

# **Whirling Disease/Habitat Interactions**

## **Federal Aid Project F-427-R4**

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Federal Aid in Fish and Wildlife Restoration

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Project Title: Whirling Disease / Habitat Interactions

Project No.: F-427-R

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

Job No 1: Identification and Reduction of *Tubifex 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 *Myxobolus cerebralis*.

Period Covered: July 1, 2006 to June 30, 2007

## INTRODUCTION

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

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

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

Nevertheless, certain areas of infectivity remain that are not reservoir related but appear to



harbor *M. cerebralis* persistently. The objectives of this study are to determine whether it is possible to remove or greatly reduce these areas of infection by physical habitat manipulation and stream habitat improvement techniques, and to determine if such manipulations result in reduced prevalence and intensity of infection among resident trout downstream of modified sites.

#### Segment Objectives:

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

## METHODS and MATERIALS

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

### Fish Sampling

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

### Oligochaete Sampling

Oligochaete sampling was conducted during this segment only at the study site on the Poudre River. Oligochaete populations were characterized by sampling what was subjectively judged to be the best oligochaete habitat at the study site and in the physically isolated backwaters on two separate occasions. Nine replicate samples were obtained on each occasion by a kicknet technique. A 0.5 m<sup>2</sup> area was selected by surrounding with a frame made of copper water pipe, and 53.5 cm<sup>2</sup> core samples were removed at the center and near each end of the area selected. Depth of the core samples was 10 cm unless the substrate prevented this depth of penetration; all core depths were measured and recorded. The core samples were collected by USGS personnel in order to examine organic content and particle size distributions and determine whether relationships exist 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- $\mu$ m 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 taken to the lab for processing. Two samples of 50 haired oligochaete worms (if available) 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. The proportion of haired versus non-haired worms during the sample selection process was also recorded in order to obtain an estimate of the percentage of the oligochaete population that was *T. tubifex*. Each worm sample was also tested by PCR for the presence of the parasite.

### **Actinospore Sampling**

Two 120-L volumes of water were filtered monthly at each study site through 20- $\mu$ m Pecap screen to concentrate actinospores. These concentrates were examined for the presence of *M. cerebralis* actinospores in the lab by established protocols (Thompson and Nehring 2000). The filtration protocol is the same that has been in use since July 2004 (Thompson 2006).

## **RESULTS**

### Beaver Creek (South Fork Rio Grande drainage)

Habitat modifications were accomplished at this site in October 2001. Monitoring below this modified site for actinospores ceased after the 2004-05 segment. During this segment the fish population was sampled for the presence of the parasite among age 1+ and YOY brown trout.

Myxospore monitoring in age 1+ brown trout suggests that prevalence and mean concentrations have not been significantly reduced among the wild brown trout inhabiting the stream. Infection prevalence reached an apparent low point in 2002 (Figure 1.01), the year after habitat modification. However, the sample in 2003 would have been the first sample that was exposed to *M. cerebralis* as newly hatched fry under the new habitat conditions. The PCR results (Table 1.01) continue to show high prevalence of infection among YOY brown and rainbow trout.

Interpretation of the myxospore data is slightly complicated by the use of two labs and two techniques to evaluate the samples. A private lab in Maine was used in 1999 and the analysis was conducted using plankton centrifuge rather than the standard pepsin-trypsin digest. The same private lab was used again in 2004, albeit with pepsin-trypsin digest, but using this technique the private lab tended to have a higher probability of detection than the state lab due to generally lower volumes of PTD product. This affects prevalence more than it does mean concentration as the additional detections occur in fish exhibiting low spore concentrations, but may help explain the highest recorded prevalence noted in 2004. It is encouraging to note that prevalence the last two years was no higher than the long-term average even though the CDOW State Aquatic Animal Health lab achieved lower volumes of PTD product during that time than in previous years.

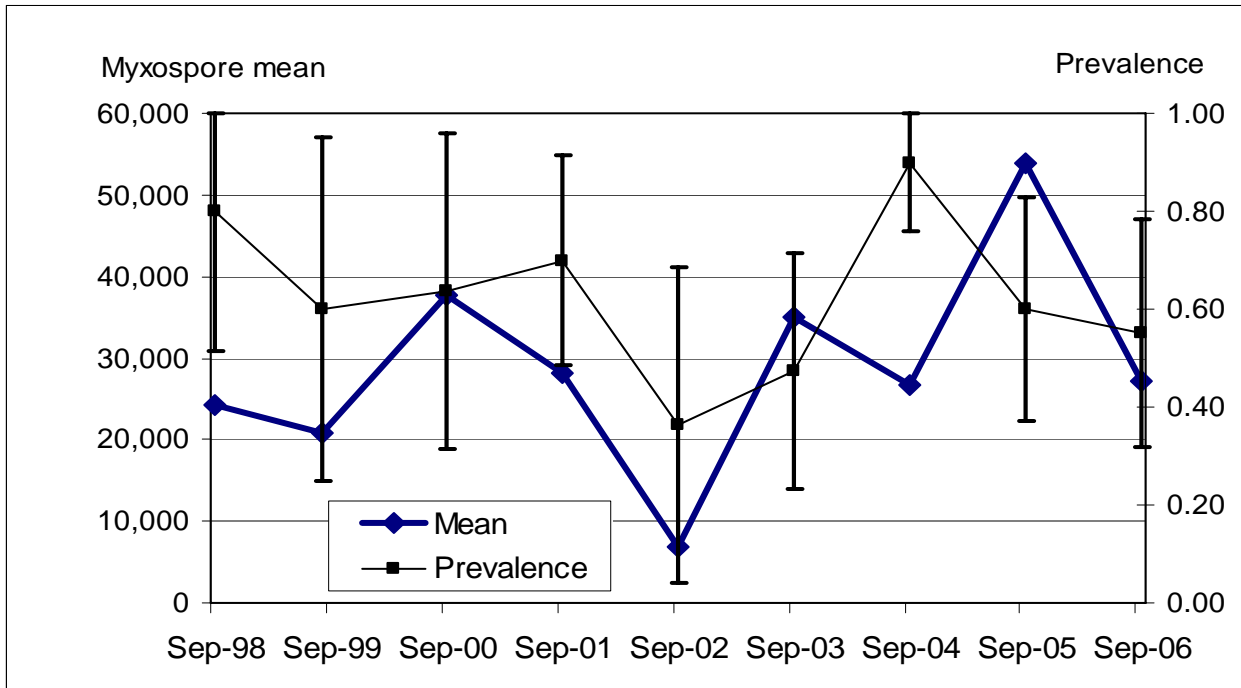


Figure 1.01. Mean myxospore concentration and prevalence of infection in samples of age 1+ brown trout (n = 11 – 20) collected from Beaver Creek below the habitat manipulation site. Error bars on the prevalence data are 95% confidence intervals from the binomial approximation.

Table 1.01. Results of PCR tests on young-of-the-year brown and rainbow trout from Beaver Creek below Beaver Creek Reservoir from 2000 to 2006. Mean scores are based on a scale from '0' (negative, no PCR signal) to '4' (very strong positive signal).

| Date     | Sample size (N) | Positive fish | Mean PCR score | Sample size (N) | Positive fish | Mean PCR score |
|----------|-----------------|---------------|----------------|-----------------|---------------|----------------|
|          | Brown trout     |               |                | Rainbow trout   |               |                |
| 09/22/00 | 11              | 9             | 2.55           | 2               | 2             | 3.50           |
| 09/26/01 | 10              | 10            | 3.50           | 10              | 10            | 2.60           |
| 09/13/02 | 13              | 8             | 2.71           | 22              | 21            | 3.68           |
| 09/23/03 | 20              | 15            | 1.80           | 15              | 10            | 1.87           |
| 09/19/04 | 20              | 18            | 2.40           | 11              | 8             | 2.82           |
| 09/22/05 | 15              | 13            | 3.20           | 15              | 12            | 1.80           |
| 09/29/06 | 10              | 9             | 3.10           | 15              | 9             | 1.93           |

### Cache la Poudre River

The Cache la Poudre River was added to the work schedule during the 2002-03 segment. Significant strides have been made in reducing *M. cerebralis* actinospores emanating from the Poudre Rearing Unit (PRU) (see Job 2 of Nehring and Thompson 2003, Schisler 2003), so additional

attention was focused on in-stream habitats near the PRU. Allen (1999) found that the main channel of the river in the low-gradient reach above PRU contained few oligochaetes, but that they were often numerous in side-pockets, alcoves, and side channels. While not detailed in Allen’s thesis, one such site identified was at Kinikinik. In the area there are two significant backwater areas that appear to be excellent habitat for *T. tubifex*.

Berms designed to isolate both of the backwater areas at Kinikinik were constructed in September and October 2004 and described in Thompson (2005). The berms were designed to preclude 90% or more of all average daily flows in this reach from entering the backwater areas, based on historic data from a discontinued gage near Rustic. Flows that overtop the berms will only occur during runoff, a time when actinospores are seldom encountered. Moreover, any actinospores produced during peak runoff would also be highly diluted if they entered the river. In 2006, the backwaters were connected to the river for six weeks or less (Figure 1.02).

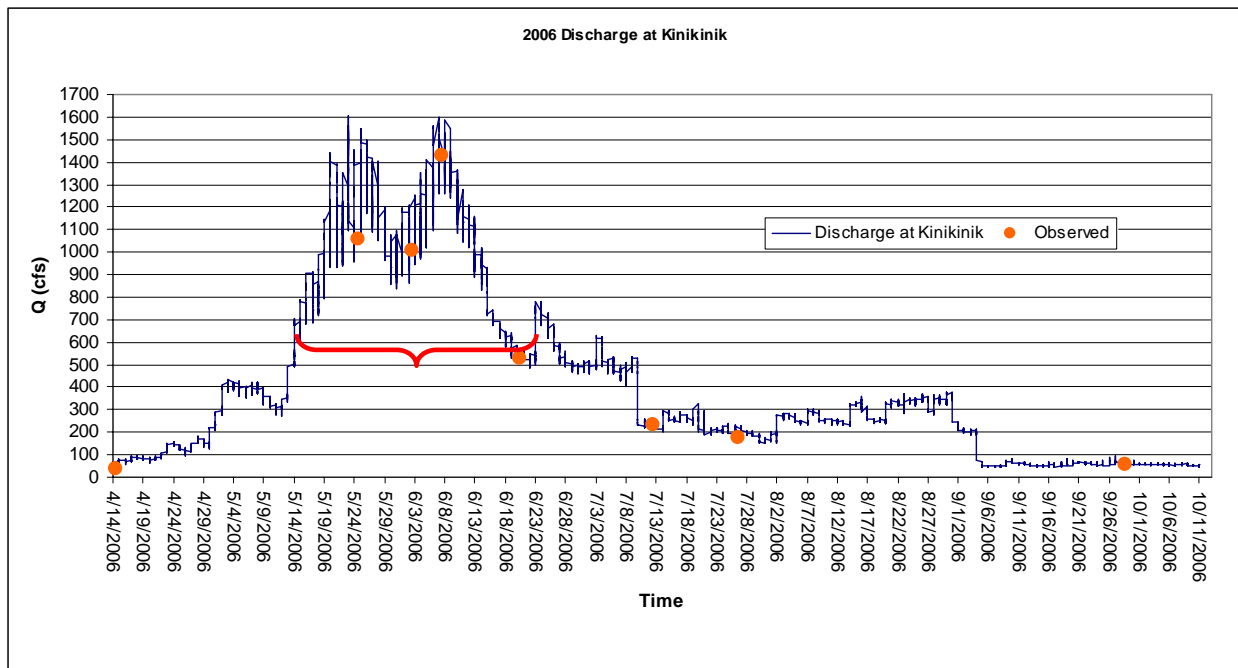


Figure 1.02. Discharge in the Poudre River at the Kinikinik study site, modeled from pressure sensors and observed flows. The approximate period of berm inundation is indicated by the bracket and extended from about May 14 to June 24, 2006. “Observed” data points are measured discharges.

