# Whirling Disease/Habitat Interactions

## Federal Aid Project F-427-R2

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

Colorado Division of Wildlife

Fish Research Section

Fort Collins, Colorado

May 2005

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*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-R

Project Objective: To investigate the influence of aquatic habitat factors on the severity of *M. cerebralis (M. 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* (*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 *M. cerebralis*.

Period Covered: July 1, 2004 to June 30, 2005

## INTRODUCTION

In the early 1990's 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 *M. cerebralis*.

Sentinel fish studies in the Colorado River and *M. cerebralis* actinospore filtration studies in numerous drainages indicate 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 2000). Reservoirs may act as such foci. Stocking Spring Creek Reservoir with catchable trout infected with *M. 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 reservoirs 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 collection of baseline oligochaete and triactinomyxon data at Cache la Poudre River study site above Poudre State Fish Rearing Unit (PRU) at Kinikinik.
2. Assist USGS personnel with survey work at Willow Creek study site (post-manipulation).
3. Implement habitat modifications at one study site (Cache la Poudre River).
4. Continue collecting post-manipulation triactinomyxon and fish data at study sites modified previously.
5. Collect post-manipulation oligochaete data at the Willow Creek study site.
6. Conduct electrofishing at standard stations on study streams.

## METHODS and MATERIALS

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

### Fish sampling

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

### Oligochaete sampling

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

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

**Actinospore sampling**

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

Another significant difference implemented during this segment was the replication of water samples at each site. This resulted from the time savings associated with filtering only 120 L of water for each sample rather than 1900 L. Commencing in July 2004 two replicate samples were taken at each monitoring site. A benefit of this strategy is that measures of precision associated with the monitoring may now be applied to the estimate of actinospores in the stream at the time the samples were taken, whereas previously the measure of precision applied only within the single sample taken at each site.

## RESULTS

Beaver Creek (South Fork Rio Grande drainage)

Habitat modifications were accomplished at this site in October 2001. Monitoring below this modified site for actinospores continued through the current segment, and indicates low actinospore densities since the habitat manipulation was completed (Figure 1.01). No actinospores have been detected since June 2003, and just one of the filtrates in this segment yielded a positive PCR test.

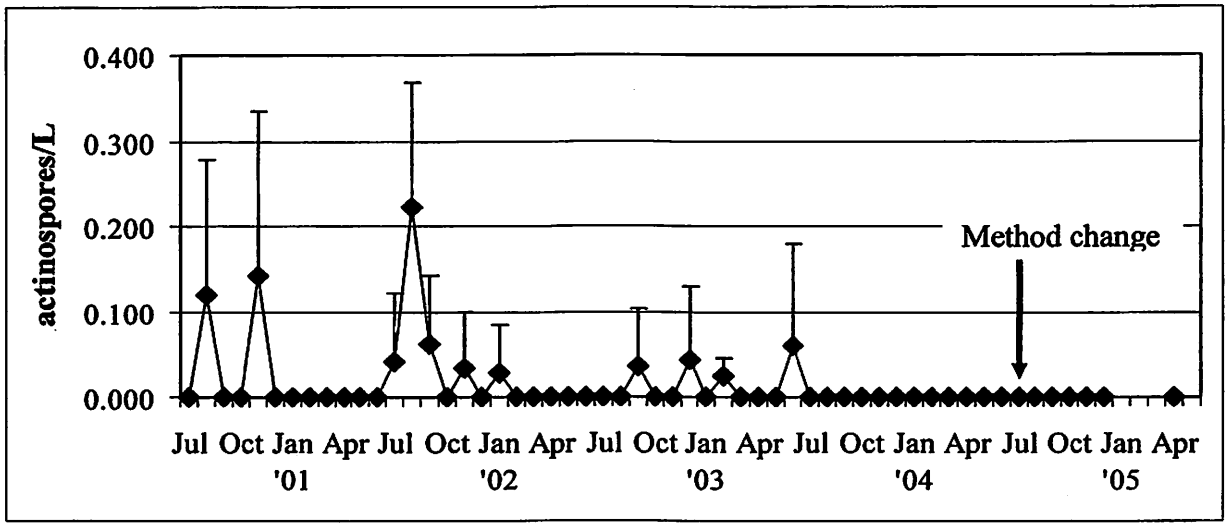


Figure 1.01. Density of actinospores of *M. cerebralis* in Beaver Creek below the side channel containing beaver ponds from July 2000 through May 2005. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later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

Samples of juvenile brown trout taken since 1998 establish that the trout population in this section of stream is substantially infected by the whirling disease parasite. Myxospore evaluations in brown trout suggest that infection prevalence reached a low point in 2002 (Figure 1.02), the year after habitat modification. Since then prevalence has been higher. 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. Results observed over the last two years suggest that despite the reduction of *M. cerebralis* actinospores in Beaver Creek, prevalence and mean myxospore concentrations have not been significantly reduced among the wild brown trout inhabiting the stream.

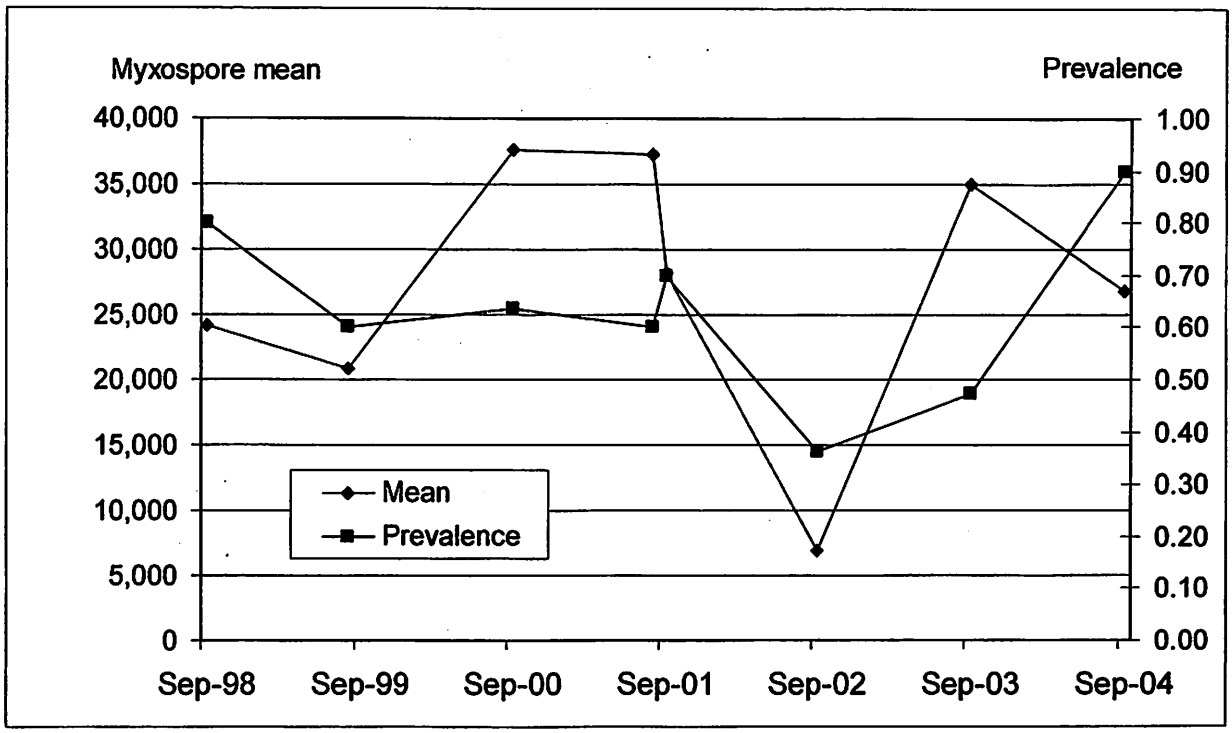


Figure 1.02. Mean myxospore concentration and prevalence of infection in samples of age 1+ brown trout (n = 11 – 20) collected from Beaver Creek below the habitat manipulation site.

Table 1.01. Trout population biostatistics (fish ≥ 15 cm) for Beaver Creek one km below Beaver Creek Reservoir from the fall (September and October) of 1998 through 2004.

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

One promising trend at this site is the reappearance of wild age 1+ rainbow trout during the last few years (Table 1.01). These rainbow trout first were detected again in 2000, prior to habitat modification. During the last two segments the highest number of rainbow trout in seven years was observed. As with the brown trout, the rainbow trout hatched in 2002 were the first to be exposed to ambient stream conditions from hatch under the new habitat conditions. Survivors

were captured as age 1+ rainbow trout in the fall of 2003. Wild age 1+ rainbow trout were observed in fall 2004 also. While gains in the wild rainbow trout population have been modest, the timing of these gains does appear to coincide with the habitat modifications implemented in 2001. The average PCR score obtained from YOY rainbow trout decreased from 3.7 in 2002 (n = 22) to 1.9 in 2003 (n = 15), but was up again in 2004 (2.8, n = 11).

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. 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 we decided 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 have been collected above and below the Kinikinik site since January 2003 (Figure 1.03). Additional samples were collected at the Bliss State Wildlife Area, about 1.5 km upstream of the Kinikinik site. Density estimates were low on all occasions, and no actinospores have been observed in water filtrates at this study site in 12 months. The samples indicate that on average there are more actinospores in the river at Kinikinik than upstream at Bliss SWA. The Bliss site is no longer being monitored regularly.

Pre-construction oligochaete sampling was completed during this segment (Table 1.02). Lineage V, considered to contain 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), 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.

This unexpected change in lineage DNA should be viewed with caution, because the proportions reflect only the DNA present in the samples and not necessarily the proportions of worms present in the samples. This is the case because differently sized worms yield differing amounts of DNA, and the individuals comprising the 50-worm samples are not necessarily the same size. Nevertheless, the changes in our samples may reflect an actual change in the worm population structure, a phenomenon currently being explored in Windy Gap Reservoir as well. If it reflects a change in the worm population structure in the river at Kinikinik, it may also help to explain the failure to detect actinospores at this site during the last 12 months. A significant

decrease in the number of susceptible lineage III individuals at this site could well translate into reduced numbers of actinospores being produced at the site.

