Whirling Disease Investigations

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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, 2005 through June 30, 2006

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), a debilitating malady of trout and salmon 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 (M. cerebralis).* WD was recognized as 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 for more than 80 years. In the early 1980s research efforts first described the complex two-host life cycle that alternately infects a tubificid worm (*Tubifex tubifex*) and a salmonid fish (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 salmonids infected by the parasite shed into the aquatic environment 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.

In Colorado, *M. cerebralis* was first detected in two public and two private trout rearing facilities in late 1987 (Walker and Nehring 1995). Although almost impossible to eradicate in aquatic environments, decades of experience with the parasite in the aquaculture industry led to management strategies that can be effective in minimizing losses (Hoffmann 1990). 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*. According to Schisler (2001), more than one million trout from *M. cerebralis*-infected hatcheries and rearing units were stocked into the Cache la Poudre River and reservoirs

tributary to the 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). Recently, a special technical report, **Colorado's Cold Water Fisheries: Whirling Disease Case Histories and Insights for Risk Management,** has been published that 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). The parasite was detected in Yellowstone cutthroat trout (*O. clarki bouvieri*) in 1998 and is now known to 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. in press).

Over the past 30 years, many species of Salmonidae have been exposed to *M. cerebralis* under a variety of circumstances. Several different metrics have been used to assess the relative level of vulnerability. Metrics of effect have included 1) quantification of cranial myxospores after five or more months post exposure (PE) (O'Grodnick 1979; Hedrick et al 1998; Hedrick et al. 1999a; Hedrick et al. 1999b; Thompson et al. 1999), 2) chronic mortality resulting from exposure to the parasite (Thompson et al. 1999) and 3) histological techniques to evaluate the relative amount of skeletal tissue abnormalities and damage caused by the parasite after 80-90 day PE (Vincent 2002).

In a large number of studies, quantification of cranial myxospores was often the primary metric of effect. Using that technique, rainbow trout (*Oncorhynchus mykiss*) are generally considered the most vulnerable species of salmonid (Hedrick et al. 1999a; O'Grodnick 1979; Thompson et al. 1999; Vincent 2002) while brook trout (*Salvelinus fontinalis)* follow as a close second (O'Grodnick 1979; Thompson et al. 1999; Vincent 2002). Similarly, sockeye salmon (*Oncorhynchus nerka*) and chinook salmon (*Oncorhynchus tshawytscha*) can be highly susceptible when exposed as alevins or very small fry (O'Grodnick 1979; Hedrick et al. 2001). In contrast, brown trout (*Salmo trutta*) are more resistant to infection (Hedrick et al. 1999a; O'Grodnick 1979; Thompson et al. 1999) as are coho salmon – *Oncorhynchus kisutch* (Hedrick et al. 2001) and Atlantic salmon - *Salmo salar* (Blazer et al. 2004). Lake trout (*Salvelinus namaycush*) have been shown to be highly resistant or refractory to infection (O'Grodnick 1979; Blazer et al.2004).

In Montana, Vincent (2002) used histological techniques to assess the relative vulnerability of numerous species of salmonids exposed to a single dose of TAM actinospores of *M. cerebralis* and then held for 80 – 90 days in specific-pathogen-free (SPF) water. Nine or 10

strains of rainbow trout, three subspecies of cutthroat trout (*Oncorhynchus clarki* ssp.), kokanee salmon, chinook salmon, brown trout, brook trout, bull trout (*Salvelinus confluentus*), and Arctic grayling (*Thymallus arcticus*) were tested in this manner . The strains of rainbow trout and eastern brook trout were the most seriously affected, followed by the three subspecies of cutthroat trout. Bull trout and chinook salmon were less seriously affected while brown trout and Arctic grayling were highly resistant.

In Colorado, Thompson et al. (1999) used both cranial myxospore concentration and chronic mortality to assess the relative susceptibility of seven species or subspecies of salmonids to infection by *M. cerebralis.* These tests were *in vivo* continuous exposures to ambient levels of TAMs completed over a number of years at a site in the upper Colorado River. In these tests brown trout, brook trout, several sizes, strains and ages of rainbow trout and four subspecies of cutthroat trout (*O. clarki* ssp.) were held in floating tanks and exposed to ambient levels of TAMs in the river for 12 to 18 months. Mortalities were monitored on a daily basis throughout all of the experiments. Rainbow trout were the most sensitive species across all tests when cranial myxospore concentrations were the metric of effect. Cutthroat trout subspecies and brook trout had intermediate levels of cranial myxospore concentrations compared to rainbow trout. Brown trout were the most resistant. These results are largely congruent with those of other investigators that tested similar strains and species (Hedrick et al. 1999a; O' Grodnick 1979; Vincent 2002).

In field exposures, mortality is not generally used as a metric of effect (O'Grodnick 1979) because of 1) the lack of a negative control and 2) other pathogens could be acting synergistically with *M. cerebralis*. However, if the ultimate objective of a research investigation is to determine if exposure to this parasite in the natural environment can have lethal consequences for certain species of fish, use of mortality is a valid measure of effect despite the aforementioned problems. This can be very important when endangered or threatened species or species of special concern are potentially facing exposure to this parasite. For these reasons Thompson et al. (1999) used total cumulative mortality as a measure of susceptibility and compared those data for brown trout, rainbow trout, brook trout, and 4 subspecies of cutthroat trout, including three subspecies native to Colorado. The cutthroat trout subspecies tested included Snake River cutthroat trout (*O. c. behnkei*), Rio Grande cutthroat trout (*O. c. virginalis*), Colorado River cutthroat (CRC) trout (*O. c. pleuriticus*) and greenback cutthroat trout (*O. c. stomias*).

As expected, in these tests brown trout suffered the least mortality in almost all exposures. In the 1995-1996 exposures, brook trout and CRC trout suffered much higher mortality than did rainbow trout exposed at the same time. One treatment group of CRC trout suffered 85% mortality 132 days PE. During the 1996-1997 exposures, one treatment group of rainbow trout (Rbt-7-p6) survived significantly better than three of five treatment groups of cutthroat trout even though the latter were larger and older than the rainbow trout at the time of initial exposure. Among the two treatment groups of cutthroat trout that survived as well as the rainbow trout treatment group (Rbt-7-p6), both groups had experienced approximately 600 degree-days (° C) more growth post-hatch than the rainbow trout prior to initial exposure (Thompson et al. 1999). On that basis the rainbow trout were considered less vulnerable to

chronic mortality than any of the four subspecies of cutthroat trout when chronically exposed to ambient levels of *M. cerebralis* in the Colorado River.

Myxobolus cerebralis is widely distributed in the mountainous regions of Colorado. It 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 has negatively impacted fry recruitment for any of Colorado's three sub-species of cutthroat trout. In reality not much testing for the presence of the parasite in cutthroat trout waters had actually been undertaken. The parasite is enzootic among CRC 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. Whether or not negative impacts will begin to occur in these two populations of cutthroat trout is unknown.

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.

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 four 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 the 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 four different lineages of *T. tubifex* make this possible (Beauchamp et al. 2001, 2002). During 2003 and 2004, a private laboratory (Pisces Molecular) developed a four probe-multiplex qPCR (quantitative polymerase chain reaction) test that allows the screening of a sample of up to 50 aquatic oligochaetes for the relative percentage of DNA for each of the four 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 five-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 PCR using the HSP 70 test to determine if DNA of *M. cerebralis* is present in the worm sample. The HSP 70 gene (heat shock protein gene 70) test developed by Pisces Molecular, LLC, Boulder, Colorado, targets a highly conserved protein sequence 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 three 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. In streams where cutthroat trout were sympatric with other salmonids, those species were sacrificed for disease testing. Cutthroat trout were taken only when they occurred allopatrically.

Aquatic Oligochaete Studies – Efforts were made to collect aquatic oligochaetes in sediment-laden microhabitats from multiple locations within a study reach on each study stream. 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 and tested by PCR in two different ways. Our protocol was to preserve at least one sample of 50 haired oligochaetes from each collection for PCR testing. 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. Each sample was screened using a four probe-multiplex qPCR technique to determine the relative percentage of DNA derived from four different lineages of *T. tubifex* in each 50-worm aliquot. Several studies have demonstrated that the relative susceptibility to the *Mc* parasite varies between the four lineages (Beauchamp et al. 2001, 2002; DuBey and Caldwell 2004; DuBey et al. 2005; Kerans et al.2004). Each sample was also screened for DNA of *M. cerebralis* from the HSP 70 gene.

RESULTS and DISCUSSION

As shown in Map 1 in the Appendix, there are nine major river basins in Colorado, including the Arkansas, Colorado, Dolores, Gunnison, North Platte, Rio Grande, San Juan, South Platte, and the Yampa-White River systems. Greenback cutthroat trout are native to the Arkansas and South Platte rivers. Rio Grande cutthroat trout are native to the Rio Grande basin. CRC trout are native to the Colorado, Dolores, Gunnison, San Juan and Yampa-White River systems. No cutthroat trout were ever native to the North Platte drainage in Colorado.

Most streams sampled during the 2005 field season were selected from the statewide list of streams identified as either 1) containing native greenback, Rio Grande or CRC trout, 2) were considered to have potential as cutthroat trout recovery streams, or 3) were in proximity of or connected to streams containing cutthroat trout. For most sampling forays, efforts were made to select streams for sampling in a small geographic area to minimize the need to travel back to the same region of the state to complete evaluations on additional streams in subsequent years. Sites sampled in 2003, 2004 and 2005 are shown on Map 1 in the Appendix. Maps 2 through 5 in the Appendix show the streams and sites sampled during the 2005 field season for the South Platte, Rio Grande, Colorado and Gunnison River basins and also indicate whether evidence of *M. cerebralis* infection was detected in the trout collected at the sampling site(s).

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 are organized by sub-species and presented in Tables 1 through 6. Data for greenback cutthroat trout for the South Platte and Cache la Poudre river basins are summarized in Tables 1 and 2. Population estimates were completed on three stream reaches in the headwaters of the Cache la Poudre basin and on one stream reach is the South Platte basin in 2005 (Table 1). In addition, single pass electrofishing surveys were completed at 7 sites on 6 different streams in the Cache la Poudre basin; however, population estimates at these sites were not completed due to the low numbers of trout captured. The results of the PTD and PCR screening for *M. cerebralis* are summarized in Table 2.

Tables 3 and 4 contain the data summaries for streams sampled in the Rio Grande basin during 2005. Electrofishing surveys were completed at 27 different sampling sites on 19 different streams in the Rio Grande drainage. Trout population estimates were completed on all streams sampled and at all sampling sites (see Table 3). Results from the PTD and PCR screening for *M. cerebralis* in the Rio Grande basin are summarized in Table 4.

Data summaries for sites surveyed in the Colorado River drainage basin during 2005 are shown in Tables 5 and 6. Electrofishing surveys were completed at 12 different sites on 10 different streams. Adequate numbers of trout were present at four sample sites for completing trout population estimates (Table 5). Data summaries for PTD and PCR disease screening are shown in Table 6.

Greenback Cutthroat Trout – There are 46 bodies of water (35 streams, eight lakes and three reservoirs that have been listed as habitats that either presently support greenback cutthroat trout populations or could in the future. All of these bodies of water occur in the Arkansas River and South Platte River basins.

Among the 10 sites across nine streams where trout were collected for disease screening in 2005, evidence of *M. cerebralis* infection was only detected in lower Sheep Creek near the confluence with the North Fork of the Cache la Poudre River, where 8 of 10 YOY wild brown trout tested positive by PCR (see Map 2 and data summaries in Table 2 for details). It is somewhat puzzling that there was no evidence of infection among the 10 juvenile (age 1) brown trout tested by PTD collected at the same location. This may indicate that the parasite has very recently become enzootic at this location.