Water samples have been collected above and below the Kinikinik site since January 2003 (Figure 1.03). Density estimates were low on all occasions when actinospores were actually observed. No actinospores were observed from the isolated backwater areas during this segment.

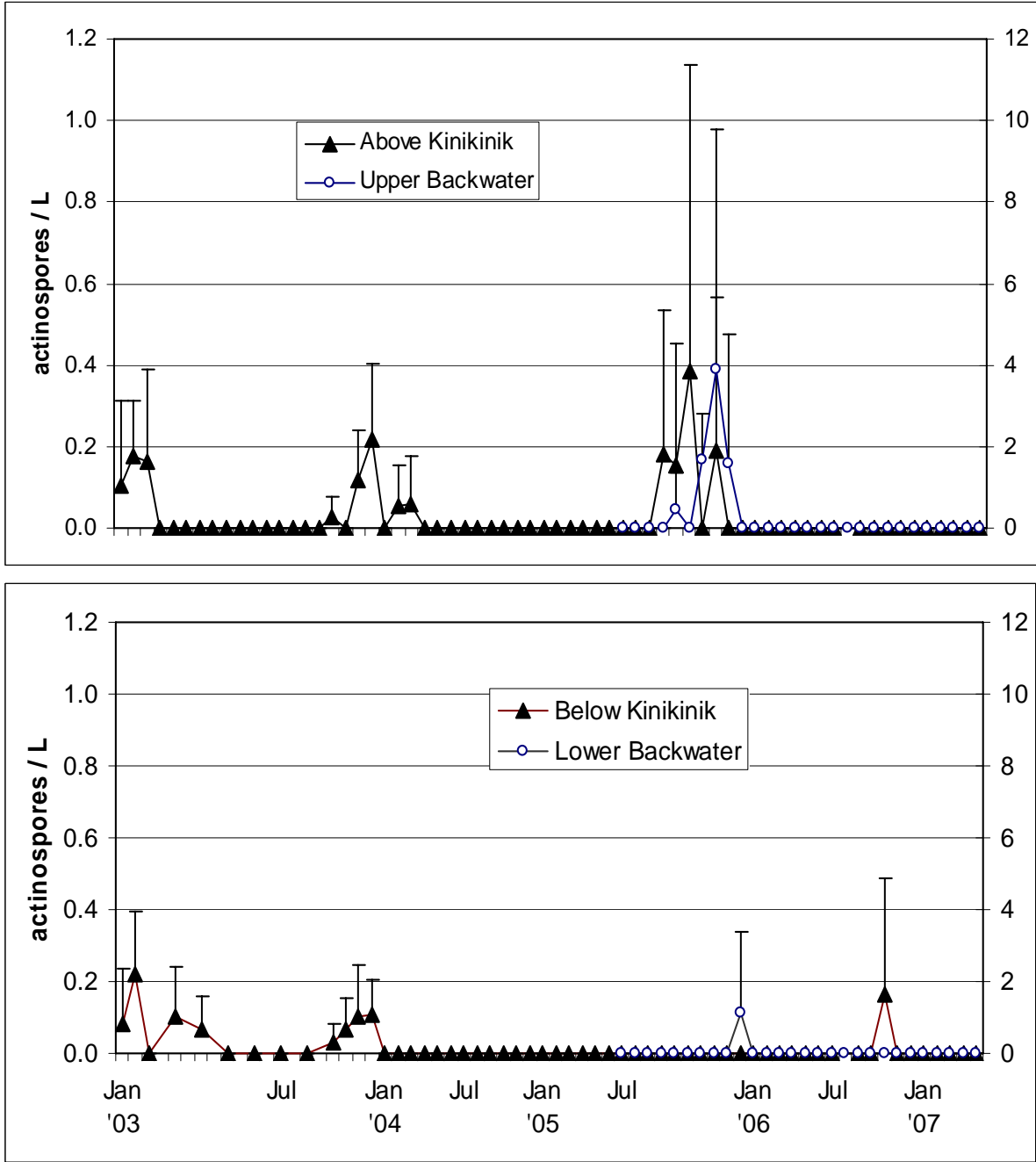


Figure 1.03. Estimates of actinospores/L in the Poudre River at above and below Kinikinik from January 2003 through May 2007. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream. Sampling frequency was twice per month early in the monitoring and during late summer and fall 2005, hence the uneven x-axis. Backwater values are referenced to the 2<sup>nd</sup> y-axis.

Baseline oligochaete sampling was completed in 2003 and 2004 (Table 1.02), and post-

construction samples were collected in 2005 and 2006. Lineage V, containing few if any susceptible individuals, has not been represented in the oligochaete samples collected from this area to date. Lineage III is presently believed to be the *T. tubifex* most susceptible to *M. cerebralis* infection (Beauchamp et al. 2002, DuBey et al. 2005), and predominated at this site in early baseline sampling. However, the proportion of lineage III DNA in the worm samples tested by qPCR showed a significant downward trend over the 13 month time span of the baseline sampling (ANOVA,  $P = 0.0002$ ), with a concomitant rise in the percentage DNA of the less-susceptible lineages I and VI.

The proportion of lineage III DNA in post construction samples has remained at about the same level as the 2004 baseline data. The other two lineages have experienced wide fluctuations; a dynamic no doubt influenced by the fact that it has been more difficult in post-construction monitoring to obtain replicate 50-worm samples from each location. Therefore the data are more sparse and the confidence intervals wider. Moreover, in many instances the qPCR test results indicate low numbers of “worm equivalents”, a measure of how many copies of the target DNA were present in the sample. This may suggest that there are now haired worms at the site that do not belong to any of the four lineages targeted by the qPCR test. None of the worm samples collected in 2006 tested positive for the presence of the parasite.

Table 1.02. Estimates of the proportion of each *Tubifex tubifex* lineage DNA found in oligochaete samples at the Kinikinik site. N refers to the number of the nine kicknet samples collected on each occasion that contained *T. tubifex*. The values in parentheses in the percent DNA composition columns are 95% confidence intervals.

| Date              | N | Approximate percent DNA composition by <i>M. cerebralis</i> lineage |             |           |             |
|-------------------|---|---|-------------|-----------|-------------|
|                   |   | I   | III         | V         | VI          |
| Pre-modification  |   |   |             |           |             |
| 8/25/03           | 9 | 2.8 (1.7)   | 73.9 (11.2) | 0.0 (0.0) | 23.3 (10.2) |
| 10/01/03          | 8 | 4.8 (2.6)   | 67.5 (10.8) | 0.0 (0.0) | 27.7 (9.9)  |
| 06/22/04          | 9 | 5.6 (6.1)   | 55.5 (25.1) | 0.0 (0.0) | 38.9 (23.3) |
| 09/13/04          | 8 | 13.7 (6.6)  | 37.3 (21.8) | 0.0 (0.0) | 48.9 (20.1) |
| Post-modification |   |   |             |           |             |
| 07/18/05          | 4 | 22.5 (54.6)   | 58.9 (62.7) | 0.0 (0.0) | 18.6 (22.3) |
| 10/24/05          | 6 | 40.6 (18.7)   | 37.7 (19.3) | 0.0 (0.0) | 21.7 (9.7)  |
| 07/18/06          | 7 | 49.7 (46.7)   | 43.3 (44.5) | 0.0 (0.0) | 7.0 (13.2)  |
| 10/11/06          | 8 | 19.7 (11.1)   | 41.7 (20.5) | 0.0 (0.0) | 38.5 (19.8) |

The results of myxospore analyses from samples of age 1+ brown trout collected over the last several years are presented in Table 1.03. On average the data suggest that a somewhat higher proportion of wild brown trout are infected with *M. cerebralis* below the Kinikinik site than above it. Mean concentrations vary considerably at both collection sites. In 2006 both sites showed a large increase in myxospore concentration; however without the outlier at the Big Bend site below Kinikinik the average for that sample decreases from 50,600 to 12,900 myxospores. In contrast, the range of positive values encountered at the site above Kinikinik was less variable (Table 1.03).

Table 1.03. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from the Poudre River.

| Date<br>mm/dd/yy                              | N  | Prevalence | Overall Mean<br>Concentration | Positive Fish |                 |
|---|----|------------|-------------------------------|---------------|-----------------|
|   |    |            |                               | Mean          | Range           |
| Bliss State Wildlife Area – above Kinikininik |    |            |                               |               |                 |
| 09/30/02                                      | 10 | 10.0%      | 2,800                         | 28,100        | 28,100          |
| 10/22/03                                      | 20 | 40.0%      | 4,400                         | 11,000        | 2,300 – 31,600  |
| 10/28/04                                      | 10 | 20.0%      | 2,600                         | 13,000        | 9,200 – 16,700  |
| 11/02/05                                      | 17 | 70.6%      | 2,000                         | 2,800         | 560 – 13,300    |
| 10/16/06                                      | 7  | 85.7%      | 30,300                        | 35,400        | 26,900 – 50,500 |
| Big Bend – below Kinikininik                  |    |            |                               |               |                 |
| 09/19/00                                      | 10 | 50.0%      | 6,300                         | 12,600        | 990 – 37,600    |
| 10/22/03                                      | 12 | 41.7%      | 3,900                         | 9,400         | 920 – 16,000    |
| 10/28/04                                      | 15 | 40.0%      | 17,100                        | 42,900        | 5,600 – 92,300  |
| 11/02/05                                      | 15 | 60.0%      | 3,600                         | 6,000         | 560 – 27,200    |
| 10/16/06                                      | 10 | 80.0%      | 50,600                        | 63,200        | 3,400 – 439,400 |

Colorado River

No habitat or other manipulations have occurred in this stream segment. Monitoring in the Colorado River at the Kemp/Breeze Wildlife Area continued during this segment for triactinomyxon and myxospore information.