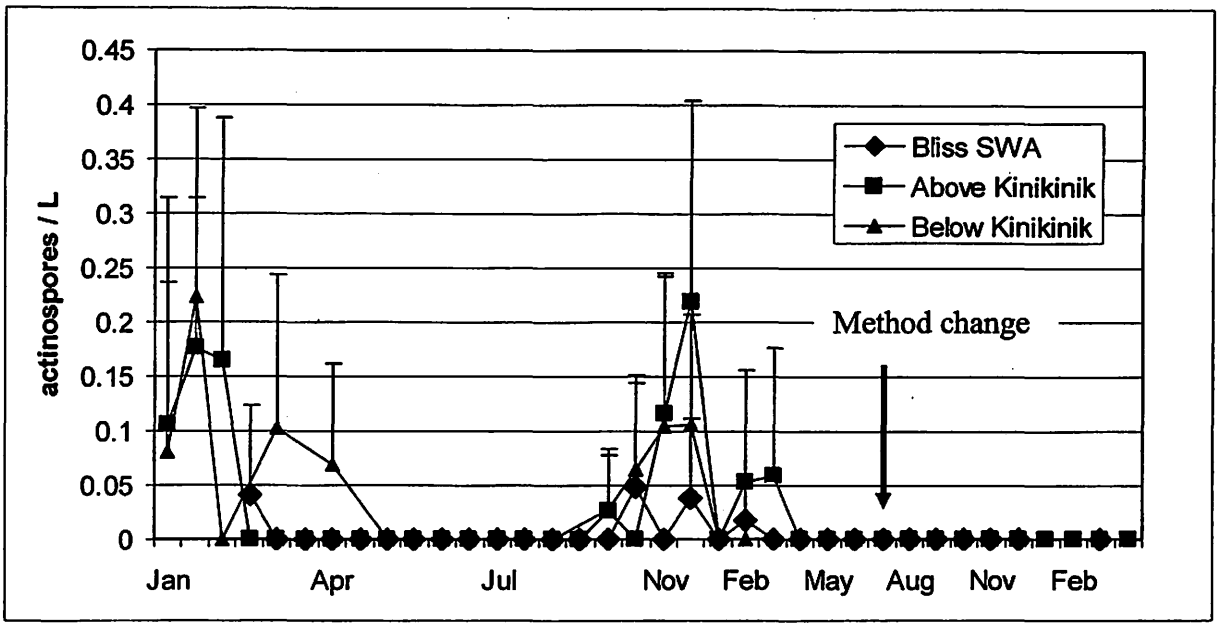


Figure 1.03. Estimates of actinospores/L in the Poudre River at three sites near Kinikinik from January 2003 through March 2005. Bliss State Wildlife Area (SWA) is upstream of the Kinikinik sites. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream. Early sampling frequency was twice per month, hence the uneven x-axis.

Table 1.02. Estimates of the proportion of each *T. tubifex* lineage DNA 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 *M. cerebralis* lineage | | | |
|---|---|---|---|---|---|
| | | I | III | V | VI |
| 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) |

The available 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 have been fairly low at both sites.

Habitat modifications designed to isolate both of the backwater areas at Kinikinik were accomplished in September and October 2004. At each backwater a berm was constructed with native fill material. Excessive groundwater input within the upper backwater necessitated the

placement of a filter section within the upper backwater isolation berm. As a precaution, a similar filter section was placed in the lower berm. These filter sections consisted of a 30.5-cm horizontal thickness of 0.45 – 0.55-mm sand 1 m deep, encased by 20.3-cm thicknesses of 6.35 mm pea gravel, after which these sections of the berm were completed with 19 – 38-mm gravel rather than the native fill material used in the remainder of the berms. Protective cobble armament sized to withstand predicted shear velocities was placed over the entire berm at both upper and lower locations. These berms were designed to preclude 90% or more of all average daily flows in this reach from entering the backwater areas, according to data from a discontinued gage near Rustic.

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

| Date mm/dd/yy | N | Prevalence | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|
| | | | | Mean | Range |
| Bliss State Wildlife Area – above Kinikinik | | | | | |
| 09/30/02 | 10 | 10% | 2,800 | 28,100 | 28,100 |
| 10/22/03 | 20 | 40% | 4,400 | 11,000 | 2,300 – 31,600 |
| 10/28/04 | 10 | 20% | 2,600 | 13,000 | 9,200 – 16,700 |
| 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 |
| 10/28/04 | 15 | 40.0% | 17,100 | 42,900 | 5,600 – 92,300 |

Colorado River

A long history of trout population evaluation on the 3.2-km reach of the Colorado River that encompasses the current Kemp/Breeze Wildlife Area clearly indicates that what was once a prodigious self-sustaining rainbow trout fishery has collapsed, beginning in the early 1990's (Figure 1.04, Table 1.04). Previous intensive study in the area points 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 appears to have benefited from reduced competition. The trend in brown trout numbers and biomass reflects substantial increase over the values observed in the 1980's (Table 1.04).

Samples of juvenile brown trout obtained since 1999 for analysis of cranial myxospore concentrations by PTD (Markiw and Wolf 1974) indicate that prevalence of infection is routinely 60% or greater (Table 1.05). Samples were collected in 2004 at the Kemp/Breeze Wildlife Area as well as at the Hitching Post Bridge downstream of Windy Gap Reservoir.

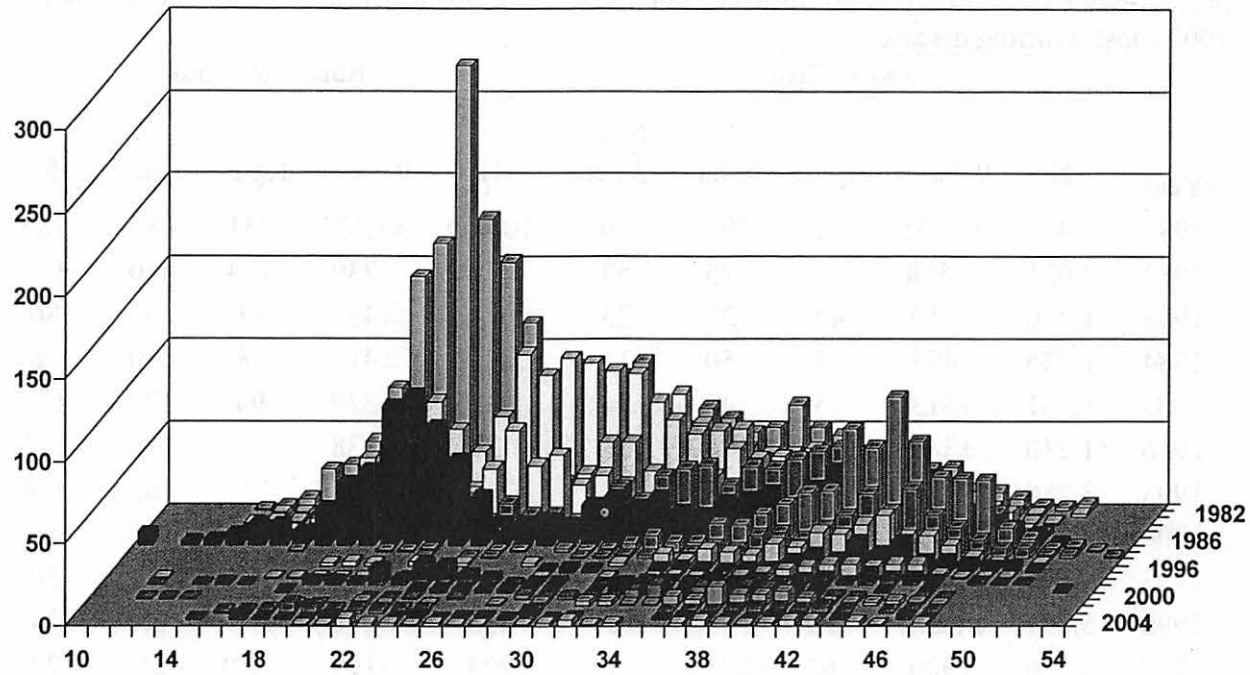


Figure 1.04. Total numbers of unmarked rainbow trout caught during mark and recapture population estimates on the Kemp/Breeze Wildlife Area of the Colorado River in 1981 – 1986 (pre whirling disease years) and 1993 – 2004 (post whirling disease years).

Baseline oligochaete sampling in the Kemp/Breeze Wildlife Area of the Colorado River was completed in 2001 (Table 1.06).

Table 1.04. 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 |
| 2004 | 9,720 | ±2,399 | 268 | 847 | 124 | 351 | ±450 | 16 | 31 | 15 |

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 estimate reported is based on the hypothetical recapture of one fish, but clearly does not satisfy the assumptions of the model for the rainbow trout component of the population.

Actinospore densities were monitored at the Breeze Bridge once each month during the last segment. *M. cerebralis* actinospores were observed on only one of nine occasions so far in this segment (Figure 1.05). This represents a higher detection rate than during the previous segment, but still lower than the earlier years of actinospore monitoring at this site.

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

| Date | N | Prevalence | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|
| | | | | Mean | Range |
| Hitching Post Bridge 1.9 km below Windy Gap Reservoir | | | | | |
| 09/29/99 | 10 | 80.0% | 6,330 | 7,920 | 1,110 – 15,550 |
| 10/12/00 | 10 | 100.0% | 58,700 | 58,700 | 8,700 – 208,700 |
| 09/13/01 | 20 | 75.0% | 20,300 | 27,500 | 4,000 – 96,000 |
| 09/27/02 | 10 | 60.0% | 12,300 | 20,400 | 3,500 – 73,800 |
| 09/29/03 | 16 | 68.8% | 11,700 | 17,000 | 2,500 – 43,700 |
| 09/27/04 | 22 | 95.5% | 19,700 | 20,700 | 560 – 96,700 |
| 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 |

Table 1.06. 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 |

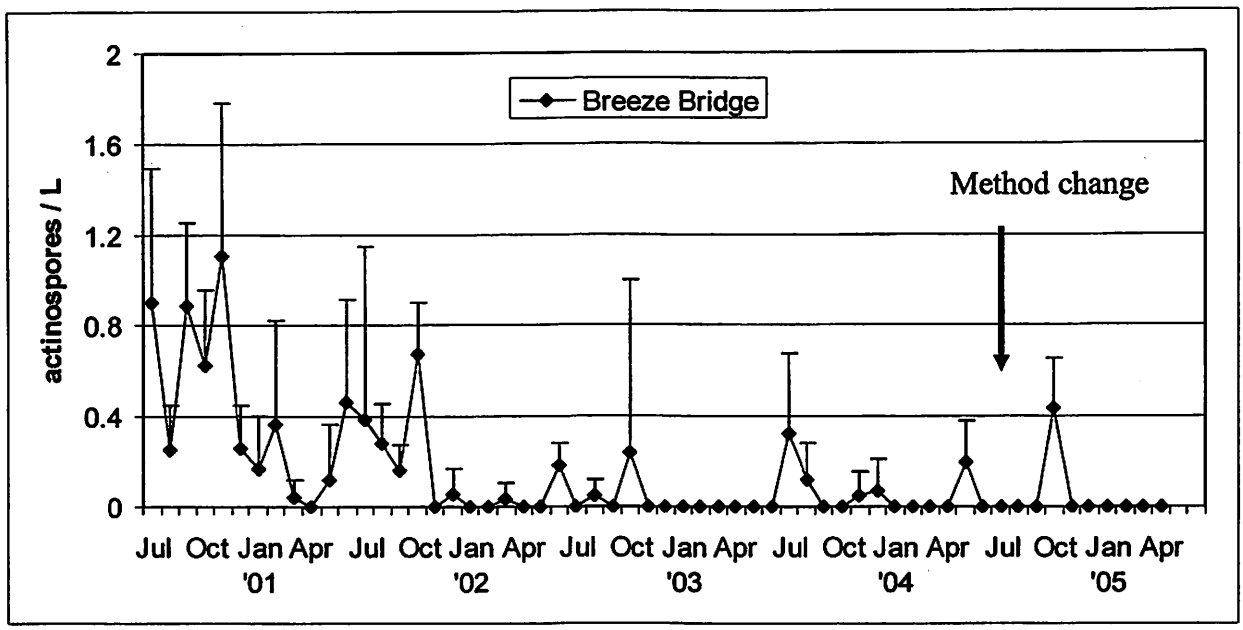


Figure 1.05. Results of water filtration to estimate ambient density of *M. cerebralis* actinospores (N/L) in the Colorado River at Breeze Bridge July 1, 2000 to May 31, 2005. Error bars represent upper 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

The Colorado River at the Kemp/Breeze Wildlife Area will continue to be monitored as a site that has not been modified to eliminate or reduce oligochaete habitat.

