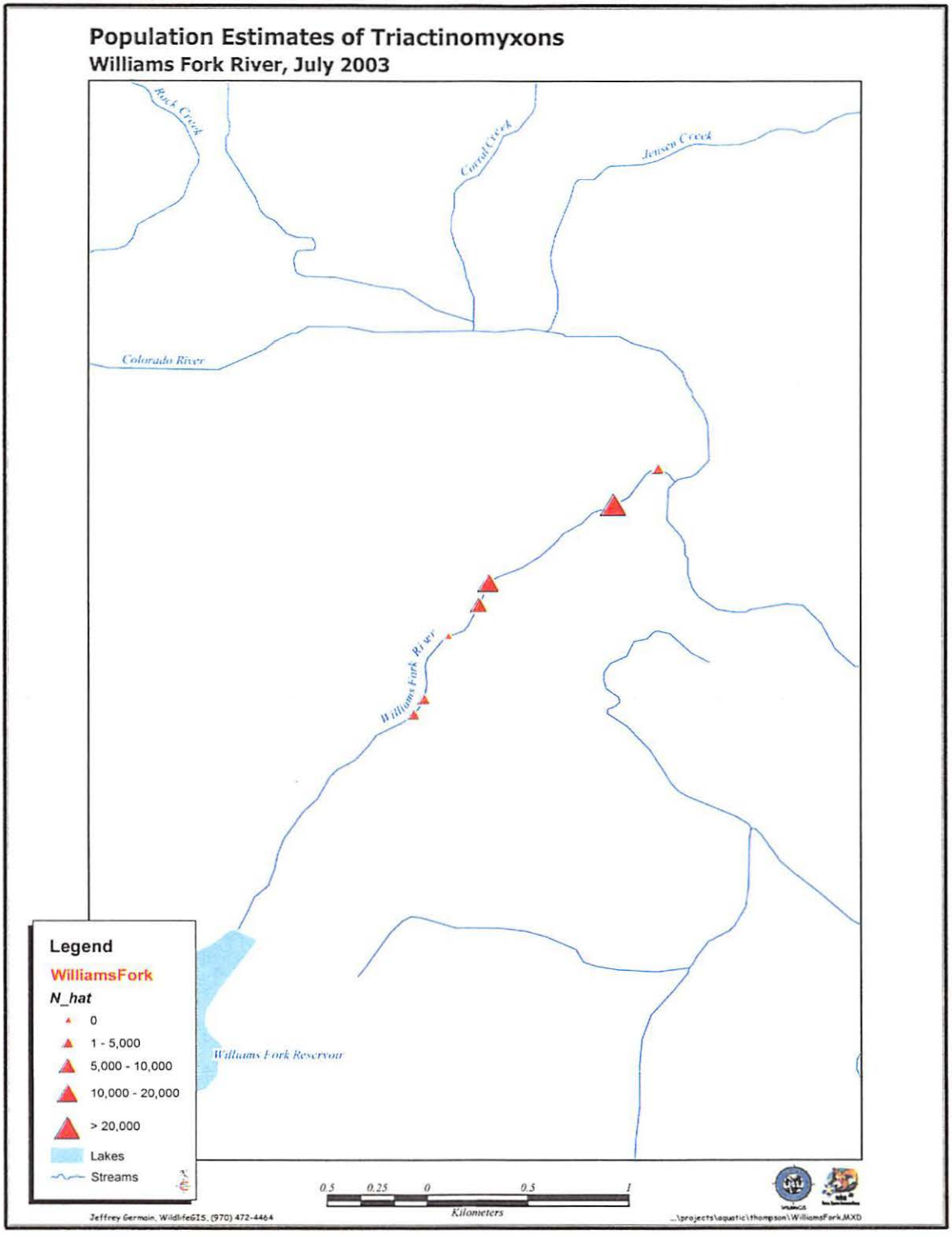

Figure 1.09. Estimates of the number of actinospores produced overnight from seven 60-second kicknet substrate samples collected from the Williams Fork River and containing infected *T. tubifex*.

Table 1.18. Oligochaete sampling results from the Williams Fork River. Biomass values are expressed in grams. Spores observed refers to the number of oligochaete samples from which actinospores were observed by microscopy over the total number of samples examined after holding the worms overnight in the lab. PCR refers to the number of filtered water samples taken from the worm samples that tested positive for the DNA of the parasite over the total number of samples examined.

Date	Mean Total Biomass	SE Mean	% likely *T. tubifex*	*T. tubifex* Mean Biomass	SE Mean	Spores observed	PCR
		Kemp/Breeze Wildlife Area irrigation diversion					
06/25/01	2.031	1.2457	83.1	1.951	1.2160	1/6	---
11/05/01	3.662	1.3799	93.9	2.865	0.9224	4/6	---
11/26/01	5.234	3.0515	93.1	4.958	3.0249	5/6	5/6
07/17/02[a]	0.327	0.1323	88.0	0.302	0.1269	0/6	1/6
10/21/02	13.097	6.1561	97.5	13.062	6.1360	3/6	3/6
11/19/02	2.8393	0.9280	92.6	2.7380	0.8958	1/6	2/5

a: The irrigation diversion was modified the first week of June 2002. The July occasion was the first post-manipulation sample.

Table 1.19. Cranial *Mc* myxospore concentrations in brown trout sampled from 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	4,944 – 141,333
		1.6 km below Williams Fork Reservoir, immediately below Treatment site			
06/04/01	20	100%	40,300	40,300	900 – 192,500
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
		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

The rapidity of oligochaete population recovery at the irrigation diversion suggested the work conducted was not a viable solution. While the total amount of suitable oligochaete habitat was reduced by the elimination of the backwater area, it appeared the habitat remaining in front of the headgates was better than any oligochaete habitat that was present before modifications to the channel. However, a confounding factor existed with the severe 2002 draw-down of Williams Fork Reservoir mentioned previously. It was possible the recovery was not as dramatic without the organically rich sediments contributed to the river by the reservoir draw-down.

Willow Creek (Colorado River drainage)

Willow Creek was added to the work schedule beginning with this segment. Extensive work was already done on this section by the U. S. Geological Survey (USGS) and the Colorado Cooperative Fish and Wildlife Research Unit (CCFWRU). Many substrate core samples were collected to characterize the oligochaete population. A backwater area initially identified as a good oligochaete habitat by Zendt (1999) was the focus of the site, and an unstable bank contributing sediment to the stream was also identified. The entire site was extensively mapped with survey-grade GPS equipment. Initial design criteria for isolating the backwater had been proposed. In cooperation with the USGS and CCFWRU grant funding was acquired to collect further baseline data with our paired core/kicknet sampling protocol and to collect similar information following habitat modification.

Baseline oligochaete sampling for Willow Creek occurred on June 23 and August 18, 2003. Oligochaete worms possessing hair and pectinate chaetae were obtained from these samples and preserved in 70% EtOH, then submitted to a laboratory for analysis by the QPCR technique to estimate percentages of worms belonging to the various lineages or strains of *T. tubifex*. Lineages III and VI were the only ones detected (Table 1.20). Lineage III was considered to contain the highest proportion of susceptible individuals (Beauchamp et al. 2002). Lineage VI also contained susceptible individuals.

Table 1.20. Estimates of the proportion of each *T. 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 *Mc* lineage			
		I	III	V	VI
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)

Collections of brown trout collected above and below the site indicated a high prevalence of *Mc* infection in the area of the backwater both by PTD from age 1+ fish (Table 1.21) and PCR from YOY fish (Table 1.22).

Portions of the Willow Creek site were re-surveyed using survey-grade GPS technology during this segment to document any changes in bed profiles surveyed during the previous two years. Habitat modifications were implemented in November 2003. The backwater was isolated

from the active channel by building a new bank of large rock. The bank excluded flows < 90 cfs, a level rarely exceeded in this stream because of the upstream Willow Creek Reservoir. The eroding bank was slightly shaped to reduce its vertical profile and a J-hook structure was placed to provide near-bank velocity reduction and redirection of high water velocities away from the bank.

