Whirling Disease Investigations

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

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Fish Research Section

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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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Job No. 1: *Myxobolus cerebralis* in Colorado's Cutthroat Trout Populations

Project Objective: To determine, and then document through professional publication, the impacts of the myxosporean parasite *Myxobolus cerebralis* on wild trout populations in selected stream ecosystems in Colorado with an overarching objective of developing risk assessment guidelines for the management of whirling disease.

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

Principal Investigator: R. Barry Nehring

Job Objective: Determine the extent of occurrence and severity of impact of *Myxobolus cerebralis* on populations of greenback *Oncorhynchus clarki stomias*, Rio Grande *O. c. virginalis*, and Colorado River cutthroat trout *O.c. pleuriticus* throughout Colorado.

INTRODUCTION

"Whirling disease" (WD), is a debilitating malady of trout and salmon that was first observed in cultured rainbow trout in Germany in 1893 (Hofer 1903). The disease gets its name from the abnormal swimming behavior (often described as tail chasing) of fry or fingerling salmonid fishes that can occur after exposure to the myxosporean parasite *Myxobolus cerebralis.* It was considered a serious problem for the aquaculture industry for much of the 20th century (Plehn 1905, 1924; Schäperclaus 1931; Uspenskaya 1957, 1982). However, the true life cycle of the parasite remained an enigma until the early 1980s when the complex 2-host life cycle that alternately infects a tubificid worm (*Tubifex tubifex*) and a salmonid fish was first described (Markiw and Wolf 1983; Wolf and Markiw 1984). The parasite produces spores in each host that are infective to the alternate host. Myxospores produced in infected salmonids can be ingested by bottom-dwelling oligochaetes. Susceptible forms of *T. tubifex* that become infected produce a triactinomyxon (TAM) actinospore that is infectious to susceptible salmonids.

Myxobolus cerebralis (Mc) was first detected in two public and two private trout rearing facilities in Colorado, late 1987 (Walker and Nehring 1995). Population level impacts among wild salmonid populations were unknown until the 1990s. However, severe losses of young rainbow trout first observed in major reaches of the upper Colorado, Cache la Poudre, Gunnison, Rio Grande, and South Platte rivers in Colorado in 1993 and 1994 were ultimately attributed to WD (Walker and Nehring 1995; Nehring and Walker 1996; Nehring et al. 1998; Nehring and Thompson 2001). The parasite became widely distributed in Colorado in the early 1990s through the stocking of millions of catchable size trout reared in waters enzootic for *M. cerebralis* (Schisler 2001). More than 1 million trout from *Mc*-infected hatcheries and rearing units were stocked into lakes, reservoirs and streams in the Cache la Poudre River drainage between 1990 and 2001. Moreover, this was not a highly unique scenario. Given such a management strategy, it is not surprising that *M. cerebralis* had been detected in feral salmonids at 118 different locations in lakes, reservoirs and major stream segments in Colorado by October

1997 and at 208 sites by spring 2000. It is estimated that *Mc* infections have negatively impacted recruitment of wild rainbow and brook trout fry in 560 – 600 km (350-400 miles) of stream in Colorado (Nehring and Thompson 2001). A recently published special technical report, **Colorado's Cold Water Fisheries: Whirling Disease Case Histories and Insights for Risk Management,** summarizes the effects of exposure to *M. cerebralis* upon Colorado's salmonid fisheries (Nehring 2006).

Debilitating effects of the parasite were documented on wild rainbow trout in major reaches of the Madison River in Montana in the 1990s (Vincent 1996a,b). Research efforts between 1994 and 2004 revealed the parasite was enzootic in many cold water habitats in Colorado (Nehring and Thompson 2003) and western Montana (Baldwin et al. 1998). It has been detected at one or more locations in almost all states west of the 100th meridian in the continental U.S. (Bartholomew and Reno 2002). Detected in Yellowstone cutthroat trout (*O. clarki bouvieri*) in 1998, *M. cerebralis* infections have had devastating impacts on spawning runs in the Yellowstone River immediately downstream of Yellowstone Lake and in Pelican Creek and Clear Creek, major spawning tributaries that drain into the northeastern corner of the lake (Koel et al. 2005, Koel et al. 2006). Recent studies suggest that *M.* cerebralis may be enzootic in one or more streams in south central Alaska near Anchorage (Arsan 2006).

Widely distributed in the mountainous regions of Colorado, *M. cerebralis* has been detected in feral salmonid populations in close proximity to areas designated as cutthroat trout recovery streams. Prior to the initiation of this study in 2003, there were no known cases where the parasite had negatively impacted fry recruitment for any of Colorado's 3 sub-species of cutthroat trout. At that time, the parasite was enzootic among Colorado River cutthroat trout in Trappers Lake in western Colorado and in greenback cutthroat trout in Zimmerman Lake in north central Colorado. Both trout populations are managed for spawn-taking operations. However, not much testing for the presence of the parasite in cutthroat trout waters had actually been undertaken.

The lack of a systematic effort to evaluate the distribution, establishment and spread of *M. cerebralis* into Colorado's aquatic ecosystems capable of supporting native cutthroat trout was the primary impetus for the initiation of this research project.

STUDY DESIGN

The primary study objective is to determine whether or not the parasite has spread into habitats capable of supporting cutthroat trout populations. A multi-faceted approach is being used to determine whether or not significant exposure and spread of *M. cerebralis* has already occurred. In the event that there has been only minimal establishment in most regions of the state, an effort is being made to determine whether introduction actually took place or not. In the event that introduction and exposure actually took place but the parasite was unable to establish itself, the objective will be to determine what factor(s) lead to a failure to complete and sustain the life cycle. A statewide systematic sampling process should provide significant insight(s) into the mechanisms and factors that facilitate the spread of *M. cerebralis*.

For the first level of assessment, in most cases trout population estimations are conducted

on one or more segments of each study stream that are at least 91 meters (300 feet) long. When possible, two population estimates are conducted, one in the headwaters and another near the downstream end of the drainage. In general, the two-pass removal estimator is used to estimate population size and determine relative density, size and approximate age structure for all species of trout in the study reach (Seber and LeCren 1967). Study reaches are selected to include fry (YOY) and juvenile habitats in the population estimation process. Studies by Thompson et al. (1999) have shown that it is during the first year of life that young cutthroat trout are particularly vulnerable to developing a lethal infection after exposure to *M. cerebralis.* Once the parasite becomes enzootic in an aquatic ecosystem, total year class failure can occur under the proper suite of environmental conditions.

After the sampling process is completed, 10 young-of-the-year (YOY) trout and 10 age 1 juvenile trout are sacrificed to test for the *M. cerebralis* parasite. The YOY trout are tested by polymerase chain reaction (PCR) for genomic DNA unique to *M. cerebralis* (Cavender et al. 2004). Yearling juvenile trout are tested for myxospores of the *Mc* parasite using the pepsin-trypsin digest (PTD) method (Markiw and Wolf 1974).

In the event that the study reveals there is little evidence of spread, there are several plausible explanations for such an eventuality. First, in many instances the particular habitat being studied may have never been exposed to the parasite. Second, the habitat in question may have been exposed, but the parasite never completed its life cycle. If the parasite did not become established there could be at least two plausible reasons. First, there may be very little stream habitat suitable for development of colonies of *T. tubifex* of sufficient density to sustain the life cycle in the aquatic oligochaete host. Second, aquatic oligochaetes may be present in the drainage but not the right species or proper lineage of *T. tubifex* that is susceptible to *M. cerebralis.* Recent studies have shown that among the 4 different lineages of *T. tubifex* (I, III, V and VI) currently known to exist in Colorado, lineage V is refractory for *M. cerebralis* (Beauchamp et al. 2001, 2002). Kerans et al. 2004 found that other tubificid oligochaetes such as *Limnodrilus hoffmeisteri* and *Ilyodrilus templetoni* do not become infected when exposed to *M. cerebralis* myxospores in a laboratory setting. Field and laboratory investigations in New Mexico suggest that only lineage III *T. tubifex* become infected when exposed to myxospores of *M. cerebralis* (DuBey and Caldwell 2004; DuBey et al. 2005).

In order to determine which possibility might be the most plausible explanation, a substantial effort is being expended to collect substrate samples containing aquatic oligochaetes in as many habitats as possible. The collections are made concurrent with the trout population estimation surveys. The samples are sorted to determine the relative abundance of "haired" and "non-haired" oligochaetes. The standard protocol is to separate and sort oligochaetes until two sub-samples of 50 "haired" worms per collection site have been identified and preserved in 70% reagent grade ethanol for quantitative PCR testing (hereafter QPCR) to determine whether or not the samples contain lineages of *T. tubifex* susceptible to *M. cerebralis*. Recent advances in testing and development of DNA-based genetic markers specific to at least 4 different lineages of *T. tubifex* make this possible (Beauchamp et al. 2001, 2002). During 2003 and 2004, a private laboratory (Pisces Molecular) developed a 4 probe-multiplex QPCR test that allows the screening of a sample of up to 50 aquatic oligochaetes for the relative percentage of DNA for each of the 4 lineages of *T. tubifex* contained in the sample. The test can also provide a relative

indication of the total amount of DNA from *T. tubifex* in the sample. Data derived from this testing procedure over the 5-year study will facilitate development of spatial and elevational distribution maps for the various lineages of *T. tubifex* by drainage basin and on a statewide basis.

In addition, each worm sample is screened by QPCR using the Hsp70 test (Cavender et al. 2004) to determine if DNA of *M. cerebralis* is present in the worm sample. The Hsp70 test targets a highly conserved region of the heat shock protein gene 70 that is found in a wide array of living organisms and also occurs in the genome for *M. cerebralis.*

METHODS

Trout Population Assessment - In most study streams, the objective was to estimate the salmonid species composition, density and size structure of the trout population at two or more sampling sites using the two-pass removal estimator as described by Seber and Le Cren (1967). Data collected during this effort were run through the Colorado Division of Wildlife's GOLDMEDL or JAKOMATIC computer software programs to develop the population estimates (N), 95% confidence limits, density (n/ha), biomass (kg/ha) and develop a relative estimate of year class abundance for the first 3 year classes based primarily on length-frequency distribution. All sampling sites were identified by GPS to facilitate mapping the collection locations using the mapping software package ARC VIEW 9.

Parasite Screening in Fish – In streams where adequate numbers of salmonids were present, we collected 10 YOY and 10 juvenile (≥age 1) trout for screening for *M. cerebralis* infection. Juvenile trout were tested for *M. cerebralis* using the PTD methodology (Markiw and Wolf 1974) while YOY trout were screened for parasite DNA using the Hsp 70 test (Cavender et al. 2004). In streams where cutthroat trout were sympatric with other salmonids, those species were sacrificed for disease testing. Cutthroat trout were taken for testing only when they occurred allopatrically.

Aquatic Oligochaete Studies –Sediment-laden microhabitats from multiple locations within a study reach on each study stream were sampled for aquatic oligochaetes. All samples were thoroughly screened for aquatic oligochaetes. Oligochaetes were examined by stereo-zoom microscopy, separated into haired and non-haired forms and preserved in 70% reagent grade ethanol and distilled water for QPCR testing. Our protocol was to preserve at least one sample of 50 haired oligochaetes from each collection to determine whether or not the sample contained DNA specific for *M. cerebralis*. Haired oligochaetes have a high probability of being *T. tubifex* (Kathman and Brinkhurst 1998). Each sample was prepped for total DNA extraction to preserve all of the genetic material in the sample. When large numbers of worms were encountered, two aliquots of 50- "haired" worms and one sample of up to 50 non-haired worms were preserved for PCR testing. First, each sample was screened using a 4 probe-multiplex QPCR technique to determine the relative percentage of DNA derived from 4 different lineages of *T. tubifex* in each 50-worm aliquot. Second, each sample was also screened for DNA of *M. cerebralis* using the Hsp 70 technique (Cavender et al. 2004).

RESULTS and DISCUSSION

As shown in Map 1 in the Appendix, there are nine major river basins in Colorado, that historically have supported native cutthroat populations. These include the Arkansas, Colorado, Dolores, Gunnison, Rio Grande, San Juan, South Platte, White and Yampa river systems. Greenback cutthroat trout are native to the Arkansas and South Platte river basins. Rio Grande cutthroat trout are native to the Rio Grande basin. Colorado River cutthroat trout are native to the Colorado, Dolores, Gunnison, San Juan and White and Yampa river systems. No cutthroat trout were ever native to the North Platte drainage in Colorado. An over view of the number of streams and sites sampled each year for each of the three sub-species of native cutthroat trout is summarized in tabular form below.

2003		2004		2005		2006	
Streams	**Sites**	**Streams**	**Sites**	**Streams**	**Sites**	**Streams**	**Sites**
			Greenback Cutthroat Trout				
9	12	3	5	9	12	--	--
			Rio Grande Cutthroat Trout				
9	13	18	26	18	24	10	18
			Colorado River Cutthroat Trout				
22	29	24	36	10	12	49	73

In 2003 (when this project was initiated), there were 46 bodies of water (35 streams, 8 lakes and 3 reservoirs) listed as habitats that were thought to support greenback cutthroat trout populations or could in the future. All occurred in the Arkansas River and South Platte River basins. Over the past four field seasons electrofishing operations have been conducted on 21 streams at 29 sites. For the most part, these streams were either classified as greenback cutthroat trout recovery streams or have direct connectivity to waters thought to support this sub-species. Detailed information regarding streams and sites sampled from 2003 through 2005 can be seen in Nehring 2004; Nehring 2005 and Nehring 2006. No electrofishing operations were conducted on streams supporting greenback cutthroat trout during the 2006 field season.

In 2003 (when this project was initiated), there were 82 bodies of water listed as habitats that either supported Rio Grande cutthroat trout populations or could in the future. The vast majority of them were either creeks or rivers; however, the list included one reservoir and 10 lakes. All streams feed into the Rio Grande drainage that flows through the San Luis Valley in south central Colorado. Over the past 4 field seasons electrofishing operations have been conducted on 55 streams at 81 sites. These streams were either classified as Rio Grande cutthroat trout recovery streams or had direct connectivity to waters thought to support this sub-species. Detailed information regarding streams and sites sampled from 2003 through 2005 can be seen in Nehring 2004; Nehring 2005 and Nehring 2006.

Historically, Colorado River cutthroat trout occurred in the majority of coldwater streams west of the Continental Divide in Colorado. In 2003, the number streams, lakes and reservoirs listed as present or future Colorado River cutthroat trout recovery areas by major drainage basin were as follows:

Major Drainage Basin	Streams	Lakes	Reservoirs
Colorado River	74	7	1
Dolores River	5	0	0
Gunnison River	10	0	2
San Juan River	11	0	0
White River	6	1	0
Yampa River	17	1	0
Totals	**123**	**9**	**3**

Over the past 4 years, we have conducted fish population estimates and/or collected trout for disease testing for evidence of *M. cerebralis* infection from 105 streams and 150 sites in the greater Colorado River drainage in western Colorado. During the 2006 field season we collected fish and/or aquatic oligochaetes from 49 streams and lakes from 73 separate locations that were considered to have potential as cutthroat trout recovery streams, or were in proximity of or connected to streams containing Colorado River cutthroat trout. Sites sampled each year between 2003 and 2006 are shown on Map 1 in the Appendix. Most of the sampling effort for fish and worm collections during 2006 was concentrated in the San Juan, White and Yampa River basins. In addition, an intensive effort was expended collecting additional worm samples in the Arkansas, Blue, Cache la Poudre, and South Platte River basins.