An allopatric population of greenback cutthroat trout was present in Herman Gulch in the headwaters of Clear Creek just 2-3 km east of the Eisenhower Tunnel near I-70. Length-frequency distribution indicates that there were a minimum of five year classes present in the sampling reach and possibly seven or more age classes. Brook trout and greenback cutthroat trout were sympatric in George Creek in the North Fork of the Cache la Poudre River drainage (Table 1). In George Creek, the length-frequency distribution for both species indicated that only two year classes were present in the population in the sampling reach. However, brook trout comprised 85% of the population.

Rio Grande Cutthroat Trout - There are 82 bodies of water listed as habitats that either presently support Rio Grande cutthroat trout populations or could in the future. The vast majority of them are either creeks or rivers; however, the list includes one reservoir and ten lakes. All streams feed into the Rio Grande drainage that flows through the San Luis Valley in south central Colorado.

During the summer of 2003 trout population surveys and collections of aquatic oligochaetes and trout were concentrated in the very headwaters of the upper Rio Grande in the vicinity of Rio Grande Reservoir, also known as Farmers Union Reservoir. Efforts were focused in this area for two reasons. First, there are 8 to 10 streams tributary to the Rio Grande in the vicinity of the reservoir. Second, the reservoir was stocked with approximately 29,000 catchable rainbow trout in 1993 and 1994 reared at the Colorado Division of Wildlife (CDOW) Roaring Judy (RJ) State Fish Rearing Unit. The RJ unit first tested positive for *M. cerebralis* in 1992. Rio Grande Reservoir (RGR) is narrow, relatively shallow and heavily sedimented, thereby potentially providing a substantial amount habitat for *T. tubifex.* Results of the trout population surveys and analyses from the PCR and PTD disease screenings for the 2003 field season were summarized in Tables 3 and 4 (Nehring 2004).

During the summer of 2004, sampling sites for Rio Grande cutthroat trout were concentrated in three areas: 1) the Saguache Creek and Carnero Creek drainages, 2) the Conejos and Rio de los Pinos river basins and 3) in the headwaters of the upper Rio Grande upstream of Rio Grande Reservoir (RGR). The results of the 2004 studies and disease testing are summarized in Tables 3 and 4 (Nehring 2005). During the summer of 2004, additional sampling in the headwaters of the Rio Grande was done at sites that were not sampled in 2003 and to collect additional aquatic oligochaete samples. The qPCR testing of the oligochaete samples collected in the upper Rio Grande during 2003 were corrupted because the samples were preserved in a 70% mixture of ethanol and tap water. It was later determined that tap water contains enough chlorine to degrade DNA in the oligochaetes, thereby invalidating the tests for determination of lineages of *T. tubifex.*

During the summer of 2005, sampling efforts in the Rio Grande basin were focused in three areas. The primary sampling area was on the east side of the San Luis Valley from the Sand Creek drainage on the northeast corner of Great Sand Dunes National Park south to the Cuates Creek drainage on the Cielo Vista Ranch only 4-5 km north of the Colorado-New Mexico stateline. The second sampling area was focused in the Saguache Creek basin, west of Saguache, Colorado to the summit of North Cochetopa Pass. In addition, collections were made in the San Francisco Creek drainage south of Del Norte and the Alder Creek drainage north of South Fork, Colorado. The stream locations and sampling sites where surveys were made in 2005 are shown on Map 3.

In 2005, Rio Grande cutthroat trout were captured at 23 of 27 sampling sites (Table 3) and were the only species of salmonid captured at 14 of 27 sites. Evidence of *M. cerebralis* infection was found in trout collected from nine streams and at ten collection sites (Table 4). In the majority of instances evidence of infection was the result of PCR testing. Evidence of significant levels of *M. cerebralis* infection was found in three streams, Middle and Sheep creeks in the Saguache Creek basin, and Placer Creek in the Sangre de Cristo Creek drainage on the east

side of the San Luis Valley. In all three cases substantial evidence of infection was documented with both PTD and PCR testing.

Historically, Placer Creek has supported one of the core conservation populations of Rio Grande cutthroat trout. Placer Creek is tributary to Sangre de Cristo Creek. *Myxobolus cerebralis* was first detected in brook trout in Sangre de Cristo Creek in 2003 (see Table 4 in Nehring 2004 for details). At that time, prevalence of infection was 70% among the brook trout tested by PTD and mean cranial myxospore concentration among the fish testing positive was 68,126. The maximum observed cranial myxospore concentration was 195,900. These data suggest the parasite was already well established in Sangre de Cristo Creek by the summer of 2003. In 2005, prevalence of infection among juvenile brook trout in lower Placer Creek was 67% and mean cranial myxospore concentration among the fish testing positive was 40,722. The maximum observed cranial myxospore level was 211,000. These fish were collected downstream of the failed migration barrier that formerly isolated the Placer Creek Rio Grande cutthroat trout from Sangre de Cristo Creek. Taken together, the 2003 and 2005 data suggest the infection level is quite high, but stable in the lower reaches of Placer Creek near the confluence with Sangre de Cristo Creek.

The PCR and PTD data for 2005 for the upstream reach of Placer Creek indicate prevalence of infection is much lower, suggesting that *M. cerebralis* has only recently become enzootic in the headwater areas since the failure of the migration barrier. Prevalence of infection among YOY brook trout tested by PCR was only 20%. Similarly, prevalence of infection among age 1 brook trout collected at the same location was 19%, with a mean cranial myxospore concentration of 16,111 among fish testing positive by the PTD methodology. The range of myxospore concentrations among the four fish (of 21) testing positive was 1,111 to 27,778 (see Table 4 for details).

Because of the unique circumstances with the apparent recent establishment of *M. cerebralis* in the upper reaches of Placer Creek, this stream may respond favorably to an innovative attempt at reclamation to control or hopefully eliminate the parasite from the drainage. The project would require quick action and several points of attack. First, it would require a large electrofishing operation during the summer of 2006 to remove most if not all of the trout from the drainage upstream of the failed migration barrier. Samples of brook and Rio Grande cutthroat trout removed during the electrofishing operation would be tested to determine the spatial distribution of the parasite in the upper reaches of the basin. Second, another migration barrier would have to be installed in the drainage during the summer of 2006 to prevent re-invasion by the brook trout. Third, the upper reaches of the drainage would be seeded with lineage 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). CDOW researchers initiated laboratory exposure experiments on various lineages of *T. tubifex* in December 2005 to test the hypothesis that lineage V and VI *T. tubifex* in Colorado have a high degree of resistance to *M. cerebralis.* These exposure experiments are scheduled for completion by June 2006. Thus far, these tests are corroborating the findings of DuBey and Caldwell (2004) and DuBey et al. (2005), i.e., lineage I, V and VI worms are not producing any TAMs more than sic months PE to 50 myxospores per worm. Rapid depopulation of the stream (preferably in 2006), together with re-installation of a

migration barrier to prevent re-invasion by the brook trout, and introduction of resistant lineage *T. tubifex* to act as biological "biofilters" to consume and deactivate *M. cerebralis* myxospores has the potential to reduce the presence or even eliminate the parasite from the drainage.

Evidence of *M. cerebralis* infection was also detected in the lower reaches of Sand Creek where it flows out into Great Sand Dunes National Park. Introduction of the parasite in the lower reaches of this drainage probably occurred during the time when the land was in private holdings and attempts were made to rear trout commercially in ponds. The ponds no longer are functional but a few brook trout were seen swimming in standing water over shallow mud flats within the area where the ponds once were (Table 4).

Finally, brook and rainbow trout collected at the lower sampling site on Middle Creek tested positive for *M. cerebralis* by both PCR and PTD testing (Table 4). However, Rio Grande cutthroat trout in East Middle Creek tested negative for the parasite. A migration barrier isolates this population from the exposed populations of salmonids in Middle Creek

Colorado River Cutthroat Trout – Historically, CRC trout occurred in the majority of coldwater streams west of the Continental Divide in Colorado. Currently, the number streams, lakes and reservoirs listed as present or future CRC trout recovery areas by major drainage basin are 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**

During the summer and fall of 2003, population survey efforts were focused in the headwaters of the Fryingpan River in west central Colorado and in the upper Colorado River basin in Middle Park (Grand County). The upper Colorado River basin in Middle Park has been a focal point for research on the impacts of *M. cerebralis* on rainbow trout for a decade (Walker and Nehring 1995; Nehring and Walker 1996; Nehring et al. 1998; Schisler et al. 1999a,b; Thompson et al. 1999; Zendt and Bergersen 2000; Nehring and Thompson 2001; Nehring and Thompson 2003; Nehring et al. 2003). Most of the research effort has occurred in three major streams in Middle Park; the Colorado, Fraser and Williams Fork rivers where *M. cerebralis* has been known to be enzootic since the early 1990s (Walker and Nehring 1995). However, it was unknown if the parasite had spread into the headwater tributary areas. The same was true for most of the Fryingpan River basin upstream of Ruedi Reservoir. Several streams in each of these major drainages have supported core conservation populations of CRC trout in the recent past.

The upper reaches of the Fryingpan and Colorado River basins also share another unique characteristic in that almost all headwater tributaries are interconnected by large transmountain water diversion and collection systems. Snowmelt and rainwater flowing into most of the tributaries of the upper Fryingpan basin are captured by a series of canals, ditches, and gravity siphons and diverted across the Continental Divide into the United States Bureau of Reclamation (USBOR) Fryingpan/Arkansas Project. Similarly, water captured by a series of canals, ditches, and gravity siphons from the headwater streams tributary to the Colorado, Fraser and Williams Fork rivers are diverted across the Continental Divide to supply water to Colorado's Front Range cities from Denver northward to Boulder, Longmont and Fort Collins. The results of studies completed in these drainages for 2003 are summarized in Nehring (2004).

During the 2004 field season, sampling of CRC trout streams focused on streams draining from the Roan Plateau north of I-70, on Battlement Mesa south of I-70, and on Grand Mesa. The results of the electrofishing surveys are summarized in Tables 5 and 7 (Nehring 2005). Results of the PCR and PTD testing on trout collected from these streams are summarized in Tables 6 and 8 (Nehring 2005). Electrofishing surveys were completed on 26 streams at 35 sampling sites. Allopatric CRC trout populations were observed in 19 streams and at 24 sampling locations (Tables 5 and 7). Densities of CRC trout ≥15 cm exceeded 1,000/ha at 10 sampling sites and biomass estimates (kg/ha) exceeding 100 kg/ha were observed at seven sampling sites. Multiple year classes of fish were evident at most sampling sites where CRC trout were found.

Among the trout from sites sampled in the Colorado River drainage and its sub-basins tested by the PCR and/or PTD methodologies in 2004, evidence of *M. cerebralis* infection was detected in only four streams (Tables 6 and 8; Nehring 2004). These streams were Crooked Creek (Fryingpan River drainage), Black Gore Creek at the top of Vail Pass (Eagle River drainage) Big Creek, draining off the north side of Grand Mesa and Carr Creek on the Roan Plateau. At the upstream sampling site on Carr Creek a single cutthroat tested positive by PCR. This may indicate the parasite is established in this drainage, or it might be a false positive resulting from cross contamination of the sample. The positive samples from Black Gore Creek and Crooked Creek were not unexpected, since samples from these sites had tested positive for *M. cerebralis* on previous occasions.