Table 1.21. Cranial *Mc* myxospore concentrations in age 1+ brown trout sampled from Willow Creek.

Date mm/dd/yy	Sample Size		Overall Mean Concentration	Positive Fish	
	N	prevalence		Mean	Range
Above Willow Creek Gage					
09/30/03	20	70%	21,400	30,60	2,600 – 194,700
Downstream of backwater site					
09/30/03	20	60%	10,700	17,90	2,000 – 41,200

Table 1.22. Results of PCR tests of samples of YOY brown trout collected from Willow Creek. Mean PCR score was 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
Below Willow Creek Gage			
09/30/03	11	7	1.7

The first post-manipulation substrate samples were collected May 18, 2004. No actinospores were observed from the water filtrates obtained after holding the samples overnight in the lab. No other results were available from these samples.

DISCUSSION

In the last report (Nehring and Thompson 2003) we recommended that searches for infectivity hot-spots included worm sampling and screening of water from those samples for actinospores. Work conducted during this segment included the use of such sampling on the Williams Fork River and Spring Creek. Although the modified sites on these streams were ones that we considered to be identifiable foci of infectivity, the sampling conducted during this segment with this method proved that one may easily find other sites that contain infected *T. tubifex*. While most of the sites thus identified were not as large as those chosen for habitat modification, and no visible worm colonies were observed, cumulatively one wondered whether such sites had just as much effect on the stream reach as the more easily identifiable sites. Continued monitoring will help to establish whether this was so. If it was so, then attempts to clean up stream reaches will become much more labor- and cost-intensive than envisioned under the hypothesis driving this job.

Job No. 2: Actinospore Hot Spot Abatement Studies.

Job Objective: Develop and test strategies to reduce, control or eliminate the production of triactinomyxon actinospores of *Mc* from man-made ponds and settling ses of infectivity.

Period Covered: 2004

DUCTION

Whirling dy of some salmonid fishes that can result from exposure of su ngerlings to the waterborne actinospore of the myxosporean parasite *Mc* (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 often display an erratic swimming behavior known as "whirling", hence the name whirling disease. Rose et al. (2000) suggests the cause of the erratic swimming pattern was 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 was now widely 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 *Mc* adequately explained the recurring losses of young wild rainbow trout observed on nearly 600 km of Colorado's premier trout streams. Occurrence of the parasite among wild salmonids in western and southwestern Montana was well documented (Baldwin et al. 1998), and in some areas was also affecting rainbow trout recruitment. Researchers now recognize that *Mc* infection was a major reason for the decline in abundance of wild rainbow trout in certain Montana trout streams (Baldwin et al. 2000).

Research in Colorado since 1994 demonstrated major declines in numbers of wild rainbow trout *Oncorhynchus mykiss* in a number of rivers that were the result of whirling disease (Walker and Nehring 1995; Nehring 1996; Nehring and Walker 1996; Nehring et al. 1998; Nehring 1998; Nehring 1999). Sentinel fish studies in the Colorado River and *Mc* actinospore filtration studies in numerous drainages have indicated that there were areas within streams that act as foci of infection for the parasite (Thompson et al. 2002, Nehring and Thompson 2001; Thompson and Nehring 2000a, Thompson and Nehring 2000b). More recently, stocking of Spring Creek Reservoir with catchable trout infected with *Mc* resulted in elevated infectivity in Spring Creek below the reservoir (Nehring et al. 2001, Nehring and Thompson 2002). 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 *Mc* infectivity emanating from a series of off-channel ponds was documented. The most severe reduction in abundance of age 1 wild rainbow trout occurred

downstream of this focus of infection, suggesting that whirling disease induced the decline (Nehring et al. 2000).

The objective of this job was to document the changes in *Mc* 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 effluent of the sand filter wetland on the Cap-K Ranch in the Fryingpan River drainage,

3. De-populate settling ponds at Pitkin and Roaring Judy hatcheries. De-populate the Roaring Judy effluent ditch between the end of the concrete raceways and the new kokanee trap, and

4. Remove brook trout from the upper two ponds on the Cap-K Ranch.

METHODS and MATERIALS

Field Filtration and Sample Collection

We sampled 1900-L volumes of water for all field samples. This volume was usually filtered in 15 to 30 minutes by pouring 19-L buckets of water through a 20 µm mesh Pecap® screen fitted over a plastic utility tub measuring 66 cm long, 51 cm wide, and 15 cm deep. The tub wall was perforated with numerous 12-mm holes to allow rapid draining of the water through the net and out of the tub. The tub provided a rigid frame for support of the 20-µm mesh screen. When filtration was complete, the screen was thoroughly rinsed down and the filtrate concentrated in one corner of the screen to minimize volume, then rinsed into a collection jar for storage in a cooler and transport to the lab for analysis. Filtrates generally ranged between 50 and 150 ml total volume.

At the laboratory we measured the volume of filtrate in a graduated cylinder. The sample was then shaken to re-suspend sediment and any actinospores. Twenty 1-mL aliquots were subsampled from the filtrate and stained with 60 µL of an aqueous solution saturated with crystal violet biological stain. A single 82.4-µL subsample was micropipetted from each aliquot onto a gridded petri dish for examination by a stereozoom dissecting microscope. The 82.4-µL sample size compensated for the addition of stain so that 80 µL of actual filtrate was the volume subjected to examination. A 24 X 40 mm microscope cover slip over the 82.4-µL drop dispersed the sample at a uniform thickness and facilitated identification and enumeration of actinospores.

Actinospores of *Mc* were identified on the basis of general appearance, overall conformation, size and shape according to descriptive criteria in El-Matbouli and Hoffmann (1998). Triactinomyxons not conforming to these descriptions were not counted for this study.

A single 1.6-mL sample (equal to the volume examined from the 20 aliquots) of filtrate from each field sample was subjected to the PCR test. From early 1998 through March 2001 we used the single round modification (Schisler et al. 2001) of the PCR assay developed by Andree et al. (1998). 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 *Mc* designated as HSP70. Each sample subjected to testing by PCR was preserved in 70% ethyl alcohol in a 15-mL centrifuge tube. Each sample was identified only with an alphanumeric code when sent to the laboratory.

RESULTS and DISCUSSION

Cap-K Ranch Ponds (Fryingpan River drainage)

The Fryingpan River was sampled on a monthly basis at four or more sites since August 1998. Water samples drawn from the Fryingpan River immediately downstream from Ruedi Dam were collected on 67 sampling occasions from July 1998 through May 2004. Actinospores were detected just once, in April 2001. None of the water samples tested positive by PCR for DNA of *Mc.*

The Cap K Ranch ponds were 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 returned to the Fryingpan River. The capability existed to divert pond water back to the Fryingpan River before it entered Pond 5. Table 2.01 contains filtration data obtained since 2000 on ponds 1 and 2. Pond 6 was the site of primary interest on the Cap K Ranch with regard to this job, since it generally returns all flow from the series of ponds to the Fryingpan River. Pond 6 was historically a source of *Mc* actinospores to the Fryingpan River, with the highest densities observed in Spring 2002 (Figure 2.01).

The terminal pond was modified during February and March 2003. A description of the filter installed was previously provided (Nehring and Thompson 2003).

Once the wetland filter was completed and the pond refilled, monitoring commenced to compare actinospore densities in the Pond 6 inlet to the effluent of the wetland filter. On fifteen paired occasions, actinospores were observed in the Pond 6 inlet while they were observed in the wetland headbox eight times and in the wetland effluent one time (Table 2.02). The single actinospore observed in the effluent may not have reflected failure of the sand filter. Capacity of the filter was quite low on this date, and it was possible that bilging the sample drew water back into the discharge pipe from the afterbay because discharge water velocity was very low. Results of PCR tests on all the filtrates also suggested that the filter was completely removing any actinospores that were introduced to it. The single occasion when an effluent sample tested positive was again suspect, because the wetland headbox controls were improperly set when the filter was placed back on line in August 2003.