Samples of juvenile brown trout obtained since 1999 for analysis of cranial myxospore concentrations by PTD (Markiw and Wolf 1974) indicate that prevalence of infection is routinely 60% or greater (Table 1.04). Samples were collected in 2006 at the Kemp/Breeze Wildlife Area as well as at the Hitching Post Bridge downstream of Windy Gap Reservoir. As with the Cache la Poudre River, 2006 saw an increase in average myxospore concentration in the samples of brown trout. The year-to-year variations in prevalence and myxospore concentration are not really different than those being observed in treated stream sections. This suggests that the habitat manipulations implemented elsewhere may be no more responsible for changes in these metrics than the random processes occurring in all the streams.

Table 1.04. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from the Colorado River during the fall in 1999-2006.

| Date  | N  | Prevalence | Overall Mean Concentration | Positive Fish |                 |
|---|----|------------|----------------------------|---------------|-----------------|
|   |    |            |                            | Mean          | Range           |
| Hitching Post Bridge 1.9 km below Windy Gap Reservoir     |    |            |                            |               |                 |
| 09/29/99  | 10 | 80.0%      | 6,330                      | 7,920         | 1,110 – 15,550  |
| 10/12/00  | 10 | 100.0%     | 58,700                     | 58,700        | 8,700 – 208,700 |
| 09/13/01  | 20 | 75.0%      | 20,300                     | 27,500        | 4,000 – 96,000  |
| 09/27/02  | 10 | 60.0%      | 12,300                     | 20,400        | 3,500 – 73,800  |
| 09/29/03  | 16 | 68.8%      | 11,700                     | 17,000        | 2,500 – 43,700  |
| 09/27/04  | 22 | 95.5%      | 19,700                     | 20,700        | 560 – 96,700    |
| 10/17/05  | 15 | 60.0%      | 4,900                      | 8,200         | 560 – 24,400    |
| 10/23/06  | 20 | 55.0%      | 40,100                     | 61,700        | 3,100 – 187,400 |
| Kemp/Breeze Wildlife Area 26 km below Windy Gap Reservoir |    |            |                            |               |                 |
| 09/29/99  | 10 | 60.0%      | 2,330                      | 3,890         | 2,220 – 6,670   |
| 09/18/01  | 19 | 36.8%      | 13,800                     | 37,300        | 1,900 – 160,600 |
| 10/08/02  | 13 | 84.6%      | 19,900                     | 23,600        | 3,300 – 68,100  |
| 09/17/03  | 15 | 93.3%      | 14,400                     | 15,400        | 3,300 – 70,100  |
| 09/30/04  | 21 | 76.2%      | 7,900                      | 10,400        | 1,100 – 50,000  |
| 10/17/05  | 14 | 78.6%      | 9,800                      | 12,500        | 560 – 25,600    |
| 10/23/06  | 20 | 65.0%      | 18,600                     | 28,600        | 1,700 – 156,500 |



Actinospore densities were monitored at the Breeze Bridge once each month during the last segment. *Myxobolus cerebralis* actinospores have been observed only once during the last two segments (Figure 1.04). In addition, only two samples have tested positive for the parasite among the 18 samples submitted for PCR since July 2005. Each of the samples was scored a “2” on the 0-4 point scale.

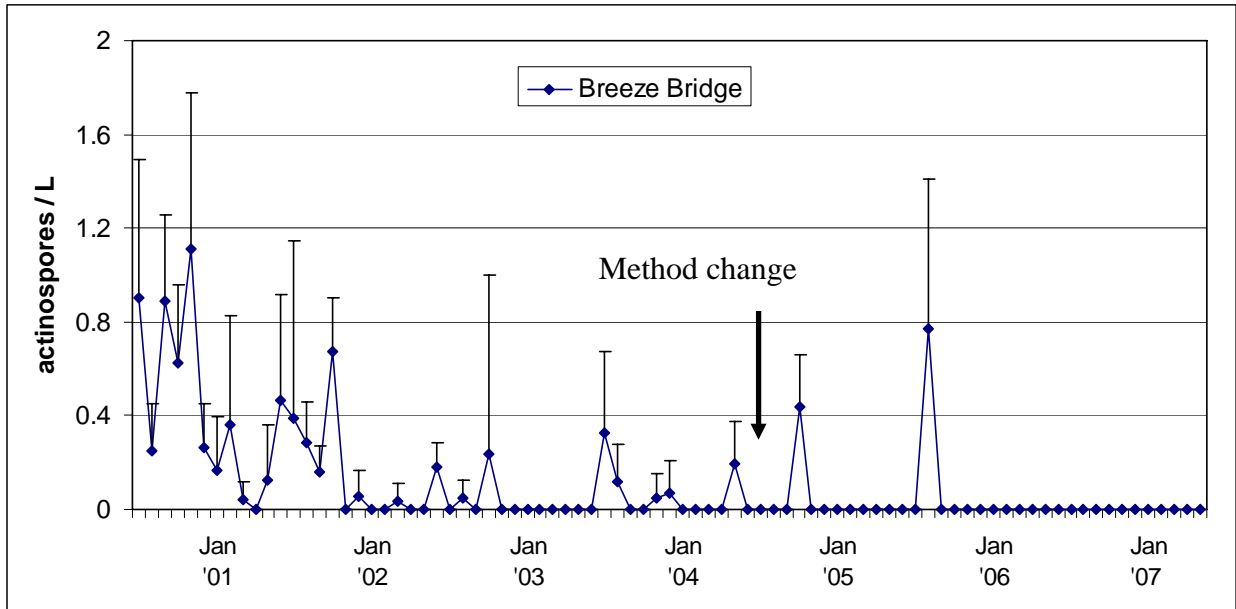


Figure 1.04. Results of water filtration to estimate ambient density of *M. cerebralis* actinospores (N/L) in the Colorado River at Breeze Bridge from July 2000 to May 2007. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Spring Creek (Taylor River drainage)

Habitat modifications occurred on this stream in October 2002. Monitoring continued in this segment at both the study site and at upstream and downstream control sites to collect data on ambient actinospore density and on prevalence of infection and myxospore concentration in brown trout. The brown trout population remains stable in this stream; the rainbow trout population is sparse and consists largely of stocked catchable trout (Table 1.05). However, at the uppermost station below Spring Creek Reservoir we captured three wild rainbow trout in 2006. One was a healthy-appearing young-of-the-year, but more importantly, the other two had recruited to the juvenile population.

Table 1.05. Trout population biostatistics for three sites upstream from, downstream from, and at Salsbury Gulch on Spring Creek, from fall electrofishing efforts.

| Year  | Brown Trout |        |       |              |             | Rainbow Trout    |        |       |              |             |
|---|-------------|--------|-------|--------------|-------------|------------------|--------|-------|--------------|-------------|
|   | N ≥ 15 cm   | 95% CI | Kg/ha | N/ha ≥ 15 cm | N/ha Age 1+ | N ≥ 15 cm        | 95% CI | Kg/ha | N/ha ≥ 15 cm | N/ha Age 1+ |
| 0.8 km downstream of Spring Creek Reservoir                             |             |        |       |              |             |                  |        |       |              |             |
| 2002  | 265         | ± 2    | 506   | 5,725        | 4,571       | 0                | ---    | 0     | 0            | 0           |
| 2003  | 246         | ± 5    | 261   | 3,173        | 4,345       | 0                | ---    | 0     | 0            | 0           |
| 2004  | 231         | ± 1    | 258   | 2,967        | 2,323       | 0                | ---    | 0     | 0            | 0           |
| 2005  | 199         | ± 4    | 178   | 2,564        | 3,193       | 2                | ± 0    | 3     | 26           | 0           |
| 2006  | 190         | ± 1    | 253   | 2,450        | 2,809       | 6                | ± 2    | 18    | 77           | 13          |
| 5 km downstream of Spring Creek Reservoir at Salsbury Gulch (treatment) |             |        |       |              |             |                  |        |       |              |             |
| 2002  | 393         | ± 1    | 329   | 2,861        | 1,182       | 0                | ---    | 0     | 0            | 0           |
| 2003  | 309         | ± 8    | 288   | 2,803        | 1,240       | 7                | ± 1    | 10    | 63           | 0           |
| 2004  | 347         | ± 2    | 315   | 3,143        | 1,875       | 72 <sup>a</sup>  | ± 8    | 99    | 649          | 205         |
| 2005  | 308         | ± 5    | 282   | 2,789        | 1,283       | 50 <sup>a</sup>  | ± 3    | 88    | 451          | 0           |
| 2006  | 273         | ± 7    | 305   | 2,473        | 853         | 63 <sup>a</sup>  | ± 1    | 114   | 589          | 36          |
| 19 km downstream of Spring Creek Reservoir (control)                    |             |        |       |              |             |                  |        |       |              |             |
| 2002  | 175         | ± 5    | 207   | 2,105        | 1,814       | 207 <sup>a</sup> | ± 1    | 427   | 2,435        | 24          |
| 2003  | 157         | ± 8    | 180   | 1,653        | 1,664       | 52 <sup>a</sup>  | ± 2    | 102   | 554          | 21          |
| 2004  | 146         | ± 5    | 124   | 1,538        | 1,245       | 71 <sup>a</sup>  | ± 4    | 124   | 748          | 0           |
| 2005  | 160         | ± 9    | 181   | 1,687        | 1,725       | 34 <sup>a</sup>  | ± 0    | 67    | 359          | 11          |
| 2006  | 169         | ± 9    | 171   | 1,781        | 2,104       | 21 <sup>a</sup>  | ± 1    | 39    | 223          | 0           |

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

The “post-treatment” samples of age 1+ brown trout collected in the last three segments indicate that there is no difference in prevalence or infection intensity compared to samples collected prior to habitat manipulation (Table 1.06). There is also no real evidence that there are any differences among the stations along the length of the study reach in prevalence and myxospore concentration. This circumstance makes it all the more surprising that there appear to be a very few rainbow trout surviving into the second year and beyond in a stream exhibiting such high infectivity for *M. cerebralis*.