Fryingpan River

The Fryingpan River between Ruedi Dam and the Roaring Fork River confluence is a stream that has a long history of trout population study to draw from. Previous study indicates that *M. cerebralis* infection has had a noticeable impact on the rainbow trout population in the lower part of the reach. One focus of infectivity has been identified as the effluent of the Cap-K Ranch ponds (Nehring 1999, Nehring et al. 2000). A search for others throughout the drainage below Ruedi Dam was completed in fall 2002. The conclusion of the study was that the Cap-K Ranch ponds were a far more significant and consistent source of infectivity than any other site studied. A potential in-river site on the Christine Unit of the Basalt Wildlife Area was investigated during previous segments, and was judged to be a minimal impact site. Consequently there are no plans to modify in-river habitats on the Fryingpan River.

Three sites continue to be monitored for ambient actinospore concentrations (Figure 1.06) and for parasite prevalence and myxospore concentration in brown trout (Table 1.07). Actinospores are rarely detected in the Fryingpan River, especially upstream of the Cap K Ranch. However, it is apparent from the fish monitoring that the parasite has increased in prevalence and severity of infection over the last several years at the uppermost monitoring site just downstream of Ruedi Dam (Table 1.07).

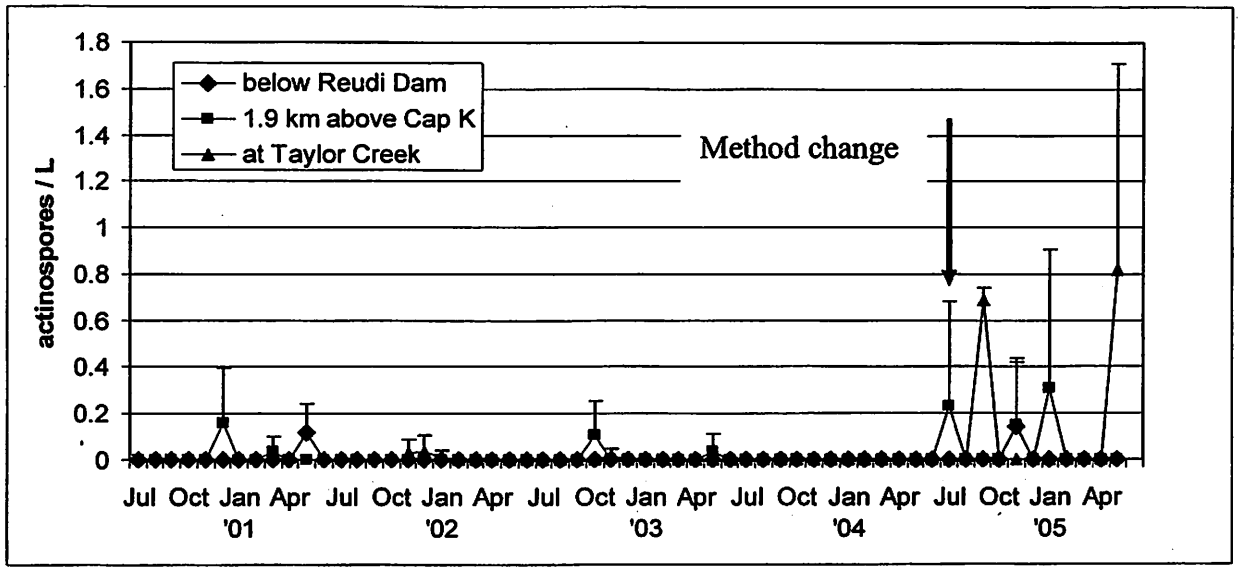


Figure 1.06. Results of water filtration to estimate ambient density of *M. cerebralis* actinospores (N/L) at three sites in the Fryingpan River from July 1, 2001 to May 31, 2005. Error bars are 95% confidence limit for the sample only based on subsample counts through June 2004. Samples taken in July 2004 and later were replicated so confidence limits apply to the stream.

Table 1.07. Cranial *M. cerebralis* myxospore concentrations in brown trout sampled from the Fryingpan River during the fall in 2000-2004.

| Date | Age | N | Prevalence | Overall Mean Concentration | Mean | Positive Fish Range |
|---|---|---|---|---|---|---|
| | | | 1 km below Ruedi Dam | | | |
| 10/28/00 | 1+ | 10 | 0% | | | |
| 10/30/01 | 1+ | 11 | 36.4% | 7,800 | 21,500 | 2,780 – 35,800 |
| 10/29/03 | 1+ | 20 | 45.0% | 38,500 | 85,600 | 4,880 – 541,300 |
| 10/26/04 | 1+ | 23 | 82.6% | 26,500 | 33,200 | 560 – 254,400 |
| | | | 1.6 km above Cap K Ranch | | | |
| 10/28/00 | 1+ | 10 | 10.0% | 26,900 | 269,300 | 269,300 |
| 10/30/01 | 1+ | 10 | 60.0% | 15,700 | 26,100 | 2,670 – 71,000 |
| 10/29/03 | 1+ | 20 | 55.0% | 21,800 | 39,600 | 4,560 – 112,300 |
| 10/26/04 | 1+ | 21 | 85.7% | 46,900 | 55,200 | 1,670 – 197,800 |
| | | | Taylor Creek Confluence | | | |
| 05/30/00 | 1 | 10 | 70.0% | 6,740 | 9,600 | 430 – 31,200 |
| 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 |

Spring Creek (Taylor River drainage)

Habitat modifications occurred on this stream in 2002. Monitoring continued in this segment at both the study site and at upstream and downstream control sites to collect data on ambient actinospore density and on prevalence of infection and myxospore concentration in brown trout. The brown trout population remains stable in this stream; the rainbow trout population sparse and consisting largely of stocked catchable trout (Table 1.08).

Table 1.08. 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 |
| 2004 | 231 | ± 1 | 258 | 2,967 | 2,323 | 0 | --- | 0 | 0 | 0 |
| | Above Spring Creek Campground (control site) | | | | | | | | | |
| 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[a] | ± 2 | 102 | 554 | 21 |
| 2004 | 146 | ± 5 | 124 | 1,538 | 1,245 | 71[a] | ± 4 | 124 | 748 | 0 |
| | At Salsbury Gulch (treatment site) | | | | | | | | | |
| 2002 | 393 | ± 1 | 329 | 2,861 | 1,182 | 0 | --- | 0 | 0 | 0 |
| 2003 | 309 | ± 8 | 288 | 2,803 | 1,240 | 7 | ± 1 | 10 | 63 | 0 |
| 2004 | 347 | ± 2 | 315 | 3,143 | 1,875 | 72[a] | ± 8 | 99 | 649 | 205 |

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

The samples of age 1+ brown trout collected during this segment for myxospore analysis are the first true "post-treatment" samples, having hatched in the spring of 2003. Results indicate that there is no difference in prevalence or infection intensity between the fish collected within and just below the treatment site versus those collected at upstream and downstream unmodified sites (Table 1.09). Also, there is no evidence at the treatment site that myxospore concentration or parasite prevalence in brown trout decreased in the first post-treatment sample compared to pre-treatment samples collected at the same site. No *M. cerebralis* was detected in a sample of 15 age 1+ brown trout collected three km above Spring Creek Reservoir (data not shown in the table).

Table 1.09. Cranial *M. cerebralis* myxospore concentrations in age 1+ brown trout sampled from Spring Creek.

| Date mm/dd/yy | N | Prevalence | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|
| | | | | Mean | Range |
| 0.8 km downstream of Spring Creek Reservoir | | | | | |
| 05/18/01 | 20 | 45% | 6,500 | 14,400 | 1,400 – 56,000 |
| 08/01/01 | 20 | 80% | 21,200 | 26,500 | 4,200 – 82,300 |
| 09/17/02 | 19 | 79% | 43,900 | 55,700 | 2,000 – 195,000 |
| 09/22/03 | 23 | 78% | 63,300 | 80,900 | 4,100 – 316,000 |
| 09/07/04 | 26 | 92% | 50,700 | 54,900 | 4,400 – 56,700 |
| 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 |
| 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 |

Samples of YOY brown trout were collected at the same three sites in September of the last three years. The YOY samples collected in 2003 were the first post-manipulation data. The heads were analyzed by the PCR technique and indicate that there is a high prevalence of infection among YOY brown trout at all three sites for all years (Table 1.10). Prevalence was 100% in the samples from all three sites over the last two years, and there is little difference in the average PCR score among the three sites within any given year. Additionally, the PCR data suggest that average scores may be increasing at all sites.

Water samples taken during the segment continued to indicate that habitat manipulation at this site did not result in reduced actinospore densities following construction. To the contrary, post-construction monitoring has resulted in a greater frequency of actinospore detection compared to pre-construction sampling (Figure 1.07) at both the treatment and control sites. In conjunction with the myxospore and PCR data this suggests that infectivity in this stream is increasing, despite the decrease in oligochaete worm biomass originally observed within the treatment section following habitat modifications (Table 1.11). This observation is tempered by

the possibility that increased sensitivity in the actinospore filtration technique may be partly responsible for the higher frequency of detection in Spring Creek.