Table 2.01. Estimates of *Mc* actinospore density in 1900-L samples of water in the effluents of Cap-K Ranch ponds 1 and 2. Values are actinospores/L followed by the half-width of 95% confidence intervals.

Month	2000-01	2001-02	2002-03	2003-04
		Outlet of Pond #1		
Jul	0.038 (0.08)	0.0 (---)	0.185 (0.17)	0.064 (0.13)
Aug	0.038 (0.07)	0.0 (---)	0.026 (0.05)	0.0 (---)
Sep	0.031 (0.06)		0.0 (---)	0.0 (---)
Oct	0.055 (0.11)	0.175 (0.14)	0.0 (---)	0.0 (---)
Nov	0.062 (0.08)	0.0 (---)	0.0 (---)	0.0 (---)
Dec	0.120 (0.16)	0.0 (---)	0.182 (0.20)	0.043 (0.08)
Jan	0.086 (0.09)	0.0 (---)	0.149 (0.20)	0.0 (---)
Feb	0.043 (0.08)	0.143 (0.15)	0.0 (---)	0.0 (---)
Mar	0.116 (0.16)	0.0 (---)	0.0 (---)	0.0 (---)
Apr	0.0 (---)	0.0 (---)	0.124 (0.24)	0.0 (---)
May	0.0 (---)	0.0 (---)	0.337 (0.66)	
Jun	0.0 (---)	0.0 (---)	0.039 (0.08)	
		Outlet of Pond #2		
Jul	0.172 (0.26)	0.067 (0.09)	1.017 (0.33)	0.0 (---)
Aug	0.617 (0.30)	0.043 (0.08)	0.318 (0.17)	0.0 (---)
Sep	0.745 (0.45)	---	0.495 (0.28)	0.0 (---)
Oct	0.981 (0.33)	0.317 (0.18)	2.588 (0.85)	0.0 (---)
Nov	0.670 (0.36)	0.855 (0.27)	1.743 (2.05)	0.125 (0.13)
Dec	1.014 (0.46)	0.950 (0.42)	1.718 (0.54)	0.0 (---)
Jan	0.803 (0.44)	1.785 (0.7)	1.082 (0.41)	0.139 (0.08)
Feb	0.361 (0.27)	0.060 (0.12)	0.291 (1.07)	0.356 (0.28)
Mar	1.010 (0.42)	0.096 (0.13)	0.351 (0.26)	0.028 (0.06)
Apr	0.423 (0.22)	0.038 (0.07)	0.624 (0.46)	0.176 (0.19)
May	0.951 (0.49)	0.370 (0.27)	0.703 (0.47)	0.317 (0.27)
Jun	0.091 (0.10)	0.0 (---)	0.874 (0.3)	

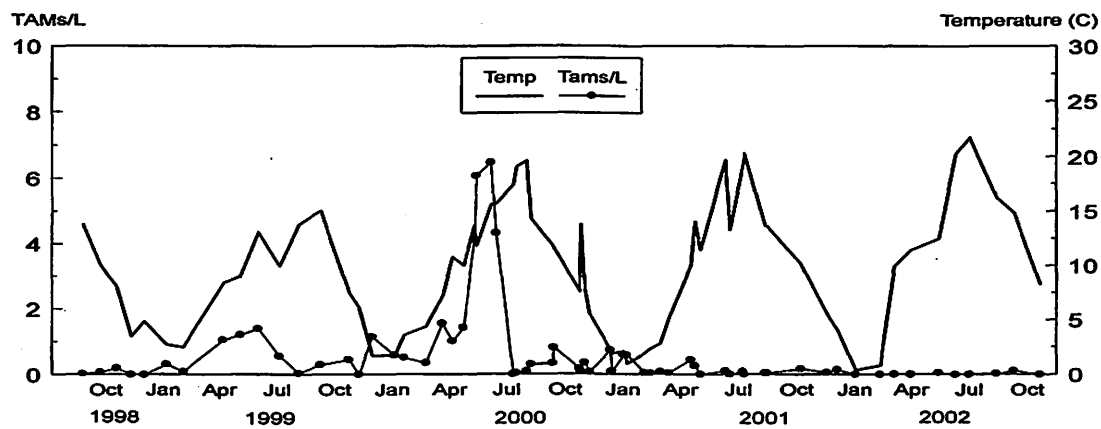

Figure 2.01. Estimated actinospore density (TAMs/L) and temperature in degrees Celsius in the outlet of pond #6 at the Cap-K Ranch on the Fryingpan River (August 1998-October 2002).

The filter rapidly lost capacity to handle the water introduced to it. Within a couple of months of the initial start-up, capacity was perhaps only 25% of the design capacity. Changes of flow direction and the introduction of compressed air while the wetland was flowing from bottom to top did not renew the flow capacity of the filter to a level that met expectations. In fact, capacity appeared to continue to drop in spite of backwash efforts and periods of total bypass to let the filter "rest". During the Spring 2004 total discharge from the filter appeared not to exceed about 80 gpm. This was difficult to measure because there was no measuring device in the overflow channel so that overflow could be separated from total discharge of the system.

Discussions with representatives of the engineering firm occurred regarding the possibility of rehabilitating the filter by chemical means. However, such an action required great care not to pollute the Fryingpan River and was subject to the scrutiny of public health agencies. Moreover, if such actions were found to be necessary on a regular basis it would have discouraged most private landowners from implementing similar filtration efforts on other ponds. The engineer who designed the filter suggested that a future filter should have less filter depth and consist of graded crushed glass rather than the masonry sand used in this one.

Table 2.02. Estimates of *Mc* actinospore density in paired equivalent-volume samples of water taken from the inlet of Pond 6 and the effluent of the wetland filter at the Cap-K Ranch during May and June 2003. The numbers in parentheses in the Tams/L column are the half-width of 95% confidence intervals. PCR result is based on a qualitative rating of the strength of the banding pattern on a scale from '0' (no signal, PCR-negative) to '4' (very strong signal).

	Pond 6 Inlet		Wetland filter effluent	
Date	Tams/L	PCR result	Tams/L	PCR result
05/07/03	0.727 (0.57)	3	0.0 (---)	0
05/20/03	0.115 (0.12)	3	0.0 (---)	0
06/04/03	0.755 (0.41)	3	0.0 (---)	0
06/17/03	0.169 (0.18)	3	0.0 (---)	0
06/23/03	0.0 (---)	4	0.0 (---)	0
08/21/03	0.0 (---)	0	0.0 (---)[a]	0
08/28/03	0.0 (---)	0	0.0 (---)[a]	2
09/2/03	0.0 (---)	2	0.0 (---)[a]	0
10/16/03	0.037 (0.07)	4	0.0 (---)	0
11/10/03	0.024 (0.05)	3	0.0 (---)	0
12/9/03	0.0 (---)	2	0.0 (---)	0
1/6/04	0.0 (---)	0	not sampled	
2/11/04	0.069 (0.09)	4	not sampled	
03/25/04	0.0 (---)	0	0.0 (---)	0
04/8/04	0.0 (---)[b]	0	0.0 (---)	0
04/22/04	0.0 (---)		0.0 (---)	
05/5/04	0.125 (0.13)		0.030 (0.06)	

a: Filter gates were improperly set. These samples were not truly filtered.
b: Sample site was moved from Pond 6 inlet to wetland filter headbox from April 8, 2004 on.

Other management actions were implemented in the Cap-K Ranch ponds during this segment to attempt to reduce infectivity in the entire system. During April and May 2004 ponds 1 and 2 were electrofished on three separate occasions to remove brook trout fry. Several thousand were removed in this effort, which was the third year of such efforts. In addition, adult brook trout were removed from the upper two ponds down to the third pond in April, where they provided fishing recreation for the landowners but were not expected to spawn successfully. Neither will they be exposed to as many actinospores as in pond 2. However, observation by crew members indicated that some of the adult brook trout might successfully negotiate the steep culvert back into pond 2.