The most dismaying test results were from the Big Creek drainage upstream of Bonham Reservoir. Big Creek was not scheduled for sampling in 2004. However, in early September 2004, a phone call from an avid fly fisherman provided the impetus to sample in the Big Creek drainage. The fly fisherman reported that he had seen trout whirling violently in Big Creek just upstream from Bonham Reservoir, having seen the behavior in videos on WD in the past. The observations were astute and correct. More than two dozen cutthroat trout juveniles whirling violently and displaying all the clinical signs of WD were netted from the stream without the aid of electrofishing gear. PCR and PTD tests subsequently performed on brook, rainbow, cutthroat and grayling collected from the stream revealed all four species were infected with *M. cerebralis* (see Table 6 in Nehring, 2005 for details). Subsequent samples collected in October 2004 revealed that wild cutthroat trout in Big Creek just 100 meters downstream of Big Creek Reservoir were also infected with *M. cerebralis.* However, cutthroat trout collected from the West Fork of Big Creek and Big Creek upstream of Big Creek Reservoir were not infected.

The infection in Big Creek appears to be a rare case where the parasite was not introduced through the stocking of trout reared in an aquaculture facility testing positive for *M. cerebralis.* There is no history of stocking of trout from a rearing unit testing positive for *M. cerebralis* in the drainage according to archived CDOW fish stocking records. Similarly, there is no private land or ponds in the Big Creek drainage and therefore no reason to believe the introduction resulted from private stocking. It is possible that the parasite was brought into the drainage by mammalian or avian predators, or by an angler that may have caught infected trout from another drainage or lake atop Grand Mesa and brought them to Big Creek before cleaning them. Unwitting anglers may have been fishing at several lakes on the Grand Mesa and cleaned the day's catch of fish in Big Creek and disposed of the entrails in the water. The parasite has been established in several lakes and reservoirs across Grand Mesa for 5 to 10 years (Schisler 1999). Fresh entrails from a dozen or more trout were observed in Big Creek on September 9, 2004, just upstream of where the road crossed the stream where the electrofishing survey was completed.

In 2005, a public information guide for anglers fishing the Grand Mesa was revised and published. The new brochure admonishes anglers **NOT** to dispose of fish entrails in the water. Rather, anglers are asked to dispose of them in a trash container or in waste destined for the landfill.

During the summer and fall of 2005, electrofishing operations were conducted at 12 sampling sites on 10 streams in the Colorado River and Gunnison River basins. For details on the locations of the collection sites see Maps 4 and 5 in the Appendix. Data summaries on trout population numbers, density, biomass, species occurrence and evidence of *M. cerebralis* infection are shown in Tables 5 and 6. Population estimates were completed on four streams. Single pass electrofishing was completed in attempts to collect enough trout for disease screening at the remaining collection sites. CRC trout were observed at three streams, including the East Fork of Big Creek, the West Fork of Big Creek draining from the north slopes of Grand Mesa and in Second Creek, a small tributary to the North Fork of the Gunnison River east of Crawford, Colorado. Trout testing positive for *M. cerebralis* were collected from 6 of 10 streams (Table 6). Fish sacrificed for disease screening tested positive by both the PCR and PTD methodologies from all six streams. There were no instances where CRC trout tested positive for *M. cerebralis* from streams sampled during 2005 including 10 CRC trout collected upstream of the migration barrier on Second Creek in May 2006. However, *M. cerebralis*-infected rainbow trout were collected immediately downstream of the migration barrier preventing rainbow and brown trout from immigrating into the stream reach supporting a CRC trout population.

Aquatic Oligochaete Sampling – Since 2001, there has been a substantial research effort to understand the population dynamics of aquatic oligochaetes and determine the relative differences in susceptibility to *M. cerebralis* among oligochaetes in general and among the four lineages of *T. tubifex* in particular (Beauchamp et al. 2001, 2002, 2005, 2006; DuBey and Caldwell 2004; DuBey et al. 2005; Kaesar and Sharpe 2006; Kerans et al. 2004). As more and more research investigations are directed at the aquatic oligochaete side of the life cycle of *M. cerebralis* it becomes increasing clear that the presence of the lineage III *T. tubifex* in an aquatic environment may 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 are currently emerging from on-going laboratory tests in Colorado (Nehring, unpublished data) and Oregon (Dr. Jerri Bartholomew, 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 multiplex 4-probe qPCR protocol that would allow for testing for the four 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).

By spring 2004, development and testing of the four-probe multiplex qPCR protocol was complete. That year greater effort was expended to collect sediment samples. Sample preservation and laboratory protocols were improved. In addition, aquatic oligochaete samples were collected during August 2004 from the streams sampled in the upper Rio Grande in 2003. Taken together, these efforts resulted in larger numbers of aquatic oligochaetes collected at most sampling sites during the 2004 field season. Additional efforts were expended during surveys in 2005 to collect sufficient numbers of aquatic oligochaete to determine which lineages of *T. tubifex* were present in the stream at the study site. Data summaries for the oligochaete sampling efforts for 2003, 2004 and 2005 are presented in Tables 7, 8 and 9.

The location of each sample site is referenced using global positioning technology (GPS). This allows all data to be plotted on a map to visually depict 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 6, 7, 8 and 9, 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 10 shows those sites where aquatic oligochaetes were collected, but there was no amplification of 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 Beregersen 2000).

During 2003 and 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. The results of those efforts are summarized in Tables 10a, 10b, 10c and 10d. The 50 worm aliquot provided the best results across a broad range of worm sizes while concurrently minimizing the reagent costs at the laboratory.

CONCLUSIONS

There are no definitive conclusions that can be drawn regarding the rate and degree of spread of *M. cerebralis* in aquatic habitats designated as present or future potential cutthroat trout recovery areas. However, it is noteworthy that the majority of the locations where *M. cerebralis* was detected at sampling sites in the Arkansas River, Colorado River, Rio Grande and South Platte River basins were also areas where catchable rainbow trout had been previously stocked on one or more occasions in one or more years from units that had tested positive for the *Mc* parasite. In contrast, in those areas where there was no record of stocking of trout from units testing positive for the parasite since the mid-1980s, YOY trout tested by PCR and juvenile trout tested by either PCR or PTD tested negative for presence of the parasite in most cases.

Recent developments in the DNA typing and testing of the various lineages of *T. tubifex* for susceptibility or resistance to *M. cerebralis* offer hope that it could be feasible to attack the worm side of the life cycle of the parasite to either reduce ambient levels of infection or possibly control or eliminate the parasite completely

Table 1 Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within greenback cutthroat trout (*Oncorhynchus clarki stomias*) recovery zones sampled during the summer of 2005.

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
Bennett Creek GPS 13T 455432//4500316												
									9 [ad]	--	67	--
Upper Black Hollow Creek GPS 13T 443716//4501762												
09/28/05	Electrofished 244 (800 feet) meters of channel. No fish seen or captured											
Lower Black Hollow Creek GPS 13T 445329//4505520												
09/28/05	--	--	--	--	1	0	44	2	--[b]	--	--	--
Cornelius Creek GPS 13T 448098//4528517												
09/28/05	--	--	--	--	11	±1	696	36	--	--	--	--
George Creek GPS 13T 446036//4527728												
09/28/05	--	--	--	--	78 [de]	±2	5,583	42	14[cd]	±2	1,011	6
Herman Gulch GPS 13S 426506//4395499												
09/26/05	--	--	--	--	--	--	--	--	43	±2	1,709	111
Pennock Creek GPS 13T 454013//4491792												
09/28/05	4	--	108	--	1	--	27	--	--	--	--	--
Headwaters of the East Fork Sheep Creek, Cache la Poudre River drainage GPS 13T 440239//4496978												
09/28/05	--	--	--	--	--	--	--	--	4 [d]	--	172	--
Headwaters of the West Fork Sheep, Cache la Poudre River drainage GPS 13T 438716//4495298												
09/28/05	--	--	--	--	--	--	--	--	1[d]	--	27	--
Sheep Creek (above Eaton Reservoir), North Fork Cache la Poudre River drainage GPS 13T 438627//4531358												
09/30/05	--	--	--	--	1 [d]	--	21	--	--	--	--	--
Sheep Creek (below Eaton Reservoir), North Fork Cache la Poudre River drainage GPS 13T 443083//4533118												
09/30/05	--	--	--	--	4 [d]	---	215	--	--	--	--	--
Sheep Creek (below George and Cornelius creeks) near the N. Fk Cache la Poudre River confluence GPS 13T 452123//4527192												
09/30/05	49	±1	1,346	113	--	--	--	--	--	--	--	--

a: All fish were rainbow trout.
b: Rainbow trout fry and juveniles were present but all were < 15 cm total length.
c: Greenback cutthroat trout fry and juveniles were present but all were < 15 cm total length.
d: Single electrofishing pass only; no population estimate.
e: 74 of 78 brook trout collected were < 15 cm total length.

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 *M. cerebralis* in drainages in the vicinity of streams designated as present or future areas for recovery of greenback cutthroat trout ***(****Oncorhynchus clarki stomias****)*** during 2005. 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.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	n+	Mean (n+) myxospores	Range Myxospores (n+)
Greenback Cutthroat Trout (*Oncorhynchus clarki stomias*) Recovery Areas and Nearby Tributary Streams									
Bennett Creek	Above Little S. Fk. Poudre River	Rainbow	--	-	--	4	0	------	------
Black Hollow Creek	1 km above of Poudre River confluence	Brook	10	0	10	7	0	------	------
Black Hollow Creek	1 km above of Poudre River confluence	Rainbow	--	-	--	10	0	------	------
Cornelius Creek	upstream of George Creek confluence	Brook	10	0	10	10	0	------	------
George Creek	upstream of Cornelius Creek confluence	Brook	10	0	10	10	0	------	------
Herman Gulch	1 km upstream S. Clear Creek confluence	Cutthroat	10	0	10	10	0	------	------
Pennock Creek	Upstream of Little South Poudre River	Brook	2	0	10	1	0	------	------
Pennock Creek	Upstream of Little South Poudre River	Brown	8	0	10	10	0	------	------
Sheep Creek, upper	Above Eaton Reservoir	Brook	10	0	10	10	0	------	------
Sheep Creek, middle	Below Eaton Reservoir	Brook	10	0	10	10	0	------	------
Sheep Creek, lower	**Near confluence with N. Fork Poudre River**	**Brown**	**10**	**8**	**32**	10	0	------	------
West Fk Sheep Creek	Headwaters above Poudre R. confluence	Cutthroat	--	-	--	1	0	------	------

Table 3 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 2005.

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
Alamosito Creek – 0.4 km upstream of N/S Ranch Road GPS 13S 473446//4104236												
07/26/05	---	---	---	---	---	---	---	---	14	±4	663	58
Alder Creek, lower site near South Fork, CO GPS 13S 355105//4173549												
08/02/05	37	±7	1,318	114	---	---	---	---	---	---	---	---
West Alder Creek GPS 13S 350780//4182270												
08/02/05	--	--	--	--	52	±11	2,169	142	3	±0	125	11
Cross Creek, upper station on Peterson Property GPS 13S 381657//4230263												
08/01/05	---	---	---	---	---	---	---	---	16	±2	1,883	128
Upper Cuates Creek on Cielo Vista Ranch GPS 13S 469490//4097123												
07/25/05	---	---	---	---	---	---	---	----	6	±1	267	2.0
Lower Cuates Creek on Cielo Vista Ranch GPS 13S 467447//4097134												
07/25/05	---	---	---	---	---	---	---	----	14	±1	930	63
Jacks Creek –SLB Property leased by Sutherland GPS 13S 378868//4228945												
08/01/05	---	---	---	---	---	---	---	---	11	±1	2,368	124
Upper Jaroso Creek on Cielo Vista Ranch GPS 13S 470999//4100003												
07/25/05	---	---	---	---	---	---	---	----	17	±3	798	56
Lower Jaroso Creek on Cielo Vista Ranch GPS 13S 468368//4100091												
07/25/05	---	---	---	---	18	±5	329	25	21	±2	384	29
Medano Creek @ road crossing below Frenchman's Cabin GPS 13S457344//4184321												
08/02/05	--	--	--	--	--	--	--	--	24	±3	685	58
Middle Creek @ Rio Grande National Forest trailhead (Saguache Creek drainage) GPS 13S 386129//7237436												
08/03/05	13	±62	389	56	15	±2	454	39	11 [a]	±12	329	36
Headwaters of East Middle Creek (Saguache Creek drainage) GPS 13S 390087//4242786												
08/03/05	--	--	--	--	--	--	--	--	10	±0	664	48
East Pass Creek, 2 km below Buffalo Creek Campground (Saguache Creek drainage) GPS 13S 369441//4227325												
08/01/05	--	--	--	--	--	--	--	--	1	±0	108	25

a: These fish were all rainbow trout.

b: All trout captured were ≤150 mm.