Trout Population Assessment and Parasite Screening -Trout population estimates and summaries of electrofishing surveys as well as PCR and PTD test results for evidence of *M. cerebralis* infection for all sampling sites for 2006 are organized by sub-species and presented in Tables 1 through 10. Odd-number tables contain bio-statistical estimates for population size, 95% confidence limits, density (N/ha) and biomass (kg/ha) for each stream sampled. Even-number tables summarize the PCR and PTD test results for fish collected and tested for evidence of *M. cerebralis* infection. No streams containing greenback cutthroat trout for the South Platte and Arkansas river basins were sampled for fish collections during 2006.

Rio Grande Cutthroat Trout **-** Tables 1 and 2 contain the data summaries for Rio Grande basin streams sampled during 2006. In addition, the field crew spent several days collaborating with Rio Grande basin Aquatic Biologist John Alves in collecting and removing brook trout from the Placer Creek drainage in an attempt to minimize the continued spread of *M. cerebralis* into the upper headwater regions.

Samples of brook and Rio Grande cutthroat trout removed during the extensive electrofishing operations in Placer Creek during the summer of 2006 (Table 2) demonstrated that the spatial distribution of *M. cerebralis* was restricted to main Placer Creek. Infection prevalence and severity was greatest in the section of Placer Creek upstream of the confluence

with the Middle Fork Placer Creek. Infected brook and Rio Grande cutthroat trout were found in the middle reaches of the drainage up to 3 km upstream of the confluence with the Middle Fork. Disease testing of fish 5 km upstream in Placer Creek as well as at all collection sites from the Middle Fork of Placer Creek tested negative for evidence of infection (Table 2). These findings support the hypothesis that the focal point of infection was a washed out beaver pond on main Placer Creek approximately 2.8 – 3 km upstream of the confluence with the Middle Fork. Despite extensive sediment sampling effort over several days, no tubificid oligochaetes were found in the Middle Fork of Placer Creek, or in Placer Creek upstream of the confluence with the Middle Fork. It is possible that ash or some other factor associated with the Malo Vega forest fire that swept through the area in late-spring 2006 may have had lethal affects for tubificid worms in the basin.

Management plans for Placer Creek in 2007 call for chemical reclamation to remove the fish population subsequent to the installation of a new barrier to prevent re-invasion by brook trout from Sangre de Cristo Creek. It is also being proposed that non-susceptible lineages of *T. tubifex* be transplanted into the Placer Creek drainage to 1) act as "bio-filters" to consume and de-activate any residual *M. cerebralis* myxospores, and 2) possibly establish dense colonies of non-susceptible lineages (I, V and VI) of *T. tubifex* that might out-compete the highly susceptible lineage III worms. The upper reaches of main Placer Creek (in the vicinity of the inactive beaver pond) would be seeded with lineage I, V and lineage VI *T. tubifex* worms that recent research has shown to be highly resistant or refractory for infection by *M. cerebralis* (Beauchamp et al. 2001, 2002; DuBey and Caldwell 2004; DuBey et al. 2005). Laboratory exposure experiments on various lineages of *T. tubifex* conducted by CDOW researchers during the winters of 2005-2006 and 2006-2007 have clearly demonstrated that lineage I, V and VI *T. tubifex* in Colorado are not susceptible to infection by *M. cerebralis*, corroborating the findings of DuBey and Caldwell (2004) and DuBey et al. (2005). Depopulation of the stream, re-installation of a migration barrier to prevent re-invasion by the brook trout, and introduction of resistant lineages of *T. tubifex* to act as biological "biofilters" to consume and deactivate *M. cerebralis* myxospores have the potential to reduce the presence or even eliminate the parasite from the Placer Creek drainage.

Colorado River Cutthroat Trout - Tables 3 through 10 summarize the bio-statistical data for the fish population sampling and the corresponding PCR and PTD disease testing results for the San Juan, Colorado, Dolores, Gunnison, Uncompahgre, White and Yampa river basins. Colorado River cutthroat trout occur in all of these river basins.

It was disturbing to find evidence of *M. cerebralis* infection among so many trout from so many collection sites in habitat(s) suitable for supporting Colorado River cutthroat trout (see Tables 4, 6,8 and 10 for details). Salmonids testing positive for *M. cerebralis* infection were collected from 7 of 19 sampling sites on 16 streams in the San Juan River sub-basin of the Colorado River drainage (Table 4). Salmonids testing positive for *M. cerebralis* infection were collected at 4 of 9 sampling sites from 9 streams in the Dolores River, Gunnison River, Uncompahgre River and Plateau Creek sub-basins of the Colorado River drainage (Table 6). Salmonids testing positive for *M. cerebralis* infection were collected from 14 of 29 sampling sites from 14 streams in the White River sub-basin of the Colorado River drainage (Table 8). Finally, salmonids testing positive for *M. cerebralis* infection were collected from 4 of 11

streams at 6 of 17 sampling sites in the Yampa River sub-basin of the Colorado River drainage (Table 10).

Most troubling is the up-ramping of *M. cerebralis* infection among brook trout and Colorado River cutthroat trout in Trappers Lake and its tributary streams, Cabin, Fraser and Heberton Creek. This lake is in the Flattops Wilderness in the headwaters of the White River basin east of Meeker and has been the mainstay of spawning-taking operations for Colorado River cutthroat by the Colorado Division of Wildlife for almost a century. Overt clinical signs of WD were evident among many of the YOY and juvenile fish collected from these three streams as well as those in Marvine Creek just upstream of the confluence with the East Fork of Marvine Creek during the summer and fall of 2006. Marvine Creek is also tributary to the White River. *M. cerebralis*-positive fish were first collected from Trappers Lake in 2003. The high level of cranial myxospore concentrations among salmonids collected from several sites on the North Fork of the White River beginning at the USFS Himes Peak Campground just downstream from Trappers Lake suggests that Trappers Lake is clearly a point source of infectivity. Cranial myxospore concentrations decrease in both prevalence and severity as distance down the White River (from Trappers Lake) increases. However, the data clearly demonstrate that there are most likely several point sources of *M. cerebralis* infectivity in the White River drainage in addition to Trappers Lake, including Marvine Creek, North Elk Creek and Big Beaver Creek.

There are several streams that are tributaries of the White River that sustain viable populations of Colorado River cutthroat trout including Big Beaver Creek upstream of Lake Avery, Fawn Creek, Lost Creek, the Middle and East Fork(s) of North Elk Creek and Ute Creek. Only the Ute Creek population of Colorado River cutthroat trout is isolated upstream of a migration barrier. It is of paramount importance that migration barriers be constructed on these streams as soon as is feasible to protect them from non-native salmonids carrying the *M. cerebralis* parasite that could migrate out of the White River, or the Big Beaver Creek inlet to Lake Avery. Although they do not produce as high a concentration of cranial myxospores as rainbow trout, Colorado River cutthroat trout are highly vulnerable to the *M. cerebralis* parasite and can suffer very high mortality when exposed to ambient levels of *M. cerebralis* actinospores as YOY fry in free-flowing streams (Thompson et al. 1999). Continued increases in ambient levels of *M. cerebralis* infection potentially pose a threat to the mountain whitefish (*Prosopium williamsoni*) population of the White River.

M. cerebralis-positive salmonids were collected from several streams in the Yampa River sub-basin of the Colorado River, including the Bear River, East Coal Creek, Green Creek and Trout Creek. Most puzzling was the fact that salmonids collected from Trout Creek approximately 2 km upstream of Sheriff Reservoir tested negative for evidence of *M. cerebralis* infection, even though infected brook trout were collected from the creek downstream of Sheriff Reservoir and lineage III *T. tubifex* worms highly infected with *M. cerebralis* were collected from Trout Creek at the inlet to Sheriff Reservoir. The Trout Creek population of Colorado River cutthroat trout are not isolated from upstream migrating fish from Sheriff Reservoir by a migration barrier.

Aquatic Oligochaete Sampling – Since 2001, substantial research efforts have been directed at developing a better understanding of the factors that affect the population dynamics

and distribution of aquatic oligochaetes in the natural environment and determining the relative differences in susceptibility to *M. cerebralis* among the 4 lineages of *T. tubifex* (Beauchamp et al. 2001, 2002, 2005, 2006; DuBey and Caldwell 2004; DuBey et al. 2005; Kaesar and Sharpe 2006; Kerans et al. 2004: Winkelman and Nehring 2007). As more and more research investigations have been directed at the aquatic oligochaete side of the life cycle of *M. cerebralis* it has become increasing clear that the presence of the lineage III *T. tubifex* in an aquatic environment may well be the primary determining factor governing whether or not *M. cerebralis* becomes established after the initial introduction occurs. In the San Juan River below Navajo Dam in New Mexico, DuBey and Caldwell (2004) found that only lineage III *T. tubifex* were infected with *M. cerebralis*, even though *T. tubifex* belonging to lineages I and VI were also present in the stream. Moreover, in a follow-up laboratory study where worms from lineages I, III and VI were exposed to myxospores of *M. cerebralis*, evidence of infection by the parasite was only detected in lineage III worms (DuBey et al. 2005). Similar outcomes have emerged from laboratory tests where lineage I, III, V and VI *T. tubifex* have been exposed to varying concentrations of *M. cerebralis* myxospores in Colorado (Nehring, unpublished data), Oregon (Dr. Jerri Bartholomew, personal communication) California (Dr. Ron Hedrick, unpublished data) and in states in the eastern U.S. (Dr. Vicki Blazer, personal communication). For these reasons, ascertaining the distribution and relative abundance of the various lineages of *T. tubifex* in Colorado's cutthroat trout streams appears to be a critically important component in assessing risk of establishment and spread of *M. cerebralis* in Colorado.

The aquatic oligochaete sampling protocol has been a "learning experience". During 2003, oligochaetes were often difficult to collect, even from sites (such as heavily sedimented beaver ponds) where habitat conditions looked optimal (Nehring 2004). Errors in protocol also ruined some samples. All of the samples from the Rio Grande basin in 2003 had no detectible DNA of *M. cerebralis* when tested by PCR. Chlorinated tap water was inadvertently used to dilute the ethanol for preservation of the samples. Minute amounts of chlorine will denature DNA, rendering PCR analysis ineffective. Development and testing of the 4-probe multiplex qPCR protocol that would allow for simultaneous testing and quantification of mitochondrial DNA from 4 different lineages of *T. tubifex* in a single sample (Beauchamp et al. 2002) was an on-going process through the summer and fall of 2003 (John Wood, Pisces Molecular; personal communication).

During 2003 and the early months of 2004, a large amount of testing was done to facilitate development and testing of the 4-probe multiplex qPCR test for quantifying the relative amount of DNA for the various lineages (I, III, V and VI) of *T. tubifex* in aquatic oligochaetes. Large numbers of worms were tested individually as well as in pooled aliquots of 5, 10, 25, 50 and 100 worms. These tests were completed to 1) develop standards for calibration of the test for the 4 lineages of worms, and 2) determine what number of worms in an individual aliquot seemed to produce the most reliable (accuracy and precision) and repeatable results. By spring 2004, development and testing of the 4-probe multiplex qPCR protocol was complete. The results of those efforts are summarized in Tables 10a, 10b, 10c and 10d in Nehring 2006 and will not be reiterated here. The 50 worm aliquot provided the best results across a broad range of worm sizes while concurrently minimizing the reagent costs at the laboratory.

Since 2003, greater effort has been expended to collect sediment samples. Skills in

recognizing probable habitats where oligochaetes are likely to occur have been improved. Sample preservation and laboratory protocols have also improved. Taken together, these efforts have resulted in larger numbers of aquatic oligochaetes collected at most sampling sites during the 2004, 2005 and 2006 field seasons. Data summaries for the oligochaete sampling efforts for 2003, 2004, 2005 and 2006 are presented in Tables 11 through 20 in the Appendix.

The location of each sample site is referenced using global positioning technology (GPS). This allows all data to be plotted on s visually depicting the distribution of both aquatic oligochaetes and fish collected and tested for *M. cerebralis* infection. It also facilitates a visual representation of the distribution of the various lineages of *T. tubifex* by drainage basin for all of Colorado. Maps summarizing the distribution of those lineages can be seen in the Appendix. The locations of collections of *T. tubifex* identified as lineage I, III, V and VI are shown on maps 2, 3, 4, and 5 respectively. Examination of these maps indicate that *T. tubifex* belonging to lineage III are the most common and widely distributed throughout the Colorado, particularly within the Colorado River basin. Map 6 shows those sites where aquatic oligochaetes were collected, but there was no amplification of mitochondrial DNA for any of the 4 lineages of *T. tubifex.* Quality control checking has shown that tubificid DNA is present in the samples. Base pair sequence comparisons of DNA from some of the tubificid samples from the Rio Grande basin as well as other areas around the state with base pair sequences stored in GENBANK indicate the DNA contained in samples that did not amplify was usually from *Limnodrilus hoffmeisteri* or *Ilyodrilus templetoni*. These two tubificid species are cosmopolitan and commonly found in both lake and stream habitats. Both occur in Windy Gap Reservoir and in the upper Colorado River basin (Zendt and Bergersen 2000).

It is unknown whether or not colder water temperatures or harsh environmental conditions found at high altitudes in the mountains of Colorado might prohibit the distribution of *T. tubifex,* particularly the lineage III worm that is the susceptible host for *M. cerebralis*. All sites where lineage III *T. tubifex* have been collected across Colorado from 2003 through 2006 were stratified into elevational zones to evaluate this possibility. The data below do not support this hypothesis. Mitochondrial DNA specific to lineage III *T. tubifex* was detected in more worm samples at each 1,000 foot elevational increment between 6,000 feet and 11,000 above mean sea level than that for lineages I, V and VI combined. It may be noteworthy that no mitochrondial DNA for any lineage of *T. tubifex* was detected in oligochaete samples collected at sites > 11,000 feet; however, oligochaetes have only been collected at seven sites at those high elevations.

Elevation (ft.)	**Number of Sites where each Lineage of *Tubifex tubifex* was present**				
	Lineage I	**Lineage III**	**Lineage V**	**Lineage VI**	**No Lineage**
6,000 – 7,000	1	10	1	2	3
7,001 – 8,000	1	16	2	4	2
8,001 – 9,000	4	20	3	8	10
9,001- 10,000	2	13	2	6	11
10,001- 11,000	1	14	1	2	10
11,001 – 12,000	0	0	0	0	7
Total	9	73	9	22	43
Percent	6	47	6	14	27

CONCLUSIONS

Recent developments in the DNA typing and testing of the various lineages of *T. tubifex* for susceptibility or resistance to *M. cerebralis* infection are very encouraging. Results of studies conducted in Colorado, California , Oregon, New Mexico, and West Virginia have consistently shown that only lineage III *T. tubifex* are susceptible to *M. cerebralis* infection. Oligochaetes belonging to lineages I, V and VI are refractory to the parasite and do not produce fish-infective *M. cerebralis* TAMs. These results offer hope lineage I, V and VI *T. tubifex* can act as "biofilters" to consume and deactivate *M. cerebralis* myxospores in habitats where the parasite is already enzootic, and dramatically reduce ambient levels of infection. Indeed, this appears to have been occurring at Windy Gap Reservoir in Colorado for the past 5-6 years (Winkelman and Nehring 2007).