In the previous segment adult and juvenile brook trout were removed from the system along with the YOY. These efforts to reduce the population of this susceptible species seemed to reduce infectivity in the system, as evidenced by the trend in actinospore densities detected in the pond 2 effluent over the last two segments (Table 2.01).

Adult and juvenile brook trout removed from the upper three ponds and examined for myxospores by the plankton centrifuge method (O'Grodnick 1975) in April 2004 indicated that infectivity continues to be high (Table 2.03).

Table 2.03. Cranial *Mc* myxospore concentrations in brook trout sampled from ponds on the Cap-K Ranch, Fryingpan River drainage. October 2002 samples were analyzed by the PTD method. April 2004 samples were analyzed by the plankton centrifuge method.

Date	Age	Sample Size		Overall Mean	Positive Fish	
mm/dd/yy	(months)	N	prevalence	Concentration	Mean	Range
			Pond 1			
10/24/02	12+	24	100%	169.500	169.50	2.700 – 980.600
10/24/02	24+	13	100%	159.500	159.50	10.450 – 749.700
04/02/04	≥12	40	82.5%	118.500	142.90	2.800 – 1.233.300
			Pond 2			
10/24/02	7	24	100%	183.000	183.00	19.700 – 858.700
10/24/02	24+	20	65%	79.700	122.60	17.600 – 456.500
04/02/04	≥12	39	76.9%	44.200	57.400	560 – 338.900
			Pond 3			
10/24/02	24+	20	100%	136.000	136.00	14.500 – 289.900
04/02/04	>12	40	77.5%	54.800	70.700	1.100 – 450.000

Pitkin Rearing Unit

According to CDOW records, trout reared at the Pitkin Rearing unit first tested positive for *Mc* 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 with the re-start of the unit.

Table 2.04. Results of water filtration to quantify actinospores (Tams) of *Mc* in 1900-L samples of water at Pitkin Hatchery July 1, 2001 through May 31, 2004. PCR results were based on a scale of '0' (negative) to '4' (very strongly positive).

Date	Tams/L (95% CI)	PCR result	Tams/L (95% CI)	PCR result
	Quartz Creek above effluent		Settling Pond effluent	
11/15/01	0.091 (0.12)		3.181 (0.92)	
12/19/01	0 (---)		3.079 (0.86)	
01/02/02	0 (---)	0	1.236 (0.49)	
02/14/02	0 (---)	0	1.51 (0.42)	
03/08/02	0 (---)	0	2.92 (0.83)	3
04/08/02	0 (---)	2	3.31 (0.95)	4
05/02/02	0 (---)	0	21.02 (3.53)	3
05/21/02	0 (---)	0	0.669 (0.45)	2
06/05/02	0 (---)	0	0.602 (0.35)	0
07/10/02	0 (---)	0	0.347 (0.13)	3
08/12/02	0 (---)	0	0.439 (0.22)	0
09/11/02	0 (---)	0	0.277 (0.18)	3
10/09/02	0 (---)	3	0.391 (0.25)	1
11/12/02	0.024 (0.05)	3	17.033 (4.78)	4
11/25/02	0.201 (0.15)	3	61.501 (3.66)	4
12/02/02	0.090 (0.18)	2	94.821 (20.80)	4
12/09/02	not sampled		45.285 (12.71)	4
12/16/02	not sampled		40.090 (3.88)	4
12/18/02	not sampled		1.392 (0.61)	4
12/20/02	not sampled		1.872 (0.71)	4
01/06/03	0 (---)	1	3.645 (0.81)	4
01/30/03	0 (---)	0	0.524 (0.31)	4
02/07/03	0 (---)	0	0.285 (0.15)	4
03/05/03	0 (---)	0	0 (---)	3
04/01/03	0.677 (0.27)	4	0 (---)	0
05/07/03	0.062 (0.12)	1	5.759 (0.69)	4
06/02/03	0 (---)	0	0.296 (0.22)	3
07/1/03	0 (---)	0	0.055 (0.11)	3
08/6/03	0 (---)	0	0 (---)	0
09/4/03	0 (---)	0	0 (---)	3
10/3/03	0 (---)	1	0.569 (0.31)	3
11/5/03	0.023 (0.05)	2	0 (---)	3
12/4/03	0 (---)	0	0 (---)	3
1/13/04	0 (---)	0	0 (---)	0
02/2/04	0.101 (0.11)	4	0.077 (0.08)	2
03/8/04	0.069 (0.07)	2	0.102 (0.08)	2
04/15/04	0 (---)		1.210 (0.37)	4
05/7/04	0 (---)		0.151 (0.20)	
06/01/04	0 (---)		0.021 (0.04)	

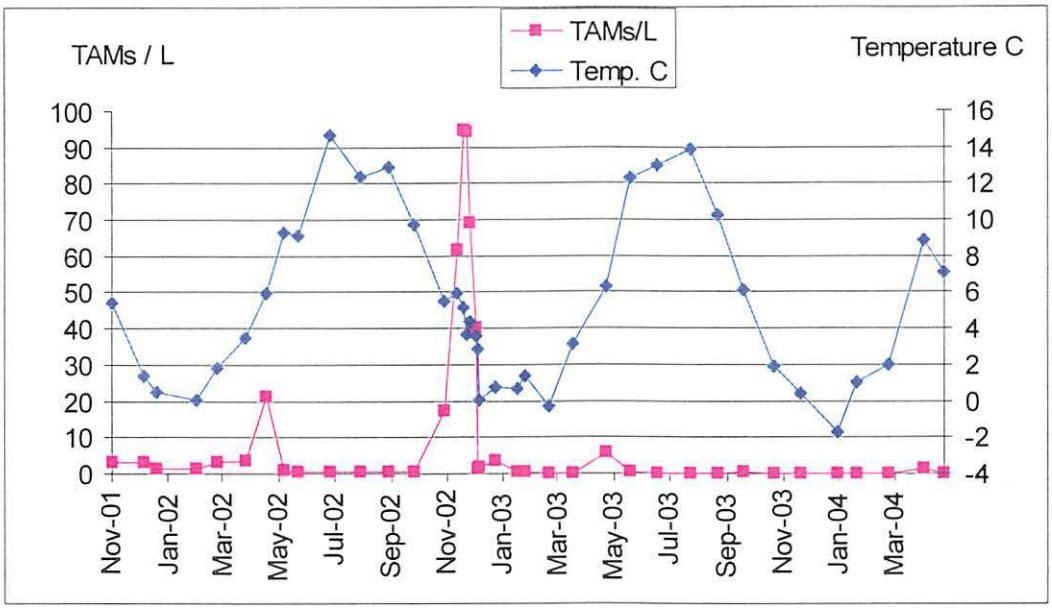

Figure 2.02. Actinospores (TAMs) per liter and temperature (degrees Celsius) in the Pitkin State Fish Hatchery effluent (November 2001-May 2004).