Table 3 (continued). 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 of 2005.

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 Pass Creek, 4 km above Buffalo Creek Campground (Saguache Creek drainage) GPS 13S 364951//4228635												
08/01/05	--	--	--	--	--	--	--	--	12	±0	1,291	128
East Pass Creek, 5 km Above Buffalo Creek Campground (Saguache Creek drainage) GPS 13S 364441//4229023												
08/01/05	--	--	--	--	--	--	--	--	8	±0	143	35
Upper Placer Creek above failed barrier GPS 13S 473015//4162508												
07/27/05	---	---	---	---	11	±1	541	49	12	±2	603	46
Lower Placer Creek-below failed barrier GPS 13S 474741//4158103												
07/27/05	---	---	---	---	1	±1	33	0.2	3	±0	98	0.7
Lower Sand Creek at Great Sand Dunes National Park near Liberty, CO GPS 13S 448776//4188650												
08/03/05	4 [a]	±0	58	11	19	±5	281	18	1	±0	15	2
San Francisco Creek on Cielo Vista Ranch GPS 13S 472143//4103473												
07/26/05	57	±2	1,873	246	---	---	---	---	---	---	---	---
San Francisco Creek south of Del Norte, Colorado GPS 13S 379070//4159804												
07/28/05	---	---	---	---	---	---	---	---	20	±1	667	9.5
Upper Torcido Creek on Cielo Vista Ranch GPS 13S 472105//4100827												
07/26/05	---	---	---	---	---	---	---	----	77	±8	3,947	73
Lower Torcido Creek on Cielo Vista Ranch GPS 13S 470703//4101333												
07/25/05	---	---	---	---	---	---	---	----	56	±4	2,488	75
North Vallejos Creek, upper station GPS 13S 474910//4108430												
07/27/05	22	±2	747	68	---	---	---	---	3	±6	100	6
North Vallejos Creek, lower station GPS 13S 473144//4108409												
07/27/05	14	±2	500	41	---	---	---	---	1	±0	35	3
Upper Vallejos Creek - 3.5 km upstream of N/S Ranch Road GPS 13S 475584//4107269												
07/26/05	20	±10	763	47	---	---	---	---	7	±10	269	17
Lower Vallejos Creek GPS 13S 472613//4107219												
07/26/05	41	±1	1,051	84	---	---	---	---	---	---	---	---
Wagon Creek upstream of confluence with Sangre de Cristo Creek GPS 13S 480861//4152417												
08/02/05	--	--	--	--	6	±62	308	25	5	±0	256	18

a: These fish were all rainbow trout, not cutthroat trout.

b: All trout captured were ≤150 mm.

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 *M. 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 2005. 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.

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									
Alamosito Creek	At Cielo Vista Ranch	Cutthroat	10	0	10	--	--	------	------
Alder Creek	Near South Fork, CO	Brown	10	0	10	10	0	------	------
West Alder Creek	**At East Fork Alder Creek confluence**	**Brook**	**10**	**2**	**15**	**20**	**0**	------	------
Cross Creek, Upper	On Peterson Ranch property	Cutthroat	7	0	10	10	0	------	------
Cross Creek, Lower	Above confluence with Jack Creek	Cutthroat	4 [a]	0	10	6	0	------	------
Cuates Creek, lower	At Cielo Vista Ranch	Cutthroat	10	0	10	11	0	------	------
Upper Jack Creek	SLB Property leased by Sutherland	Cutthroat	--	-	--	10	0	------	------
Lower Jack Creek	Above Cross Creek confluence	Cutthroat	5	0	10	5	0	------	------
Lower Jack Creek	Above Cross Creek confluence	Brook	--	-	--	1	0	------	------
Jaroso Creek, lower	At Cielo Vista Ranch	Brook	8	0	10	20	0	------	------
Jaroso Creek, lower	At Cielo Vista Ranch	Cutthroat	2	0	10	2	0	------	------
Medano Creek, lower	At Great Sand Dunes National Park	Cutthroat	10	0	10	11	0	------	------
Middle Creek	**At USFS trail head**	**Brook**	**10**	**9**	**33**	**10**	**2**	**30,000**	**7,778 –52,222**
Middle Creek	**At USFS trail head**	**Rainbow**	**2**	**1**	**20**	**6**	**4**	**24,028**	**12,222 – 53,333**
Middle Creek, East	3 km above Middle Creek confluence	Cutthroat	4	0	10	10	0	------	------
East Pass Creek	2 km below Buffalo Creek Campground	RGNCut	--	-	--	4	0	------	------
East Pass Creek	**4 km ↑ Buffalo Creek Campground**	**RGNCut**	**3**	**1**	**17**	10	0	------	------
Upper Placer Creek	**Approx. 3 km above barrier**	**Brook**	**10**	**2**	**17**	**21**	**4**	**16,111**	**1,111 – 27,778**
Lower Placer Creek	**Approx. 0.5 km below barrier**	**Brook**	**1**	**1**	**30**	**15**	**10**	**40,722**	**556 – 211,111**
Lower Placer Creek	**Approx. 0.5 km below barrier**	**Cuttroat**	**10**	**7**	**22**	--	--	------	------
Sand Creek, lower	**At Great Sand Dunes National Park**	**Brook**	**10**	**4**	**22**	**16**	**0**	------	------
Sand Creek, lower	At Great Sand Dunes National Park	Rainbow	--	-	--	14	0	------	------
Sand Creek, upper [b]	Headwaters above National Park	Cutthroat	--	-	--	20	0	------	------
Upper Sand Crk Lake [b]	Headwaters above National Park	Cutthroat	--	-	--	20	0	------	------
Lower Sand Crk Lake [b]	Headwaters above National Park	Cutthroat	--	-	--	20	0	------	------
Kerr Lake	NE of Platoro, CO	Cutthroat	--	-	--	60	0	------	------

a: These four fish were YOY brook trout.

b: These samples collected by John Alves, Monte Vista Area (Rio Grande basin) fisheries biologist.

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 *M. cerebralis* in drainages in the vicinity of s1treams designated as present or future areas for recovery of Rio Grande cutthroat (*Oncorhynchus clarki virginalis*) trout in 2005. 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.

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									
San Francisco Creek	Approx. 10 km south of Del Norte, CO	Cutthroat	2	0	10	9	0	------	------
San Francisco Creek	Approx. 10 km south of Del Norte, CO	Cutthroat	--	-	--	9	8 [c]	22,292	556 – 118,889
San Francisco Creek	On Cielo Vista Ranch SE of San Luis	Brown	3	0	10	22	0	------	------
Sheep Creek	**Above Spruce Creek confluence**	**Brown**	**10**	**10**	**41**	**10**	**7**	**2,778**	**556 – 6,111**
Torcido Creek	On Cielo Vista Ranch SE of San Luis	Cutthroat	--	-	--	10	0	------	------
Vallejos Creek, lower	On Cielo Vista Ranch SE of San Luis	Brown	10	0	10	11	0	------	------
Vallejos Creek, upper	**On Cielo Vista Ranch SE of San Lius**	**Brown**	10	0	10	**10**	**1**	**556**	**556**
N. Vallejos Creek	**CieloVista Ranch 2 km ↑road crossing**	**Brown**	**--**	**-**	**--**	**10**	**1**	**556**	**556**
Wagon Creek	Upstream of Sangre de Cristo Creek	Brook	--	-	--	7	0	------	------
Wagon Creek	**Upstream of Sangre de Cristo Creek**	**RGNCut**	**10**	**1**	**12**	3	0	------	------

c: Cranial myxospores that look similar to *Henneguya salminicola* or *H. zschokkei*, but probably an as yet undescribed species.

Table 5. Trout population biostatistics for trout ≥ 15 cm collected in streams and sampling sites within CRC trout (*Oncorhynchus clarki pleuriticus*) recovery zones in the Colorado River basin sampled during 2005.

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 of Big Creek, upstream of Big Creek confluence GPS 13S 250741//4332495												
08/29/05	--	--	--	--	--	--	--	--	69 [a]	±1	3,065	25
West Fork of Big Creek upstream of Bonham Reservoir GPS 13S 248795//43329863												
09/16/05	--	--	--	--	--	--	--	--	7 [b]	--	222	**??**
Buzzard Creek on Grand Mesa GPS 13S 267359//434 and 13S 270108//4328625												
09/15/05	No trout captured or seen at two separate stations; only dace and fathead minnows											
Cow Creek upstream of Overland Reservoir GPS 13S 271856//4329904												
09/15/05	7 [c]	±1	218	**??**	17	±1	535	**??**	--	--	--	--
Main Hubbard Creek GPS 13S 276239//4326308												
10/25/05	--	--	--	--	21	±0	580	**??**	--	--	--	--
Middle Hubbard Creek GPS 13S 276003//4325386												
10/25/05	--	--	--	--	15	±1	1,367	**??**	--	--	--	--
Upper Plateau Creek upstream of Vega Reservoir GPS 13S 260674//4345015												
09/16/05	20 [abc]	--	1,435	--	--	--	--	--	--	--	--	--
Lower Plateau Creek downstream of Vega Reservoir GPS 13S 256485//4235743												
09/16/05	3 [b]	--	40	--	7 [b]	--	94	--	--	--	--	--
Second Creek upstream of barrier for fish migration GPS 13S 281885//4289935												
10/24/05	--	--	--	--	--	--	--	--	10 [b]	--	362	--
Second Creek downstream of migration barrier GPS 13S 281556//428865												
10/24/05	10 [bc]	--	368	--	--	--	--	--	--	--	--	--
Smith Fork of the Gunnison River upstream of Second Creek confluence GPS 13S 283741//4288865												
10/24/05	10 [bd]	--	--	--	3 [bd]	--	--	--	12 [cd]	--	--	--
Unnamed tributary to Bonham Reservoir GPS 13S 248300//4330026												
08/29/05	--	--	--	--	20 [bd]	--	--	--	7 [bde]	--	--	--

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 Arctic grayling *Thymallus thymallus*, not cutthroat trout.

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 *M. 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 Colorado River basin on Battlement Mesa, Grand Mesa and in the North Fork of the Gunnison River basin in 2005. 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.