Results of the aquatic oligochaete sampling and testing over the past four field seasons have demonstrated that the *M. cerebralis*-susceptible lineage III *T. tubifex* is the most widely distributed of the four lineages *T. tubifex* known to occur in Colorado. Mitochondrial DNA specific to the lineage III oligochaete has been detected in more worm samples at all elevation zones between 6,000 and 11,000 feet in Colorado than that for worms belonging to lineages I, V and VI combined. These findings indicate that the risk of establishment of *M. cerebralis* is quite high, once introduced into a previously unexposed aquatic ecosystem, given the wide distribution and high abundance of the lineage III worms.

The number of sites visited in 2006 where trout tested positive for *M. cerebralis* infection was considerably higher than in any of the previous three field seasons. It was particularly troublesome to see the high degree of prevalence and severity of infection evident in both brook trout and Colorado River cutthroat trout in Trappers Lake in the Flattops Wilderness. As is the case for Yellowstone Lake (Koel et al. 2006) , the mechanism by which *M. cerebralis* was vectored into Trappers Lake remains a mystery. However, in both instances there has been a significant up-ramping in the degree of infection among cutthroat trout in both of these pristine ecosystems. The number of potential point sources of *M. cerebralis* TAM production in the White River sub-basin of the Colorado River could prove to be problematic for the mountain whitefish population in that drainage.

After four field seasons of study, it is becoming evident that *M. cerebralis* has become established in several aquatic habitats that support native cutthroat trout populations in Colorado. How serious a threat this poses for the recovery of Colorado's three sub-species of cutthroat trout is unknown. However, given the degree of spread of the parasite into high elevation habitats in the White and Yampa River basins that have direct connectivity to streams supporting excellent populations of Colorado River cutthroat trout, it is important that efforts be increased to construct barriers to isolate these populations and prevent invasion by non-native salmonids carrying the parasite from other areas where it is already enzootic.

Table 1. Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within **Rio Grande** cutthroat trout (*Oncorhynchus clarki virginalis*) recovery zones sampled during the summer and fall of 2006.

Date	Brown Trout				Brook Trout				RGN Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Alberta Creek below Alberta Park Reservoir GPS 13S343916//4148625												
07/14/06	--	--	--	--	5	--	163	12	--	--	--	--
Big Spring Creek (north Clear Creek drainage above Mesa Creek confluence) GPS 13S 310994//4196330												
08/14/06	--	--	--	--	9	--	461	26	--	--	--	--
North Fork Culebra Creek – lower station (near confluence with S. Fork Culebra Creek) GPS 13S 475030//4117880												
08/17/06	32	± 5	646	98	3	±7	61	1	4	±3	82	20
North Fork Culebra Creek – upper station (above confluence with S. Fork Culebra Creek) GPS 13S 475464//4119408												
08/17/06	65	±7	826	85	5	±0	64	1	--	--	--	--
West Indian Creek (on the Trinchera Ranch) GPS 13S 479120//4143135												
08/16/06	--	--	--	--	35	±1	1,256	73	7	±10	251	28
Pass Creek (South Fork Rio Grande east of Big Meadows Reservoir) GPS 13S344398//4150542												
07/14/06	--	--	--	--	39	±5	1,072	65	--	--	--	--
Trinchera Creek (on the Trinchera Ranch) GPS 13S 472959//4136610												
08/15/06	--	--	--	--	2	± 0	72	5	--	--	--	--
North Fork of Trinchera Creek (on the Trinchera Ranch) GPS 13S 477129//4137176												
08/16/06	--	--	--	--	26	±3	922	70	1	± 0	36	3
South Fork of Trinchera Creek (on the Trinchera Ranch) GPS 13S 476906//4132186												
08/15/06	--	--	--	--	15	± 2	545	49	4	± 3	144	17

Table 2. Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of **Rio Grande** cutthroat (*Oncorhynchus clarki virginalis*) trout in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" ≤ 9 or "n" ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold.

An "ns" abbreviation in any data column indicates no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	N +	Score	N	n+	Mean (n+) myxospores	Range Myxospores (n+)
Rio Grande Cutthroat Trout *(Oncorhynchus clarki virginalis*) Recovery Areas and Nearby Tributary Streams and Reservoirs									
Alberta Creek	Below Alberta Park Reservoir	Brook	10	0	10	10	0	0	0
Big Spring Creek	Near summit of Spring Creek Pass	Brook	10	0	10	10	0	0	0
N. Fk. Culebra Creek	Site # 2 (upstream sampling site)	Brook	1	0	10	10	0	0	0
N. Fk. Culebra Creek	Site # 2 (upstream sampling site)	Brown	10	0	10	10	0	0	0
N. Fk. Culebra Creek	Site #1 (downstream sampling site)	Brown	9	0	10	10	0	0	0
West Indian Creek	Forbes Trinchera Ranch	Brook	10	0	10	9	0	0	0
West Indian Creek	Forbes Trinchera Ranch	RGN cut	ns	ns	ns	1	0	0	0
Pass Creek	↓ confl. w/ Alberta Park Rsvr outflow	Brook	ns	ns	ns	10	0	0	0
Placer Creek	**Sangre de Cristo Crk to 1st Rd. Cross.**	**Brook**	ns	ns	ns	**14**	**1**	**56,983**	**56,986**
Placer Creek	**Sangre de Cristo Crk to 1st Rd. Cross.**	**RGN cut**	ns	ns	ns	**6**	**3**	**76,419**	**8,267 – 195,689**
Placer Creek	**1 km below Greyback Creek confl.**	**Brook**	**10**	**10**	**50**	**20**	**0**	**0**	**0**
Placer Creek	**1 km below Greyback Creek confl.**	**RGN cut**	**ns**	**ns**	**ns**	**10**	**4**	**42,250**	**8,528 – 95,950**
Placer Creek	**0.5 km ↓ Middle Fork confluence**	**Brook**	**10**	**10**	**48**	**ns**	**ns**	**ns**	**ns**
Placer Creek	**0.3 km ↑ Middle Fork confluence**	**Brook**	**7**	**7**	**50**	**ns**	**ns**	**ns**	**ns**
Placer Creek	**3 km ↑confluence w/ Middle Fork**	**Brook**	**10**	**9**	**21**	**10**	**5**	**68,170**	**2,156 – 123,889**
Placer Creek	**3 km ↑confluence w/ Middle Fork**	**RGN cut**	**ns**	**ns**	**ns**	**10**	**8**	**53,153**	**4,244 – 150,167**
Placer Creek	5 km ↑confluence w/ Middle Fork	Brook	10	0	10	10	0	0	0
Placer Creek	5 km ↑confluence w/ Middle Fork	RGN cut	ns	ns	ns	11	0	0	0
Middle Fk. Placer Crk.	0.8 km ↓ confluence w. South Fork	Brook	10	0	10	20	0	0	0
Middle Fk. Placer Crk.	0.5 km ↑ confluence w/ South Fork	Brook	10	0	10	20	0	0	0
Middle Fk. Placer Crk.	4.7 km ↑ confluence w/ Placer Creek	Brook	10	0	10	20	0	0	0
Trinchera Creek	Forbes Trinchera Ranch	Brook	10	0	0	ns	ns	ns	ns
N.Fk. Trinchera Creek	Forbes Trinchera Ranch – site 1	Brook	10	0	10	10	0	0	0
N.Fk. Trinchera Creek	Forbes Trinchera Ranch – site 2	Brook	10	0	10	10	0	0	0

Table 3. Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **San Juan River** basin sampled during 2006.

Date	Brown Trout				Brook Trout				CRN Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Castle Creek (Rio Blanco River drainage) GPS 13S 342405//4118605												
No fish collected or seen. 2 km of the creek upstream of the road crossing were walked and checked for fish.												
Corral Creek (headwaters of the Hermosa Creek drainge) GPS 13S 241676//4173368												
07/10/06	2 [c]	± 0	42 [c]	2 [c]	13	± 1	274	23	7	± 1	148	11
Fish Creek (at trailhead – Rio Blanco Basin) GPS 13S 347063//4120773												
09/19/06	--	--	--	--	15	±1	414	27	--	--	--	--
Florida River (100 m below Lemon Reservoir) GPS 13S 264340//4140216												
07/10/06	8 [bcd]	--	69 [bcd]	4 [bcd]	--	--	--	--	--	--	--	--
Hermosa Creek near Hermosa, CO GPS 13S 13S 247945//4146165												
06/26/06	10 [bcd]	--	154 [bcd]	18 [bcd]	1 [bcd]	--	15 [bcd]	1 [bcd]	--	--	--	--
East Fork Hermosa Creek near Purgatory Ski Area GPS 13S 246612//4168800												
07/11/06	--	--	--	--	--	--	--	--	10	± 0	536	45
Piedra River – 2 km upstream of U.S. Hwy 160 GPS 13S 292221//4123812												
09/21/06	6 [bd]	--	22 [bd]	3 [bd]	2 [bcd]	--	7 [bcd]	0	--	--	--	--
Piedra River – 2 km below confluence of East and Middle Forks GPS 13S 305894//4144596												
09/20/06	3 [bd]	--	16 [bd]	1 [bd]	2 [bcd]	--	11 [bcd]	1 [bcd]	--	--	--	--
East Fork of the Piedra River upstream of confluence with Middle Fork GPS 13S 311137//4148008												
07/11/06	12 [bd]	--	65 [bd]	1 [bd]	6 [bd]	--	3 [bd] 2	1 [bd]	2 [bcd]	--	11 [bcd]	4 [bcd]
Middle Fork of the Piedra River upstream of confluence with East Fork GPS 13S 308185//4150342												
07/11/06	19 [bd]	--	102 [bd]	2 [bd]	2 [bd]	--	11 [bd]	0	--	--	--	--
Rito Blanco River (Blanco River Basin) GPS 13S 335159//4127187												
09/20/06	--	--	--	--	--	--	--	--	13	± 1	278	20

[a]: Almost all trout captured were ≤150 mm. **[b]** Single electrofishing pass only; no population estimate. **[c]:** Rainbow trout, not brown, brook or cutthroat trout. **[d]:** electrofishing for PTD and PCR samples only**.**

Table 3 (continued). Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **San Juan River** basin sampled during 2006.

Date	Brown Trout				Brook Trout				Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
East Fork San Juan River headwaters, above falls GPS 13S 346149//4142267												
07/13/06	1 [bcd]	--	2 [bcd]	0	--	--	--	--	9 [bd]	--	22 [bd]	3 [bd]
East Fork San Juan River, below Sand Creek confluence GPS 13S 335536//4138930												
07/13/06	25 [bd]	--	117 [bd]	1 [bd]	10 [bcd]	--	47 [bcd]	9 [bcd]	--	--	--	--
West Fork of the San Juan River GPS 13S 330960//4146490												
07/13/06	3 [bd]	--	31 [bd]	15 [bd]	6 [bcd]	--	62 [bcd]	56 [bcd]	--	--	--	--
South Branch of South Creek, Rio Blanco River basin GPS 13S 343475//4118966												
09/19/06	--	--	--	--	8 [bd]	--	287 [bd]	14 [bd]	--	--	--	--
White Creek – tributary to the Rio Blanco GPS 13S 342978//4119185												
09/19/06	--	--	--	--	8 [bd]	--	1,148 [bd]	103 [bd]	--	--	--	--
Weminuche Creek headwaters GPS 13S 302918//4149094												
07/12/06	8 [bd]	--	27 [bd]	1 [bd]	4 [bd]	--	13 [bd]	1 [bd]	1 [bcd]	--	3 [bcd]	0
East Fork Weminuche Creek upstream of confluence with Weminuche Creek GPS 13S 298168//4159605												
07/12/06	7 [bd]	--	44 [bd]	6 [bd]	11 [bd]	--	70 [bd]	4 [bd]	--	--	--	--
Williams Creek 1 km upstream of Williams Creek Reservoir GPS 13S 305760//4156844												
07/11/06	--	--	--	--	20 [bd]	--	215 [bd]	4 [bd]	--	--	--	--
Williams Creek at USFS Bridge Campground below Williams Creek Reservoir GPS 13S 305366//4149844												
09/20/06	20 [bd]	--	215 [bd]	1 [bd]	--	--	--	--	--	--	--	--

[a]: Almost all trout captured were ≤150 mm. [b] Single electrofishing pass only; no population estimate. [c]: Rainbow trout, not brown, brook or cutthroat trout. [d]: electrofishing for PTD and PCR samples only.

Table 4. Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*) trout in the **San Juan River** basin in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" was ≤ 9 or ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold. Letters ns indicate no sample collected. An "ns" in any column means no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	N+	Mean (n+) myxospores	Range Myxospores (n+)
Corral Creek	↑ confl. E. Fk. Hermosa Crk	Brook	10	0	10	10	0	0	0
Corral Creek	↑ confl. E. Fk. Hermosa Crk	Rainbow	ns	ns	ns	1	0	0	0
Fish Creek	End of road (Rio Blanco R. basin)	Brook	10	0	10	10	0	0	0
Florida River	**100 m below Lemon Reservoir**	**Rainbow**	ns	ns	ns	**8**	**8**	**89,722**	**31,111 – 270,000**
Hermosa Creek	**Near Hermosa, CO**	**Rainbow**	ns	ns	ns	**10**	**2**	**5,000**	**2,222 – 7,778**
E. Fk. Hermosa Crk.	↑ USFS Sig Creek Campground	CRN cut	10	0	10	7	0	0	0
E. Fk. Hermosa Crk.	↓ USFS Sig Creek Campground	CRN cut	3	0	10	ns	ns	ns	Ns
Piedra R.	2 km ↑ U.S. Hwy 160 bridge	Brown	ns	ns	ns	10	0	0	0
Piedra R. #3	**2 km ↓E. Fk. & Md. Fk. Confl.**	**Brown**	**10**	**1**	**14**	8	0	0	0
Piedra R. #3	2 km ↓E. Fk. & Md. Fk. Confl.	Rainbow	ns	ns	ns	2	0	0	0
E. Fork Piedra R.	**Above confluence w/ Middle Fk.**	**Rainbow**	**ns**	**ns**	**ns**	**2**	**0**	**0**	**0**
E. Fork Piedra R.	**Above confluence w/ Middle Fk.**	**Brown**	**10**	**3**	**15**	**5**	**0**	**0**	**0**
E. Fork Piedra R.	**Above confluence w/ Middle Fk.**	**Brook**	**ns**	**ns**	**ns**	**3**	**1**	**68,889**	**68,889**
Md. Fk. Piedra R.	Above confluence w/ E. Fork	Brown	10	0	10	8	0	0	0
Md. Fk. Piedra R.	Above confluence w/ E. Fork	Brook	ns	ns	ns	2	0	0	0
Rito Blanco River	2 km ↓ Mariposa Creek confluence	CRN cut	10	0	10	10	0	0	0
E. Fork San Juan R.	Below Sand Creek confluence	Brown	10	0	10	10	0	0	0
E. Fork San Juan R.	Below Sand Creek confluence	Rainbow	ns	ns	ns	10	0	0	0
E. Fork San Juan R.	Above barrier falls	Rainbow	ns	ns	ns	1	0	0	0
E. Fk. San Juan R.	**Above barrier falls**	**CRN cut**	ns	ns	ns	**9**	**1**	**556**	**556**
W. Fk. San Juan R	**Above E. Fork confluence**	**Brown**	**2**	**0**	**10**	**3**	**0**	**0**	**0**
W. Fk. San Juan R	**Above E. Fork confluence**	**Rainbow**	**1**	**0**	**10**	**5**	**2**	**1,667**	**556 – 2,778**
S. Branch S. Creek	Rio Blanco R. basin	Brook	ns	ns	ns	10	0	0	0

Table 4 (continued). Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*) trout in the **San Juan River** basin in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" was ≤ 9 or ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold. Letters ns indicate no sample collected. An "ns" in any column means no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	N+	Mean (n+) myxospores	Range Myxospores (n+)
Weminuche Creek	↓private ranch –Piedra R. basin	Brook	7	0	10	1	0	0	0
Weminuche Creek	↓private ranch –Piedra R. basin	Brown	ns	ns	ns	8	0	0	0
Weminuche Creek	↓private ranch –Piedra R. basin	Rainbow	ns	ns	ns	1	0	0	0
E. Fk. Weminuche C.	↑ private ranch - Piedra River basin	Brook	ns	ns	ns	6	0	0	0
E. Fk. Weminuche C.	↑ private ranch - Piedra River basin	Brown	ns	ns	ns	4	0	0	0
White Creek	wilderness bndry. Rio Blanco R.	Brook	10	0	10	10	0	0	0
Williams Creek	↑Williams Creek Reservoir	Brook	10	0	10	10	0	0	0
Williams Creek	**Below Williams Creek Reservoir**	**Brown**	**10**	**1**	**11**	10	0	0	0

Table 5. Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **Colorado, Dolores, Gunnison** and **Uncompahgre River** basins sampled during 2006.