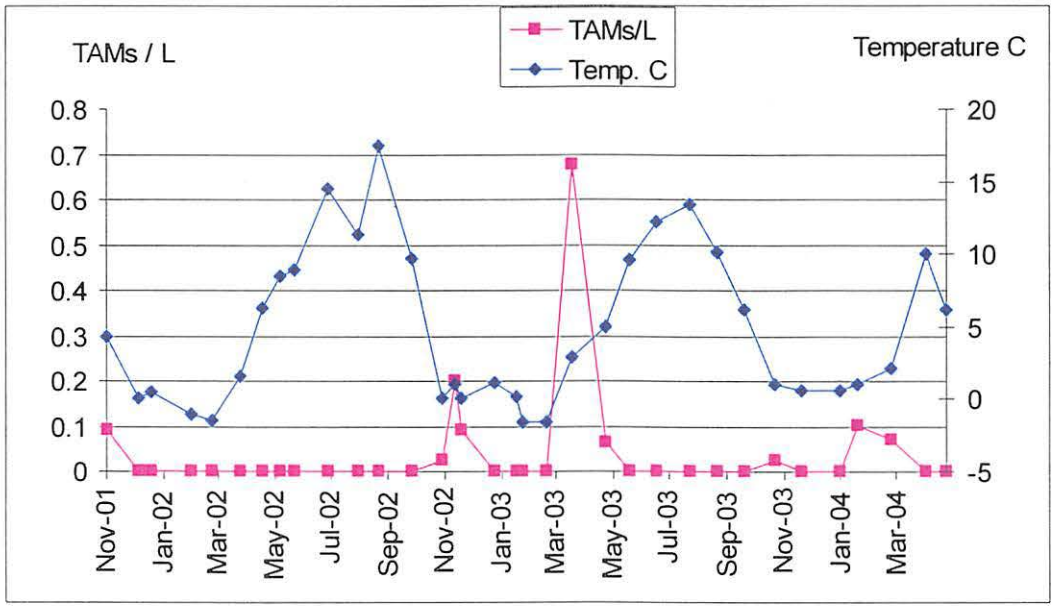

Figure 2.03 Actinospores (TAMs) per liter and temperature (degrees Celsius) in Quartz Creek upstream of the Pitkin State Fish Hatchery effluent (November 2001-May 2004).

Monitoring of actinospore densities began at the Pitkin Rearing Unit in November 2001. Actinospores of *Mc* were routinely observed in the effluent of the settling pond, including a large "pulse" during November and December 2002. Actinospores were detected in the effluent on six occasions during this segment. Actinospores were also observed in Quartz Creek above the settling

pond effluent on nine occasions (Table 2.04, Figures 2.02 – 2.03), with three occurring during this segment. These results, as well as PCR tests on the water samples, indicated the hatchery effluent remained far more infected than Quartz Creek water upstream of the effluent.

Pitkin Unit personnel attempted to remove all feral fish from the unit's settling pond during unit renovation in 2001-02. Initially accomplished by opening the pond to anglers, the effort continued in Spring 2002 by gillnetting. A 30-fish rainbow trout sample acquired by gillnet in May 2002 was submitted for PTD analysis and proved to be 80% positive for *Mc*, with an average concentration of 210,600 myxospores per head. In contrast, a 20-fish brown trout sample acquired about 2 km downstream of the hatchery showed a prevalence of 60% and a mean myxospore concentration of 10,000. Brook trout samples acquired above the hatchery were negative in South Quartz Creek (n=11) and 20% positive in North Quartz Creek (n=10, mean myxospores = 12,400). During this segment samples were collected from Quartz Creek approximately one mile above and below Pitkin Rearing Unit. Twenty-fish samples showed 10% prevalence with a mean myxospore concentration of 2,940 above the Unit and 45% prevalence with a mean myxospore concentration of 10,200 below the Unit. The best management practice in this case was clearly to keep the settling pond free of trout that serve as a source of myxospores to the *T. tubifex* population.

Poudre Rearing Unit

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

Actinospores of *Mc* were still encountered frequently at the filtration sites on the PRU during this segment (Table 2.05, Figures 2.04 – 2.06). Estimated densities remained low in the PRU effluent compared to the historic high numbers seen in 1999-2000 (Figure 2.06). Poudre River water was diverted into the water supply lake for the PRU through a culvert in the dike for the lake, just upstream from a roller dam on the river. Water samples from the supply pond contained actinospores on 10 of 11 occasions compared to 6 of 11 occasions in the Poudre River inflow to the supply pond. The densities of actinospores observed in the supply pond were significantly higher than those in the Poudre River inflow (mean 0.29/L vs. 0.04/L, paired *t*-test, $p = 0.042$, 95% C.I. for mean difference 0.011, 0.483). In fact, the mean density in the supply pond tended to be higher than the mean density in the PRU effluent (mean 0.29/L vs. 0.09/L, paired *t*-test, $p = 0.058$, 95% C.I. for mean difference -0.008, 0.402).

It is very possible that once the supply pond is bypassed with a direct pipeline for most of each year, the Unit effluent actinospore densities will decrease to a level consistent with the background densities in the Poudre River. The modification of the entire supply pipeline system at the Poudre Unit is scheduled for construction during Fall 2004. Actinospore monitoring in the Summer and Fall of 2005 should indicate whether the bypass of the supply pond will allow the Poudre Rearing Unit to achieve the goal of actinospore densities at or below the background level.

Table 2.05. Results of water filtration to quantify actinospores (N/L) of *Mc* in 1900-L (500-gallon) samples of water at the PRU from July 1, 2003 through May 31, 2004. Values in parentheses are the half-width of 95% confidence intervals on the density of actinospores in that sample, not the source of the sample.

Month	Poudre River at Unit inflow	Supply pond outflow	Unit effluent
Jul	0. 0 (---)	0.324 (0.29)	0.150 (0.29)
Aug	0. 0 (---)	0.586 (0.31)	0.093 (0.18)
Sep	0. 0 (---)	0.971 (0.32)	0.099 (0.19)
Oct	0.048 (0.09)	0.758 (0.35)	0.207 (0.28)
Nov	0.058 (0.11)	0.091 (0.08)	0.135 (0.18)
Dec	0.203 (0.19)	0.226 (0.19)	0.125 (0.14)
Jan	0.033 (0.07)	0.088 (0.09)	0.046 (0.06)
Feb	0. 0 (---)	0.061 (0.12)	0.110 (0.22)
Mar	0. 0 (---)	0. 0 (---)	0.054 (0.11)
Apr	0.075 (0.10)	0.032 (0.04)	0.0 (---)
May	0.056 (0.11)	0.053 (0.07)	0.0 (---)

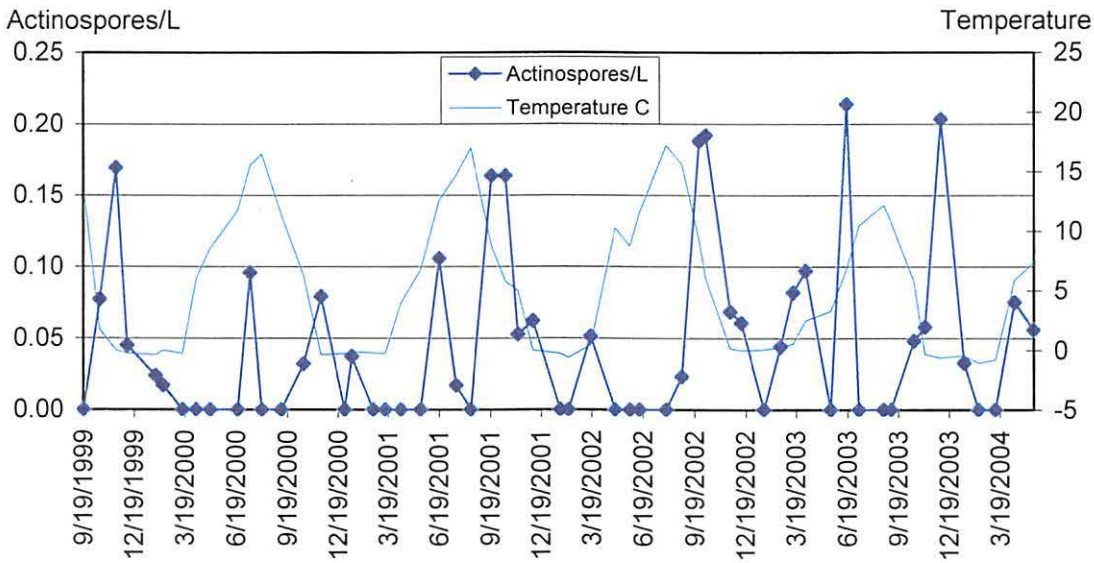

Figure 2.04. Comparison of temperature in degrees Celsius versus actinospores (TAMs) per liter from the inlet to the PRU water supply lake (September 1999-May 2004).