Stream Name	Approximate Collection Location	PCR (YOY)				PTD (≥ Age 1)			
		Species	N	n+	Score	N	n+	Mean (n+) myxospores	Range Myxospores (n+)
Buzzard Creek	Upstream of Cheney Creek	No trout	--	-	--	--	-	------	------
East Fork Big Creek	Above Big Creek confluence	Cutthroat	10	0	10	10	0	------	------
Unnamed Creek	**Tributary to Bonham Reservoir**	**Brook**	**15**	**11**	**37**	**10**	**5**	**98,877**	**20,611 – 243,556**
Cow Creek	**2 km above Overland Reservoir**	**Brook**	**10**	**3**	**22**	**10**	**2**	**18,986**	**6,333 – 31,639**
Cow Creek	below Overland Reservoir outlet	Rainbow	--	-	--	10	0	------	------
Hubbard Creek	**Above Middle Hubbard Creek**	**Brook**	**10**	**10**	**48**	**10**	**9**	**138,648**	**12,167 – 280,889**
Mid. Hubbard Crk	**Above Hubbard Creek**	**Brook**	**10**	**8**	**34**	**10**	**10**	**109,009**	**10,867 – 231,933**
Plateau Creek	Upstream of Vega Reservoir	Rainbow	--	-	--	20	0	------	------
Plateau Creek	Below Vega Reservoir	Brook	--	-	--	7	0	------	------
Plateau Creek	Below Vega Reservoir	Rainbow	--	-	--	3	0	------	------
Second Creek	above cutthroat trout barrier	Cutthroat	--	-	--	10	0	------	-----
Second Creek	**Below cutthroat trout barrier**	**Rainbow**	**--**	**-**	**--**	**10**	**6**	**32,098**	**4,133 – 80,944**
Smith Fork of the Gunnison River	**Upstream of the Second Creek confluence**	**Brown**	**10**	**0**	**10**	**--**	**--**	**------**	**------**
		Brook	**2**	**1**	**20**	**--**	**--**	**------**	**------**
		Rainbow	**2**	**1**	**30**	**10**	**4**	**54,838**	**1,667 – 160,467**

Table 7. Aquatic oligochaete collections from the summers of 2003, 2004 and 2005 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 *T. 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
Big Thompson River	10/18/04		150	150	0	0	0	0	0
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
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 2 km E. of Eisenhower Tunnel	08/04/04	13S424248//4393335	111	111	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
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
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
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
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

a: nd – not determined, but highly probable that most worms were haired given that the DNA tested out as 100% lineage III worms.

Table 8. Aquatic oligochaete collections from the summer of 2004 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 *T. 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
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
Tuttle Creek (Saguache Creek basin)	07/21/04	13S392159//4234536	213	192	21	0	0	0	0
Ford Creek (Saguache Creek basin)	07/21/04	13S377887//4199158	13	0	13	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
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
Weminuche Creek	08/09/04	13S296398//4174715	337	224	113	0	0	0	0
Pole Creek	08/10/04	13S282910//4186664	219	125	94	0	0	0	0
Rio Grande below Quartzite Creek	08/10/04	13S279530//4182908	325	112	113	0	0	0	0
Big Flint Lake	08/12/04	13S283623//4167121	125	1	124	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
Rio de los Pinos River ↓ Trujillo Meadows	09/21/04	13S371564//4100909	453	129	324	0	0	0	0

Table 8 (continued). Aquatic oligochaete collections from the summer of 2005 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 *T. 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
West Alder Creek	08/03/05	13S351282//4181417	1200	100	1100	0	100	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
Jaroso Creek	07/25/05	13S468638//4100091	54	4	50	0	100	0	0
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
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
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

Table 9. Aquatic oligochaete collections from the summers of 2003, 2004 and 2005 for streams considered to be present recovery areas or future locations for CRC 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 *T. 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
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
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
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
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

Table 9 (continued). Aquatic oligochaete collections from the summers of 2003, 2004 and 2005 for streams considered to be present recovery areas or future locations for CRC 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 *T. 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
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
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
Gunnison River Basin									
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
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

Table 9 (continued). Aquatic oligochaete collections from the summers of 2003, 2004 and 2005 for streams considered to be present recovery areas or future locations for CRC 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 *T. 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
Roan Plateau Area (Lower Colorado River Basin)									
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 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 10a. Summary of test results using the 4-probe multiplex qPCR test to evaluate levels of accuracy, precision and preferred sample size for determination of the percentage of DNA for lineage I, III, V and VI *T. tubifex* in composite samples of 5, 10, 25, 50 and 100 "haired" aquatic oligochaetes collected from a single benthic sample in the Colorado River at Breeze Bridge on the CDOW Kemp-Breeze State Wildlife Area.

No. Worms/Sample	Percent Lineage I	Percent Lineage III	Percent Lineage V	Percent Lineage VI
5	0	1.5	0	98.5
5	100	0	0	0
5	73.7	0	0	26.3
5	41.1	0	58.9	0
5	100	0	0	0
Mean (Range)	**63.0 (09 -100)**	**0.30 (0 – 1.5)**	**11.8 (0 – 58.9)**	**25.0 (0 – 98.5)**
10	31.1	39.9	29.0	0
10	28.9	62.0	0	9.2
10	29.5	0	28.9	41.6
10	18.0	44.5	0	37.5
10	58.4	0	0	41.6
Mean (Range)	**33.2 (18.0 – 58.4)**	**29.3 (0 – 62.0)**	**11.6 (0 – 29.0)**	**26.0 (0 – 41.6)**
25	62.6	0	0.1	37.3
25	29.2	0	35.3	35.5
25	9.1	38.2	12.4	40.3
25	37.9	21.5	0	40.6
25	34.1	28.1	0	37.9
Mean (Range)	**34.6**	**17.6**	**9.6**	**38.3**
50	45.0	8.4	0	46.6
50	34.8	15.9	0	49.3
50	36.2	0	35.3	28.0
50	33.1	6.2	12.4	60.7
50	36.4	0	0	63.6
Mean (Range)	**37.1**	**6.1**	**7.2**	**49.6**
100	39.6	0	15.4	44.9
100	30.0	0	33.3	36.6
100	31.4	9.3	26.3	33.0
100	37.6	14.2	11.1	37.0
100	1.9	0.8	95.5	1.7
Mean (Range)	**28.1**	**4.9**	**36.3**	**30.6**

Table 10b. Summary of test results using the 4-probe multiplex qPCR test to evaluate levels of accuracy, precision and preferred sample size for determination of the percentage of DNA for lineage I, III, V and VI *T. tubifex* in composite samples of 5, 10, 25, 50 and 100 "haired" aquatic oligochaetes collected from a single benthic sample from the Williams Fork River downstream of Williams Fork Reservoir.

No. Worms/Sample	Percent Lineage I	Percent Lineage III	Percent Lineage V	Percent Lineage VI
5	0	0	100	0
5	0	0	100	0
5	0	0	100	0
5	0	0	100	0
5	0	0	100	0
Mean (Range)	**0**	**0**	**100**	**0**
10	0	0	100	0
10	0	0.1	99.9	0
10	0	0	100	0
10	0	0	100	0
10	0	0	100	0
Mean (Range)	**0**	**0**	**100**	**0**
25	0	0	100	0
25	0	0	100	0
25	0	0	100	0
25	0	0	100	0
25	0	0	100	0
Mean (Range)	**0**	**0**	100	**0**
50	0	0	100	0
50	0	0	100	0
50	0	0	97.6	2.4
50	0	0	94.2	5.8
50	0	0	100	0
Mean (Range)	**0**	**0**	**98.4**	**1.6**
100	0	0	100	0
100	0	0	99.6	0.4
100	0	0	100	0
100	0	0	100	0
100	0	0	100	0
Mean (Range)	**0**	**0**	**100**	**0**

Table 10c. Summary of test results using the four-probe multiplex qPCR test to evaluate levels of accuracy, precision and preferred sample size for determination of the percentage of DNA for lineage I, III, V and VI *T. tubifex* in composite samples of 10 and 50 aquatic oligochaetes taken from a single core sample collected from the Windy Gap Reservoir.

No. Worms/Sample	**Percent Lineage I**	**Percent Lineage III**	**Percent Lineage V**	**Percent Lineage VI**
10	13.1	14.6	46.8	25.6
10	23.6	76.4	0	0
10	0	72.1	0	27.9
10	4.0	74.3	0	20.8
10	6.4	43.7	0	49.8
Mean (Range)	**9.6 (0 – 23.6)**	**56.2 (14.6 – 76.4)**	**9.4 (0-46.8)**	**24.8 (0 – 49.8)**
50	10.7	50.4	3.4	35.4
50	8.4	28.2	35.7	27.7
50	0.3	31.1	38.6	30.0
50	2.4	84.7	0	12.9
50	1.0	28.4	31.7	38.9
Mean (Range)	**4.6 (0.3 – 10.7)**	**44.6 (28.2 – 84.7)**	**21.9 (0 – 38.6)**	**29.0 (12.9 – 38.9)**

Table 10d. Summary of test results using the 4-probe multiplex qPCR test for determination of the percentage of DNA for lineage I, III, V and VI *Tubifex tubifex* in composite samples of 50 aquatic oligochaetes taken from single core samples collected from various sites in Windy Gap Reservoir on April 26, 2004 to assess variations in accuracy and precision in relation to sample size.

Site/ No. Worms/Sample	Percent Lineage I	Percent Lineage III	Percent Lineage V	Percent Lineage VI
1 50	0	0	0	100
1 50	3	1	0	96
1 50	0	7	0	93
1 50	10	6	0	84
1 50	0	5	0	95
1 50	19	0	0	81
Mean (Range)	**5.3 (0 – 19)**	**3.2 (0 – 7)**	**0 (0)**	**91.5 (81 – 100)**
3 50	40	0	42	18
3 50	34	0	39	27
3 50	23	4	54	19
3 50	35	5	42	18
3 50	22	3	45	30
3 50	24	4	39	33
3 50	32	2	45	21
3 50	22	0	56	22
3 50	15	4	61	20
3 50	17	4	37	41
Mean (Range)	**26.4 (15 – 40)**	**2.7 (0 – 5)**	**46.0 (37 – 61)**	**24.9 (18 – 41)**
4 50	66	6	0	28
4 50	61	5	0	34
4 50	29	6	0	64
4 50	48	9	19	25
4 50	64	4	10	22
4 50	35	15	8	41
4 50	53	8	0	38
4 50	55	2	8	35
4 50	48	16	0	37
4 50	28	13	29	30
Mean (Range)	**48.8 (28 – 66)**	**8.4 (2 – 16)**	**7.4 (0 – 29)**	**35.4 (22 – 64)**

LITERATURE CITED

Baldwin, T. J., J. E. Peterson, G. C. McGhee, K. D. Staigmiller, E. S. Motteram, C. C. Downs, and D. R. Stanek. 1998. Distribution of *Myxobolus cerebralis* in salmonid fishes in Montana. Journal of Aquatic Animal Health 10:361-371.

Bartholomew, J. L., and P. W. Reno. 2002. The history and dissemination of whirling disease. In Bartholomew, J. L., and J. C. Wilson (editors). 2002. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland USA.

Beauchamp, K. A., M. El-Matbouli, M. Gay, M. P. Georgiadis, R. B. Nehring, and R. P. Hedrick. 2006. The effect of cohabitation of *Tubifex tubifex* (Oligochaeta:Tubificidae) populations on infections to *Myxobolus cerebralis* (Myxozoa:Myxobolidae). Journal of Invertebrate Pathology 91:1-8.

Beauchamp, K. A., G. O. Kelley, R. B. Nehring, and R. P. Hedrick. 2005. The severity of whirling disease among wild trout populations corresponds to differences in genetic composition of *Tubifex tubifex* populations in central Colorado. Journal of Parasitology 91(1):53-60.

Beauchamp, K. A., M. Gay, G. O. Kelley, M. El-Matbouli, R. D. Kathman, R. B. Nehring, and R. P. Hedrick. 2002. Prevalence and susceptibility of infection of *Myxobolus cerebralis*, and genetic differences among populations of *Tubifex tubifex*. Diseases of Aquatic Organisms 51:113-131.

Beauchamp, K. A., R. D. Kathman, T. S. McDowell, and R. P. Hedrick. 2001. Molecular phylogeny of tubificid oligochaetes with special emphasis on *Tubifex tubifex*. Molecular Phylogenetics and Evolution 19 (2):216-224.