Date	Brown Trout				Brook Trout				Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Big Creek, below Bonham Reservoir on Grand Mesa GPS 13S 246775//4336667 (Colorado River)												
06/26/06	--	--	--	--	2 [bd]	--	120 [bd]	2 [bd]	3 [bd]	--	179 [bd]	15 [bd]
West Fork of Big Creek above Bonham Reservoir 13S 246612//4168800 (Colorado River)												
06/26/06					6 [bd]	--	108 [bd]	20 [bd]	--	--	--	--
Unnamed tributary to Big Creek upstream of Bonham Reservoir GPS 13S 248826//4330469 (Colorado River)												
06/26/06	--	--	--	--	10 [bd]	--	359 [bd]	46 [bd]	--	--	--	--
Dolores River at USFS Barlow Creek Campground GPS 13S 236903//4184364												
08/10/06	4	--	17	2	9 [bcd]	--	39 [bcd]	4 [bcd]	--	--	--	--
Scotch Creek upstream of Dolores River confluence GPS 12S 760909//4171452												
08/10/06	17	--	1,830	59	3	--	323	8	--	--	--	--
West Fork Cebolla Creek near Spring Creek Pass GPS 13S 308645//4203293 (Gunnsion River)												
08/14/06	--	--	--	--	21 [bd]	--	753 [bd]	12 [bd]	--	--	--	--
Beaver Dams Creek (Uncompahgre River Basin) GPS 12S 753660//4243910												
06/13/06	--	--	--	--	--	--	--	--	7	±1	628	29
East Fork Dry Creek near Montrose (Uncompahgre River Basin) GPS 12S 758030//4249908												
06/13/06	7	±0	314	47	5 [c]	±2	224 [c]	17 [c]	--	--	--	--

[a]: Almost all trout captured were ≤150 mm. **[b]** Single electrofishing pass only; no population estimate. **[c]:** Rainbow trout, not brown, brook or cutthroat trout. **[d]:** electrofishing for PTD and PCR samples only**.**

Table 6. Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*) trout in the **Colorado River** basin in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" was ≤ 9 or ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold. An "ns" abbreviation in any data column indicates no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	n+	Mean (n+) myxospores	Range Myxospores (n+)
Unnamed Stream	**Big Creek ↑ Bonham Reservoir**	**Brook**	**3**	**0**	**10**	**10**	**1**	**1,944**	**1,944**
Unnamed Stream	**Big Creek ↑ Bonham Reservoir**	CRN cutt	ns	ns	Ns	1	0	0	0
Beaver Dams Creek	Uncompahgre R. near Montrose	CRN cutt	ns	ns	ns	10	0	0	0
West Fk. Big Creek	**Big Creek ↑ Bonham Reservoir**	**Brook**	**5**	**5**	**40**	**10**	**9**	**84,507**	**1,667 – 268,889**
Big Creek	**Big Creek ↓ Bonham Reservoir**	**Brook**	ns	ns	ns	**2**	**1**	**60,000**	**60,000**
Big Creek	**Big Creek ↓ Bonham Reservoir**	**CRN cut**	ns	ns	ns	**10**	**1**	**3,333**	**3,333**
W. Fk. Cebolla Ck.	**Gunnison River headwaters**	**Brook**	10	0	10	**10**	**2**	**12,084**	**1,111 – 23,056**
Dolores River	@ USFS Barlow Creek Campgrd.	Brown	ns	ns	ns	4	0	0	0
Dolores River	@ USFS Barlow Creek Campgrd.	Rainbow	3	0	10	6	0	0	0
East Fork Dry Creek	Uncompahgre R. near Montrose	Brown	ns	ns	ns	10	0	0	0
East Fork Dry Creek	Uncompahgre R. near Montrose	Rainbow	ns	ns	ns	1	0	0	0
Scotch Creek	@ USFS Barlow Creek Campgrd.	Brook	ns	ns	ns	3	0	0	0
Scotch Creek	@ USFS Barlow Creek Campgrd.	Brown	11	0	10	7	0	0	0
Second Creek	↑ barrier near confl. w/ Smith Fk.	CRN cut	ns	ns	ns	10	0	0	0

Table 7. Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **White River** basin sampled during 2006.

Date	Brown Trout				Brook Trout				Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Big Beaver Creek, above Lake Avery and below Allen Creek confluence GPS 13T 277427//4439387												
09/05/06	--	--	--	--	--	--	--	--	7	±1	251	13
Cabin Creek @ Little Trappers Lake outlet GPS 13S 311247//4429101												
10/09/06	--	--	--	--	--	--	--	--	30 [bd]	--	897 [bd]	3 [bd]
Fawn Creek upper station GPS 13T 283840//4438256												
09/09/06	--	--	--	--	--	--	--	--	14 [bd]	±0	273 [bd]	24 [bd]
Fawn Creek lower station at Moeller Creek confluence GPS 13T 284218//4436000												
09/07/06	--	--	--	--	--	--	--	--	50	±3	538	59
Fraser Creek upstream of inlet to Big Trappers Lake GPS 13S 309580//4427548												
10/06/06	--	--	--	--	15 [bd]	--	538 [bd]	4 [bd]	16 [bd]	--	574 [bd]	1 [bd]
Heberton Creek upstream of inlet to Big Trappers Lake GPS 13S 309209//4428322												
10/06/06	--	--	--	--	51 [e]	±3	2,025 [e]	33 [e]	108 [e]	±14	4,254 [e]	18 [e]
Lost Creek, upper station GPS 13T 289524//44378981												
09/07/06	--	--	--	--	--	--	--	--	12	±1	248	21
Lost Creek, lower station GPS 13T 300414//4439256												
09/07/06	--	--	--	--	1 [c]	±0	25 [c]	4 [c]	17	±1	427	37
Marvine Creek upstream of East Fork of Marvine Creek confluence GPS 13T 292709//4431588												
09/07/06	26 [bde]	--	466 [bde]	29 [bde]	11 [bde]	--	197 [bde]	10 [bde]	--	--	--	--
North Elk Creek @ USFS boundary adjacent to private land GPS 13S271719//4421341												
10/11/06	1	±0	36	19	2	±0	72	10	31 [c e]	±1	1,115 [c e]	200 [c e]
Middle Fork North Elk Creek above East Fork North Elk Creek confluence GPS 13S 274475//4416545												
10/11/06	--	--	--	--	7 [e]	±1	239 [e]	17 [e]	44 [e]	±10	1,495 [e]	13 [e]
Snell Creek, lower station 1 km upstream of confluence with N. Fk. White River GPS 13T 299872//4438689												
09/06/06	--	--	--	--	19 [e]	±12	269 [e]	14 [e]	11 [e]	±8	160 [e]	10 [e]
Snell Creek, 2 km above road and upstream of lower station GPS 13T 300728//4439425												
09/06/06	--	--	--	--	39 [e]	±13	851 [e]	44 [e]	64 [e]	±8	1,401 [e]	35 [e]

[a]: Almost all trout captured were ≤150 mm. **[b]** Single electrofishing pass only; no population estimate. **[c]:** Rainbow trout, not brown trout. **[d]:** electrofishing for PTD and PCR samples only. **[e]:** estimates include young-of-the-year.

Table 7 (continued). Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **White River** basin sampled during 2006.

Date	Brown Trout				Brook Trout				Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Ute Creek, lower station GPS 13S 286915//4433634												
09/05/06	--	--	--	--	57 [e]	± 16	1,488 [e]	28 [e]	--	--	--	--
Ute Creek, upper station GPS 13S 287421//4430615												
09/05/06	--	--	--	--	35 [e]	± 9	903 [e]	34 [e]	--	--	--	--
White River, upper end of Sleepy Cat SWA to South Fork Bridge GPS 13S 273967//4427272												
10/19/06	1	±	0.2	0.03	175 [c]	±79	39 [c]	21	918 [f]	±324	205 [f]	150 [f]
White River, from County Rd 54 Bridge to Sleepy Cat SWA ponds GPS 13S 269781//4425716												
10/19/06	22	±17	5	5.6	391 [c]	±219	95 [c]	59 [c]	2,673 [f]	±565	648 [f]	352 [f]
White River near Wakara Ranch Bridge east of Meeker GPS 13T 258769//4432538												
11/09/06	20 [bd]	--	215 [bd]	4 [bd]	--	--	--	--	--	--	--	--
White River near Franklin Ranch Bridge east of Meeker GPS 13T 253672//4435718												
11/09/06	20 [bd]	--	215 [bd]	7 [bd]	--	--	--	--	--	--	--	--
North Fork of the White River @ USFS Himes Peak Campground GPS 13T 305869//4433332												
10/24/06	1 [bcd]	--	5 [bcde]	0 [bcde]	7 [bde]	--	38 [bde]	2 [bde]	13 [bde]	--	70 [bde]	1 [bde]
North Fork of the White River below the confluence with Snell Creek GPS 13T 299265//4438307												
10/24/06	2 [bcd]	--	11 [bcd]	1 [bcd]	--	--	--	--	12 [de]	--	65 [de]	1 [de]
North Fork of the White River @ USFS North Fork Campground GPS 13T 291940//4437116												
10/24/06	1 [bcd]	--	5 [bcd]	0 [bcd]	18 [bde]	--	97 [bde]	2 [bde]	--	--	--	--
South Fork of the White River at Flat Tops Wilderness boundary trailhead GPS 13S 283344//4415526												
10/24/06	1 [bcd]	--	18 [bcde]	2 [bcde]	5 [bde]	--	90 [bde]	5 [bde]	14 [bde]	--	251 [bde]	3 [bde]

[a]: Almost all trout captured were ≤150 mm. **[b]** Single electrofishing pass only; no population estimate. **[c]:** Rainbow trout, not brown, brook or cutthroat trout. **[d]:** electrofishing for PTD and PCR samples only. **[e]:** estimates include young-of-the-year. **[f]:** Mountain whitefish, not cutthroat trout.

Table 8. Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*) trout in the **White River** basin in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" was ≤ 9 or ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold. An "ns" abbreviation in any data column indicates no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	n+	Mean (n+) myxospores	Range Myxospores (n+)
S. Fk.White River	**Bel-Aire SWA ponds effluent**	**Brook**	ns	ns	ns	**10**	**8**	**61,444**	**3,072 – 156,683**
Big Beaver Creek	**0.1 km above Lake Avery**	**rainbow**	ns	ns	ns	**21**	**18**	**82,716**	**12,500 – 210,000**
Big Beaver Creek	**0.1 km above Lake Avery**	**CRN cut**	ns	ns	ns	**2**	**2**	**17,778**	**13,333 -22,222**
Big Beaver Creek	Below Allen Creek confluence	CRN cutt	10	0	10	10	0	0	0
North Elk Creek	**Wetland seep to White River**	**Brook**	**8**	**8**	**50**	**ns**	**ns**	**ns**	**ns**
North Elk Creek	**Wetland seep to White River**	**Rainbow**	**2**	**2**	**50**	**ns**	**ns**	**ns**	**ns**
North Elk Creek	**@USFS Boundary fence**	**Brook**	**ns**	**ns**	**Ns**	**2**	**1**	**20,833**	**20,833**
North Elk Creek	**@USFS Boundary fence**	**RXCutt**	**12**	**1**	**14**	**8**	**3**	**7,407**	**2,222 – 12,222**
Mid Fk. N.Elk Crk	@ confluence w/East Fork N. Elk	Brook	ns	ns	ns	6	0	0	0
Mid Fk. N.Elk Crk	@ confluence w/East Fork N. Elk	CRN Cut	10	0	10	4	0	0	0
Lower Fawn Creek	**Below Moeller Creek confluence**	**CRN cut**	**10**	**0**	**10**	**10**	**1**	**5,556**	**5,556**
Upper Fawn Creek	2 km ↑ Moeller Creek confluence	CRN cut	10	0	10	10	0	0	0
Fraser Creek	**Above Trapper's Lake inlet**	**Brook**	**9**	**7**	**32**	**7**	**6**	**21,019**	**2,778 – 20,000**
Fraser Creek	**Above Trapper's Lake inlet**	**CRN cut**	**10**	**3**	**19**	**4**	**0**	**0**	**0**
Heberton Creek	**Above Trapper's Lake inlet**	**CRN cut**	**20**	**0**	**10**	**ns**	**ns**	**ns**	**ns**
Heberton Creek	**Above Trapper's Lake inlet**	**Brook**	**10**	**10**	**49**	**10**	**6**	**49,046**	**1,667 – 208,333**
Lower Lost Creek	N. Fk. White River near Buford	Rainbow	ns	ns	ns	1	0	0	0
Lower Lost Creek	**N. Fk. White River near Buford**	**CRN cut**	**8**	**2**	**16**	9	0	0	0
Upper Lost Creek	N. Fk. White River near Buford	Rainbow	ns	ns	ns	1	0	0	0
Upper Lost Creek	N. Fk. White River near Buford	CRN cut	ns	ns	ns	9	0	0	0
Marvine Creek	**Above E. Fk. Marvine Creek**	**Brook**	**1**	**1**	**15**	**10**	**2**	**36,111**	**24,444 – 47,778**
Marvine Creek	**Above E. Fk. Marvine Creek**	**Rainbow**	**13**	**13**	**44**	**12**	**11**	**131,655**	**556 – 480,000**
Lower Snell Creek	N. Fork White River Basin	CRN cut	ns	ns	ns	1	0	0	0
Lower Snell Creek	N. Fork White River Basin	Brook	4	0	10	9	0	0	0
Upper Snell Creek	N. Fork White River Basin	CRN cut	10	0	10	5	0	0	0
Upper Snell Creek	N. Fork White River Basin	Brook	10	0	10	5	0	0	0