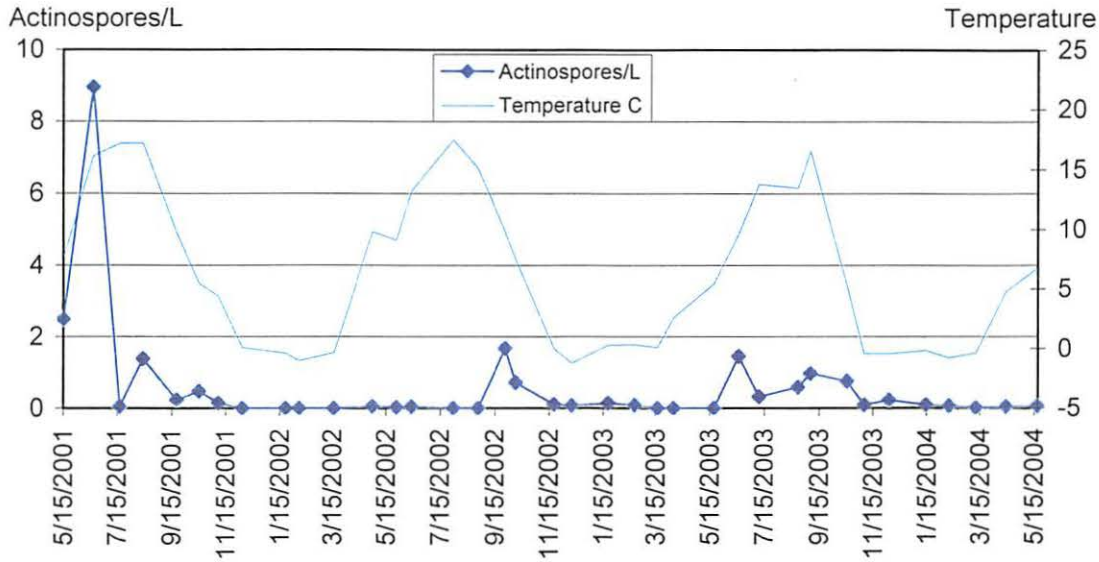

Figure 2.05. Comparison of temperature in degrees Celsius versus actinospores per liter from the water supply lake outlet to the PRU (May 2001- May 2004).

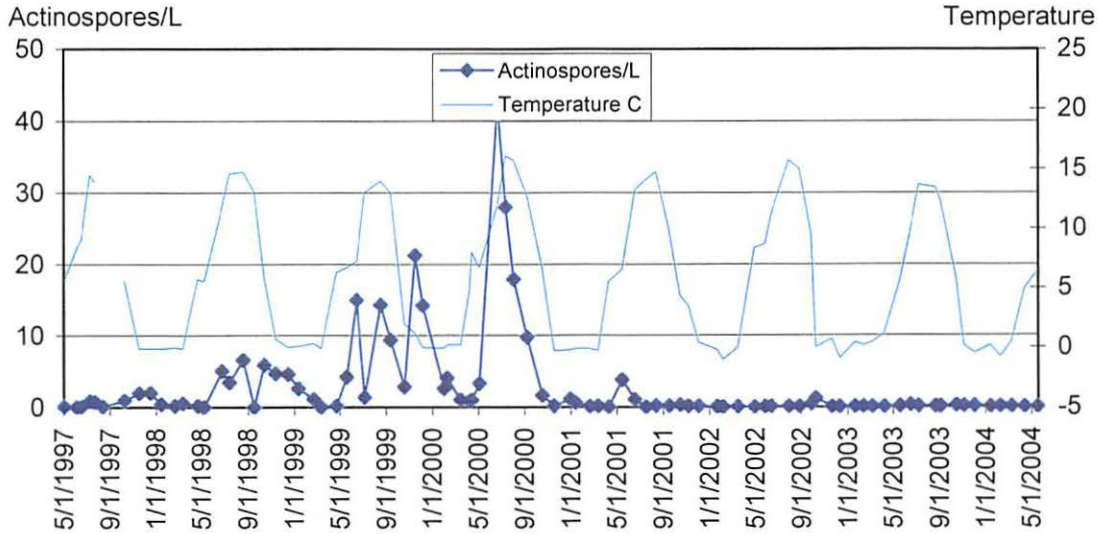

Figure 2.06. Comparison of temperature in degrees Celsius versus actinospores (TAMs) per liter from the northeast outlet of the PRU (April 1997- May 2004).

During late Summer 2000 the earthen rearing ponds were taken out of production and dried up. They have not been used for catchable trout production since. The northeast settling pond was dried up in the Spring 2001. Accumulated sediments were cleaned out and a liner was installed to keep effluent water from the concrete raceways on the north side of the unit from contacting the mud bottom. The results of implementing these changes were dramatic as can be seen from the summaries of filtration data for the effluent of the northeast pond in Table 2.06. The average density of actinospores observed in the settling pond effluent during 2001 and 2002 declined by

94% and 98% compared to the average for the year 2000, the last year the earthen ponds were used for production of catchable trout.

Table 2.06. Summary of estimated density of actinospores of *Mc* (N/L) detected in 1900-L samples of water drawn from the effluent of the northeast settling pond on the CDOW Poudre Rearing Unit, January 1998 through December 2003.

Month	1998	1999	2000	2001	2002	2003
Jan	0.399	2.61	2.63	0.634	0.065	0.105
Feb	0.148	1.11	4.08	0.094	0	0.104
Mar	0.547	0.018	1.04	0.133	0.036	0.034
Apr	0.18	0.16	0.91	0	0	0.065
May	0	4.30	3.35	3.85	0.067	0.129
Jun	5.07	8.67	42.9	1.08	0.077	0.299
Jul	3.49	1.36	28.0	0	0.080	0.150
Aug	6.58	14.3	17.9	0.193	0.108	0.093
Sep	2.83	9.36	9.75	0.182	0.452	0.099
Oct	5.92	2.83	1.63	0.315	1.255	0.207
Nov	4.70	21.2	0.162	0.135	0.063	0.135
Dec	4.62	14.2	1.10	0.147	0.032	0.125
Average	2.87	6.68	9.45	0.564	0.186	0.129

Roaring Judy Rearing Unit

According to inspection records at the CDOW Aquatic Animal Health Laboratory, trout from the Roaring Judy (RJ) State Fish Rearing Unit first tested positive for the presence of *Mc* in early 1992. Those same records indicated 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, Colorado) in 1988. Meridian Lake is approximately 25 km upstream of the RJ Unit. Meridian Lake was stocked with rainbow trout by a private aquaculturist whose facility tested positive for the parasite in late 1987.

Monthly actinospore monitoring at various locations commenced at RJ in April 1997. This effort continued through May 2004. Results of sampling to estimate densities of *Mc* actinospores at several locations on the RJ Unit during this segment are summarized in Table 2.07 and Figures 2.07 – 2.10.

In the Fall 2001 and 2002, members of the Gunnison Angling Society Trout Unlimited Chapter, RJ hatchery staff, and permanent and temporary personnel from the CDOW Aquatic Research section staff collaborated to regularly remove kokanee salmon carcasses from the west settling ponds and connecting waterways. Monitoring of the west settling ponds effluent suggested this action helped reduce the density of actinospores emanating from the ponds (Figure 2.10). However, the scheduled removal of kokanee carcasses from the ponds in Fall 2003 did not occur because TU volunteers and hatchery staff experienced difficulty connecting on the first several appointed pick-up days. Subsequently the research staff decided to refrain from removing the

carcasses during this segment to see if a rise in actinospore densities would result. To date , no such rise appears to have occurred (Figure 2.10). However, it is possible an increase will become apparent later in 2004, during the release that usually occurs during August to October.

Table 2.07. Results of water filtration to quantify actinospores (N/L) of *Mc* in 1900-L (500-gallon) samples of water drawn from various sites at the CDOW RJ Unit from July 1, 2003 to May 31, 2004.