Blazer, V.S., C.L. Densmore, W. B. Schill, D.D. Cartwright, and S. J. Page. 2004. Comparative susceptibility of Atlantic salmon, lake trout and rainbow trout to *Myxobolus cerebralis* in controlled laboratory exposures. Diseases of Aquatic Organisms 58:27-34

DuBey, R., and C. A. Caldwell. 2004. Distribution of *Tubifex tubifex* lineages and *Myxobolus cerebralis* infection in the tailwater of the San Juan River, New Mexico. Journal of Aquatic Animal Health 16:179-185.

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

Hedrick, R. P., M. El-Matbouli, M. A. Adkison, and E. MacConnell. 1998. Whirling disease: re-emergence among wild trout. Immunological Reviews 166:365-376.

Hedrick, R. P., T. S. McDowell, K. Mukkatira, M. P. Georgiadis, and E. MacConnell. 2001. Susceptibility of three species of anadromous salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. Journal of Aquatic Animal Health 13:43-50.

Hedrick, R. P., T. S. McDowell, M. Gay, G. D. Marty, M. P. Georgiadis, and E. MacConnell. 1999a. Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to *Myxobolus cerebralis*, the cause of salmonid whirling disease. Diseases of Aquatic Organisms 37:173-183.

Hedrick, R. P., T. S. McDowell, K. Mukkatira, and M. P. Georgiadis. 1999b. Susceptibility of selected inland salmonids of experimentally induced infections with *Myxobolus cerebralis,* the causative agent of whirling disease. Journal of Aquatic Animal Health 11:330-339.

Hofer, B. 1903. Ueber die Drehkrankheit der Regenbogenforelle. Allgemeine Fischerei Zeitschrift 28:7-8.

Hoffmann, G. L. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. Journal of Aquatic Animal Health 2:30-37.

Kaesar, A. J., and W. E. Sharpe. 2006. Patterns of distribution and abundance of *Tubifex tubifex* and other aquatic oligochaetes in *Myxobolus cerebralis* enzootic areas in Pennsylvania, Journal of Aquatic Animal Health 18:64-78.

Kathman, R. D., and R. O. Brinkhurst. 1998. Guide to the freshwater oligochaetes of North America. Aquatic Resources Center. Thompson Station, Tennessee.

Kerans, B.L., C. Rasmussen, R. Stevens, A.E.L. Colwell, and J. R. Winton. 2004. Differential propagation of the metazoan parasite *Myxobolus cerebralis* by *Limnodrilus hoffmeisteri, Ilyodrilus templetoni* and genetically distinct strains of *Tubifex tubifex*. Journal of Parasitology 90(6):1366-1373.

Koel, T. M., P. E. Bigelow, P. D. Doepke, B. D. Ertal, and D. L. Mahoney. 2005. Nonnative lake trout in Yellowstone cutthroat trout decline and impacts to bears and anglers. Fisheries 30(11): 10-17.

Koel, T.M., D. L. Mahoney, K.L. Kinnan, C. Rasmussen, C.J. Hudson, S. Murcia, and B. L. Kerans. *In press* **.** *Myxobolus cerebralis in native* cutthroat trout of the Yellowstone Lake ecosystem. Journal of Aquatic Animal Health.

Markiw, M. E. and K. Wolf. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiologic agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. Journal of Protozoology 30(3):561-564.

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

Nehring, R. B. 2006. Colorado's cold water fisheries: whirling disease case histories and insights for risk management. Colorado Division of Wildlife Special Technical Report Number 79. DOW—R-S-79-06. 46 pages.

Nehring, R. B. 2005 . Whirling disease investigations. *Myxobolus cerebralis* in Colorado's cutthroat trout populations. Colorado Division of Wildlife Job 1 Progress Report, Federal Aid Project F-237-R12. Fort Collins.

Nehring, R. B. 2004. Whirling disease investigations. *Myxobolus cerebralis* in Colorado's cutthroat trout populations. Colorado Division of Wildlife Job 1 Progress Report, Federal Aid Project F-237-R11. Fort Collins.

Nehring, R. B., and K. G. Thompson. 2003. Whirling disease investigations. Colorado Division of Wildlife Final Report, Federal Aid Project F-237-R-10. Fort Collins.

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

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

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

O'Grodnick, J. J. 1979. Susceptibility of various salmonids to whirling disease (*Myxosoma cerebralis*). Transactions of the American Fisheries Society 108:187-190.

Plehn, M. 1924. Prakitum der Fischkrankheiten. Stuttgart, Germany.

Plehn, M. 1905. Uber die Drehkrankheit der salmoniden [(*Lentospora cerebralis*) (Höfer) Plehn]. Archiv Protistenkunde 5:145-166.

Schäperclaus, W. 1931. XXI Die Drehkrankheit in der Forrellenzucht und ihre Bekämpfung. Zeitschrift Fischerei 29:521-567.

Schisler, G. J. 2001. Whirling disease investigations. Rapid assessment technique evaluation. Colorado Division of Wildlife Federal Aid Job Number 5 Progress Report. Federal Aid Project F-237-R-8. Fort Collins.

Schisler, G. J. 1999. Whirling disease investigations. Colorado Division of Wildlife Federal Aid Job Number 5 Progress Report. Federal Aid Project F-237-R-6. Fort Collins.

Schisler, G. J., E. P. Bergersen, and P.G. Walker. 1999a. Evaluation of chronic gas supersaturation on growth, morbidity, and mortality of fingerling rainbow trout infected with *Myxobolus cerebralis*. North American Journal of Aquaculture 61:175-183.

Schisler, G. J., P. G. Walker, L. A. Chittum, and E. P. Bergersen. 1999b. Gill ectoparasites of juvenile rainbow trout and brown trout in the upper Colorado River. Journal of Aquatic Animal Health 11:170-174.

Seber, G.A.F., and E.D. LeCren. 1967. Estimating population parameters from catches large relative to the population. Journal of Animal Ecology. 36:631-643.

Thompson, K. G., R. B. Nehring, D. C. Bowdin, and T. Wygant. 1999. Field exposure of seven species or subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. Journal of Aquatic Animal Health 11:312-329.

Uspenskaya, A.V. 1957. The ecology and spreading of the pathogen of trout whirling disease – *Myxosoma cerebralis* (Hofer, 1903, Plehn, 1905) in the fish ponds of the Soviet Union. Pages 47-55 *In* G. K. Petrushevskii, editor. Parasites and diseases of fish. Bulletin All-Union Institute of Freshwater Fisheries. Leningrad.

Uspenskaya, A.V. 1982. New data on the life cycle and biology of Myxosporidia. Archiv fur Protistenkunde 126:309-338.

Vincent, E. R. 2002. Relative susceptibility of various salmonids to whirling disease with emphasis on rainbow and cutthroat trout. *In* Bartholomew, J. L., and J. C. Wilson (editors). 2002. Whirling disease: reviews and current topics. American Fisheries Society, Symposium 29, Bethesda, Maryland USA.

Vincent, E. R. 1996a. Whirling disease - the Montana experience, Madison River. Page 159 *In* E. P. Bergersen and B. A. Knopf, editors. Proceedings: Whirling disease workshop-where do we go from here? Colorado Cooperative Fish and Wildlife Research Unit. Fort Collins, Colorado.

Vincent, E. R. 1996b. Whirling disease and wild trout: the Montana experience. Fisheries. 21(6):32-33.

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

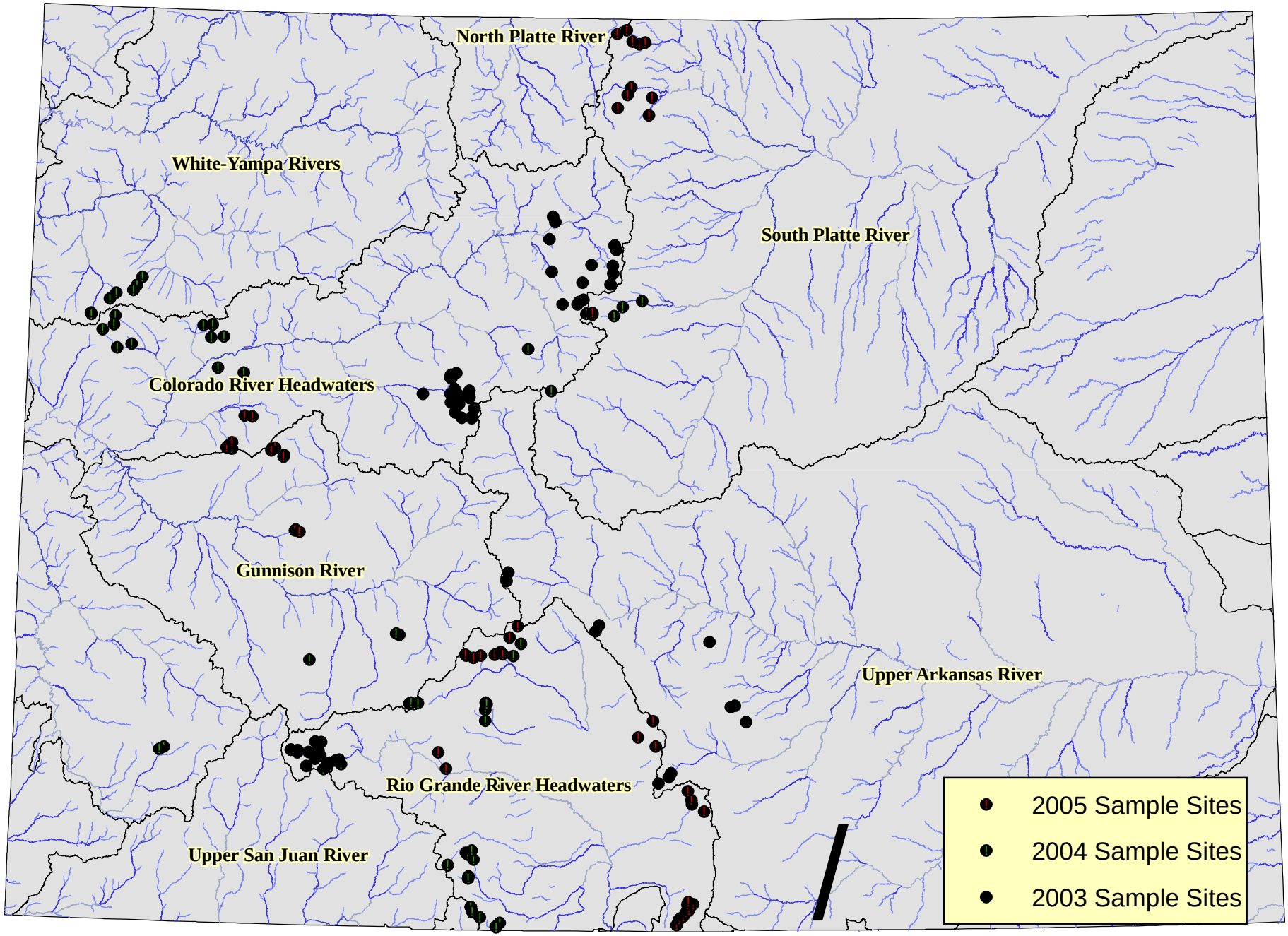
Wolf, K., and M. E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science 225:1449-1452.

Zendt, J. S., and E. P. Bergersen. 2000. Distribution and abundance of the aquatic oligochaete host *Tubifex tubifex* for the salmonid whirling disease parasite *Myxobolus cerebralis* in the upper Colorado River basin. North American Journal of Fisheries Management 20:502-512.