Table 1.10. Results of PCR tests of samples of YOY brown trout collected from Spring Creek. Mean PCR score is based on assigning numerical values to the qualitative score given to indicate strength of signal as follows: negative = 0, weak positive = 1, positive = 2, strong positive = 3, and very strong positive = 4.

| Date | Sample size (N) | Positive fish | Mean PCR score |
|---|---|---|---|
| 0.8 km downstream of Spring Creek Reservoir | | | |
| 09/17/02 | 18 | 14 | 1.6 |
| 09/22/03 | 20 | 20 | 2.8 |
| 09/07/04 | 25 | 25 | 3.8 |
| 5 km downstream of Spring Creek Reservoir at Treatment site | | | |
| 09/18/02 | 21 | 18 | 1.9 |
| 08/22/03 | 20 | 20 | 3.1 |
| 09/08/04 | 20 | 20 | 3.4 |
| 19 km downstream of Spring Creek Reservoir at Control site | | | |
| 09/18/02 | 10 | 10 | 2.3 |
| 09/23/03 | 20 | 20 | 2.7 |
| 09/08/04 | 20 | 20 | 3.9 |

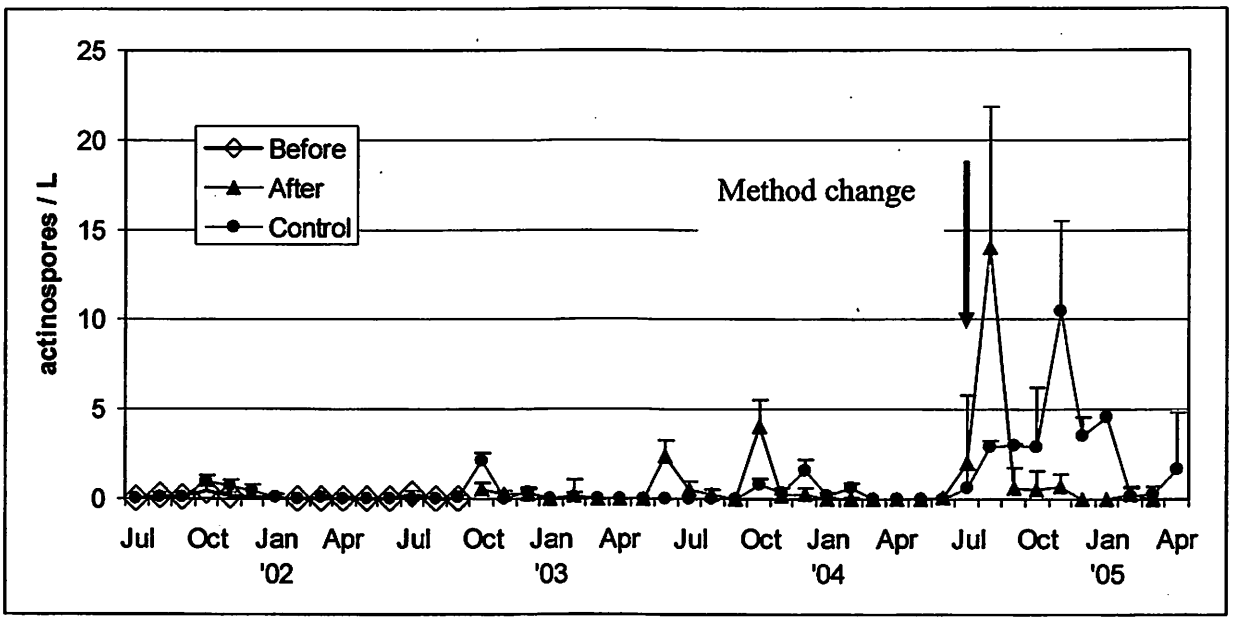


Figure 1.07. Density of actinospores observed in concentrates of surface water samples collected at the Spring Creek treatment and control sites. "Before" designates the 15 months (13 samples) immediately preceding construction, and "After" the 30 months following construction.

The planned pre- and post-manipulation oligochaete sampling completed in earlier segments suggested that the habitat manipulations in Spring Creek had at least a short-term effect of reducing oligochaete biomass (Table 1.11). Exploratory sampling during July 2003 and July

2004 confirmed other oligochaete habitats throughout the 19 km reach from Spring Creek Dam down to the control site adjacent to Spring Creek Campground, 3.2 km above the Taylor River confluence. Testing of replicate oligochaete samples from each site for the DNA of four lineages of *T. tubifex* reveals that the entire length of Spring Creek between Spring Creek Reservoir and Spring Creek Campground is dominated by the very susceptible lineage III. Twenty-eight samples representing all 12 sites yielded 100% lineage III DNA.

Table 1.11. 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 *M. cerebralis* 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 *M. cerebralis* 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.

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.

Trout population data have been collected from the Williams Fork River for the past four years (Table 1.12). The rainbow trout population remains sparse. Densities of juvenile rainbow

trout remain consistently higher just below Williams Fork Dam versus below the habitat modification site. This circumstance supports the hypothesis that the majority of present-day infectivity comes from within the river rather than the reservoir.

Table 1.12. Trout population biostatistics for two 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 |
| 2004 | 473 | ± 4 | 197 | 2490 | 560 | 39 | ± 2 | 65 | 188 | 104 |
| | 1.6 km below Williams Fork Reservoir, below Kemp/Breeze Wildlife Area irrigation | | | | | | | | | |
| 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 |
| 2004[d] | 472 | ± 8 | 191 | 1202 | 1336 | 24 | ± 2 | 21 | 54 | 23 |

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 high actinospore density observed 12 months post-construction still appears to be an aberration, although low densities of actinospores have been observed on three other occasions (Figure 1.08). Overall, we continue to detect actinospores less frequently than was the case before habitat modification.

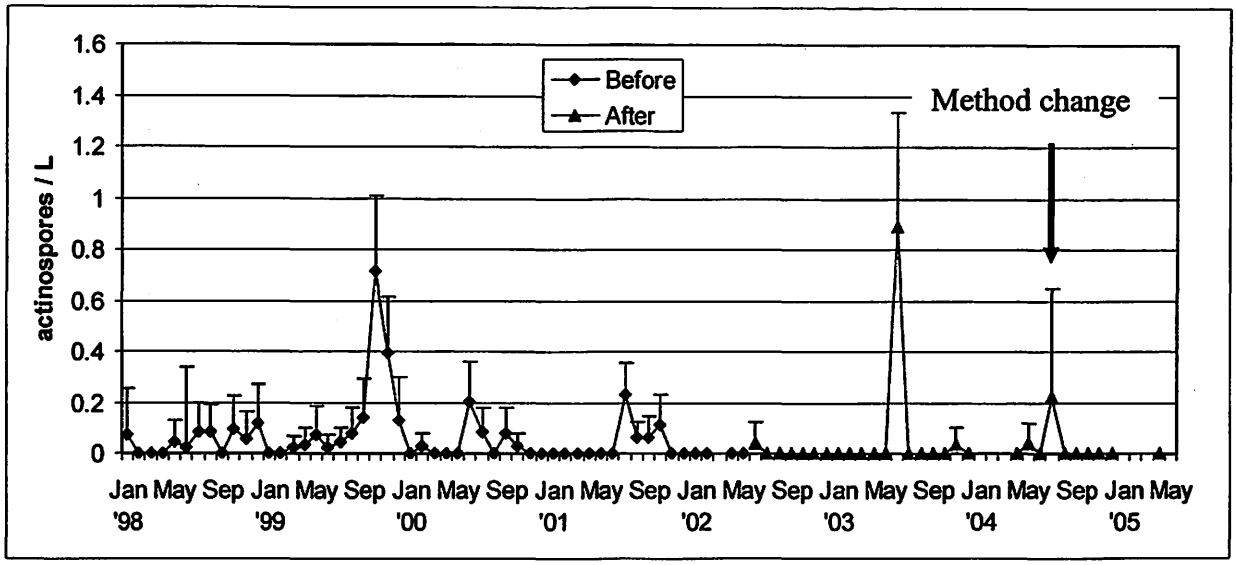


Figure 1.08. Density of actinospores observed in concentrates of surface water samples collected at the Williams Fork treatment site from January 1998 through April 2005. "Before" designates samples collected prior to construction and "After" the samples collected following construction.

Previously collected baseline and post-construction oligochaete monitoring data are presented in Table 1.13 for completeness.

Table 1.13. 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.

The brown trout collected in the fall of 2004 represent the first true post-manipulation samples, having hatched in 2003. Over the last couple of years prevalence of infection and average myxospore concentration have been higher at the downstream sites compared to just

below the Williams Fork Dam (Table 1.14). In the samples from 2004 there is no evidence that the habitat modifications resulted in lower prevalence or lesser average myxospore concentrations compared to previous years or the other monitoring sites. Although actinospores are detected less frequently, it does not appear to be resulting in significantly lower prevalence or spore count.

Table 1.14. Cranial *M. cerebralis* 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 |
| 11/16/04 | 21 | 43% | 714 | 1,667 | 556-4,444 |
| 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 |
| 11/16/04 | 21 | 76% | 12,249 | 16,077 | 556-66,667 |
| 2.6 km below Williams Fork Reservoir | | | | | |
| 09/12/01 | 20 | 55% | 21,600 | 39,200 | 4,300 – 113,700 |
| 11/18/02 | 15 | 53% | 3,600 | 6,700 | 1,600 – 13,600 |
| 11/18/03 | 20 | 90% | 14,300 | 15,800 | 2,900 – 61,500 |
| 11/16/04 | 20 | 60% | 31,538 | 52,546 | 5,556-240,000 |

Willow Creek (Colorado River drainage)

Willow Creek was added to the work schedule in the 2003-04 segment to take advantage of extensive baseline work accomplished by the U. S. Geological Survey (USGS) and the Colorado Cooperative Fish and Wildlife Research Unit. Further baseline oligochaete sampling occurred during the summer of 2003 (Table 1.15). Habitat modifications occurred during the 2003-04 segment. Both before and after the habitat changes lineages III and VI were the only ones detected. Lineage III is considered to contain the highest proportion of susceptible individuals (Beauchamp et al. 2002), but lineage VI also contains susceptible individuals. The average proportion of lineage III DNA in the qPCR samples was less in the samples collected after habitat modification than in those collected before (Kruskal-Wallis non-parametric ranks

test, $p = 0.0745$, Table 1.15). Whether this decrease is attributable to the habitat modification is unclear, since a decrease in lineage III DNA proportion was also observed in the Poudre River before any habitat modifications occurred. It is also possible that the changes observed in Willow Creek were brought about by oligochaete response to chronic *M. cerebralis* infectivity.

Table 1.15. 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 *M. cerebralis* lineage | | | |
|---|---|---|---|---|---|
| | | I | III | V | VI |
| | | Prior to habitat modification | | | |
| 6/23/03 | 8 | 0.0 (0.0) | 22.1 (21.0) | 0.0 (0.0) | 77.9 (21.0) |
| 8/18/03 | 9 | 0.0 (0.0) | 19.2 (14.2) | 0.0 (0.0) | 80.8 (14.2) |
| | | After habitat modification | | | |
| 5/18/04 | 9 | 0.0 (0.0) | 5.3 (4.7) | 0.0 (0.0) | 94.7 (4.7) |
| 8/16/04 | 9 | 0.0 (0.0) | 9.3 (9.7) | 0.0 (0.0) | 90.7 (9.7) |

Table 1.16. Cranial *M. cerebralis* myxospore concentrations in age 1+ brown trout sampled from Willow Creek.

| Date mm/dd/yy | Age | N | Prevalence | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|---|
| | | | | | Mean | Range |
| | | | Above Willow Creek Gage | | | |
| 09/30/03 | 1+ | 20 | 70% | 21,400 | 30,600 | 2,600 – 194,700 |
| 09/29/04 | 1+ | 15 | 40% | 8,100 | 20,100 | 5,000 – 64,500 |
| | | | 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 |

Table 1.17. Results of PCR tests of samples of YOY 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 |
| | Downstream of backwater site | | |
| 09/30/03 | 11 | 7 | 1.7 |
| 09/29/04 | 20 | 16 | 2.9 |

Collections of brown trout collected above and below the site indicate a high prevalence of *M. cerebralis* infection in the area of the backwater both by PTD from age 1+ fish (Table 1.16) and PCR from YOY fish (Table 1.17). The 2004 PTD samples are considered pre-modification samples because of the timing of exposure. The age 1+ brown trout were much more difficult to collect in 2004 than in 2003. Beaver activity through the project area resulted in extensive ponds that reduced electrofishing efficiency. Those fish that were collected in 2004 exhibited lower prevalence than similarly aged fish collected in 2003. Only three of ten fish were found to be infected below the backwater, but all had substantial myxospore concentrations. The 2004 PCR fish samples are post-modification samples and indicate that there was no decrease in infectivity to YOY brown trout for the 2003 year class.