Table 8 (continued). Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*) trout in the **White River** basin in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" was ≤ 9 or ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold. An "ns" abbreviation in any data column indicates no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	n+	Mean (n+) myxospores	Range Myxospores (n+)
Cabin Creek	Little Trapper's Lake outlet	Brook	12	0	10	20	0	0	0
Cabin Creek	Little Trapper's Lake outlet	CRN cut	23	0	10	8	0	0	0
Cabin Creek	**Inlet to Big Trappers Lake**	**CRN cut**	**3**	**2**	**36**	**ns**	**ns**	**ns**	**ns**
Little Trappers Lake	Lake sample	CRN Cut	23	0	10	43	0	0	0
Little Trappers Lake	Lake sample	Brook	11	0	10	15	0	0	0
Lower Ute Creek	N. Fork White River	Brook	10	0	10	10	0	0	0
Upper Ute Creek	N. Fork White River	Brook	10	0	10	10	0	0	0
N. Fk. White River	**@ USFS Himes Peak Camprnd.**	**Brook**	**ns**	**ns**	**ns**	**7**	**2**	**39,945**	**10,000 – 63,889**
N. Fk. White River	**@ USFS Himes Peak Camprnd.**	**CRN cut**	**6**	**4**	**45**	**2**	**2**	**24,722**	**22,500 – 26,944**
N. Fk. White River	**@ USFS Himes Peak Camprnd.**	**Rainbow**	**5**	**5**	**44**	**1**	**1**	**305,556**	**305,556**
N. Fk. White River	**↓Snell Creek confluence**	**Rainbow**	**5**	**4**	**36**	**2**	**1**	**461,111**	**461,111**
N. Fk. White River	**↓Snell Creek confluence**	**CRN cut**	**4**	**4**	**35**	**2**	**1**	**57,222**	**57,222**
N. Fk. White River	**@ USFS N. Fork Campground**	**Brook**	**6**	**5**	**45**	**9**	**4**	**16,389**	**556 – 55,000**
N. Fk. White River	**@ USFS N. Fork Campground**	**Rainbow**	**4**	**4**	**48**	**1**	**1**	**46,111**	**46,111**
S. Frk White River	**@ Flatops Wilderness Bndry**	**Brook**	**1**	**1**	**50**	5	0	0	0
S. Frk White River	**@ Flatops Wilderness Bndry**	**CRN cut**	**2**	**2**	**17**	**4**	**0**	**0**	**0**
S. Frk White River	**@ Flatops Wilderness Bndry**	**Rainbow**	**8**	**2**	**12**	**1**	**0**	**0**	**0**
White River	**Upper Sleepy Cat SWA**	**Whitefish**	**ns**	**ns**	**ns**	**16**	**0**	**0**	**0**
White River	**Upper Sleepy Cat SWA**	**Rainbow**	**ns**	**ns**	**ns**	**2**	**1**	**34,444**	**34,444**
White River	**Lower Sleepy Cat SWA**	**Whitefish**	**ns**	**ns**	**ns**	**19**	**1**	**17,778**	**17,778**
White River	**Lower Sleepy Cat SWA**	**Rainbow**	**ns**	**ns**	**ns**	**1**	**0**	**0**	**0**
White River	**@ Wakara Ranch Bridge**	**Brown**	**10**	**2**	**26**	**10**	**1**	**556**	**0**
White River	**@ Franklin Ranch Bridge**	**Brown**	**10**	**9**	**39**	**10**	**0**	**0**	**0**

Table 9. Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **Yampa River** basin sampled during 2006.

Date	Brown Trout				Brook Trout				Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Bear River headwaters, station # 1 above meadow approx. 3 km upstream of Stillwater Reservoir GPS 13S316278//4429593												
08/23/06	--	--	--	--	5 [e]	±0	136 [e]	24 [e]	--	--	--	--
Bear River, station #2; below beaver ponds in meadow approx. 2 km ↑ Stillwater Reservoir GPS 13T316639//4430655												
08/23/06	--	--	--	--	2	±0	69	6	--	--	--	--
Bear River, station #3, approx. 1 km above Stillwater Reservoir GPS 13T317198//4431273												
08/23/06	2 [c]	±0	56	4	57	±2	1,618	167	4	±0	113	22
Bear River, station #4, below Yamcolo Reservoir GPS 13T 328223//4436450												
08/23/06	4 [bd]	--	43 [bd]	3 [bcd]	3 [bd]	--	32 [bcd]	5 [bcd]	1 [bcd]	--	11 [bcd]	1 [bcd]
Circle Creek near confluence with Elkhead Creek GPS 13T 319552//4515074												
10/04/06	--	--	--	--	10	±0	54	8	3	±0	54	3
East Coal Creek, (Bear River drainage) Station 1 GPS 13T 325192//4436693												
08/21/06	1	±0	18	5	48	±0	852	80	3	±0	53	9
East Coal Creek, (Bear River drainage) Station 2 GPS 13T 325033//4437368												
08/22/06	--	--	--	--	81	±9	1,889	41	--	--	--	--
East Coat Creek, (Bear River drainage) Station 3 GPS 13T 324912//4437879												
08/22/06	--	--	--	--	12	±1	359	28	1	±0	30	2
West Coal Creek, (Bear River drainage) Station 1 GPS 13T 325028//4436641												
08/21/06	--	--	--	--	--	--	--	--	6	±0	416	22
West Coal Creek, (Bear River drainage) Station 3 GPS 13T 323847//4436771												
08/21/06	--	--	--	--	--	--	--	--	8	±0	224	17
West Coal Creek, (Bear River drainage) Station 4 GPS 13T 323482//4437504												
08/22/06	--	--	--	--	--	--	--	--	13	±2	512	25
Elkhead Creek upstream of Circle Creek confluence GPS 13T 319711//4514372												
10/04/06	--	--	--	--	7	±0	251	15	4	±0	144	24

[a]: Almost all trout captured were ≤150 mm. **[b]** Single electrofishing pass only; no population estimate. **[c]:** Rainbow trout, not brown trout or cutthroat trout. **[d]:** electrofishing for PTD and PCR samples only. **[e]:** young-of-the-year included in estimate.

Table 9 (continued). Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the **Yampa River** basin sampled during 2006.

Date	Brown Trout				Brook Trout				Cutthroat Trout			
MMDDYY	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha	N	95% CI	N/Ha	Kg/Ha
Green Creek (lower end of upper meadow) GPS 13T 351481//4464043												
08/24/06	--	--	--	--	129	±16	3,011	148	--	--	--	
Green Creek Ranch GPS 13T 317198//4431273												
08/25/06	--	--	--	--	15	±20	423	22	--	--	--	
Little Cottonwood Creek (0.5 km upstream of Freeman Reservoir GPS 13T 295542//4515506												
10/12/06	--	--	--	--	--	--	--	--	271 [e]	±7	13,868 [e]	35 [e]
Poose Creek 0.5 km downstream of Vaughn Lake GPS 13T 307939//4445249												
10/02/06	--	--	--	--	--	--	--	--	13 [e]	±2	474 [e]	8 [e]
Sand Creek (tributary to Sheriff Reservoir) GPS 13T 318556//4446339												
10/03/06	--	--	--	--	43	±1	1,017	58	--	--	--	--
Slater Creek (Snake River basin) 13T 320084//4522952												
10/04/06	--	--	--	--	45	±4	1,027	77	--	--	--	--
Trout Creek 0.5 km downstream of Sheriff Reservoir GPS 13T 318439//4447622												
10/03/06	--	--	--	--	11	±0	1,579	150	--	--	--	--
Trout Creek 1 km upstream of Sheriff Reservoir GPS 13T 318008//4445060												
10/03/06	--	--	--	--	17	±1	308	17	6	±0	108	4

[a]: Almost all trout captured were ≤150 mm. **[b]** Single electrofishing pass only; no population estimate. **[c]:** Rainbow trout, not brown trout. **[d]:** electrofishing for PTD and PCR samples only. **[e]:** young-of-the-year included in the estimates.

Table 10. Results of polymerase chain reaction (PCR) testing of young-of-the-year (YOY) salmonids and pepsin-trypsin digest (PTD) testing of salmonids ≥ age 1 for evidence of infection by *Myxobolus cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of Colorado River cutthroat (*Oncorhynchus clarki pleuriticus*) trout in the **Yampa River** basin in 2006. PCR score is the cumulative total for 10 fish (or standardized to 10 fish if "n" was ≤ 9 or ≥ 11) where a negative score= 1, weak positive (w+) =2, + = 3, ++ = 4, and +++ = 5. A cumulative score of 10 indicates all fish were negative and a score of 50 indicates all fish were rated 5 (+++). Fish from sites testing positive are highlighted in bold. An "ns" abbreviation in any data column indicates no sample was collected.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	N+	Mean (n+) myxospores	Range Myxospores (n+)
Bear River #1	Headwaters above Stillwater Rsrvr	Brook	ns	ns	ns	4	0	0	0
Bear River #2	**Beaver ponds ↑Stillwater Rsvr**	**Brook**	ns	ns	ns	**10**	**2**	**1,389**	**278 – 2,500**
Bear River #3	**Below ↓Yamcolo Reservoir**	**Brown**	**10**	**9**	**44**	**10**	**5**	**15,667**	**556 – 36,667**
Bear River	**Lower meadow↓ steep riffle**	**Brook**	**10**	**0**	**10**	**10**	**1**	**56**	**556**
Circle Creek	Above Elkhead Creek confluence	Brook	ns	ns	ns	10	0	0	0
East Coal Creek	**Site # 1**	**Brook**	**10**	**0**	**10**	**10**	**2**	**1,250**	**833 – 1,667**
East Coal Creek	Site # 3	Brook	10	0	10	10	0	0	0
West Coal Creek	Upstream headwaters site	CRN cutt	4	0	10	10	0	0	0
Little Cottonwood Crk.	0.1 km ↑ Freeman Reservoir	CRN cutt	10	0	10	10	0	0	0
ElkHead Creek	Above Circle Creek confluence	Brook	ns	ns	ns	7	0	0	0
Green Creek	@ lower end upper meadow	Brook	10	0	10	10	0	0	0
Green Creek	**@ Green Creek Ranch**	**Brook**	**10**	**10**	**49**	**10**	**1**	**190,000**	**190,000**
Poose Creek	1 km below Vaugh Lake outlet	CRN Cut	10	0	10	10	0	0	0
Sand Creek	1 km ↑ inlet to Sheriff Rsvr	Brook	10	0	10	10	0	0	0
Slater Creek	Yampa River Basin	Brook	10	0	10	10	0	0	0
Trout Creek	1 km ↑inlet to Sheriff Reservoir	Brook	10	0	10	10	0	0	0
Trout Creek	**1 km ↓inlet to Sheriff Reservoir**	**Brook**	**10**	**10**	**50**	**10**	**7**	**28,413**	**4,444 – 143,889**

Table 11. Aquatic oligochaete collections from the summers of 2003, 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for greenback cutthroat trout (*Oncorhynchus clarki stomias*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis (Beauchamp et al. 2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Arkansas River Basin									
Chalk Creek below Wright's Lake	08/29/05	13S398393//4287466	100	100	0	0	99	0	1
Chalk Cliff Unit effluent pond	08/29/05	13S401933//4289271	100	100	0	0	5	0	95
Clear Creek ↑ Clear Creek Reservoir	07/24/06	13S383797//4317744	254	133	121	0	100	0	0
East Fk. Arkansas R. near Climax	07/24/06	13S394876//4356112	108	106	2	0	0	0	0
Fooses Creek ↑ S. Fk. S. Arkansas R. confl.	06/26/06	13S389418//4265170	100	100	0	0	100	0	0
Halfmoon Crk, @ beaver pond ↑ 1st cpgrnd	07/18/06	13S379108//4335241	349	0	349	0	0	0	0
Huerfano River @ Huerfano SWA	07/30/03	13S0464696/4171153	140	nd[a]	nd[a]	0	100	0	0
S. Fork Huerfano R. @ High Mesa Ranch	07/30/03	13S0458606/4166244	559	nd[a]	nd[a]	0	100	0	0
Lake Creek ↑ Twin Lakes	07/24/06	13S381192//4326919	156	152	4	0	0	83	17
Leadville Nat'l Fish Hatchery Pond # 1	09/28/06	13S380300//4342615	200	143	57	0	0	0	0
Leadville Nat'l Fish Hatchery Pond # 2	09/28/06	13S380300//4342615	409	147	262	0	100	0	0
Mt. Massive Lakes near Leadville	09/15/06	13S386932//4335031	167	100	67	88	0	0	12
South Fork, South Arkansas River (ARUF)	09/01/05	13S384670//4264250	100	100	0	0	46	0	54
South Fork, South Arkansas River (ARBP)	09/01/05	13S384670//4264250	100	100	0	0	85	0	15
South Fork, South Arkansas River (ARMP)	09/01/05	13S384670//4264250	100	100	0	0	75	0	25
S. Cottonwood Creek ↑ Cottonwood Lake	06/22/06	13S387571//4293150	23	2	21	0	100	0	0
Tennessee Creek near Leadville, CO	07/18/06	13S384279//4346950	75	64	11	0	100	0	0
Trout Creek – East of Buena Vista, CO	05/22/06	13S413401//4299239	114	112	3	0	100	0	0
Trout Creek – Forest Rd 26 crossing	06/22/06	13S414300//4299828	295	109	186	0	100	0	0
Trout Creek USFS Rd 26 crossing	11/16/06	13S414300//4299828	154	153	1	0	100	0	0
Trout Creek – Micturation station	11/16/06	13S413401//4299239	127	121	6	0	100	0	0

nd[a] **:** Haied or non-haired traits were not determined.