Table 1.06. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from Spring Creek. Samples collected in 2004 and later comprise the post-manipulation data.

| Date<br>mm/dd/yy  | N  | Prevalence | Overall Mean<br>Concentration | Positive Fish |                  |
|---|----|------------|-------------------------------|---------------|------------------|
|   |    |            |                               | Mean          | Range            |
| 0.8 km downstream of Spring Creek Reservoir                             |    |            |                               |               |                  |
| 05/18/01  | 20 | 45%        | 6,500                         | 14,400        | 1,400 – 56,000   |
| 08/01/01  | 20 | 80%        | 21,200                        | 26,500        | 4,200 – 82,300   |
| 09/17/02  | 19 | 79%        | 43,900                        | 55,700        | 2,000 – 195,000  |
| 09/22/03  | 23 | 78%        | 63,300                        | 80,900        | 4,100 – 316,000  |
| 09/07/04  | 26 | 92%        | 50,700                        | 54,900        | 4,400 – 56,700   |
| 09/21/05  | 20 | 65%        | 59,700                        | 91,800        | 2,800 – 590,700  |
| 09/06/06  | 20 | 95%        | 80,400                        | 84,600        | 1,700 – 500,000  |
| 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  |
| 09/07/04  | 20 | 100%       | 41,000                        | 41,000        | 560 – 191,100    |
| 09/21/05  | 21 | 86%        | 64,900                        | 76,900        | 7,100 – 422,500  |
| 09/06/06  | 20 | 95%        | 50,200                        | 52,900        | 1,700 – 168,300  |
| 19 km downstream of Spring Creek Reservoir (control)                    |    |            |                               |               |                  |
| 05/18/01  | 20 | 95%        | 57,000                        | 60,000        | 15,200 – 173,200 |
| 08/01/01  | 20 | 90%        | 76,400                        | 84,900        | 6,600 – 225,300  |
| 09/17/02  | 20 | 95%        | 13,200                        | 13,900        | 1,300 – 30,300   |
| 09/23/03  | 20 | 90%        | 40,900                        | 45,400        | 7,700 – 153,100  |
| 09/07/04  | 20 | 95%        | 53,300                        | 56,100        | 4,400 – 212,200  |
| 09/21/05  | 20 | 90%        | 46,600                        | 51,800        | 3,300 – 208,400  |
| 09/06/06  | 20 | 95%        | 52,300                        | 55,000        | 1,700 – 178,400  |

Samples of young-of-the-year (YOY) brown trout were collected at the same three sites in September of the last six years (Table 1.07). The YOY samples collected in 2003 were the first post-manipulation data. The heads were individually analyzed by the PCR technique and indicate that there is a high prevalence of infection among YOY brown trout at all three sites for all years. The severity of infection among YOY at all stations for the last several years has been at or very near the top of the scale used to judge it.

Table 1.07. Results of polymerase chain reaction (PCR) tests of samples of young-of-the-year brown trout collected from Spring Creek. Samples collected in 2003 and later comprise the post-manipulation data. Mean PCR score is based on assigning numerical values to the qualitative score given to indicate strength of signal as follows: negative = 0, weak positive = 1, positive = 2, strong positive = 3, and very strong positive = 4.

| Date  | Sample size (N) | Positive fish | Mean PCR score |
|---|-----------------|---------------|----------------|
| 0.8 km downstream of Spring Creek Reservoir                 |                 |               |                |
| 09/26/01  | 10              | 10            | 3.4            |
| 09/17/02  | 18              | 14            | 1.6            |
| 09/22/03  | 20              | 20            | 2.8            |
| 09/07/04  | 25              | 25            | 3.8            |
| 09/19/05  | 16              | 16            | 3.9            |
| 09/06/06  | 10              | 10            | 4.0            |
| 5 km downstream of Spring Creek Reservoir at Treatment site |                 |               |                |
| 09/26/01  | not sampled     |               |                |
| 09/18/02  | 21              | 18            | 1.9            |
| 08/22/03  | 20              | 20            | 3.1            |
| 09/08/04  | 20              | 20            | 3.4            |
| 09/21/05  | 15              | 15            | 3.8            |
| 09/06/06  | 10              | 10            | 3.9            |
| 19 km downstream of Spring Creek Reservoir at Control site  |                 |               |                |
| 09/26/01  | 10              | 10            | 3.8            |
| 09/18/02  | 10              | 10            | 2.3            |
| 09/23/03  | 20              | 20            | 2.7            |
| 09/08/04  | 20              | 20            | 3.9            |
| 09/22/05  | 15              | 15            | 3.9            |
| 09/06/06  | 10              | 10            | 4.0            |

Water samples taken during the segment continued to indicate that habitat manipulation at this site did not result in reduced actinospore densities following construction. To the contrary, post-construction monitoring resulted in a greater frequency of actinospore detection compared to pre-construction sampling for several years (Figure 1.05) at both the treatment and control sites. Only in this segment has the frequency of detection and density fallen to levels similar to those seen prior to construction.

One potential reason for the increased actinospore detections in the period after habitat manipulation could be the presence of infected rainbow trout catchables in the stream in 2003. We tested 14 catchable trout captured at the control station in the fall of 2003 for *M. cerebralis* by the PTD method. Eight fish were found to be positive, and the average for the sample was 101,700 myxospores per head. Stocking records indicate that 1500 catchable rainbow trout were stocked into the stream on August 28, 2003. These very likely comprised the fish population that was sampled. Being stocked late in the summer, it is also possible that many of these fish were not creel before fishing pressure dropped for the fall and winter. If so, a number of these

rainbow trout perishing in the stream would have provided an extra measure of myxospores to the environment in addition to what the resident brown trout population contributes. In contrast, we found little or no infectivity in stocked rainbow trout sampled in 2004 – 2006.

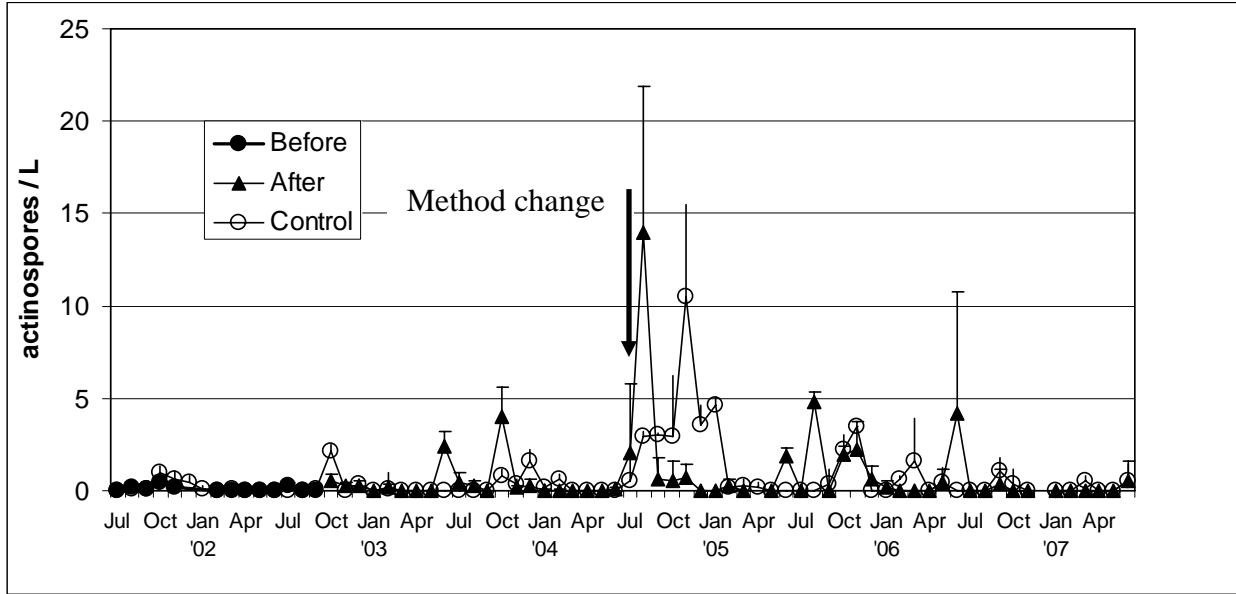


Figure 1.05. Density of actinospores observed in surface water samples collected at the Spring Creek treatment and lower control sites. “Before” designates the 15 months preceding construction at the treatment site.

Williams Fork River (Colorado River drainage)

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

Trout population data have been collected from the Williams Fork River for the past five years (Table 1.08). The rainbow trout population remains sparse. Biomass and overall density of rainbow trout remain consistently higher just below Williams Fork Dam versus below the habitat modification site. This is consistent with the hypothesis that the majority of present-day infectivity comes from within the river rather than Williams Fork Reservoir.

Table 1.08. Trout population biostatistics for two sites on the Williams Fork River below Williams Fork Reservoir.

| Year   | Brown Trout |        |       |                 |                | Rainbow Trout |        |       |                 |                  |
|--|-------------|--------|-------|-----------------|----------------|---------------|--------|-------|-----------------|------------------|
|  | N           | 95% CI | Kg/ha | N/ha ≥<br>15 cm | N/ha<br>Age 1+ | N             | 95% CI | Kg/ha | N/ha ≥<br>15 cm | N/ha<br>Age 1+   |
| 0.3 km below Williams Fork Reservoir   |             |        |       |                 |                |               |        |       |                 |                  |
| 2002   | 269         | ± 6    | 279   | 1559            | 522            | 30            | ± 1    | 56    | 174             | 93               |
| 2003   | 999         | ± 9    | 816   | 5779            | 1003           | 24            | ± 3    | 45    | 138             | 74               |
| 2004   | 430         | ± 4    | 455   | 2490            | 213            | 33            | ± 2    | 70    | 188             | 54               |
| 2005   | 523         | ± 13   | 383   | 3028            | 666            | 24            | ± 5    | 55    | 137             | 71               |
| 2006   | 408         | ± 15   | 281   | 2361            | 456            | 25            | ± 2    | 40    | 147             | 29               |
| 1.6 km below Williams Fork Reservoir, below Kemp/Breeze Wildlife Area irrigation |             |        |       |                 |                |               |        |       |                 |                  |
| 2002 <sup>a</sup>  | 593         | ± 15   | 651   | 2952            | 1600           | 25            | ± 1    | 56.8  | 125             | 55               |
| 2003   | 711         | ± 7    | 360   | 1811            | 1172           | 32            | ± 2    | 21    | 80              | 42               |
| 2004   | 472         | ± 8    | 373   | 1202            | 1336           | 21            | ± 2    | 21    | 54              | 3                |
| 2005   | 403         | ± 24   | 214   | 1026            | 796            | 33            | ± 7    | 13    | 83              | 79               |
| 2006   | 353         | ± 13   | 162   | 900             | 646            | 49            | ± 14   | 21    | 126             | 202 <sup>b</sup> |

a: Station length 385 feet in 2002; 813 feet on all other occasions

b: The Williams Fork received nearly 10,000 rainbow trout fingerlings averaging 4.28 inches about 6 weeks before the electrofishing date; this circumstance is the explanation for the large estimate of age 1+ rainbow trout.