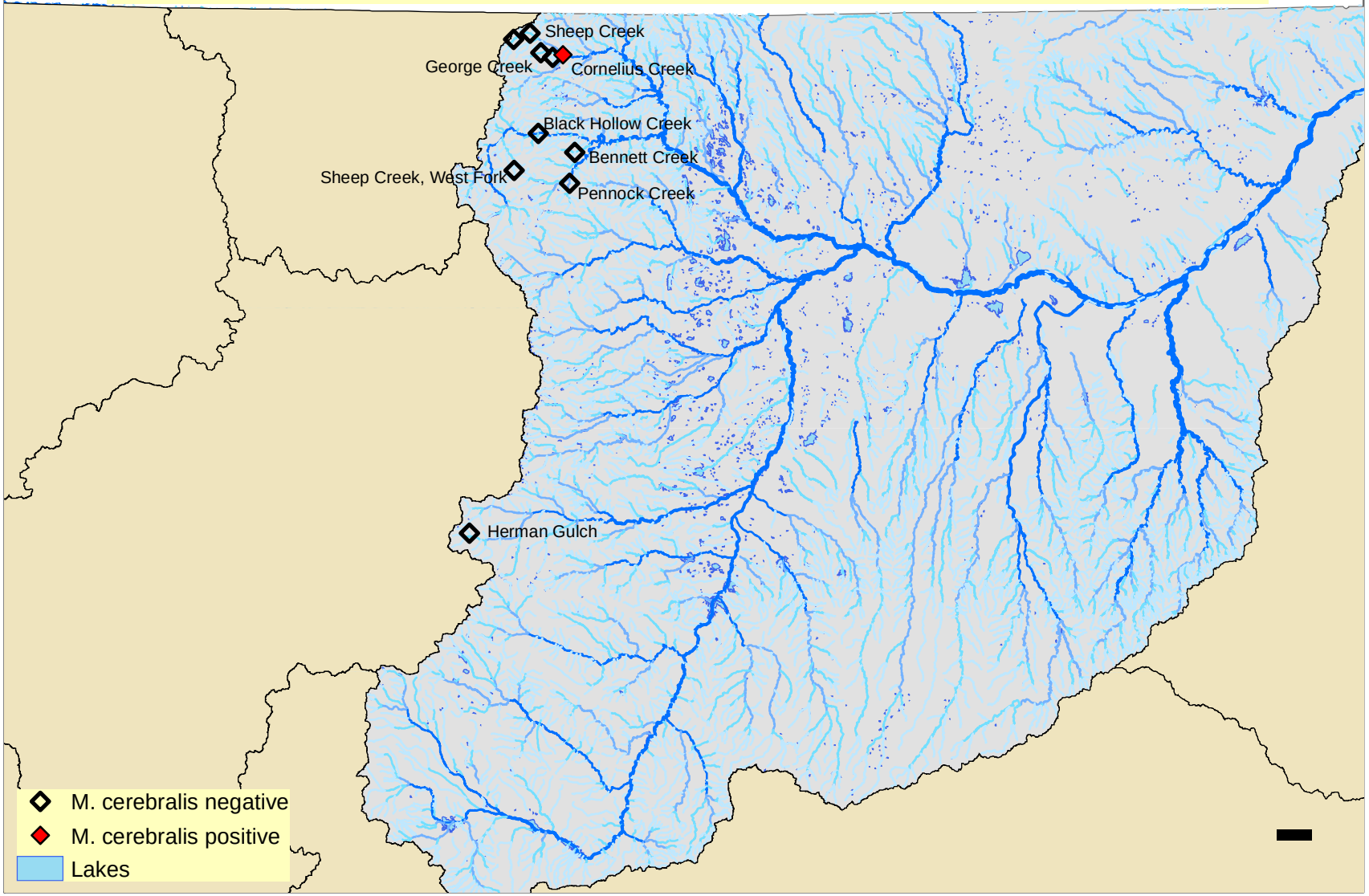
Appendix

Site Maps

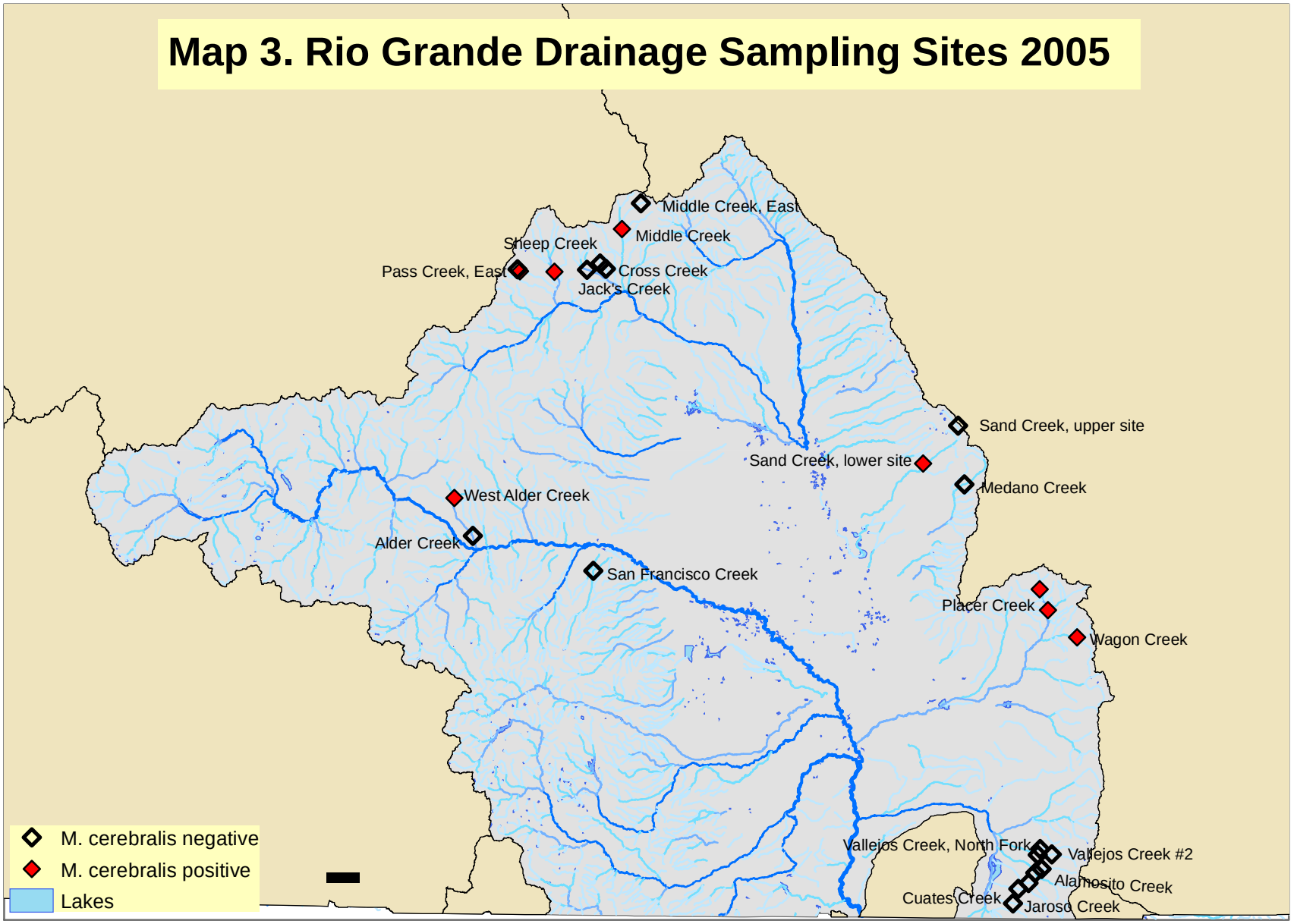
Map 1. Colorado's Cutthroat Trout Populations Sampled for *Myxobolus cerebralis* in 2003, 2004, and 2005



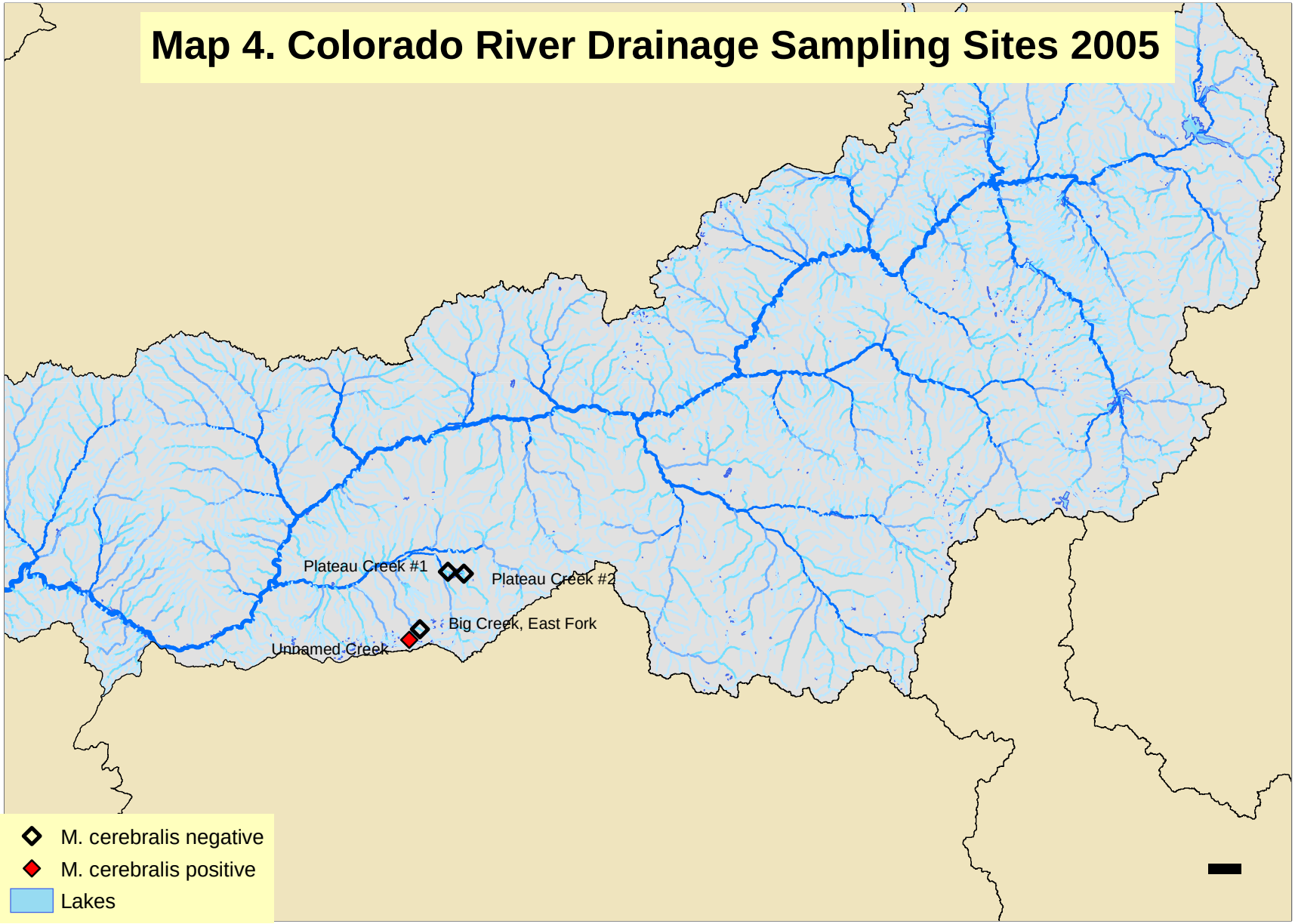
Map 2. South Platte River Drainage Sampling Sites 2005