Table 12. Aquatic oligochaete collections from the summers of 2003, 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for greenback cutthroat trout (*Oncorhynchus clarki stomias*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis (Beauchamp et al. 2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Cache la Poudre River Basin/North Platte River Basin									
Cache la Poudre @ CDOW Bliss SWA	08/25/03	13T437589//4506865	100	100	0	3	74	0	23
Cache la Poudre @ CDOW Bliss SWA	10/01/03	13T437589//4506865	100	100	0	5	67	0	28
Cache la Poudre @ CDOW Bliss SWA	06/22/04	13T437589//4506865	100	100	0	6	55	0	39
Cache la Poudre @ CDOW Bliss SWA	09/13/04	13T437589//4506865	100	100	0	14	37	0	49
Cache la Poudre @ CDOW Bliss SWA	07/18/05	13T437589//4506865	100	100	0	0	67	0	33
N. Fk. Cache la Poudre R. ↑ Sheep Creek	08/07/06	13T443823//4521266	323	127	1	0	100	0	0
George Creek ↑ Cornelius Creek confluence	08/01/06	13T446492//4627983	739	1	738	0	35	65	0
Joe Wright Creek ↓ Joe Wright Reservoir	06/15/06	13T427826//4492579	194	7	187	0	0	0	0
Laramie River @ Hohnholz Lakes SWA	06/15/06	13T427595//4497611	12	10	2	0	0	0	100
Laramie River ↓ Chambers Lake	09/01/06	13T418378//4532093	388	27	361	0	0	19	81
Sheep Creek ↑Fk Poudre R. confluence	08/01/06	13T446268//4531241	150	129	21	0	98	0	2

Table 13. Aquatic oligochaete collections from the summers of 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for greenback cutthroat trout (*Oncorhynchus clarki stomias*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis (Beauchamp et al. 2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
South Platte River Basin									
Big Thompson River	10/18/04	13T471264//4475846	150	150	0	0	0	0	0
Big Thompson River @ Idlewild Dam	05/26/06	13T473264//4475449	295	92	193	0	0	0	0
Big Thompson River @ Lake Estes inlet	05/26/06	13T458073//4468898	134	117	11				
Big Thompson R. N. Fk @ Drake Hatchery	05/26/06	13T470479//4476096	219	116	103	0	0	0	0
Clear Creek @ Loveland Ski Area	07/28/06	13S424226//4393289	1,130	126	4	0	100	0	0
Caribou Creek ↑ N. Boulder Creek confl.	08/14/06	13S449994//4426195	14	12	2	0	0	0	0
Clear Creek 2 km E. of Eisenhower Tunnel	08/04/04	13S424248//4393335	111	111	0	0	100	0	0
Clear Creek - ↓Loveland Ski Area	11/28/06	13S424248//4393335	100	100	0	0	100	0	0
Dry Gulch (3 km E. of Eisenhower Tunnel)	07/28/04	13S424285//4397851	133	0	133	0	0	0	0
Fish Creek Inlet to Lake Estes	05/26/06	13T458073//4468898	134	123	11	25	31	27	17
Georgetown Reservoir (Clear Creek)	07/28/04	13S440861//4398372	50	50	0	0	23	0	77
Georgetown Reservoir (Clear Creek)	07/28/04	13S440861//4398372	50	50	0	0	16	0	84
Georgetown Reservoir (Clear Creek)	07/28/04	13S440861//4398372	50	50	0	0	43	0	57
Georgetown Reservoir (Clear Creek)	07/28/04	13S440861//4398372	50	50	0	0	75	0	25
Georgetown Reservoir (Clear Creek)	07/28/04	13S440861//4398372	5	0	5	0	0	0	100

Table 13 (continued). Aquatic oligochaete collections from the summers of 2003, 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for greenback cutthroat trout (*Oncorhynchus clarki stomias*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis (Beauchamp et al. 2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
South Platte River Basin									
Kenosha Creek near Kenosha Pass	05/22/06	13S436484//4361719	123	123	0	0	100	0	0
Middle Boulder	08/14/06	13S448929//4422654	1,000	150	1	0	100	0	0
Middle Boulder Creek	11/28/06	13S448929//4422654	100	100	0	0	100	0	0
Middle Fork S. Platte (Platte Gulch)	07/27/04	13S406227//4357377	402	102	297	0	0	0	0
Middle Fork S. Platte (1 km ↓ reservoir)	07/27/04	13S408215//4356068	293	101	190	0	100	0	0
Middle Fork S. Platte (4 km ↓ reservoir)	07/27/04	13S408755//4352843	220	102	118	0	100	0	0
Middle Fork S. Platte (4 km ↓ reservoir)	09/13/06	13S408272//4355961	401	100	301	0	100	0	0
South Fk. S. Platte R. @ Knight/Imler SWA	05/22/06	13S415724//4325224	112	100	12	0	59	0	41
South Platte R. @ Spinney Mountain Rsvr.	05/22/06	13S446747//4313671	286	100	186	0	49	0	51
St. Vrain R. ↓ falls @ Longmont Rsvr.	05/26/06	13T470627//4453196	135	125	4	0	0	100	0
Tarryall Creek on Pike Nat'l Forest	05/22/06	13S419541//4355508	107	107	0	0	100	0	0
Tarryall Creek ↓ Tarryall Reservoir	05/22/06	13S447843//4341647	638	77	6	0	21	44	35
West Chicago Creek near Idaho Springs	06/01/06	13S444918//4393115	120	107	13	0	75	10	15

Table 14. Aquatic oligochaete collections from the summers of 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date Mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Rio Grande Basin									
Alberta Creek 1 km ↓ Alberta Park Rsvr	07/14/06	13S343916//4148625	75	1	74	0	0	0	0
West Alder Creek	08/03/05	13S351282//4181417	1200	100	1100	0	100	0	0
Big Spring Creek ↓ Spring Creek Pass	08/14/06	13S310994//4196330	223	6	1	0	0	0	0
South Fork Carnero Creek	07/20/04	13S374248//4196810	373	164	209	0	100	0	0
North Fork Carnero Creek	07/20/04	13S377887//4199158	450	1	449	0	0	0	0
Middle Fork Carnero Creek	07/20/04	13S374146//4202051	554	337	217	0	0	0	0
Lake Fork Conejos River (below Big Lake)	08/07/04	13S365590//4131567	113	3	110	0	0	0	0
Lake Fork Conejos River (in Rock Lake)	08/07/04	13S367101//4131019	105	5	100	0	0	0	0
Middle Fork Conejos River	09/20/04	13S355888//4126683	173	170	3	0	0	0	0
Upper Cross Creek	08/01/05	13S381657//4230263	0	0	0	0	0	0	0
Cuates Creek	07/25/05	13S467447//4097134	0	0	0	0	0	0	0
N. Fk. Culebra Creek	08/17/06	13S475030//4117880	36	28	8	0	0	0	0
Big Flint Lake	08/12/04	13S283623//4167121	125	1	124	0	0	0	0
Ford Creek (Saguache Creek basin)	07/21/04	13S377887//4199158	13	0	13	0	0	0	0
West Indian Creek @ Trinchera Ranch	08/16/06	13S479120//4143135	132	122	10	0	100	0	0
Jaroso Creek	07/25/05	13S468638//4100091	54	4	50	0	100	0	0
Lost Trail Creek above W. Lost Trail Creek	08/08/04	13S293419//4185965	100	0	100	0	0	0	0
West Lost Trail Creek	08/08/04	13S291413//4186961	599	104	495	0	0	0	0
Rio de los Pinos River ↓ Trujillo Meadows	09/21/04	13S371564//4100909	453	129	324	0	0	0	0

Table 14 (continued). Aquatic oligochaete collections from the summers of 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for Rio Grande cutthroat trout (*Oncorhynchus clarki virginalis*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Rio Grande Basin									
Lower Medano Creek	08/02/05	13S457344//4184321	15	2	13	0	0	0	0
East Middle Creek	08/03/05	13S390087//4242786	490	188	302	0	0	0	0
Middle Creek	08/03/05	13S386129//4237436	233	126	107	0	100	0	0
Middle Creek	07/27/06	13S386129//4237436	114	103	11	0	100	0	0
Lower East Pass Creek	08/01/05	13S368441//4227325	550	117	433	0	0	0	0
Placer Creek	07/27/05	13S473015//4162508	107	105	2	0	100	0	0
San Francisco Creek (Cielo Vista Ranch)	07/26/05	13S472143//4103473	106	103	3	0	0	0	0
San Francisco Creek south of Del Norte, CO	07/28/05	13S379070//4159804	112	102	10	0	100	0	0
Torcido Creek	07/26/05	13S470703//4101333	392	105	287	0	0	0	0
N. Fork Trinchera Creek on Forbes Ranch	08/16/06	13S477129//4137176	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
S. Fork Trinchera Creek on Forbes Ranch	08/15/06	13S476906//4132186	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Tuttle Creek (Saguache Creek basin)	07/21/04	13S392159//4234536	213	192	21	0	0	0	0
Ute Creek 3 km south of West Ute Creek	08/13/04	13S283613//4167122	657	57	600	0	0	0	0
Vallejos Creek	07/26/05	13S475584//4107269	2	0	2	0	0	0	0
North Vallejos Creek	07/27/05	13S473144/4108409	35	0	35	0	100	0	0
Wagon Creek	08/02/05	13S480861//4152417	250	112	138	0	0	0	0
Weminuche Creek	08/09/04	13S296398//4174715	337	224	113	0	0	0	0

nw[a] : nw signifies there were no worms in the sample.

Table 15. Aquatic oligochaete collections from the summers of 2003, 2004 and 2005 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sediment laden areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Upper Colorado River Basin (Middle Park and Grand County)									
Colorado River @ Breeze Bridge SWA	03/01/03	13T398294//4435218	500	500	0	35	5	36	31
Colorado River @ Breeze Bridge SWA	03/01/03	13T398294//4435218	250	250	0	37	6	7	50
South Fork Ranch Creek near Fraser, CO	09/11/03	13S0435224/4416136	113	112	1	0	0	100	0
Williams Fork River	03/01/03	13T398165//4433619	125	125	0	0	0	100	0
Williams Fork River	03/01/03	13T398165//4433619	250	250	0	0	0	98	2
Williams Fork River	03/01/03	13T398165//4433619	400	400	0	0	0	100	0
Williams Fork River	07/08/03	13T398165//4433619	100	100	0	0	35	23	42
Williams Fork River	07/08/03	13T398165//4433619	100	100	0	0	65	25	10
Williams Fork River	09/30/04	13T398165//4433619	400	400	0	0	0	98	2
Willow Creek ↓ Willow Creek Reservoir	06/23/03	13T419956//4444139	650	650	0	0	22	0	78
Willow Creek ↓ Willow Creek Reservoir	08/18/03	13T419956//4444139	650	650	0	0	19	0	81
Willow Creek ↓ Willow Creek Reservoir	05/18/04	13T419956//4444139	691	691	0	0	5	0	95
Windy Gap Reservoir	06/27/05	13T416336//4440004	113	102	11	31	5	15	49

Table 15 (continued). Aquatic oligochaete collections from the summers of 2003, 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date Mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Lower Colorado River (Battlement Mesa Area)									
Lower Beaver Creek (Battlement Mesa)	08/18/04	13S256352//4367761	0	0	0	--	--	--	--
Battlement Creek near Battlement Mesa	10/05/04	13S234029//4368811	175	166	9	0	100	0	0
Lower Colorado River (Grand Mesa Area)									
Big Creek – Above Big Creek Reservoir	10/04/04	13S251249//4329187	145	126	19	0	100	0	0
Big Creek – 100 m upstream Bonham Rsvr	09/09/04	13S249097//4330977	100	100	0	0	100	0	0
Big Creek – below Bonham Reservoir	06/27/06	13S246775//4336667	128	117	11	0	100	0	0
East Fork Big Creek	08/29/05	13S250741//4332495	106	91	15	0	100	0	0
West Fork Big Creek –Grand Mesa	09/09/04	13S248766//4330172	106	0	106	0	0	0	0
Cow Creek at Overland Reservoir	09/15/05	13S270108//4328625	115	114	1	0	100	0	0
Main Hubbard Creek	10/25/05	13S276239//4326308	112	105	7	0	100	0	0
Middle Hubbard Creek	10/25/05	13S276003//4235386	29	29	0	47	53	0	0
Plateau Creek above Vega Reservoir	09/16/05	13S260674//4345015	125	125	0	0	88	0	12
Unnamed tributary to Bonham Reservoir	09/15/05	13S248300//4330026	20	10	10	0	100	0	0

Table 15 (continued). Aquatic oligochaete collections from the summers of 2003, 2004 and 2005 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Lower Colorado River Basin (Roan Plateau Area)									
Lower Black Sulphur Creek (Roan Plateau)	10/25/04	12S720728//4410720	208	100	108	0	95	0	5
Upper Black Sulphur Creek (Roan Plateau)	10/25/04	12S716806//4404130	6	6	0	0	100	0	0
Brush Creek (Roan Plateau)	08/19/04	12S751767//4368471	131	121	10	0	100	0	0
Lower Carr Creek (Roan Plateau)	08/23/04	12S714603//4382692	195	195	0	0	100	0	0
Upper Carr Creek (Roan Plateau	08/23/04	12S714600//4382658	108	108	0	0	100	0	0
Lower Roan Creek (Roan Plateau)	08/24/04	12S702275//4385888	238	100	238	0	100	0	0
Upper Roan Creek (Roan Plateau)	08/24/04	12S702259//4385969	259	101	158	0	100	0	0
E. Middle Parachute Creek-below falls	10/06/04	12S752065//4389163	130	130	0	0	100	0	0
E.Parachute Creek Roan Plateau above falls	09/16/04	13S246911//4383989	100	100	0	0	100	0	0
E.Parachute Creek Roan Plateau above falls	06/24/06	13S246911//4383989	100	100	0	0	100	0	0
E.Parachute Creek Roan Plateau below falls	10/06/04	12S756188//4383483	125	125	0	0	100	0	0
Trapper Creek (Roan Plateau)	09/15/04	12S756759//4389979	60	10	50	0	100	0	0
Soldier Creek – upper reach	09/13/04	12S708638//4402080	594	63	531	0	0	0	0
Upper East Douglas Creek	09/14/04	12S697157//4391049	199	1	198	0	100	0	0

Table 16. Aquatic oligochaete collections from the summers of 2003, 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Blue River Basin									
Clinton Creek ↑ Clinton Reservoir	06/06/06	13S399999//4362350	400	104	296	0	0	0	0
Swan River above Dillon Reservoir	08/10/06	13S418098//437667	1,144	144	0	0	88	0	12
Swan River above Dillon Reservoir	11/28/06	13S418098//437667	100	0	0	0	95	0	5
West Ten Mile Creek @ Vail Pass	06/06/06	13S395110//4375982	50	44	6	0	64	0	36
Dolores River Basin									
Dolores R. @ USFS Barlow Crk. Cpgrnd.	08/10/06	13S236903//4184364	6	4	2	0	0	0	0
Scotch Crk. 0.5 km ↑ Dolores R. confluence	08/10/06	12S760909//4171452	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Dolores River 0.2 km ↓ McPhee Reservoir	08/10/06	12S713978//4161453	296	140	3	0	92	8	0
Dolores R. @ Lone Dome SWA weir	08/10/06	12S707204//4166274	212	26	22	0	50	5	45
Eagle River Basin									
Black Gore Creek (below Black Lakes)	07/27/04	13S395083//4377862	261	210	51	0	67	10	23
Fryingpan River Basin									
Fryingpan River @ Nast Bridge	10/08/03	13S0361642/4351214	100	100	0	0	100	0	0
Fryingpan R. @ Taylor Creek island pool	06/08/06	13S332919//4360451	118	100	18	0	25	0	75
Fryingpan R. @ Beaver pond island channel	06/08/06	13S328383//4360327	179	34	145	0	38	0	62
Little Lime Creek near Crooked Creek Rsvr	10/05/04	13S357426//4365362	180	162	18	0	4	0	96
Rocky Fork Creek near Ruedi Reservoir	10/07/03	13S0344030/4356176	100	95	5	0	0	100	0
Cap K Ranch channel between ponds 1 & 2	11/08/06	13S337444//4360598	134	132	2	0	20	0	80
Cap K Ranch pond # 2 north side	11/08/06	13S337418//4360596	163	161	2	0	4	0	96

nw[a]**:** nw indicates no worms in the sediment sample.