## DISCUSSION

The final project designed to isolate or remove discrete areas of good *T. tubifex* habitat from streams was constructed during this segment on the Poudre River near Kinikinik. While evaluation will continue for at least two more segments, early indications are that the 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. If improvement in the fish population could be asserted to have occurred on any study stream, it would be Beaver Creek, where somewhat higher age 1 rainbow trout densities have been observed the last couple of years compared to prior years.

At the annual Whirling Disease Symposium convened in Denver in February 2005, infectious disease authority and keynote speaker Dr. Paul Ewald (University of Louisville) noted that the two spores involved in the transmission of the parasite from host to host employ differing strategies. The myxospore is thought to be rather immobile once it is deposited, thus the transmission technique is to "sit and wait" for a suitable host to encounter it. Typically, disease agents characterized by this sort of strategy have a high impact on the host (Ewald 1994). In contrast, the actinospore is waterborne and disease agents characterized by this method of transmission generally have a lesser impact on the host. 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 (this study, also see 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.

Work on lab and field experiments to assess the viability of this approach as a practical management tool is anticipated to begin in the next segment under a new job of this project.

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

Period Covered: July 1, 2004 to June 30, 2005

## 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 *M. 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 often 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 *M. cerebralis* infectivity emanating from a series of off-channel ponds was documented (Nehring et al. 2000). The most severe reduction in abundance of age 1 wild rainbow trout has occurred downstream of this focus of infection, suggesting that whirling disease induced the decline.

Fish rearing facilities may also contribute infectivity to waters receiving settling pond effluent. The number of State-owned rearing units experiencing parasite infestations peaked in 1998 at 11 facilities. Currently the number stands at just five. However, in some cases rearing units are free of the parasite but the settling ponds are not. In other cases there is no expectation of ever succeeding in freeing the rearing unit of the parasite.

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

Segment Objectives:

1. Continue to monitor triactinomyxon densities at established study sites.

2. Monitor triactinomyxon densities in the 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.

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

## METHODS and MATERIALS

### Field Filtration and Sample Collection

The technique for collecting field filtration samples was changed at the beginning of this segment based on experiments conducted late in the previous segment (see Job 1, Materials and Methods: Actinospore sampling). Rather than sampling a single 1900-L volume of water at each site, we sampled duplicate 120-L samples. In some cases the samples were reprocessed in the lab through a large mesh screen to remove debris, then a small packed bed filter and centrifuge to concentrate the samples further. No distinction is made between samples that were additionally processed and those that were not, since the experiments indicated that point estimates are virtually the same between the methods.

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). Triactinomyxons not conforming to these descriptions were not counted for this study. It is possible that in some instances this resulted in an underestimate of triactinomyxons because recent evidence shows that there may be 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 each field sample was subjected to the polymerase chain reaction (PCR) test. From early 1998 through March 2001 we used the single round modification (Schisler et al. 2001) of the 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 *M. cerebralis* designated as HSP70. Each sample tested by PCR was preserved in 70% ethyl alcohol in a 15-mL centrifuge tube, and was identified only by alphanumeric code when sent to the laboratory.

### Fish Removal

Electrofishing equipment was used to accomplish fish removal at the Roaring Judy Rearing

Unit and from the Cap K Ranch ponds. Pitkin Rearing Unit personnel have handled fish removal efforts in the Pitkin settling pond using gill-nets.

## RESULTS and DISCUSSION

Cap-K Ranch Ponds (Fryingpan River drainage)

The Fryingpan River has been sampled each month at four or more sites since August 1998. Water samples drawn from the Fryingpan River immediately downstream from Ruedi Dam were collected on 67 sampling occasions from July 1998 through May 2004. Actinospores were detected twice, in April 2001 and again in November 2004. One water sample tested weakly positive by PCR for DNA of *M. cerebralis* in August 2004.

Table 2.01. Estimates of *M. cerebralis* 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 | 2004-05 |
|---|---|---|---|---|---|
| | | | 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 (---) | 0.0 (---) |
| Dec | 0.120 (0.16) | 0.0 (---) | 0.182 (0.20) | 0.043 (0.08) | 0.0 (---) |
| Jan | 0.086 (0.09) | 0.0 (---) | 0.149 (0.20) | 0.0 (---) | 0.0 (---) |
| Feb | 0.043 (0.08) | 0.143 (0.15) | 0.0 (---) | 0.0 (---) | 0.0 (---) |
| Mar | 0.116 (0.16) | 0.0 (---) | 0.0 (---) | 0.0 (---) | 0.0 (---) |
| Apr | 0.0 (---) | 0.0 (---) | 0.124 (0.24) | 0.0 (---) | 0.0 (---) |
| May | 0.0 (---) | 0.0 (---) | 0.337 (0.66) | --- | 0.831 (0.53) |
| 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 (---) | 0.0 (---) |
| Aug | 0.617 (0.30) | 0.043 (0.08) | 0.318 (0.17) | 0.0 (---) | 0.0 (---) |
| Sep | 0.745 (0.45) | --- | 0.495 (0.28) | 0.0 (---) | 0.0 (---) |
| Oct | 0.981 (0.33) | 0.317 (0.18) | 2.588 (0.85) | 0.0 (---) | 0.0 (---) |
| Nov | 0.670 (0.36) | 0.855 (0.27) | 1.743 (2.05) | 0.125 (0.13) | 0.0 (---) |
| Dec | 1.014 (0.46) | 0.950 (0.42) | 1.718 (0.54) | 0.0 (---) | 0.385 (0.75) |
| Jan | 0.803 (0.44) | 1.785 (0.7) | 1.082 (0.41) | 0.139 (0.08) | 0.0 (---) |
| Feb | 0.361 (0.27) | 0.060 (0.12) | 0.291 (1.07) | 0.356 (0.28) | 0.0 (---) |
| Mar | 1.010 (0.42) | 0.096 (0.13) | 0.351 (0.26) | 0.028 (0.06) | 0.603 (1.18) |
| Apr | 0.423 (0.22) | 0.038 (0.07) | 0.624 (0.46) | 0.176 (0.19) | 0.0 (---) |
| May | 0.951 (0.49) | 0.370 (0.27) | 0.703 (0.47) | 0.317 (0.27) | 2.037 (2.81) |
| Jun | 0.091 (0.10) | 0.0 (---) | 0.874 (0.3) | 0.045 (0.06) | |

The Cap K Ranch ponds are in a series designated by numbers 1 – 6, with pond 1 at the

top and Pond 6 at the terminus of the series. The effluent from Pond 6 returns to the Fryingpan River, although the capability exists to divert pond water back to the Fryingpan River before it enters Pond 5. Table 2.01 contains filtration data obtained since July 2000 on ponds 1 and 2.

During March and April 2005 Ponds 1 and 2 were electrofished on five occasions to remove brook trout fry. Thirty-two hundred were removed, the fourth year of brook trout fry removal. In this segment no adult brook trout were removed from the upper two ponds. Reducing the population of this susceptible species may be reducing infectivity in the system, as actinospores have been detected in the Pond 2 effluent in the last two segments less frequently than before (Table 2.01).

Pond 6 has historically been a source of *M. cerebralis* actinospores to the Fryingpan River (Thompson 2004). This pond was modified during February and March of 2003. A description of the filter installed was previously provided (Nehring and Thompson 2003). Monitoring of the inlet and outlet of the filter continued during this segment and occurred most frequently in July, August, and March through May as those are the months historically during which actinospores were frequently detected in Pond 6. Actinospores were documented in the filter inlet on 11 occasions (two points in Figure 2.01 are not clearly evident because of the compressed scale). A single actinospore was observed in the effluent on May 5, 2004. In late April and early May 2005 multiple actinospores were observed in the filter effluent. On three occasions filtrates from the effluent have tested positive for *M. cerebralis* by PCR, twice during the current segment (Figure 2.02). Even so, comparison of the paired filtrates and PCR samples indicates that the filter was effective in removing triactinomyxons from Pond 6 water until the last two occasions shown (Figure 2.01). The appearance of substantial numbers of actinospores in the filter effluent water suggests that the filter may be failing. Another possibility is that oligochaetes have become established inside the outlet pipes; however for actinospores to be produced there would need to have been myxospores available to those oligochaetes.

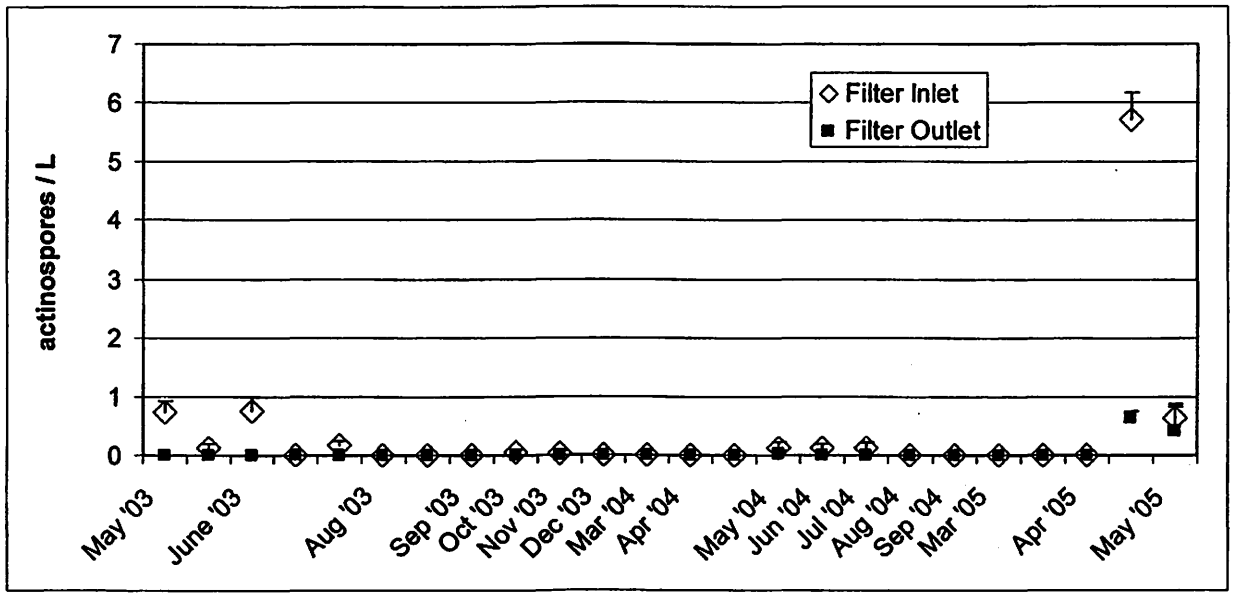


Figure 2.01. Paired water filtrations from the inlet and outlet of the wetland filter installed in Pond 6 on the Cap-K Ranch. Bars indicate +1 SE.