Month	Concrete Raceway effluent	Kokanee Trap	West Pond effluent
Jul	0.0 (---)	0.098 (0.13)	0.127 (0.10)
Aug	0.0 (---)	0.0 (---)	1.624 0.14 ()
Sep	0.0 (---)	0.0 (---)	0.328 (0.16)
Oct	0.0 (---)	0.0 (---)	0.0 (---)
Nov	0.0 (---)	0.0 (---)	0.200 (0.16)
Dec	0.0 (---)	0.056 (0.11)	0.106 (0.10)
Jan	0.0 (---)	0.0 (---)	0.0 (---)
Feb	0.0 (---)	0.0 (---)	0.083 (0.11)
Mar	0.0 (---)	0.0 (---)	0.403 (0.27)
Apr	0.0 (---)	0.0 (---)	0.735 (0.35)
May	0.0 (---)	0.0 (---)	0.494 (0.27)
May (2)	not sampled	not sampled	0.033 (0.07)

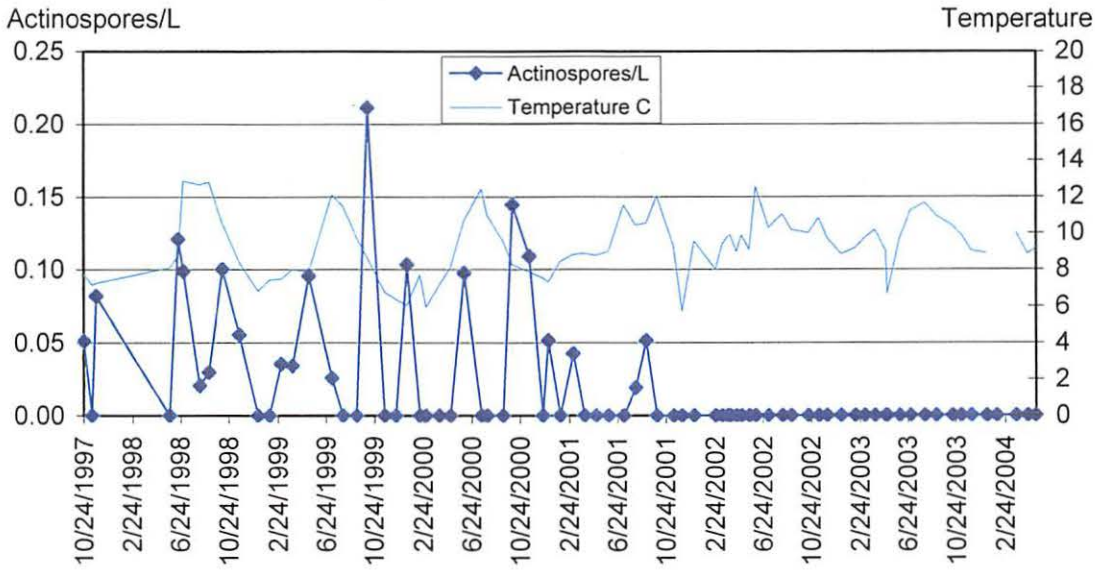

Figure 2.07. Comparison of temperature in degrees Celsius versus actinospores per liter from the concrete raceway effluent at the Roaring Judy State Fish Hatchery (June 1997-May 2004).

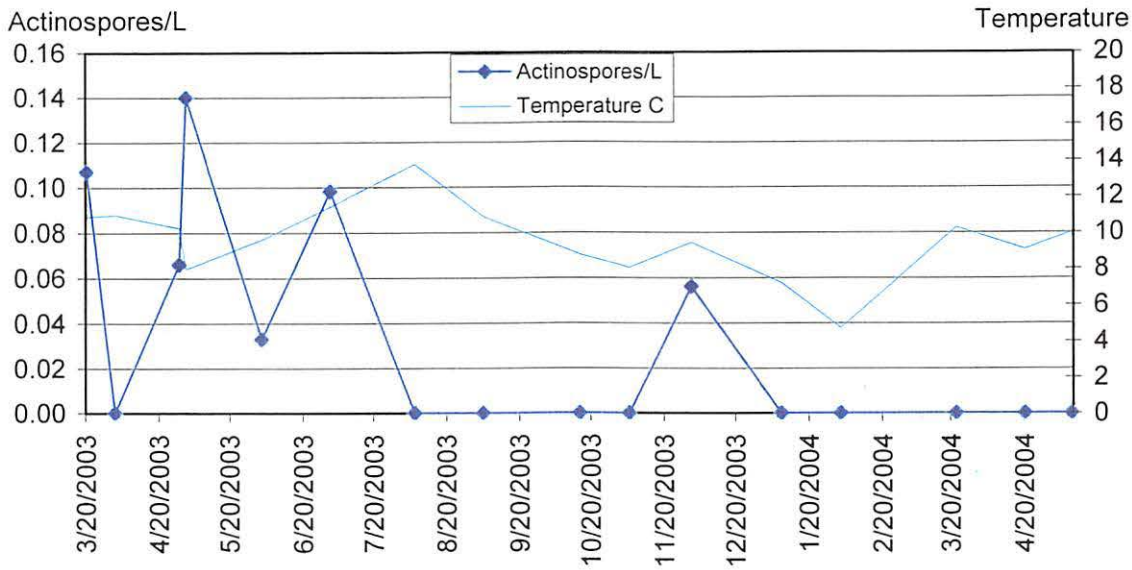

Figure 2.08. Comparison of temperature in degrees Celsius versus actinospores per liter from the kokanee conveyance channel above the kokanee trap at the Roaring Judy State Fish Hatchery (March 2003-May 2004).

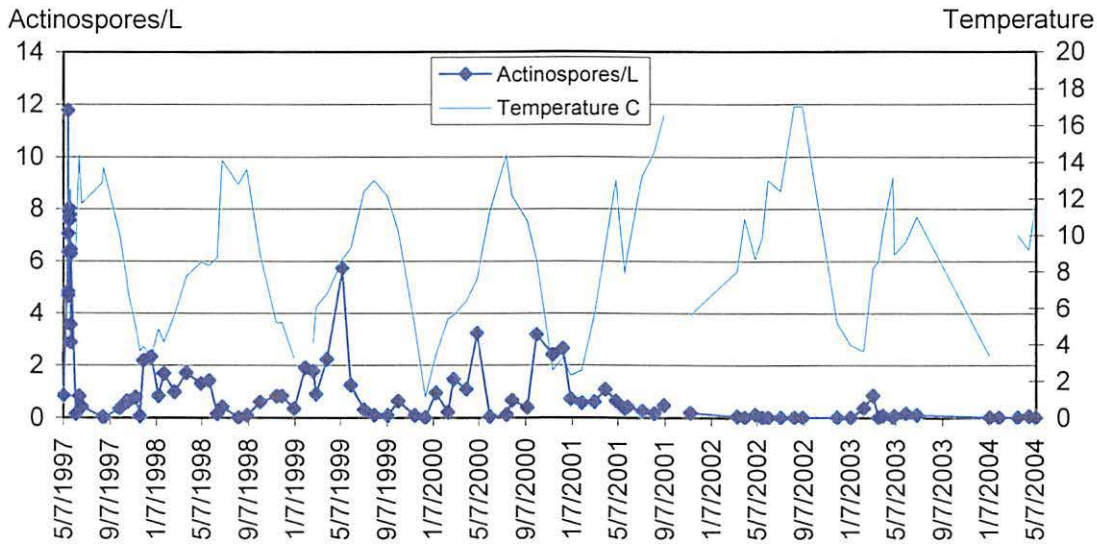

Figure 2.09. Comparison of temperature in degrees Celsius versus actinospores per liter from the east settling ponds outlet at the Roaring Judy State Fish Hatchery (May 1997-May 2004).