Table 17. Aquatic oligochaete collections from the summers of 2003, 2004, 2005 and 2006 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date Mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Gunnison River Basin									
West Fork Cebolla Creek ↓ Spring Crk. Pass	08/14/06	13S308645//4203293	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Cochetopa Creek-top station	06/29/04	13S337057//4205427	31	17	14	0	0	0	0
Gunnison River – Ute Park	09/02/04	13S252211//4283595	222	17	215	0	18	0	82
East Fork Dry Creek	06/27/06	12S753631//4244168	164	100	14	0	100	0	0
Lake Fork Cochetopa Creek	06/28/04	13S341342//4205695	848	247	175	0	0	0	0
South Beaver Creek – upper site	06/24/04	13S326079//4258088	280	50	230	0	0	0	0
South Beaver Creek – lower site	06/24/04	13S326079//4258088	1,150	103	1,047	0	15	0	85
Spring Creek below Spring Creek Reservoir	11/11/05	13S351965//4302442	100	100	0	0	100	0	0
Spring Creek at Salisbury Gulch	07/16/03	13S349982//4298595	100	100	0	0	100	0	0
Spring Creek at Spring Creek Campground	11/11/05	13S346483//4290398	100	100	0	0	100	0	0
Spring Creek at Spring Creek Campground	11/11/05	13S346483//4290398	100	100	0	0	100	0	0
Uncompahgre R. @ Baldridge Park # 1	02/07/07	13S248708//4261407	105	103	2	0	88	0	12
Uncompahgre R. @ Baldridge Park # 2	02/07/07	13S248471//4261591	105	105	0	0	85	0	15

[a]: nw indicates no worms were visible in the sediment sample.

Table 18. Aquatic oligochaete collections from the San Juan River sub-basin of the Colorado River during 2006 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
San Juan River Basin									
Castle Creek	09/19/06	13S342405//4118605	142	114	26	0	0	0	0
Corral Creek ↑ E. Fk. Hermosa Crk. Confl.	07/10/06	13S241676//4173368	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Fish Creek @ Fish Creek trailhead	09/19/06	13S347114//4120667	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Florida River 0.1 km ↓ Lemon Rsvr.	07/10/06	13S264340//4140216	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
East Fk. Hermosa Creek @ ski area	06/26/06	13S246612//4168801	320	110	49	0	0	0	0
Piedra River 2 km↓ E. Fk. & Middle Frk.	09/20/06	13S305894//4144596	117	115	2	0	100	0	0
Piedra River 2 km↓ E. Fk. & Middle Frk.	11/28/06	13S305894//4144596	100	100	0	0	99	0	1
East Fork Piedra River	07/11/06	13S311137//4148008	25	24	1	0	100	0	0
Middle Fork Piedra River	07/11/06	13S308185//4150342	381	1	380	0	100	0	0
Rito Blanco @ FR024 road crossing	09/20/06	13S335236//4127230	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
East Fork San Juan R. ↓ Sand Creek confl.	07/13/06	13S346149//4142267	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
West Fork San Juan R.	07/13/06	13S339960//4146490	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
South Branch of South Creek	09/19/06	13S343559//4118913	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
E. Fk. Weminuche Creek	07/12/06	13S298168//4159605	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Weminuche Creek	07/12/06	13S302918//4149094	3	0	3	0	0	0	0
Weminuche Creek (ranch gravel ponds)	07/12/06	13S301218//4151638	205	101	104	0	98	0	2
White Creek – Rio Blanco Rive basin	09/19/06	13S343008//4119129	169	120	49	0	0	0	0
Williams Creek 1 km ↑Wms Creek Rsvr	07/11/06	13S305760//4156844	143	118	25	0	0	0	0
Williams Creek @ USFS Bridge Cpgrnd	09/20/06	13S305366//4149984	140	103	37	0	13	0	87

[a]: nw indicates no worms were visible in the sediment sample

Table 19. Aquatic oligochaete collections from the White River sub-basin of the Colorado River during 2006 and early 2007 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
White River Basin									
Bel-Aire SWA South unit pond	10/26/06	13S275240//4428071	21	7	14	0	100	0	0
Bel-Aire SWA East Pond inlet area	11/09/06	13S275240//4428071	158	132	26	0	100	0	0
Bel-Aire SWA East Pond outlet area	11/09/06	13S275229//4428060	215	101	114	0	100	0	0
Bel-Aire SWA West Pond outlet area	11/09/06	13S275202//4428061	175	148	27	0	100	0	0
Bel-Aire SWA spawning house channel	11/09/06	13S275168//4428025	112	101	11	0	100	0	0
Bel-Aire SWA North unit pond	02/06/07	13S275557//4428680	152	102	50	0	0	0	0
Big Beaver Creek ↓ Allen Creek confl.	09/05/06	13T277427//4439387	233	115	118	0	100	0	0
Big Beaver Creek 0.2 km ↑ Lake Avery	09/05/06	13T274242//4429675	192	55	137	0	60	0	40
Big Beaver Creek 0.2 km ↑ Lake Avery	10/02/06	13T274242//4429675	117	104	13	0	77	0	23
Cabin Creek outlet @ Little Trappers Lake	10/09/06	13S311247//4429101	113	100	13	0	100	0	0
Fawn Creek – lower site	11/28/06	13T284218//4436000	150	148	2	0	100	0	0
Fraser Creek 0.5 km ↑ Trappers Lake	10/06/06	13S309580//4427548	252	50	202	0	100	0	0
Heberton Creek 0.2 km ↑ Trappers Lake	10/06/06	13S309029//4428322	104	2	102	0	0	0	0
Little Trappers Lake Inlet	10/09/06	13S311659//4428896	283	29	264	0	0	0	0
Lost Creek – N. Fork White River	09/07/06	13T289628//4436543	113	106	7	0	100	0	0
Marvine Creek↑ E. Fk. Marvine Creek	09/07/06	13T292709//4431588	42	40	2	0	100	0	0

Table 19 (continued). Aquatic oligochaete collections from the White River sub-basin of the Colorado River during 2006 and early 2007 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
White River Basin									
North Elk Creek @ USFS boundary fence	10/11/06	13S271719//4421341	168	139	29	0	100	0	0
North Elk Creek @ USFS boundary fence	11/28/06	13S271719//4421341	100	100	0	0	100	0	0
North Elk Creek @ Elk Creek Ranch	10/11/06	13S271018//4425263	118	117	1	0	71	0	29
Snell Creek ↑ confl. N. Fork White River	09/06/06	13T299872//4438689	153	122	31	0	100	0	0
Ute Creek – ↑N. Fk. White R. confluence	09/06/06	13T286915//4433634	36	6	30	0	100	0	0
South Fork White River – 3km ↓Wilderness	12/07/06	13S283344//4415526	100	100	0	0	69	0	31
White R @ County Road 54 Bridge	10/11/06	13S269781//4425716	126	126	0	5	23	0	72
White R. 0.5 km ↓ County Rd. 54 Bridge	12/07/06	13S269449//4425614	198	188	10	0	1	0	99
White River @ Ducey's Resort	04/18/07	13S266524//4425370	103	103	0	0	18	0	82
White River @ K-Slash-K Ranch Bridge	04/18/07	13S263278//4425371	108	101	7	0	17	0	83
White River @ Highland Ditch Diversion	04/18/07	13S259849//4429145	111	33	78	5	51	0	44
Off channel in Highland Ditch Diversion	04/18/07	13S259853//4429093	117	114	3	0	73	0	27
White River on Seely Ranch	04/18/07	13S258680//4431486	104	104	0	0	51	0	49
White River on K-Bar-T Ranch	04/18/07	13T258346//4433019	100	100	0	0	53	0	47
White River @ Coal Creek confluence	04/18/07	13T257354//4435197	100	100	0	1	10	0	89
White River @ Franklin Ranch Bridge	04/18/07	13T254003//4435805	108	102	6	0	59	0	41

Table 20. Aquatic oligochaete collections from the Yampa River sub-basin of the Colorado River during 2006 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Yampa River Basin									
Bear River headwaters	08/24/06	13T316278//4429593	132	132	0	0	100	0	0
Bear River below Yamcolo Reservoir	08/21/06	13T328223//4436450	135	133	2	40	60	0	0
Bear River lower meadow ↓ reservoir	08/23/06	13T317198//4431273	146	141	5	0	100	0	0
Circle Creek ↑ Elkhead Creek confluence	10/04/06	13T319552//4515074	272	49	223	0	0	0	0
Little Cottonwood Creek ↑ Freeman Rsvr	10/12/06	13T295542//4515506	75	7	68	0	0	0	0
Elkhead Creek # 3 ↑ Circle Creek confl.	10/04/06	13T319711/4514372	217	50	167	0	100	0	0
East Coal Creek	08/23/06	13T334844//4437970	308	137	171	0	0	0	0
West Coal Creek	08/22/06	13T324911//4436831	167	0	167	0	0	0	0
Green Creek above Green Creek Ranch	08/24/06	13T351481//4464043	205	50	155	0	0	0	0
Green Creek @ Green Creek Ranch	08/25/06	13T346333//4465725	111	110	1	84	16	0	0
Poose Creek below Vaughn Lake	10/02/06	13T307939//4445249	153	138	15	0	100	0	0
Poose Creek below Vaughn Lake	11/28/06	13T307939//4445249	100	100	0	0	100	0	0

Table 20 (continued). Aquatic oligochaete collections from the Yampa River sub-basin of the Colorado River during 2006 for streams considered to be present recovery areas or future locations for Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) recovery. Numbers of oligochaetes represent the number of tubificid worms enumerated in qualitative kick screen samples taken from sedimented areas in the stream. Percentage of DNA by strain type represents the proportion of DNA for each of the four lineages of *Tubifex tubifex* detected by a multi-plex (four probe) quantitative PCR test developed by Pisces Molecular LLC, Boulder, Colorado using genetic markers developed at the University of California-Davis as published in Beauchamp et al. (2002).

Stream Name	Sample Date mmddyy	GPS Coordinates	Number of Oligochaetes			Percent DNA by Strain Type			
			Total	Haired	Non-Haired	I	III	V	VI
Yampa River Basin									
Sand Creek 0.5 km↑ inlet to Sheriff Resrvr.	10/03/06	13T318566//4446339	0	0	0	nw[a]	nw[a]	nw[a]	nw[a]
Sarvis Creek 1 km ↑ Yampa R. confluence	07/17/06	13T346832//4462379	103	103	0	98	2	0	0
Slater Creek 3 km ↑ Crawford Crk confl.	10/04/06	13T320084//4522952	126	21	105	0	0	0	0
Trout Creek 1 km ↓ Sheriff Reservoir	10/03/06	13T318439//4447622	139	100	39	76	24	0	0
Trout Creek @ Sheriff Reservoir inlet	10/03/06	13T318008//4445060	148	145	3	0	100	0	0
Trout Creek @ Sheriff Reservoir inlet	11/28/06	13T318008//4445060	100	100	0	0	100	0	0
Yampa River 0.3 km ↑ Sarvis Creek	11/16/06	13T346618//4462234	144	144	0	12	55	0	33
Yampa River 0.4 km ↑ Sarvis Creek	11/16/06	13T346552//4462197	123	123	0	7	46	0	47
Yampa River 0.5 km ↑ Sarvis Creek	11/16/06	13T346438//4462205	163	161	2	18	71	0	11

[a]: nw indicates no worms were visible in the sediment sample

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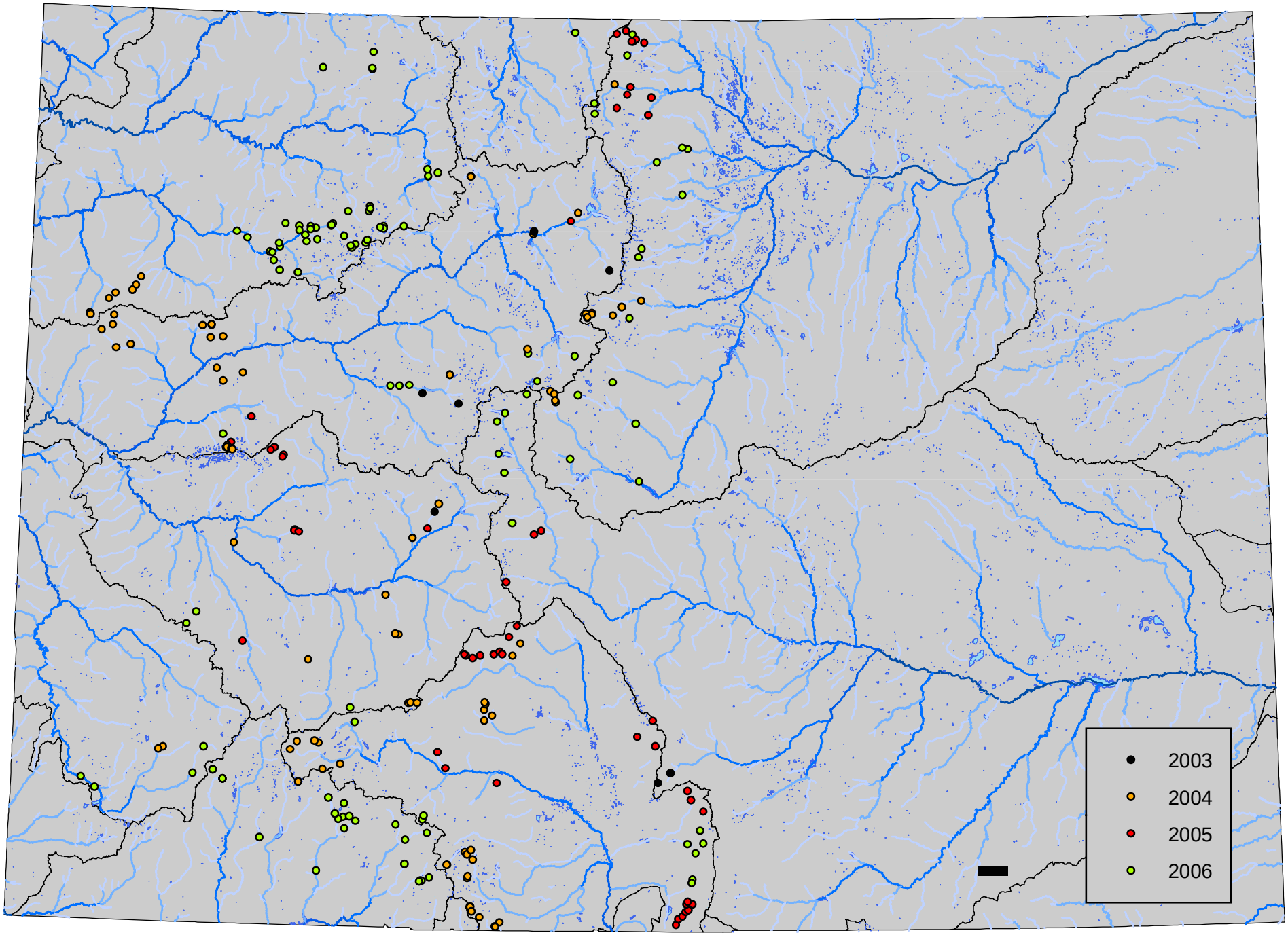
Appendix 1