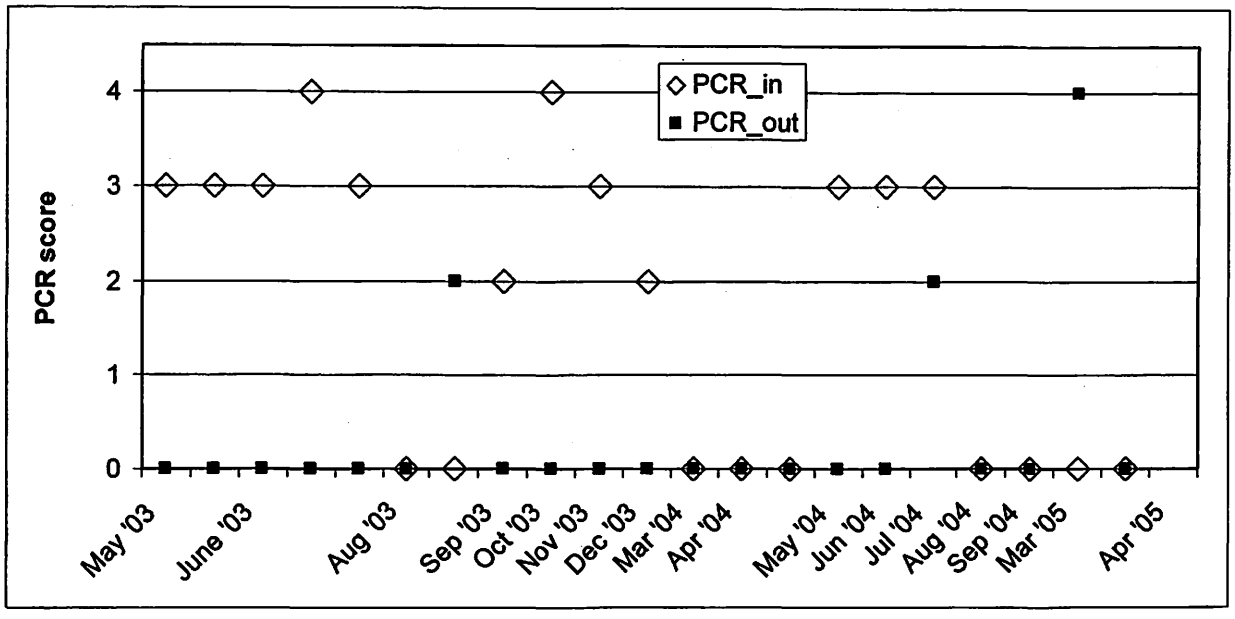


Figure 2.02. Comparison of PCR score for filtrates from the inlet and outlet of the wetland filter installed in Pond 6 on the Cap-K Ranch. PCR score is based on a scale from '0' (no PCR signal) to '4' (a very strong PCR signal). The first occasion on which the effluent tested positive was probably caused by improperly set control gates that allowed unfiltered water to exit the filter.

The filter rapidly lost capacity to handle water, and backwashing met with little success in restoring capacity (Thompson 2004). A knife-edge weir was installed on the overflow channel around the filter so reliable estimates of filter capacity could be gained. The wetland was "turned on" again on March 16, 2005. With freshly cleaned screens the wetland is currently capable of passing close to 300 gpm (Figure 2.03). The screens plug quickly however, and the flow through the filter is commonly less than 100gpm before the screens are cleaned.

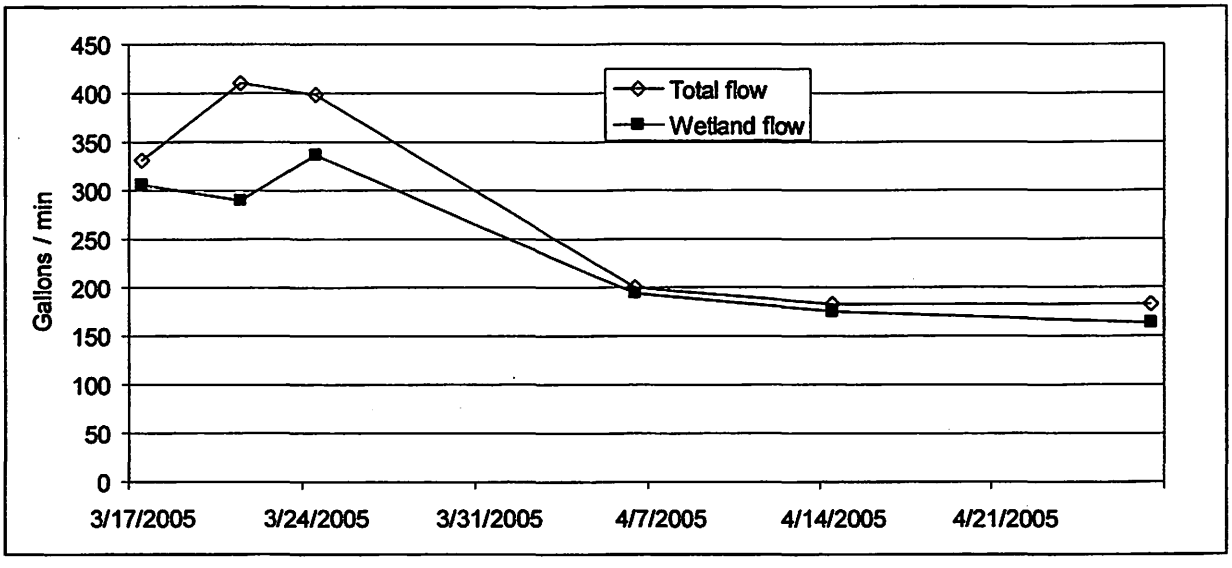


Figure 2.03. Total flow versus wetland flow with newly cleaned screens.

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 2.02). This is the fourth year out of five that the samples have been taken and the first year that the brown trout comprising the samples would have been hatched in the river after the construction of the filter in Pond 6. These samples suggest that prevalence of parasite infection may be increasing, especially at the upstream site. At the Taylor Creek site downstream of Cap-K prevalence is virtually unchanged from the previous year, but the average spore concentration is lower. Whether the lower concentration may be regarded as a result of the filter preventing actinospores from entering the Fryingpan River remains open to question. The trend in myxospore concentration has overall been downward at the Taylor Creek site according to the available data, even though prevalence has been trending higher. The notorious variability and non-normality of myxospore counts also renders it difficult to statistically detect differences among occasions or sites, even with 20-fish samples.

Table 2.02. Cranial *M. cerebralis* myxospore concentrations in brown trout sampled from locations in the Fryingpan River above and below Cap-K Ranch.

| Date mm/dd/yy | Age | N | Prevalence | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|---|
| | | | | | Mean | Range |
| | 1.6 km above Cap K Ranch | | | | | |
| 10/28/00 | 1+ | 10 | 10.0% | 26,900 | 269,300 | 269,300 |
| 10/30/01 | 1+ | 10 | 60.0% | 15,700 | 26,100 | 2,670 – 71,000 |
| 10/29/03 | 1+ | 20 | 55.0% | 21,800 | 39,600 | 4,560 – 112,300 |
| 10/26/04 | 1+ | 21 | 85.7% | 46,900 | 55,200 | 1,670 – 197,800 |
| | Taylor Creek Confluence | | | | | |
| 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 |

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.

Monitoring of actinospore densities began at the Pitkin Rearing Unit in November 2001. Actinospores of *M. cerebralis* were routinely observed in the effluent of the settling pond, including a large "pulse" during November and December 2002. No actinospores were detected in the effluent during this segment, and were observed just once in Quartz Creek above the settling pond

Table 2.03. Results of water filtration to quantify actinospores of *M. cerebralis* in samples of water at Pitkin Hatchery, August 2002 through May 2005. PCR results are 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 | |
| 05/02/02 | 0 (---) | 0 | 21.011 (3.53) | 3 |
| 05/21/02 | 0 (---) | 0 | 0.602 (0.35) | 0 |
| 06/05/02 | 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 |
| 12/16/02 | not sampled | | 40.090 (3.88) | 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 (---) | 0 | 1.210 (0.37) | 4 |
| 05/7/04 | 0 (---) | 0 | 0.151 (0.20) | 0 |
| 06/01/04 | 0 (---) | --- | 0.021 (0.04) | --- |
| 07/01/04 | 0 (---) | 0 | 0 (---) | 3 |
| 08/03/04 | 0 (---) | 0 | 0 (---) | 0 |
| 09/06/04 | 0 (---) | 0 | 0 (---) | 0 |
| 10/04/04 | 0 (---) | 0 | 0 (---) | 4 |
| 11/17/04 | 0 (---) | 0 | 0 (---) | 0 |
| 11/29/04 | 0 (---) | 0 | 0 (---) | 0 |
| 01/04/05 | 0.192 (0.38) | 0 | 0 (---) | 0 |
| 02/09/05 | 0 (---) | 0 | 0 (---) | 0 |
| 03/01/05 | 0 (---) | 0 | 0 (---) | 0 |
| 04/04/05 | 0 (---) | | 0 (---) | |
| 05/05/05 | 0 (---) | | 0 (---) | |

effluent (Table 2.03). These results, as well as PCR tests on the water samples, indicate that the hatchery effluent appears no more infected than Quartz Creek upstream of the effluent.

Pitkin Unit personnel removed all feral fish from the unit's settling pond during unit renovation in 2001-02. Since then, annual efforts on the part of unit staff to keep the settling pond free of fish have paid off in the lower actinospore densities apparent in Table 2.03. A 30-fish rainbow trout sample acquired by gillnet in May 2002 was 80% positive for *M. cerebralis*, with an average concentration of 210,600 myxospores per head. To no longer have such a myxospore source available to the *T. tubifex* community residing in the settling pond has had a positive impact on the infectivity observed in the effluent.

During this segment samples were collected from Quartz Creek approximately one mile above and below Pitkin Rearing Unit. Prevalence and spore concentration were both substantially higher than at those same locations in 2003 (Table 2.04). The tremendous increase in prevalence and average concentration upstream of Pitkin Rearing Unit suggest that the parasite is spreading in Quartz Creek. If the clean up of the Pitkin settling pond has any effect on infection prevalence in Quartz Creek it should be evident in 2006, when fish hatched in spring 2005 will be sampled as age 1+ fish, assuming the current trend of no detection of actinospores continues.

Table 2.04. Cranial *M. cerebralis* myxospore concentrations in brown trout sampled from Quartz Creek above and below the Pitkin Fish Rearing Unit.

| Date mm/dd/yy | Age | N | Prevalence | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|---|
| | | | | | Mean | Range |
| | | | Upstream of Pitkin Rearing Unit | | | |
| 08/28/03 | 1+ | 20 | 10.0% | 2,900 | 29,400 | 25,300 – 33,500 |
| 08/09/04 | 1+ | 20 | 85.0% | 15,400 | 18,100 | 1,700 – 50,100 |
| | | | 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 |

Poudre Rearing Unit

Actinospore monitoring began at numerous sites on the Poudre River in 1997. The data from 1997 through June 2001 clearly indicated that the 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 as compared to upstream (Nehring et al. 2001; Schisler 2001).