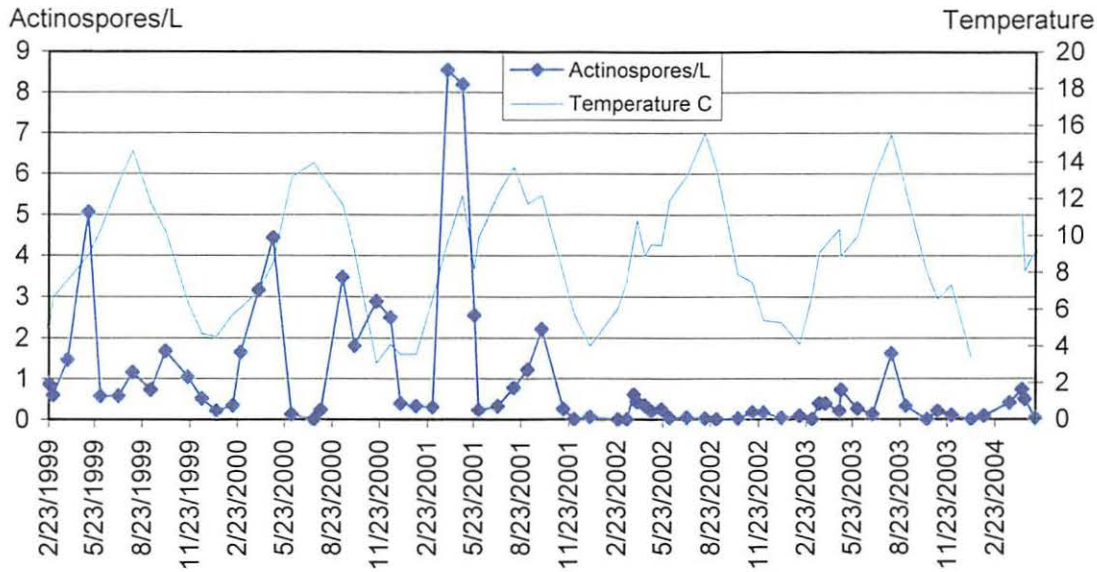

Figure 2.10. Comparison of temperature in degrees Celsius versus actinospores per liter from the west settling ponds outlet at the Roaring Judy State Fish Hatchery (February 1999-May 2004).

During this segment additional effort was made to remove fish from the effluent channel between the concrete raceway outlet and the top of the kokanee spawning facility. A two-pass removal estimate conducted in late November and early December resulted in the capture and removal of 250 brown trout, 100 rainbow trout, and seven Snake River cutthroat trout. Capture probabilities were ≥ 0.75 for fish ≥ 15 cm of each species. Consequently, there were an estimated 17 trout ≥ 15 cm remaining in the channel after the removals were completed. Boards were kept in place throughout the Winter at the top of the kokanee trap to prevent fish from the settling ponds from repopulating the channel. Even so, another single pass effort in May 2004 captured an additional 158 brown and rainbow trout ≥ 15 cm in the channel. Of these, 29 were ≥ 30 cm total length. It was possible many of these large fish were unavailable for capture during the Fall because they were further up into the concrete raceway drain pipes than the crew was able to electrofish. It certainly suggested that repeated efforts were necessary to keep the fish population reduced in the effluent channel to test the effect of this strategy on the infectivity above the kokanee trap.

Samples of the trout removed from the effluent channel showed that prevalence and intensity of *Mc* infection was high (Table 2.08). Prevalence and myxospore concentration values did not change appreciably from Spring to Fall for brown trout, but for rainbow trout both values were higher in Spring samples than in Fall samples. Additional samples were collected in May 2004, but have not yet been analyzed.

Table 2.08. Cranial *Mc* myxospore concentrations in trout sampled from the RJ Unit.

Date mm/dd/yy	Species	Sample Size		Overall Mean Concentration	Positive Fish	
		N	prevalence		Mean	Range
		Effluent Channel				
05/16/03	Brown	12	75%	39.900	53.200	2.000 – 177.750
11/25/03	Brown	20	70%	29.700	42.400	4.400 – 150.700
05/16/03	Rainbow	21	100%	367.400	367.400	3.700 – 2.242.500
11/25/03	Rainbow	22	50%	57.700	115.300	5.400 – 597.400
		Kokanee trap (fish followed kokanee from settling ponds)				
11/04/03	Bellaire[a]	28	28.6%	5,100	17,800	3,300 – 40,000
11/04/03	Tasmanian[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

a: Bellaire strain rainbow trout were from the *Mc*-negative Crystal Rearing Unit, and the Tasmanian strain rainbow trout were from the *Mc*-negative Rifle Rearing Unit. Since then, the Crystal Rearing Unit tested positive for the *Mc* parasite.

b: Unmarked rainbow trout, presumed to be feral inhabitants of the ponds or immigrants from the East River.

The suggested depopulation of the settling ponds did not occur. Rather, aquatic staff decided to stock a limited number of rainbow trout and later investigate the feasibility of removing trout from the ponds after the kokanee spawning season. The two west settling ponds were stocked with fin-clipped catchable rainbow trout in June 2003 with Bellaire strain from the Crystal Rearing Unit and Tasmanian strain from the Rifle Rearing Unit. Samples of the remaining fish were collected in November from the kokanee trap during the kokanee spawn, having followed the kokanee into the trap. Another sample of unmarked feral rainbow trout was also collected. The analysis of these samples indicated the feral trout exhibited far higher prevalence and intensity of infection than the stocked catchable rainbow trout (Table 2.08). Of the two strains stocked, the Bellaire rainbow trout exhibited a lower prevalence of infection than the Tasmanian rainbow trout, although the difference was not statistically significant (test of 2 proportions, 95% confidence interval for the difference in proportions = -0.412, 0.114).

Population estimates on the west settling ponds during early December indicated that very few of the stocked catchable rainbow trout remained in the ponds. On the other hand, population estimates for brown trout were 1330 fish (± 309) in the upper pond and 976 (± 234) in the lower pond. Consequently, it appeared the annual stocking of a moderate number of catchable rainbow trout into the settling ponds for the purpose of providing recreational fishing opportunity did not appreciably influence the density of actinospores in the pond effluent. 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.

The overall population of trout in the ponds indicated the effort to remove them each Fall would be extensive. Apart from a reliable way to exclude immigrants from the East River throughout the year except for the kokanee spawning season and a reliable method of keeping any exclusion device clean it appeared the idea to depopulate the settling ponds and to keep them free of fish was unrealistic.

RECOMMENDATIONS and CONCLUSIONS

Filtration studies at the CDOW's Pitkin, Poudre and RJ trout rearing units identified earthen bottom settling ponds as major sources of actinospore production that doubltless 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 were successful, with progress continuing toward bringing effluent actinospore densities at these units into equilibrium with the adjacent streams.

It was recommended that the settling pond at Pitkin continue to be kept as free of fish as possible. Since it appeared impractical to depopulate the settling ponds at RJ, it was 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.

Although there has been to date no measured increase in actinospore density in the RJ effluent as a result of not removing kokanee carcasses during this segment, we recommended that efforts continue to collect and remove the carcasses of kokanee salmon from the ponds and stream channel in the future. This was particularly important if actinospore density in the effluent increased during the early Fall period of the next segment.

The Cap-K Ranch sand filter proved to be a disappointment primarily due to the loss of water capacity experienced over such a short period of use. 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. Perhaps other strategies for reducing infectivity from the Cap-K Ranch ponds are more appropriate. We will continue to research the effect of removing susceptible brook trout from the system.

Job No. 3: Technical Assistance.

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

Period Covered: July 1, 2003 to June 30, 2004

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

1) Monitored three sites each month on the Blue Valley Ranch to evaluate triactinomyxon presence and abundance with regard to the exemption to operate a *Mc*-positive facility in salmonid habitat,

2) Monitored two sites each month at the Aquatic Alternatives private facility near Nathrop as part of exemption process for operating a *Mc*-positive facility in salmonid habitat,

3) Monitored one site below Antero Reservoir from March through June on behalf of Rod VanVelson to determine whether actinospores of *Mc* were present prior to stocking the fish channel constructed below Antero,

4) Peer-reviewed one paper submitted to North American Journal of Fisheries Management,

5) Reviewed a proposal at the request of Tom Powell regarding a long-term fisheries study below San Juan Reservoir, and

6) Fulfilled several requests for reprints of recent publications.

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