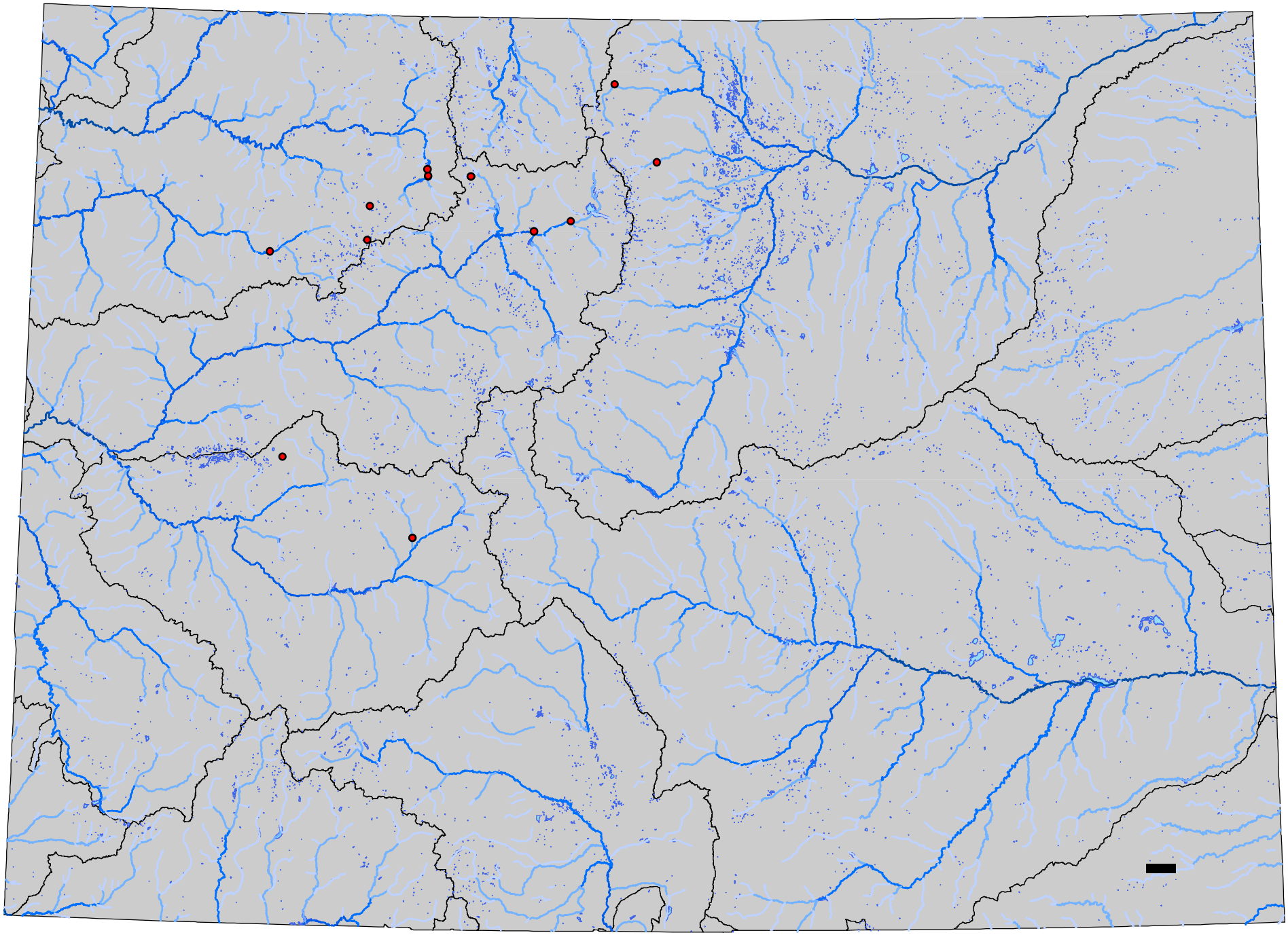
MAPS

Oligochaete Worm Sample Sites and Oligochaete Worm Lineages

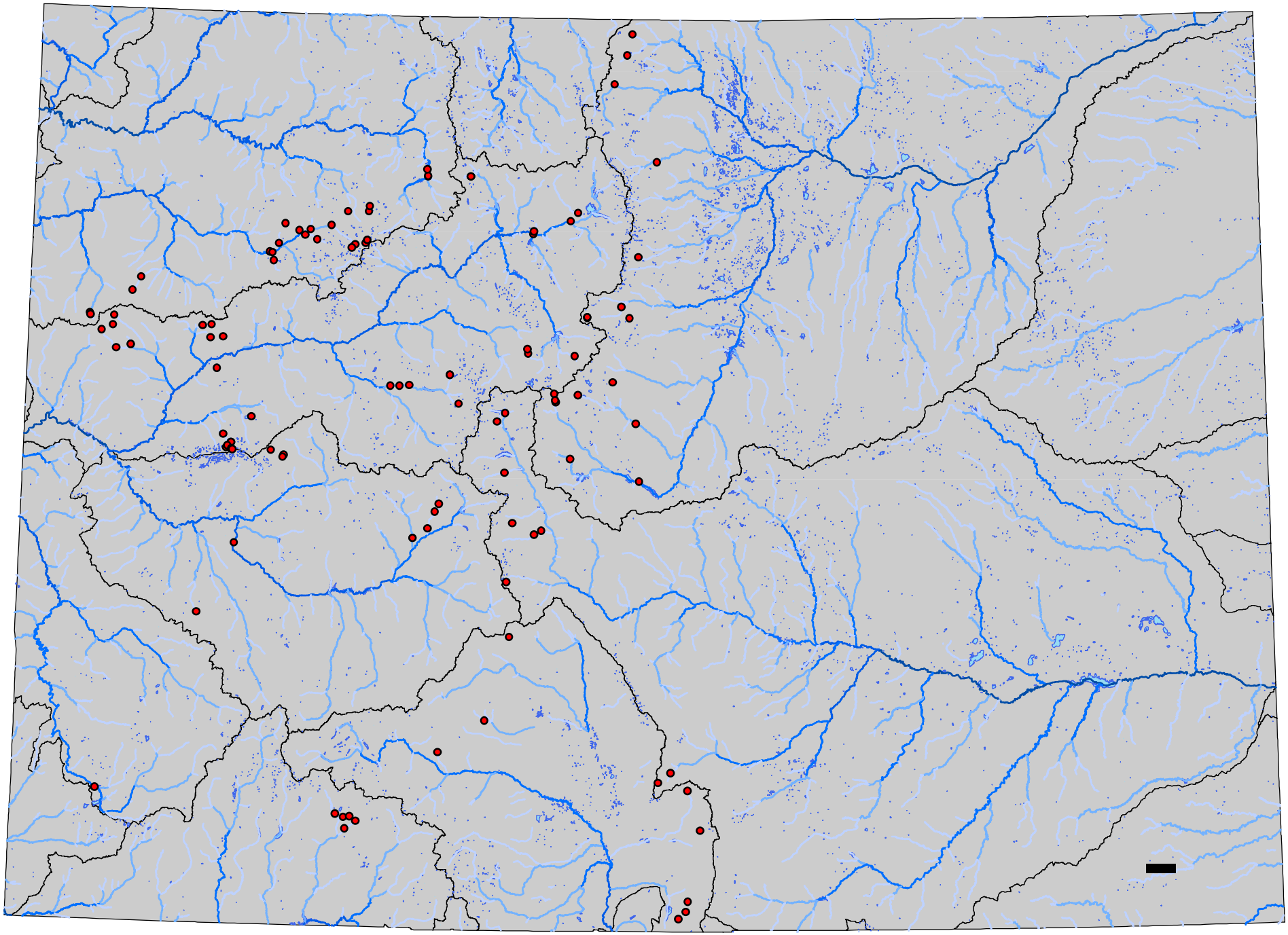
Map 1. Locations of sample sites for 2003, 2004, 2005 and 2006



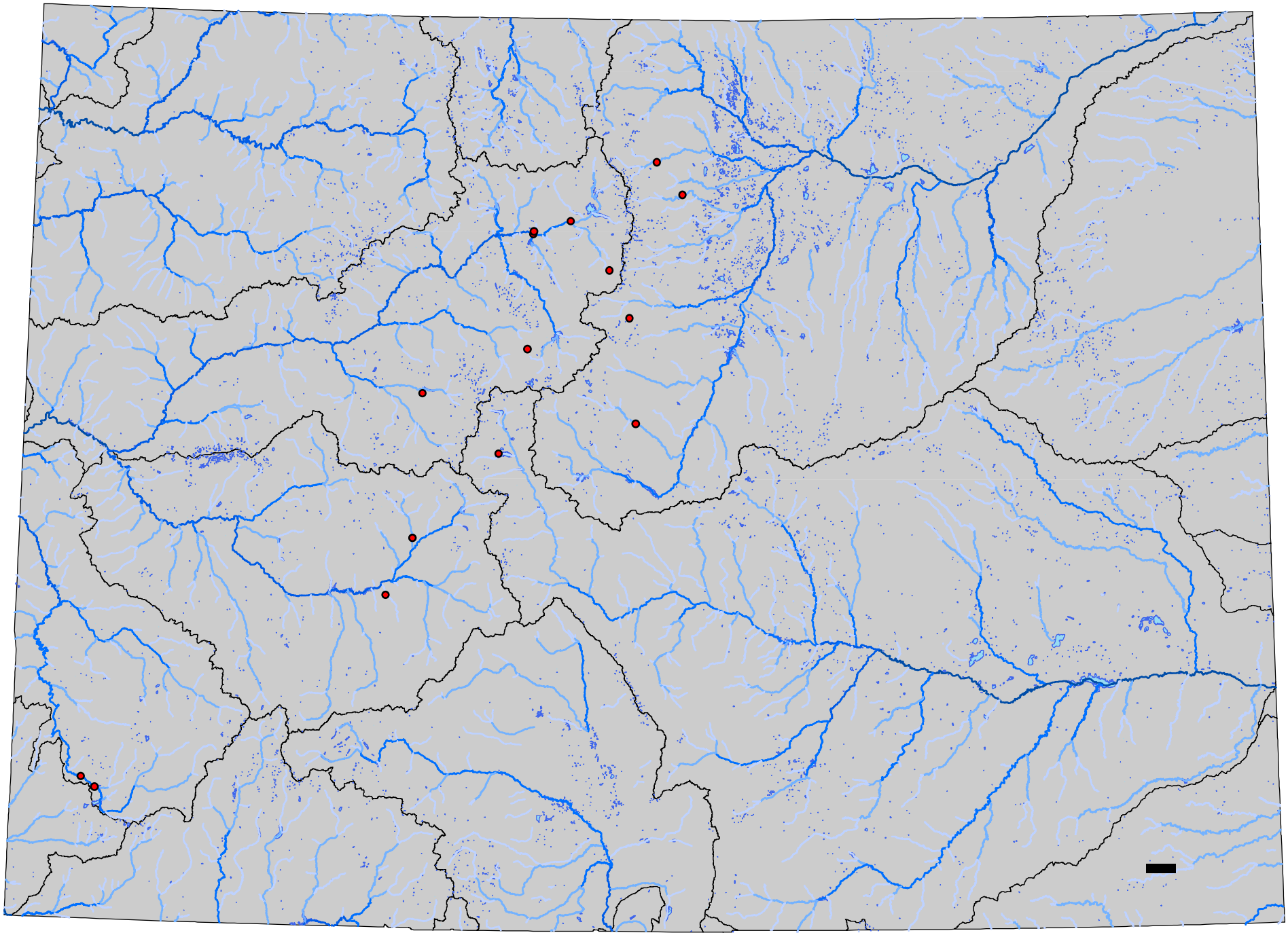
Map 2. Collection sites where lineage I worms were present (2003-2006)



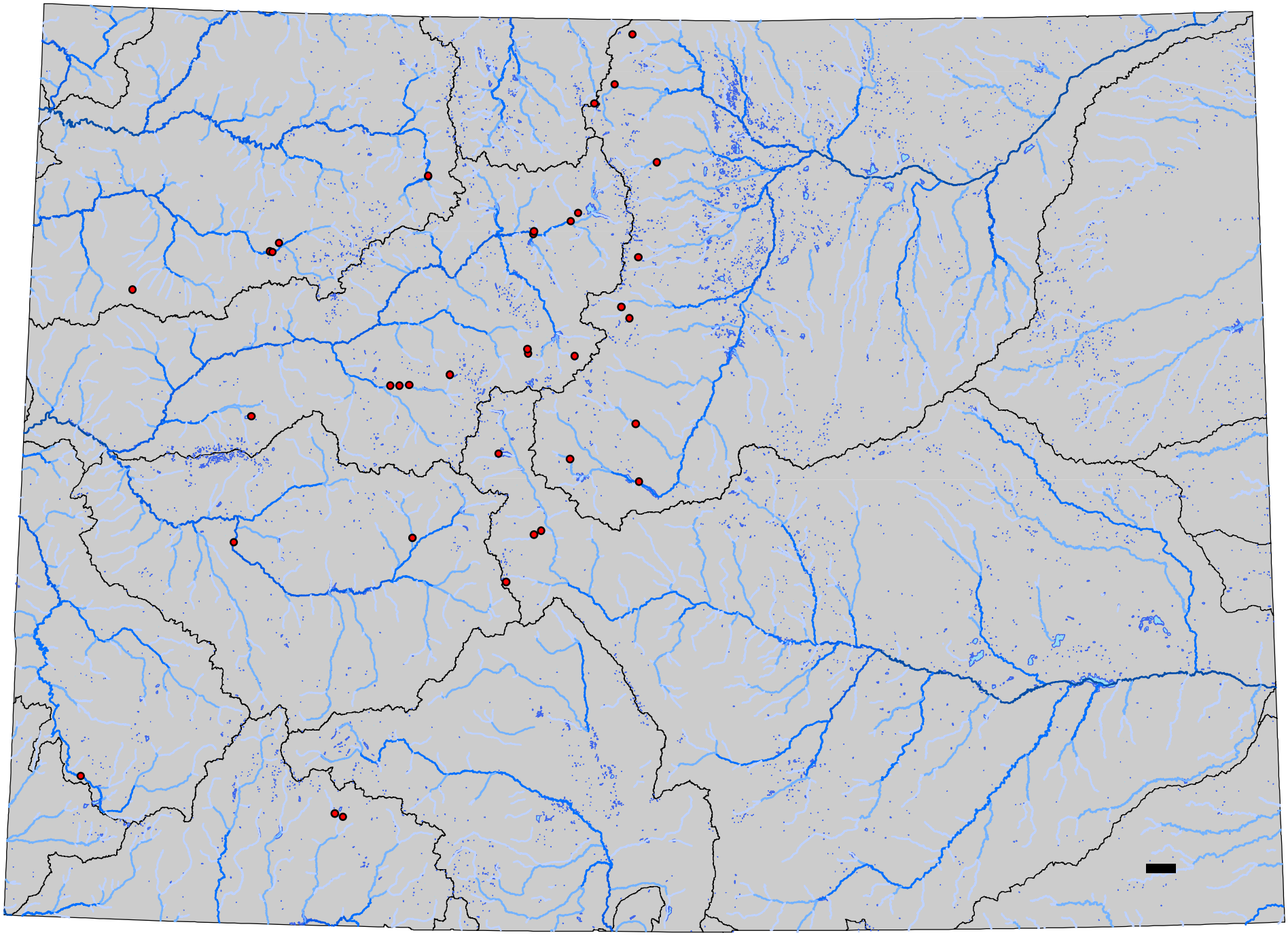
Map 3. Collection sites where lineage III worms were present (2003-2006)



Map 4. Collection sites where lineage V worms were present (2003-2006)



Map 5. Collection sites where lineage VI worms were present (2003-2006)



Map 6. Collection sites where oligochaete worms were present but no DNA was detected for any of the 4 lineages (2003-2006)