Actinospores of *M. cerebralis* were still encountered frequently at the filtration sites on the PRU during this segment (Table 2.05). Estimated densities remained low in the PRU effluent compared to the historic high numbers seen in 1999-2000 (Figure 2.04). Water samples from the unit supply pond contained actinospores on 4 of 10 occasions compared to 1 of 10 occasions in the Poudre River inflow to the supply pond; both of these values represent decreased detection

compared to the previous segment. The densities of actinospores observed in the supply pond were significantly higher than those in the Poudre River inflow (mean 0.19/L vs. 0.015/L, paired *t*-test, 1-tailed $p = 0.043$). Average actinospore density was lower in the Poudre River inflow and in the supply pond compared to the previous segment, but somewhat higher in the Unit effluent. None of the differences were significant. The increased efficiency of the filtration technique compared to previous years may in part explain the increased density in the effluent even though actinospores were detected on fewer occasions in this segment compared to the previous one. In contrast, it is encouraging that average actinospore densities decreased in the river and the supply pond despite the more efficient filtration technique.

Table 2.05. Results of water filtration to quantify actinospores (N/L) of *M. cerebralis* in 1900-L (500-gallon) samples of water at the Poudre Rearing Unit from July 1, 2003 through May 31, 2004. Values in parentheses are the half-width of 95% confidence intervals.

| Month | Poudre River at Unit inflow | Supply pond outflow | Unit effluent |
|---|---|---|---|
| Jul | 0.00 (---) | 0.65 (0.36) | 0.64 (0.51) |
| Aug | 0.00 (---) | 0.00 (---) | 0.39 (0.77) |
| Sep | 0.15 (0.30) | 0.00 (---) | 0.00 (---) |
| Oct | 0.00 (---) | 0.64 (1.25) | 0.43 (0.84) |
| Nov | 0.00 (---) | 0.22 (0.44) | 0.53 (1.03) |
| Dec | 0.00 (---) | 0.40 (0.78) | 0.00 (---) |
| Jan | 0.00 (---) | 0.00 (---) | 0.00 (---) |
| Feb | 0.00 (---) | 0.00 (---) | 0.00 (---) |
| Mar | 0.00 (---) | 0.00 (---) | 0.00 (---) |
| Apr | 0.00 (---) | 0.00 (---) | 0.00 (---) |
| May | | | |

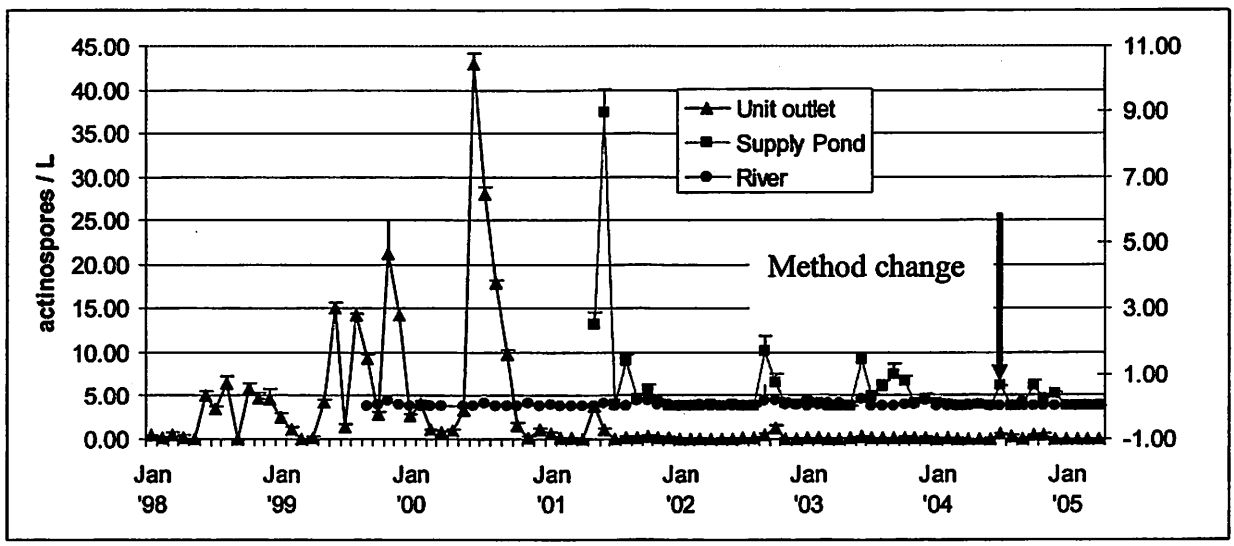


Figure 2.04. Comparison of actinospore densities from the Poudre River, the Supply pond and the Unit effluent through April 2005. The Supply pond and River values are referenced to the second y axis.

The modification of the entire supply pipeline system at the Poudre Unit should be completed in 2005. Once the supply pond is bypassed with a direct pipeline for most of each year, Unit effluent actinospore densities should further decrease to a level approaching the background densities in the Poudre River. Actinospore monitoring in the summer and fall of 2005 should indicate whether the bypass of the supply pond will allow the PRU to achieve the goal of actinospore densities at or below the background level.

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 of 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 have been dramatic as can be seen from the summaries of filtration data for the effluent of the northeast pond in Figure 2.04.

Roaring Judy Rearing Unit

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

In the spring of 2005 the ROJ regained certification as a *M. cerebralis*-free facility. However, the effluent channel, new kokanee spawning unit, and settling ponds remain enzootic habitats. Research continues on methods and management strategies to minimize the number of actinospores in the settling pond effluent. Monthly monitoring during this segment resulted in the detection of actinospores in the unit effluent on the fewest occasions since monitoring began, just 4 of 11 months (Table 2.06, Figure 2.05).

Table 2.06. Results of water filtration to quantify actinospores (N/L) of *M. cerebralis* in 1900-L (500-gallon) samples of water drawn from various sites at the CDOW Roaring Judy Fish Rearing Unit from July 1, 2003 to May 31, 2004.

| Month | Concrete Raceway effluent | Kokanee Trap | West Pond effluent |
|---|---|---|---|
| Jul | 0.000 (---) | 0.387 (0.01) | 0.976 (1.10) |
| Aug | 0.000 (---) | 0.000 (---) | 0.077 (0.15) |
| Sep | 0.000 (---) | 0.000 (---) | 0.323 (0.48) |
| Oct | 0.019 (0.04) | 0.038 (0.08) | 0.000 (---) |
| Nov | 0.236 (0.46) | 0.000 (---) | 0.000 (---) |
| Dec | 0.000 (---) | 0.000 (---) | 0.000 (---) |
| Jan | 0.000 (---) | 0.450 (0.88) | 0.000 (---) |
| Feb | 0.000 (---) | 0.000 (---) | 0.000 (---) |
| Mar | 0.000 (---) | 0.000 (---) | 0.000 (---) |
| Apr | 0.000 (---) | 0.000 (---) | 0.000 (---) |
| May | 0.000 (---) | 0.000 (---) | 0.000 (---) |

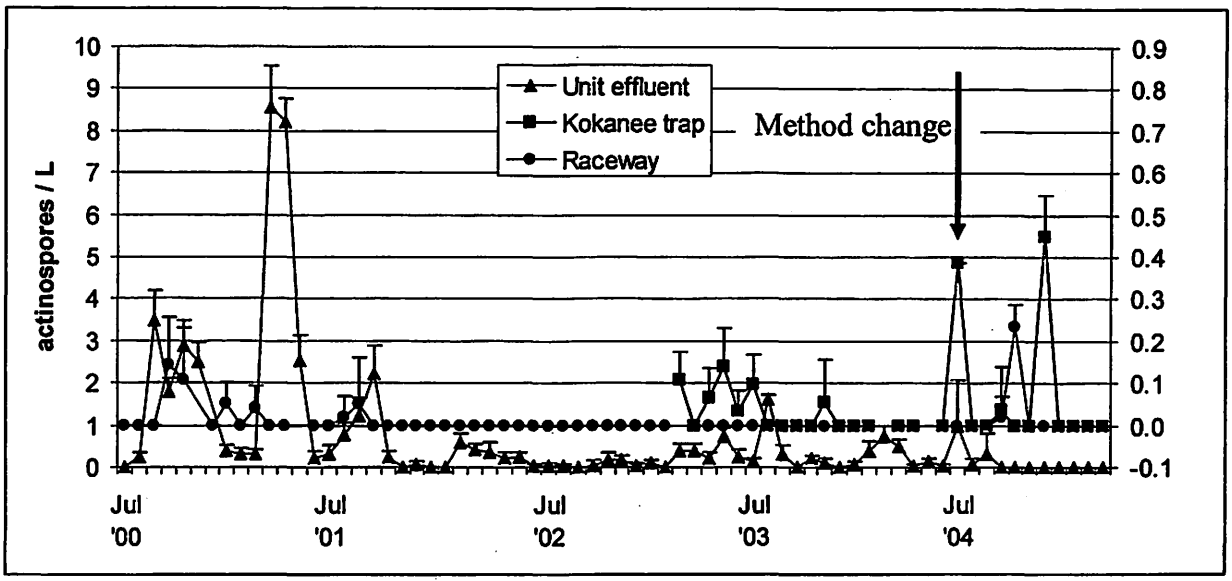


Figure 2.05. Comparison of actinospore densities from the ROJ concrete Raceway tailbox, the kokanee trap (downstream of the concrete raceways), and the Unit effluent through May 2005. The Kokanee trap and Raceway values are referenced to the second y axis.

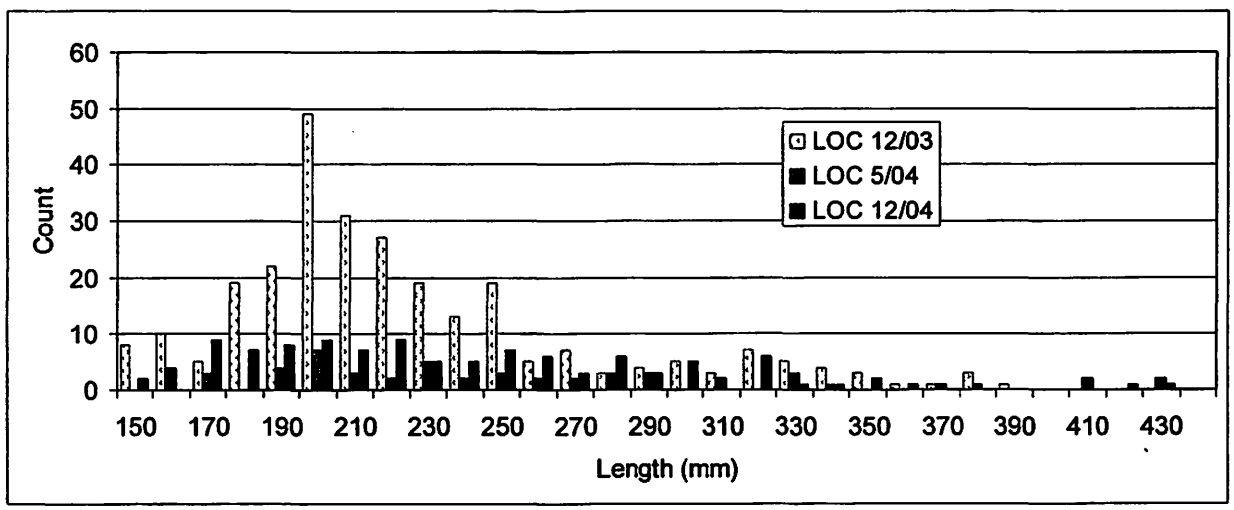


Figure 2.06. Number of brown trout removed from the ROJ effluent channel on three occasions.