Construction at the Williams Fork River site occurred during the first week of June 2002. Details of the habitat modifications and initial actinospore and oligochaete monitoring were presented previously (Nehring and Thompson 2003). The high actinospore density observed 12 months post-construction still appears to be an aberration (Figure 1.06). Overall, we continue to detect actinospores less frequently than was the case before habitat modification. Although four of the eight highest actinospore densities observed have occurred after habitat modification,

three of the samples were collected with the more sensitive technique used since July 2004 (Thompson 2005).

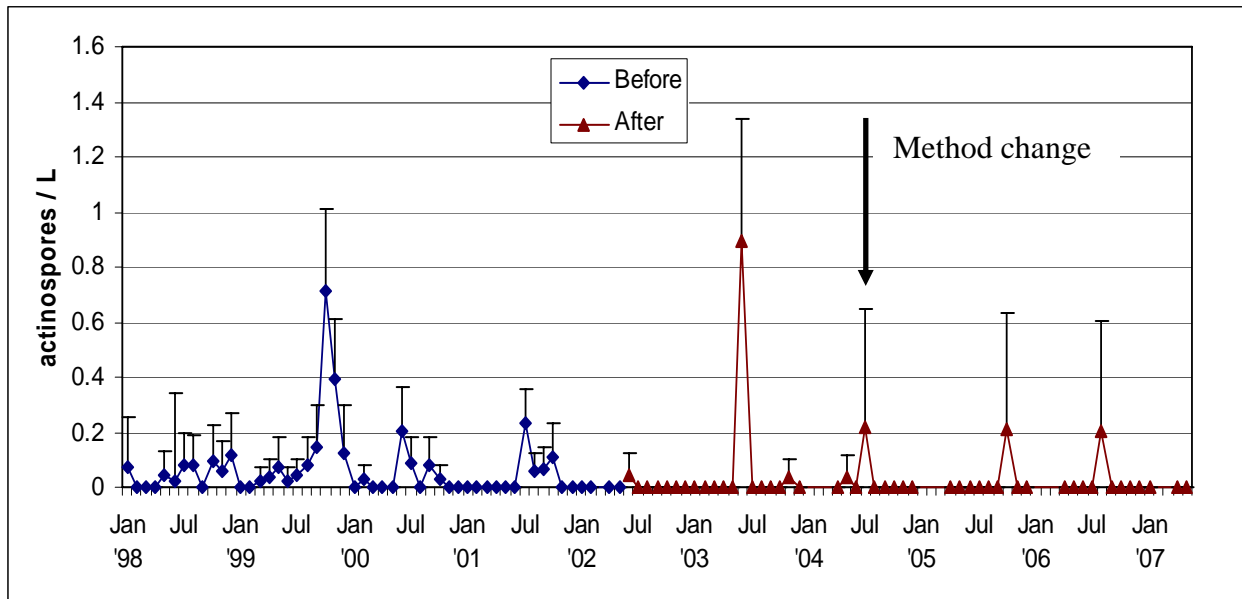


Figure 1.06. Density of actinospores observed in concentrates of surface water samples collected at the Williams Fork treatment site from January 1998 through May 2007. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream. “Before” designates samples collected prior to construction and “After” the samples collected following construction.

The brown trout collected in the fall of 2004 and later represent post-manipulation samples (Table 1.09). In recent years the prevalence and average myxospore concentrations have been moderate or low at all three sampling sites.

Table 1.09. Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from three sites in the Williams Fork River.

| Date<br>mm/dd/yy   | N  | Prevalence | Overall Mean<br>Concentration | Positive Fish |                 |
|--|----|------------|-------------------------------|---------------|-----------------|
|  |    |            |                               | Mean          | Range           |
| 0.3 km below Williams Fork Reservoir, above Treatment site |    |            |                               |               |                 |
| 09/13/01   | 15 | 13%        | 970                           | 7,300         | 6,400 – 8,100   |
| 11/18/02   | 10 | 60%        | 6,900                         | 10,400        | 2,000 – 42,400  |
| 11/18/03   | 20 | 35%        | 10,500                        | 30,000        | 4,900 – 141,300 |
| 11/16/04   | 21 | 43%        | 710                           | 1,700         | 560 – 4,400     |
| 11/15/05   | 20 | 55%        | 5,100                         | 9,300         | 560 – 32,200    |
| 11/20/06   | 20 | 25%        | 3,300                         | 13,100        | 1,700 – 43,800  |

Table 1.09 (continued). Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from three sites in the Williams Fork River.

| Date<br>mm/dd/yy  | N  | Prevalence    | Overall Mean<br>Concentration | Positive Fish |                 |
|---|----|---------------|-------------------------------|---------------|-----------------|
|   |    |               |                               | Mean          | Range           |
| <u>1.6 km below Williams Fork Reservoir, immediately below Treatment site</u> |    |               |                               |               |                 |
| 08/06/01  | 20 | 45%           | 12,600                        | 28,000        | 5,600 – 57,900  |
| 11/18/02  | 15 | 53%           | 26,900                        | 50,500        | 1,900 – 342,700 |
| 11/18/03  | 20 | 80%           | 18,800                        | 23,500        | 2,100 – 99,200  |
| 11/16/04  | 21 | 76%           | 12,200                        | 16,100        | 560 – 66,700    |
| 11/15/05  | 20 | 55%           | 1,900                         | 3,500         | 560 – 15,000    |
| 11/20/06  | 20 | 40%           | 2,500                         | 6,300         | 1,700 – 20,200  |
| <u>2.6 km below Williams Fork Reservoir</u>                                   |    |               |                               |               |                 |
| 09/12/01  | 20 | 55%           | 21,600                        | 39,200        | 4,300 – 113,700 |
| 11/18/02  | 15 | 53%           | 3,600                         | 6,700         | 1,600 – 13,600  |
| 11/18/03  | 20 | 90%           | 14,300                        | 15,800        | 2,900 – 61,500  |
| 11/16/04  | 20 | 60%           | 31,500                        | 52,500        | 5,600 – 240,000 |
| 11/15/05  |    | Not collected |                               |               |                 |
| 11/20/06  | 20 | 40%           | 6,000                         | 14,900        | 3,400 – 33,700  |



Willow Creek (Colorado River drainage)

The American beaver *Castor canadensis* activity mentioned in the last report became so extensive that the entire project area is inundated and will remain so for the foreseeable future. No oligochaete collections were made in 2006 because it is no longer possible to evaluate the effect of the backwater isolation due to the flooding of the area by beaver ponds. It has become quite difficult to obtain fish samples, and no YOY or age 1+ brown trout were encountered below the backwater in 2006 during this segment (Table 1.10, Table 1.11). Therefore it is impossible to compare above versus below myxospore concentrations.

Table 1.10. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from Willow Creek.

| Date<br>mm/dd/yy             | Age | N  | Prevalence | Overall Mean<br>Concentration | Positive Fish |                  |
|------------------------------|-----|----|------------|-------------------------------|---------------|------------------|
|                              |     |    |            |                               | Mean          | Range            |
| Above Willow Creek Gage      |     |    |            |                               |               |                  |
| 09/30/03                     | 1+  | 20 | 70%        | 21,400                        | 30,600        | 2,600 – 194,700  |
| 09/29/04                     | 1+  | 15 | 40%        | 8,100                         | 20,100        | 5,000 – 64,500   |
| 10/17/05                     | 1+  | 15 | 73%        | 3,800                         | 5,200         | 560 – 12,200     |
| 09/26/06                     | 1+  | 14 | 79%        | 13,900                        | 17,800        | 1,700 – 47,100   |
| Downstream of backwater site |     |    |            |                               |               |                  |
| 09/30/03                     | 1+  | 20 | 60%        | 10,700                        | 17,900        | 2,000 – 41,200   |
| 09/29/04                     | 1+  | 10 | 30%        | 29,200                        | 97,400        | 57,700 – 128,800 |
| 10/17/05                     | 1+  | 13 | 38.5%      | 1,300                         | 3,400         | 560 – 7,800      |
| 09/26/06                     |     |    |            | Not Collected                 |               |                  |

Table 1.11. Results of polymerase chain reaction (PCR) tests of samples of young-of-the-year brown trout collected from Willow Creek. Mean PCR score is based on assigning numerical values to the qualitative score given to indicate strength of signal as follows: negative = 0, weak positive = 1, positive = 2, strong positive = 3, and very strong positive = 4.

| Date                         | Sample size (N)  | Positive fish | Mean PCR score |
|------------------------------|------------------|---------------|----------------|
| Above Willow Creek Gage      |                  |               |                |
| 09/30/03                     | 10               | 10            | 2.6            |
| 09/29/04                     | 13               | 11            | 2.4            |
| 10/17/05                     | none encountered |               |                |
| 09/26/06                     | 10               | 9             | 2.7            |
| Downstream of backwater site |                  |               |                |
| 09/30/03                     | 11               | 7             | 1.7            |
| 09/29/04                     | 20               | 16            | 2.9            |
| 10/17/05                     | 1                | 0             | 0.0            |
| 09/26/06                     | none encountered |               |                |

## DISCUSSION

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

Over the last two segments the frequency of actinospore detection and often the estimated densities of actinospores have fallen at virtually all of the monitored study sites. This is certainly an encouraging development; however there has not been a concurrent drop in average prevalence or density of myxospores in individual brown trout heads. The reasons for this are unclear but the situation suggests that low levels of actinospore density are sufficient to maintain moderate to high prevalence of infection in wild brown trout populations.

At the annual Whirling Disease Symposium convened in Denver in February 2005,

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

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

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 *Myxobolus cerebralis* from man-made ponds and settling ponds known to be focuses of infectivity.

Period Covered: July 1, 2006 to June 30, 2007

## INTRODUCTION

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

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

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

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

## Segment Objectives:

1. Continue to monitor triactinomyxon densities at established study sites.
2. Collect worm samples from Chalk Cliffs Rearing Unit ponds to establish lineage composition baseline data. Test the worm samples for *Mc* presence.
3. Monitor infectivity in trout from the Roaring Judy effluent ditch between the end of the concrete raceways and the new kokanee trap.
4. Remove brook trout fry from the upper two ponds on the Cap-K Ranch.
5. Obtain estimates of rainbow trout remaining in Roaring Judy ponds after the kokanee spawn-take. Remove all stocked rainbow trout and submit samples for analysis of myxospore concentration by PTD.
6. Collect samples of age 1+ brown trout above and below off-channel sources of *Mc* infectivity on Quartz Creek, East River, and Fryingpan River.

## METHODS and MATERIALS

### Field Filtration and Sample Collection

Two 120-L volumes of water were filtered monthly at each study site through 20-um Pecap screen to concentrate actinospores. These concentrates were examined for the presence of *M. cerebralis* actinospores in the lab by established protocols (Thompson and Nehring 2000). The filtration protocol is the same that has been in use since July 2004 (Thompson 2006).