The removal of kokanee salmon carcasses from the west settling ponds and connecting waterways continued during this segment, accomplished by Division of Wildlife (CDOW) employees. Carcasses were disposed in an upland area. Trout also were removed on two occasions from the effluent channel between the concrete raceway outlet and the top of the kokanee spawning facility (Figures 2.06 – 2.07). Boards were intended to be in place throughout the winter at the top of the kokanee trap to prevent fish from the settling ponds from repopulating the channel; however the hatchery manager found them removed in the spring, possibly by fishermen. While the brown trout population was depleted, the rainbow trout population was not. The number of rainbow trout present in December 2004 after two earlier removal occasions can only point to fish escapement

from the rearing unit. It certainly suggests that repeated efforts will be necessary to keep the fish population reduced in the effluent channel in order to test the effect of this strategy on the infectivity above the kokanee trap. To help preclude future escapement incidents of these catchable-size fish, ROJ personnel installed a screen in an old kokanee trap immediately downstream of the raceways. Any unintentionally released fish will hopefully be retained by this screen before entering the effluent channel.

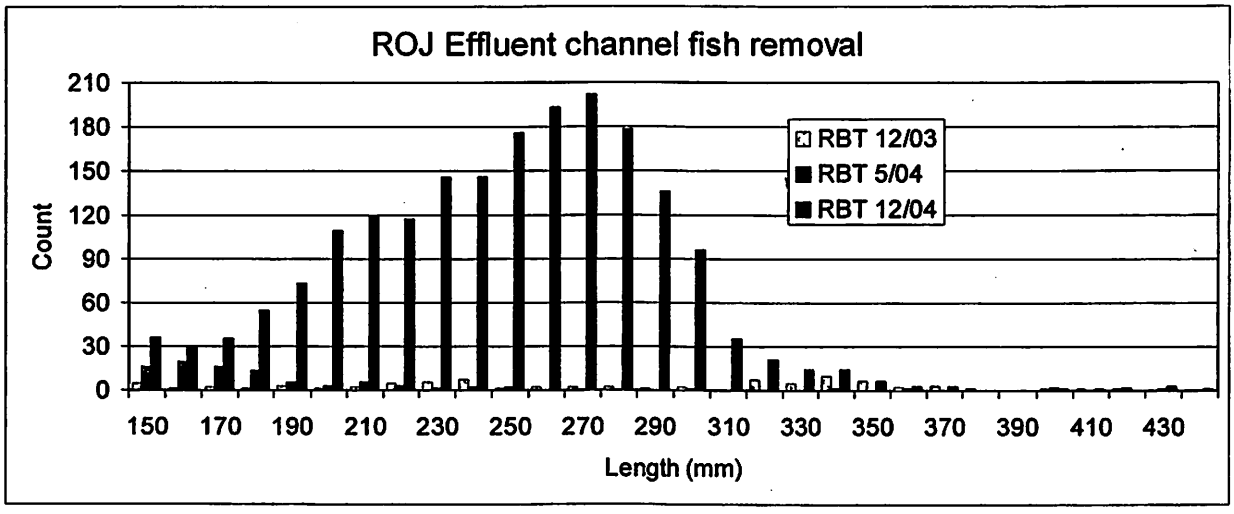


Figure 2.07. Number of rainbow trout removed from the ROJ effluent channel on three occasions.

Table 2.07. Cranial *M. cerebralis* myxospore concentrations in trout sampled from the ROJ effluent channel.

| Date mm/dd/yy | Age (yrs) | Sample Size | | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|---|
| | | N | prevalence | | Mean | Range |
| | | | | Brown trout | | |
| 05/16/03 | 1 | 12 | 75% | 39,900 | 53,200 | 2,000 – 177,750 |
| 11/25/03 | 1+ | 20 | 70% | 29,700 | 42,400 | 4,400 – 150,700 |
| 05/24/04 | 1 | 20 | 75% | 23,500 | 31,300 | 300 – 161,900 |
| 11/30/04 | 1+ | 20 | 75% | 25,700 | 34,300 | 1,100 – 193,300 |
| | | | | 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 | Results not available | | | |
| 11/30/04 | 2+ | 25 | Results not available | | | |

Samples of the trout removed from t7). 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.

Table 2.08. Cranial *M. cerebralis* myxospore concentrations in trout sampled from the ROJ settling ponds.

| Date mm/dd/yy | Species or Strain | Sample Size | | Overall Mean Concentration | Positive Fish | |
|---|---|---|---|---|---|---|
| | | N | prevalence | | Mean | Range |
| | | Settling Ponds | | | | |
| 11/04/03 | Tasmanian[a] | 28 | 28.6% | 5,100 | 17,800 | 3,300 – 40,000 |
| 11/04/03 | Bellaire[a] | 23 | 43.5% | 17,200 | 39,600 | 3,300 –136,500 |
| 11/04/03 | Rainbow[b] | 16 | 93.8% | 365,700 | 390,100 | 7,200 – 1,387,400 |
| 11/30/04 | Brown[c] | 20 | 90% | 22,600 | 25,100 | 560 – 142,200 |
| 11/30/04 | Brown[d] | 20 | 85% | 11,800 | 14,100 | 1,100 – 64,400 |
| 11/30/04 | Erwin[e] | 18 | Results not available | | | |
| 11/30/04 | Bellaire[e] | 20 | Results not available | | | |
| 11/30/04 | Rainbow[b] | 6 | Results not available | | | |

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

The two west settling ponds were stocked with fin-clipped catchable rainbow trout in May and June 2004 with Bellaire strain from the Durango Rearing Unit and Erwin 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. The analysis of these samples is still pending (Table 2.08).

In the previous progress report (Thompson 2004) the 2003 catchable rainbow trout myxospore results were misreported. Contrary to what was reported, the Tasmanian strain rainbow trout actually showed lower prevalence than the Bellaire as well as lower average spore counts (Table 2.08). This result was surprising because based on previous experience it was expected that the Tasmanian rainbow trout would exhibit greater vulnerability to the parasite. A request to the respective hatchery managers for the age of the fish at the time they were stocked revealed that the Bellaire strain fish averaged 9.04 inches and were about eight months of age. The Tasmanian strain fish were 10.24 inches and about 17 months of age. It is probable that the increased size and age of the Tasmanian strain fish conferred an extra degree of resistance to the parasite compared to the smaller and younger Bellaire strain fish. Ryce et al. (2005) demonstrated that both size and age are

important in conferring resistance to *M. cerebralis* in rainbow trout at less than three months age. At older ages, increased size resulted in increased resistance. At larger sizes, increased age resulted in increased resistance. A similar response in these older, catchable size trout would help to explain why the strain we expected to be more vulnerable actually appeared to be the less vulnerable. In the upcoming segment a similar trial is planned, using the same strains, but they will be much more closely matched in both size and age at stocking.

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 2.09). On the other hand, population estimates for brown trout were 1330 fish in the upper pond and 976 in the lower pond. It would appear that the annual stocking of a moderate number of catchable rainbow trout into the settling ponds for the purpose of providing recreational fishing opportunity will probably 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 indicates that 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 appears that the idea to depopulate the settling ponds and to keep them free of fish is not realistic.

Table 2.09. Trout population estimates from the ROJ 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 | 18 | 17 | 8 | 1163 | 252 | 334 |
| | | | Lower pond | | | |
| 12/03/03 | 8[a] | --- | 4 | 924 | 220 | 625 |
| 11/23/04 | 10[a] | --- | 6 | 1436 | 305 | 1230 |

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 ROJ at this time, it is further recommended that any catchable rainbow trout stocked into these ponds be stocked no later than the end of June. Such stocked fish should continue to be sampled and monitored following the kokanee spawning season to determine prevalence and intensity of infection of the different strains used.

The removal of kokanee salmon carcasses from the ponds and stream channel during the kokanee spawning period at ROJ should continue. Efforts should also continue to remove as many trout as possible from the upper effluent channel at ROJ, and to prevent further trout entry into the channel. Encouragement of angling harvest in the effluent channel would result in beneficial use of the trout resources that do occupy that area, and would seem preferable to removing them by electrofishing. Use by anglers may be restricted to the area downstream of the fence that crosses the effluent channel at the parshall flume that measures unit effluent.

The Cap-K Ranch sand filter has proven to be a disappointment first in the loss of water capacity experienced over a short period of use. Now, at the end of this segment it appears possible that the filter is no longer effectively capturing actinospores. Any further efforts to construct sand filtration systems must include changes to filter design as recommended by the engineering proponent of the previous filter, namely, that the filter media be graded crushed glass, probably in a thinner layer than was used for the existing filter, and finally, that backwash air lines be laid in a much higher density than was the case with the existing filter. Other strategies for reducing infectivity from the Cap-K Ranch ponds and similar habitats appear more appropriate at this time. The evidence gathered recently on the Cap-K Ranch suggests that the removal of brook trout from the upper, more highly enzootic ponds has resulted in a lessening of the actinospore production in the system. This reduction is apparent throughout the pond system. 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 Colorado Division of Wildlife Management and Hatchery Sections and to other interested agencies or publics.

Period Covered: July 1, 2004 to June 30, 2005

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 *M. cerebralis*-positive facility in salmonid habitat. Disseminated the information to the managing consultant of the property.
2) Monitored two sites each month at the Aquatic Alternatives private facility near Nathrop as part of exemption process for operating a *M. cerebralis*-positive facility in salmonid habitat. Disseminated the information to the owner of the property.
3) Monitored one site below Antero Reservoir from July through December on behalf of Rod VanVelson to determine whether actinospores of *M. cerebralis* were present in the fish channel constructed below Antero Reservoir.
4) Fulfilled several requests for reprints of recent publications.
5) Consulted with DWM and Aquatic Biologist on potential impacts with regard to *M. cerebralis* of a plan to build off-channel oxbow fishery ponds in the floodplain of the upper Blue River. Provided the data collected on the Blue Valley Ranch to CDOW personnel as a potential predictive scenario.
6) Provided input to the "Kaeser-Reno" whirling disease survey to help in providing meaningful initial parameter estimates for some of the predictor variables they will use to model *M. cerebralis* epidemiology.

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