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

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

### Fish Removal

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



km above Cap K Ranch and near Taylor Creek confluence serve as upstream and downstream evaluation sites for the manipulations that occur on the Cap K Ranch (Figure 2.02). Actinospores were detected five times below and two times above Cap K Ranch during this segment.

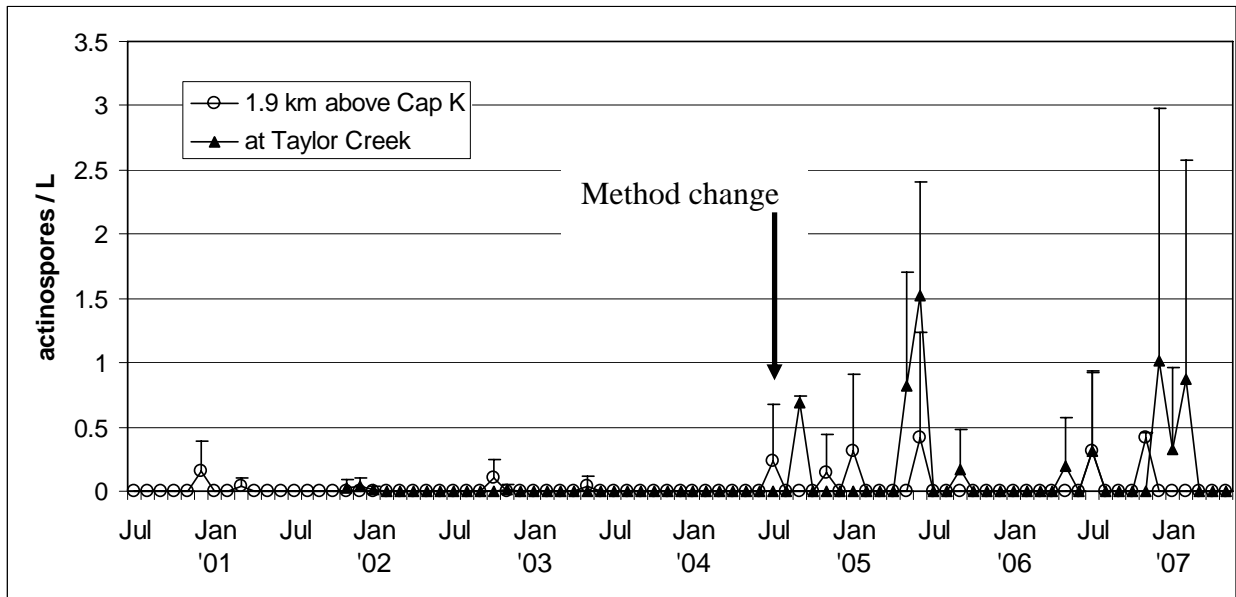


Figure 2.02. Results of water filtration to estimate ambient density of *M. cerebralis* actinospores (N/L) at three sites in the Fryingpan River from July 2001 to May 2007. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Samples of age 1+ brown trout were obtained during this segment from sites in the Fryingpan River above and below the Cap-K Ranch (Table 2.01). The samples acquired in 2006 are the third year-class of brown trout hatched in the river after the construction of the filter in Pond 6. These samples indicate that prevalence of parasite infection remains high and fairly stable at the two lower collection sites. Average spore concentrations were somewhat higher at each site in 2006 compared the previous year.

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

| Date   | Age | N  | Prevalence | Overall Mean  | Positive Fish |                 |
|--|-----|----|------------|---------------|---------------|-----------------|
|  |     |    |            | Concentration | Mean          | Range           |
| <u>1 Km below Ruedi Dam</u>                  |     |    |            |               |               |                 |
| 10/28/00                                     | 1+  | 10 | 0%         | ---           | ---           | ---             |
| 10/30/01                                     | 1+  | 11 | 36.4%      | 7,800         | 21,500        | 2,780 – 35,800  |
| 10/29/03                                     | 1+  | 20 | 45.0%      | 38,500        | 85,600        | 4,880 – 541,300 |
| 10/26/04                                     | 1+  | 23 | 82.6%      | 26,500        | 33,200        | 560 – 254,400   |
| 11/02/05                                     | 1+  | 15 | 53.3%      | 3,700         | 6,900         | 560 – 20,600    |
| 11/02/06                                     | 1+  | 20 | 50.0%      | 10,600        | 21,200        | 1,700 – 55,600  |
| <u>1.6 Km above Cap-K Ranch</u>              |     |    |            |               |               |                 |
| 10/28/00                                     | 1+  | 10 | 10.0%      | 26,900        | 269,300       | 269,300         |
| 10/30/01                                     | 1+  | 10 | 60.0%      | 15,700        | 26,100        | 2,670 – 71,000  |
| 10/29/03                                     | 1+  | 20 | 55.0%      | 21,800        | 39,600        | 4,560 – 112,300 |
| 10/26/04                                     | 1+  | 21 | 85.7%      | 46,900        | 55,200        | 1,670 – 197,800 |
| 11/02/05                                     | 1+  | 20 | 90.0%      | 18,100        | 20,100        | 1,100 – 173,300 |
| 11/02/06                                     | 1+  | 20 | 95.0%      | 71,800        | 75,600        | 3,400 – 244,100 |
| <u>Taylor Creek 4.8 km below Cap K Ranch</u> |     |    |            |               |               |                 |
| 10/31/00                                     | 1+  | 9  | 55.6%      | 37,700        | 67,800        | 9,300 – 181,900 |
| 10/30/01                                     | 1+  | 11 | 63.6%      | 35,900        | 56,400        | 2,500 – 147,500 |
| 10/29/03                                     | 1+  | 20 | 80.0%      | 29,600        | 37,000        | 1,500 – 189,900 |
| 10/27/04                                     | 1+  | 20 | 85.0%      | 16,000        | 18,800        | 1,100 – 60,000  |
| 11/02/05                                     | 1+  | 20 | 85.0%      | 10,600        | 12,400        | 1,100 – 82,200  |
| 11/02/06                                     | 1+  | 19 | 78.9%      | 21,400        | 27,200        | 1,700 – 104,400 |

### Chalk Cliffs Rearing Unit

Several of the rearing ponds and the effluent pond at Chalk Cliffs were sampled during this segment to characterize the *Tubifex* populations in them. The material that composed each sample was a composite from several areas in each individual pond so that resulting data would more closely characterize the subject pond rather than an isolated location within the pond. From each composite sample two worm samples were obtained by selecting 50 haired worms (if available) for characterization of the genetic material by quantitative real-time PCR (Table 2.02).

The worm population has been sampled at Chalk Cliffs on three occasions now; the one thing that is certain is that lineage V worms are not present. The others vary considerably both between ponds and over time within a pond. Despite our efforts to collect worms in each pond from



a variety of places, it is clearly possible that the figures presented in Table 2.02 are distorted by the patchiness that characterizes oligochaete populations as well as the small sample size in each pond. One encouraging note on the sampling done over the last couple of years is the generally high proportion of non-susceptible worms occurring in the settling pond.

Table 2.02. *Tubifex* lineage composition estimated by qPCR on replicate 50-haired worm samples from each of four production ponds and the settling pond at Chalk Cliffs Rearing Unit.

| Location      | N | Approximate percent DNA composition by <i>M. cerebralis</i> lineage |      |     |      |
|---------------|---|---|------|-----|------|
|               |   | I   | III  | V   | VI   |
| 12/13/2005    |   |   |      |     |      |
| Pond 1        | 2 | 32.5  | 41.5 | 0.0 | 25.5 |
| Pond 3        | 2 | 0.0   | 46.0 | 0.0 | 54.0 |
| Pond 5        | 2 | 0.0   | 28.5 | 0.0 | 71.5 |
| Pond 7        | 2 | ---   | ---  | --- | ---  |
| Settling Pond | 2 | 6.0   | 1.0  | 0.0 | 93.0 |
| 04/26/2006    |   |   |      |     |      |
| Pond 1        | 2 | 17.5  | 39.0 | 0.0 | 43.5 |
| Pond 3        | 2 | 1.5   | 75.0 | 0.0 | 23.0 |
| Pond 5        | 2 | 0.0   | 84.0 | 0.0 | 16.0 |
| Pond 7        | 2 | 0.0   | 41.0 | 0.0 | 59.0 |
| Settling Pond | 2 | 8.0   | 29.0 | 0.0 | 63.0 |
| 12/18/2006    |   |   |      |     |      |
| Pond 1        | 2 | 35.0  | 9.0  | 0.0 | 56.0 |
| Pond 3        | 2 | 0.0   | 42.5 | 0.0 | 57.5 |
| Pond 5        | 2 | 0.0   | 49.5 | 0.0 | 50.5 |
| Pond 7        | 2 | 0.0   | 6.5  | 0.0 | 93.5 |
| Settling Pond | 2 | 40.5  | 11.0 | 0.0 | 48.5 |

### Pitkin Rearing Unit

Trout reared at the Pitkin Rearing unit first tested positive for *M. cerebralis* in March 1997. The unit was taken out of production in 2001 and extensive renovation, modernization and securing of springs and well-water supplies was accomplished. The use of Quartz Creek surface water for rearing fish was discontinued upon re-start of the unit. The unit regained *M. cerebralis*-negative certification in January 2007.

Monitoring of actinospore densities began at the Pitkin Rearing Unit in November 2001 and continued during this segment. Actinospores of *M. cerebralis* were observed on two occasions in the effluent of the settling pond and on one occasion in Quartz Creek above the hatchery effluent (Figure 2.03). On all occasions the estimated densities were low.

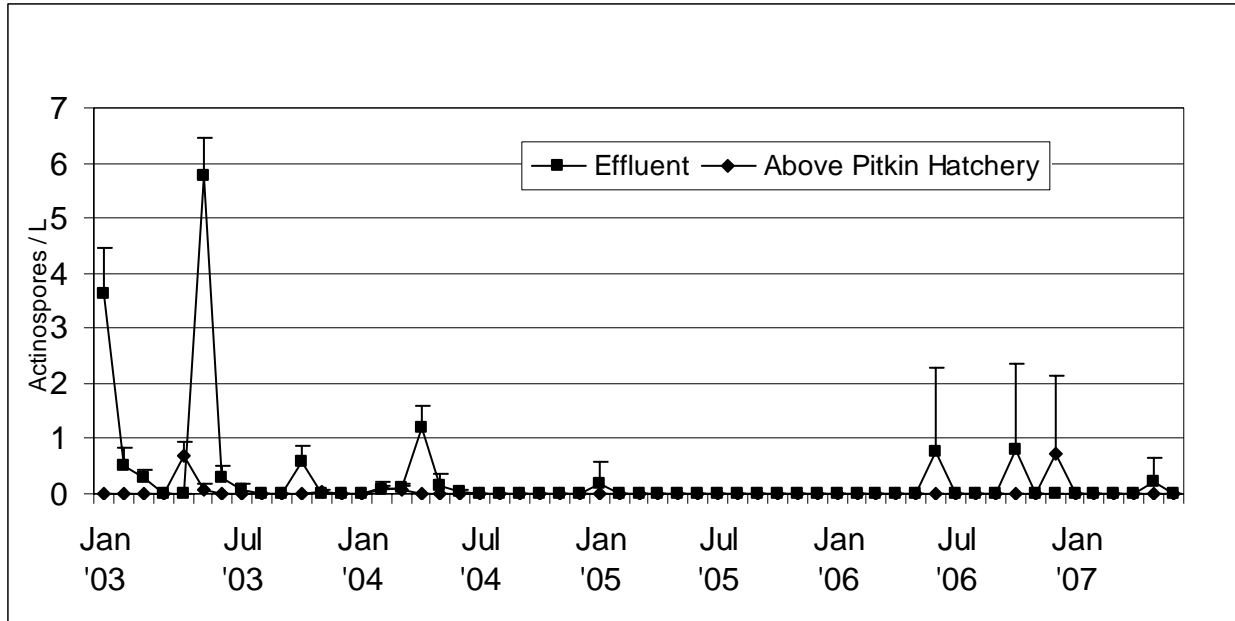


Figure 2.03. Results of water filtration to quantify actinospores of *M. cerebralis* in samples of water at Pitkin Hatchery, January 2003 through June 2007.

Pitkin Unit personnel removed all feral fish from the unit’s settling pond during unit renovation in 2001-02. It remains essentially free of fish. To no longer have a myxospore source available to the *T. tubifex* community residing in the settling pond has had a positive impact on the infectivity observed in the effluent. The oligochaete population in the settling pond is robust and the pond was sampled once during this segment in order to determine the composition of the *Tubifex* community. The results are not yet available.

Table 2.03. Cranial *Myxobolus cerebralis* myxospore concentrations in brown trout sampled from Quartz Creek above and below the Pitkin Fish Rearing Unit.

| Date<br>mm/dd/yy                  | Age | N  | Prevalence | Overall Mean<br>Concentration | Positive Fish |                 |
|-----------------------------------|-----|----|------------|-------------------------------|---------------|-----------------|
|                                   |     |    |            |                               | Mean          | Range           |
| Upstream of Pitkin Rearing Unit   |     |    |            |                               |               |                 |
| 08/28/03                          | 1+  | 20 | 10.0%      | 2,900                         | 29,400        | 25,300 – 33,500 |
| 08/09/04                          | 1+  | 20 | 85.0%      | 15,400                        | 18,100        | 1,700 – 50,100  |
| 08/17/05                          | 1+  | 20 | 40.0%      | 17,400                        | 43,600        | 2,500 – 151,500 |
| 09/05/06                          | 1+  | 20 | 70.0%      | 30,900                        | 44,100        | 1,700 – 222,200 |
| Downstream of Pitkin Rearing Unit |     |    |            |                               |               |                 |
| 08/28/03                          | 1+  | 20 | 45.0%      | 10,200                        | 22,700        | 4,900 – 59,400  |
| 08/09/04                          | 1+  | 20 | 95.0%      | 67,200                        | 70,700        | 1,500 – 489,300 |
| 08/17/05                          | 1+  | 20 | 60.0%      | 10,400                        | 17,300        | 2,800 – 68,800  |
| 09/05/06                          | 1+  | 20 | 60.0%      | 22,500                        | 37,500        | 1,700 – 200,300 |

During this segment brown trout samples were collected from Quartz Creek approximately one mile above and below Pitkin Rearing Unit. In contrast to last year, prevalence was higher above the unit and stable below the unit (Table 2.03). The prevalence and average concentration upstream of Pitkin Rearing Unit over the last three years suggests that the parasite is becoming better established in Quartz Creek above Pitkin Rearing Unit.

Poudre Rearing Unit

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

The frequency that actinospores of *M. cerebralis* were encountered during this segment was greatly diminished compared to previous years (Figure 2.04). Estimated densities remained low in the PRU effluent compared to the historic high numbers seen in 1999-2000 when it was common to observe > 10 / L.

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

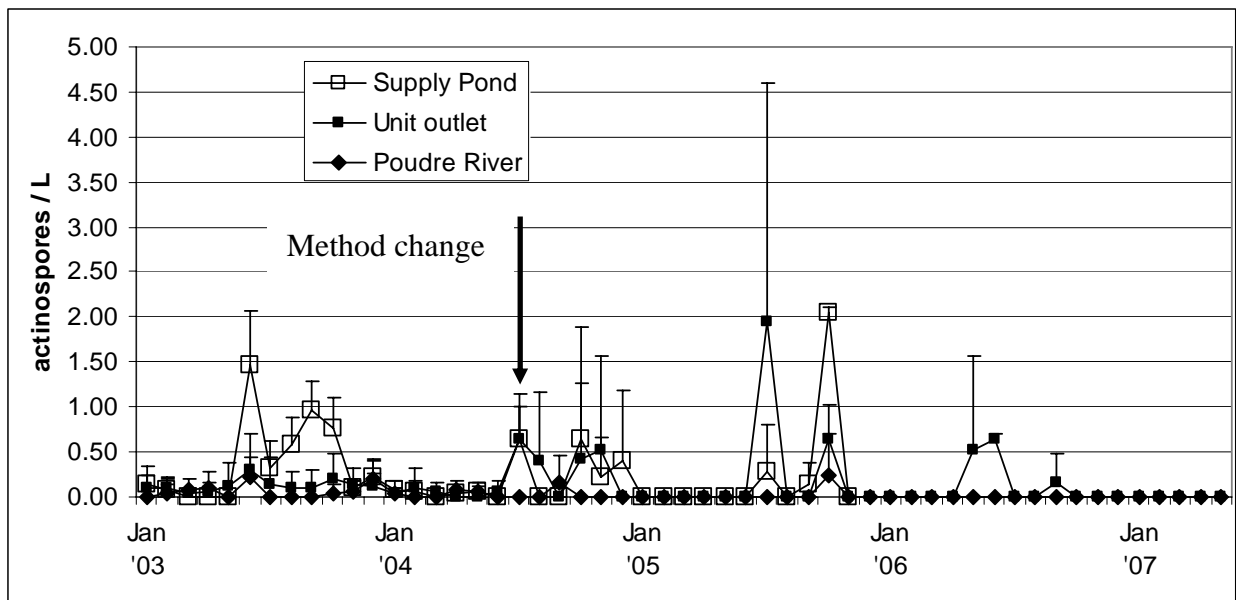


Figure 2.04. Comparison of actinospore densities from the Poudre River, the Supply pond, and the Unit effluent through May 2007. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the body of water.

Samples of brown trout were obtained above and below PRU again during this segment (Table 2.04). Prevalence of infection was higher at both sites this year compared to last year. The average myxospore concentration was much higher in the sample from above PRU, however the one outlier had a large effect on the mean. Without that outlier at the Big Bend site the average for that sample decreases from 50,600 to 12,900 myxospores. This figure is quite close to the average for the sample below PRU.

Table 2.04. Cranial *Myxobolus cerebralis* myxospore concentrations in age 1+ brown trout sampled from the Poudre River above and below the Poudre Rearing Unit (PRU).

| Date<br>mm/dd/yy              | N  | Prevalence | Overall Mean<br>Concentration | Positive Fish |                 |
|-------------------------------|----|------------|-------------------------------|---------------|-----------------|
|                               |    |            |                               | Mean          | Range           |
| Big Bend – above PRU          |    |            |                               |               |                 |
| 09/19/00                      | 10 | 50%        | 6,300                         | 12,600        | 990 – 37,600    |
| 10/22/03                      | 12 | 41.7%      | 3,900                         | 9,400         | 920 – 16,000    |
| 10/28/04                      | 15 | 40.0%      | 17,100                        | 42,900        | 5,600 – 92,300  |
| 11/02/05                      | 15 | 60.0%      | 3,600                         | 6,000         | 560 – 27,200    |
| 10/16/06                      | 10 | 80.0%      | 50,600                        | 63,200        | 3,400 – 439,400 |
| Pasquinel’s cabin – below PRU |    |            |                               |               |                 |
| 09/19/00                      | 9  | 22.2%      | 4,300                         | 21,000        | 3,900 – 35,100  |
| 10/22/03                      | 21 | 14.3%      | 1,800                         | 12,600        | 6,900 – 21,000  |
| 10/28/04                      | 6  | 0%         | ---                           | ---           | ---             |
| 11/02/05                      | 15 | 60.0%      | 3,500                         | 5,900         | 560 – 27,200    |
| 10/16/06                      | 7  | 100.0%     | 13,500                        | 13,500        | 1,700 – 60,600  |

## Roaring Judy Rearing Unit

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

While the Roaring Judy Unit regained certification as a *M. cerebralis*-free facility in the spring of 2005, that designation was lost in 2006. Research continues on methods and management strategies to minimize the number of actinospores in the settling pond effluent. Monthly monitoring during this segment resulted in the detection of actinospores in the unit effluent on the fewest occasions since monitoring began, just once in 12 months (Figure 2.05).

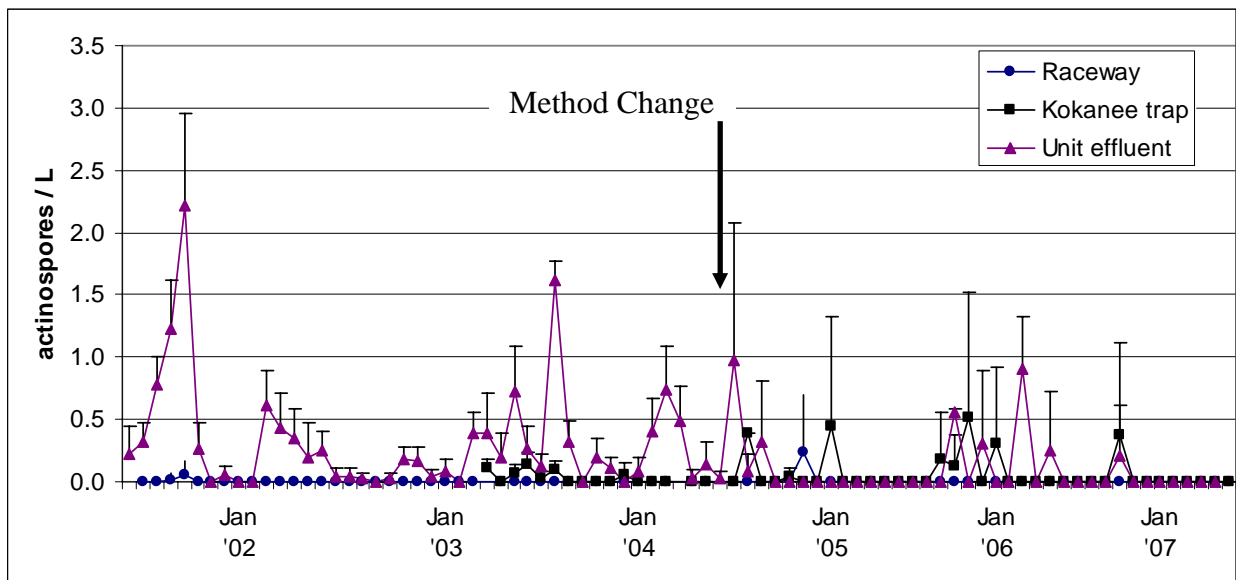


Figure 2.05. Comparison of actinospore densities from the ROJ concrete Raceway tailbox, the kokanee trap (downstream of the concrete raceways), and the Unit effluent through May 2007. Error bars are 95% confidence limits for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Samples of the trout removed from the effluent channel show that prevalence and intensity of *M. cerebralis* infection can be substantial (Table 2.05). Recent samples of older rainbow trout show lower prevalence and intensity of infection after the removal efforts that occurred in 2003 and 2004. This population will continue to be monitored for myxospore concentration and parasite prevalence. If these metrics rise in the future, additional fish removals may be prudent to eliminate heavily infected fish from the channel.

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

| Date<br>mm/dd/yy | Age (yrs) | Sample Size |            | Overall Mean<br>Concentration | Positive Fish |                   |
|------------------|-----------|-------------|------------|-------------------------------|---------------|-------------------|
|                  |           | N           | prevalence |                               | Mean          | Range             |
| Brown trout      |           |             |            |                               |               |                   |
| 05/16/03         | 1         | 12          | 75%        | 39,900                        | 53,200        | 2,000 – 177,750   |
| 11/25/03         | 1+        | 20          | 70%        | 29,700                        | 42,400        | 4,400 – 150,700   |
| 05/24/04         | 1         | 20          | 75%        | 23,500                        | 31,300        | 300 – 161,900     |
| 11/30/04         | 1+        | 20          | 75%        | 25,700                        | 34,300        | 1,100 – 193,300   |
| 11/22/05         | 1+        | 19          | 68%        | 37,500                        | 54,700        | 2,900 – 335,800   |
| 11/30/06         | 1+        | 20          | 35%        | 25,500                        | 39,200        | 3,100 – 101,400   |
| Rainbow trout    |           |             |            |                               |               |                   |
| 05/16/03         | 2         | 21          | 100%       | 367,400                       | 367,400       | 3,700 – 2,242,500 |
| 11/25/03         | 1+        | 22          | 50%        | 57,700                        | 115,300       | 5,400 – 597,400   |
| 05/24/04         | 1         | 20          | 5%         | 100                           | 1,850         | 1,850             |
| 05/24/04         | 2         | 20          | 80%        | 458,000                       | 572,200       | 4,200 – 3,111,800 |
| 11/30/04         | 1+        | 20          | 20%        | 9,600                         | 47,800        | 5,500 – 157,000   |
| 11/30/04         | 2+        | 25          | 12%        | 3,700                         | 30,600        | 5,000 – 80,400    |
| 11/22/05         | 2+        | 25          | 36%        | 21,400                        | 59,400        | 7,400 – 234,600   |
| 11/30/06         | 2+        | 25          | 20%        | 7,500                         | 37,500        | 3,100 – 76,800    |

The two west settling ponds were stocked with 4000 fin-clipped catchable Tasmanian strain rainbow trout from the Roaring Judy Rearing Unit in May and June 2006. Samples of the remaining fish were collected in November during the kokanee spawn, having followed the kokanee into the trap. As in 2005, the samples showed low prevalence and myxospore concentrations (Table 2.06).

Table 2.06. Cranial *Myxobolus cerebralis* myxospore concentrations in trout sampled from the Roaring Judy State Fish Rearing Unit settling ponds.

| Date<br>mm/dd/yy | Species or<br>Strain   | Sample Size |            | Overall Mean<br>Concentration | Positive Fish |                   |
|------------------|------------------------|-------------|------------|-------------------------------|---------------|-------------------|
|                  |                        | N           | prevalence |                               | Mean          | Range             |
| Settling Ponds   |                        |             |            |                               |               |                   |
| 11/04/03         | Tasmanian <sup>a</sup> | 28          | 28.6%      | 5,100                         | 17,800        | 3,300 – 40,000    |
| 11/04/03         | Bellaire <sup>a</sup>  | 23          | 43.5%      | 17,200                        | 39,600        | 3,300 – 136,500   |
| 11/04/03         | Rainbow <sup>b</sup>   | 16          | 93.8%      | 365,700                       | 390,100       | 7,200 – 1,387,400 |
| 11/30/04         | Brown <sup>c</sup>     | 20          | 90%        | 22,600                        | 25,100        | 560 – 142,200     |
| 11/30/04         | Brown <sup>d</sup>     | 20          | 85%        | 11,800                        | 14,100        | 1,100 – 64,400    |
| 11/30/04         | Erwin <sup>e</sup>     | 18          | 72.2%      | 35,800                        | 49,500        | 4,400 – 199,500   |
| 11/30/04         | Bellaire <sup>e</sup>  | 30          | 20%        | 34,700                        | 173,400       | 6,300 – 942,200   |
| 11/30/04         | Rainbow <sup>b</sup>   | 6           | 50%        | 93,800                        | 187,600       | 44,500 – 370,300  |
| 11/08/05         | Tasmanian              | 25          | 8%         | 3,100                         | 38,900        | 3,800 – 74,000    |
| 11/08/05         | Bellaire               | 25          | 4%         | 315                           | 7,900         | 7,900             |
| 11/07/06         | Tasmanian              | 15          | 20%        | 8,600                         | 10,800        | 1,700 – 26,900    |
| 11/07/06         | Rainbow <sup>b</sup>   | 16          | 38%        | 12,400                        | 50,200        | 3,100 – 202,700   |

a: Tasmanian strain rainbow trout were from the *M. cerebralis*-negative Crystal Rearing Unit, and the Bellaire strain rainbow trout were from the *M. cerebralis*-negative Rifle Rearing Unit.

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

c: Captured in upper pond.

d: Captured in lower pond.

e: Erwin strain rainbow trout were from the *M. cerebralis*-negative Rifle Rearing Unit, and the Bellaire strain rainbow trout were from the *M. cerebralis*-negative Durango Rearing Unit.

Population estimates on the west settling ponds were again conducted during early December, and indicated that very few of the stocked catchable rainbow trout remained in the ponds, (Table 2.07). Many of the rainbow trout represented in the lower pond estimate were unmarked, indicating they were feral fish or escapees from the rearing unit. It would appear that the annual stocking of 3-4000 catchable rainbow trout into the settling ponds for the purpose of providing recreational fishing opportunity will not appreciably influence the density of actinospores in the pond effluent because most catchables are removed by anglers before they develop myxospores. Stocking in the future should continue to be completed prior to July to ensure that most catchable trout are removed from the system each year.

Table 2.07. Trout population estimates from the Roaring Judy Fish Rearing Unit settling ponds for fish 15 cm and greater.

| Date       | Rainbow trout   |        |       | Brown trout |        |       |
|------------|-----------------|--------|-------|-------------|--------|-------|
|            | N               | 95% CI | Kg/ha | N           | 95% CI | Kg/ha |
| Upper pond |                 |        |       |             |        |       |
| 12/03/03   | 30              | 23     | 8     | 1135        | 269    | 310   |
| 11/23/04   | 12              | 14     | 5     | 1132        | 249    | 315   |
| 11/22/05   | 39              | ---    | 15    | 944         | 169    | 234   |
| 11/27/06   | 34              | 40     | 18    | 605         | 225    | 166   |
| Lower pond |                 |        |       |             |        |       |
| 12/03/03   | 8 <sup>a</sup>  | ---    | 4     | 924         | 220    | 625   |
| 11/23/04   | 10 <sup>a</sup> | ---    | 6     | 1355        | 296    | 1098  |
| 11/22/05   | 31              | 65     | 53    | 620         | 101    | 459   |
| 11/27/06   | 131             | 163    | 161   | 584         | 355    | 491   |

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

### RECOMMENDATIONS and CONCLUSIONS

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

It is recommended that the settling pond at Pitkin continue to be kept as free of fish as possible. Since it appears impractical to depopulate the settling ponds at Roaring Judy at this time, it is further recommended that catchable rainbow trout plants for these ponds continue to 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. Once rainbow trout incorporating resistance to *M. cerebralis* become available they should be used for stocking the Roaring Judy ponds.

Encouragement of angling harvest in the effluent channel at Roaring Judy would result in beneficial use of the trout resources that do occupy that area, and would seem preferable to removing them by electrofishing. Signs were posted in 2005 to encourage angler use; these should remain in place.



The Cap-K Ranch sand filter proved to be a disappointment in the loss of water capacity experienced over a short period of use. Now, it is clear that such filters will not effectively capture actinospores as a long-term solution to *M. cerebralis* infectivity. 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. Even with such changes implemented there is still considerable question whether the life of the filter would be acceptable. Other strategies for reducing infectivity from the Cap-K Ranch ponds and similar habitats appear more appropriate at this time.

Job No. 3: Technical Assistance.

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

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

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

- 1) Sent *Tubifex tubifex* worms to researchers at the National Fish Health Laboratory in West Virginia for use in whirling disease research projects.
- 2) Accommodated a number of internal requests from researchers, hatchery managers, and biologists for actinospore density, temperature, and myxospore concentration data.
- 3) Reviewed one unsubmitted manuscript for Robert Milhous of USGS.
- 4) Provided reprints of a recent paper on using occupancy models for disease research to Dr. Karen McCoy, a researcher for the French government.
- 5) Responded to information and reprint request on the subject of whirling disease to Dr. Betsy Von Holle of the US EPA.
- 6) Responded to spatial whirling disease data request from Dr. Robert DuBey at New Mexico State University.
- 7) Provided comment to the US Bureau of Reclamation regarding flow regimes from Reudi Reservoir and their potential impact on trout.
- 8) Provided temperature data for several years at several sites on the Fryingpan River to Dr. Kurt Fausch at Colorado State University.
- 9) Assisted in providing triactinomyxons of *M. cerebralis* to CSU graduate student Eric Fetherman after funding precluded the University of California-Davis from fulfilling the request for spores.

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