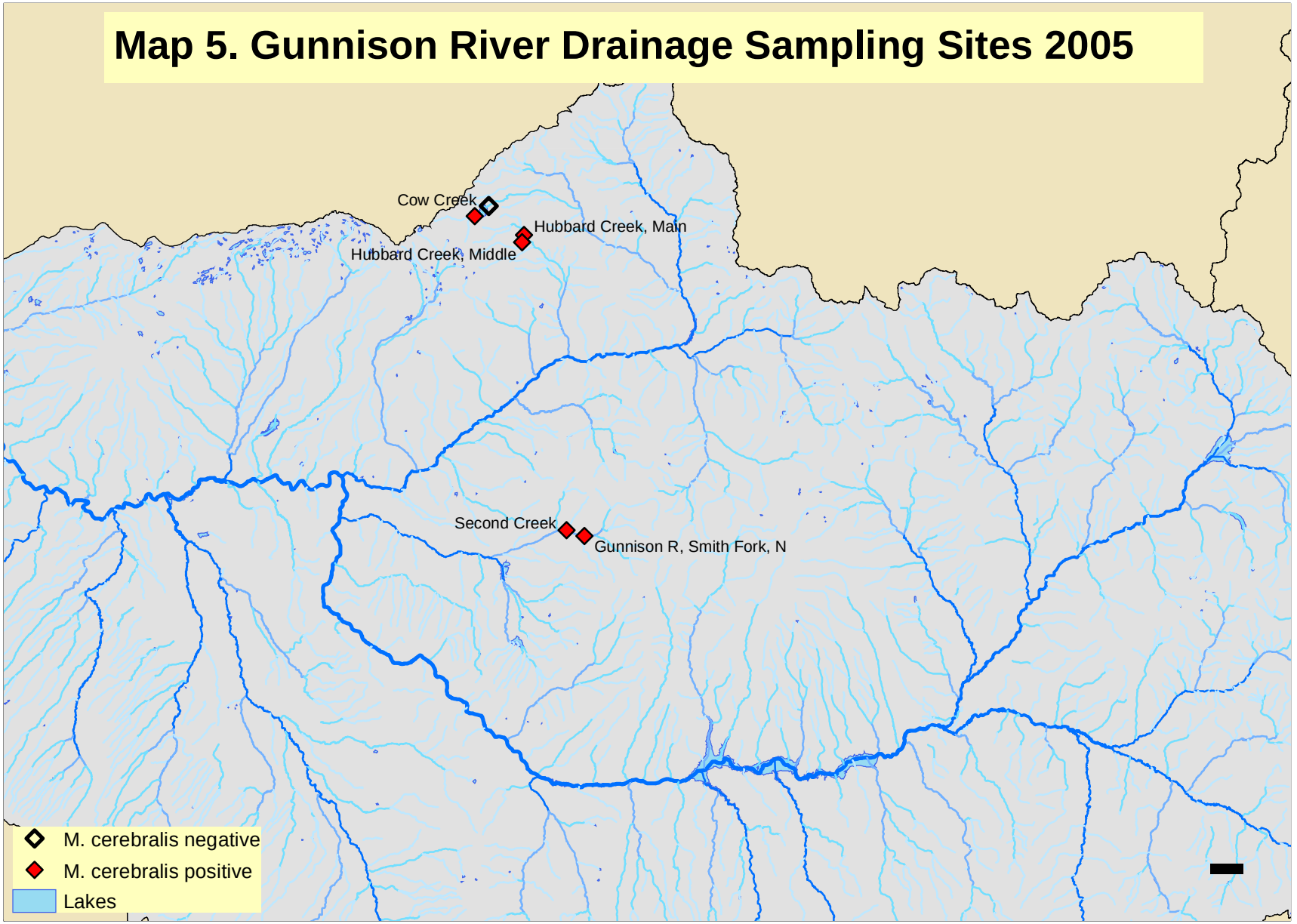
Map 3. Rio Grande Drainage Sampling Sites 2005



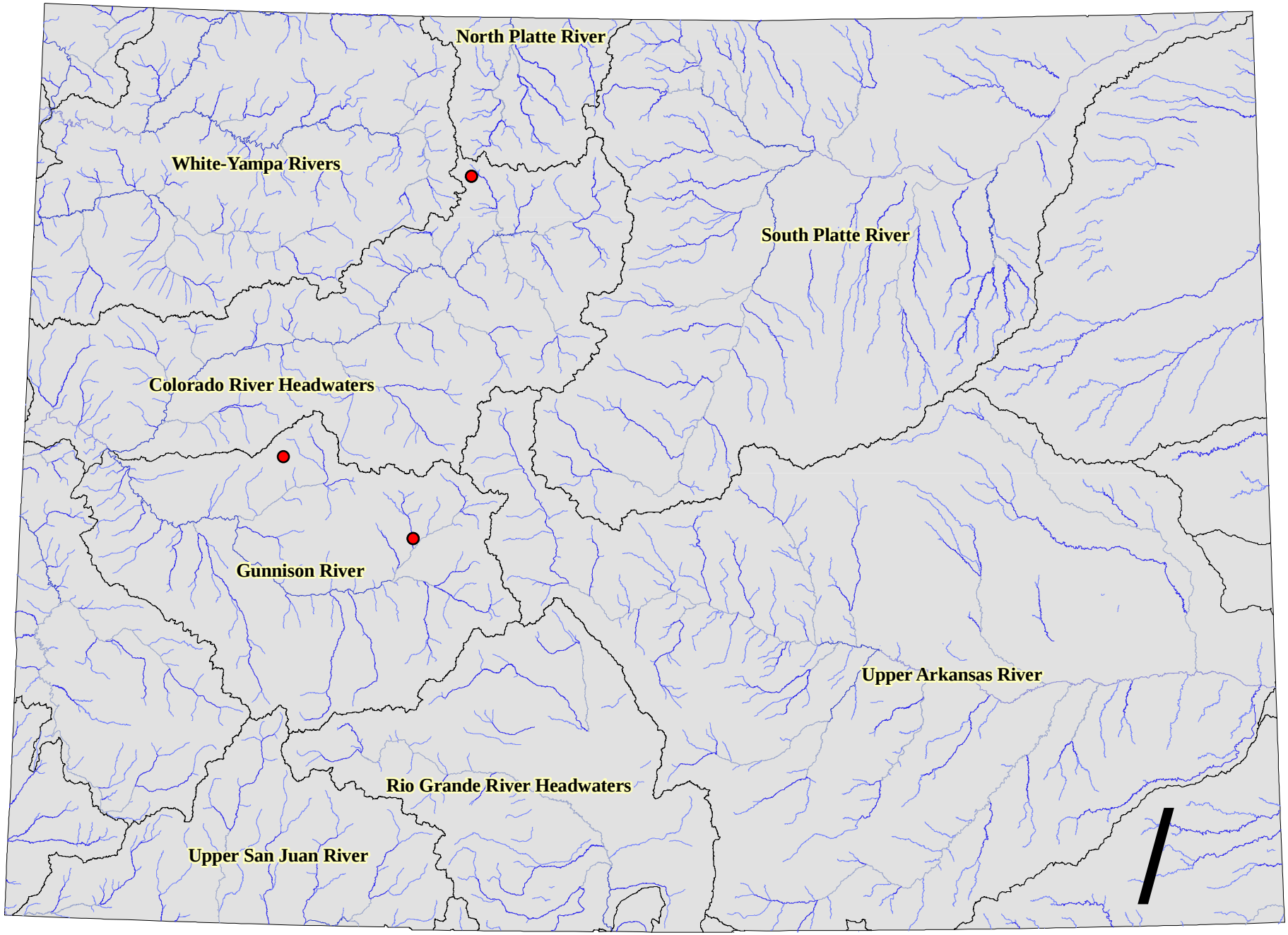
Map 4. Colorado River Drainage Sampling Sites 2005



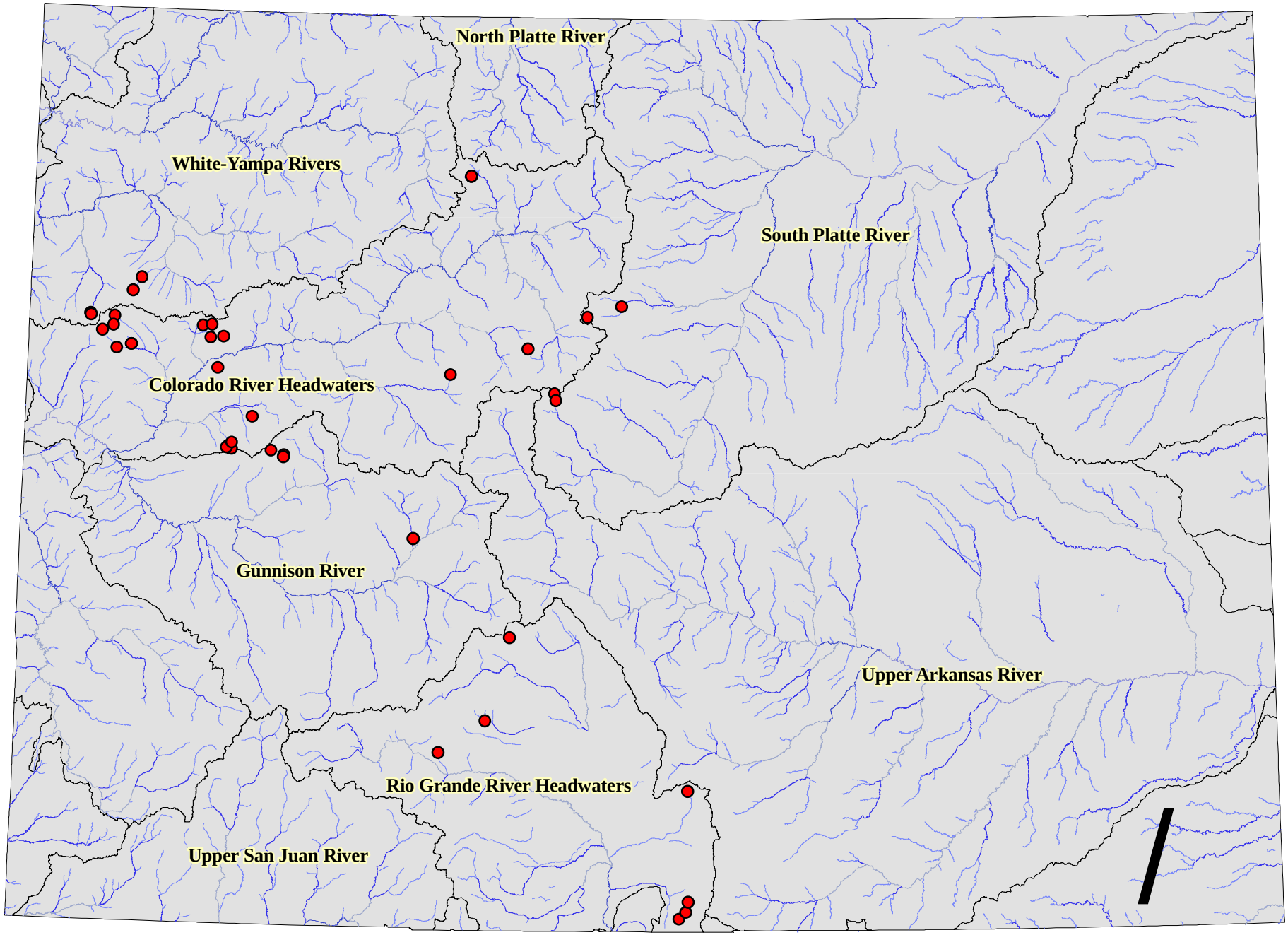
Map 5. Gunnison River Drainage Sampling Sites 2005



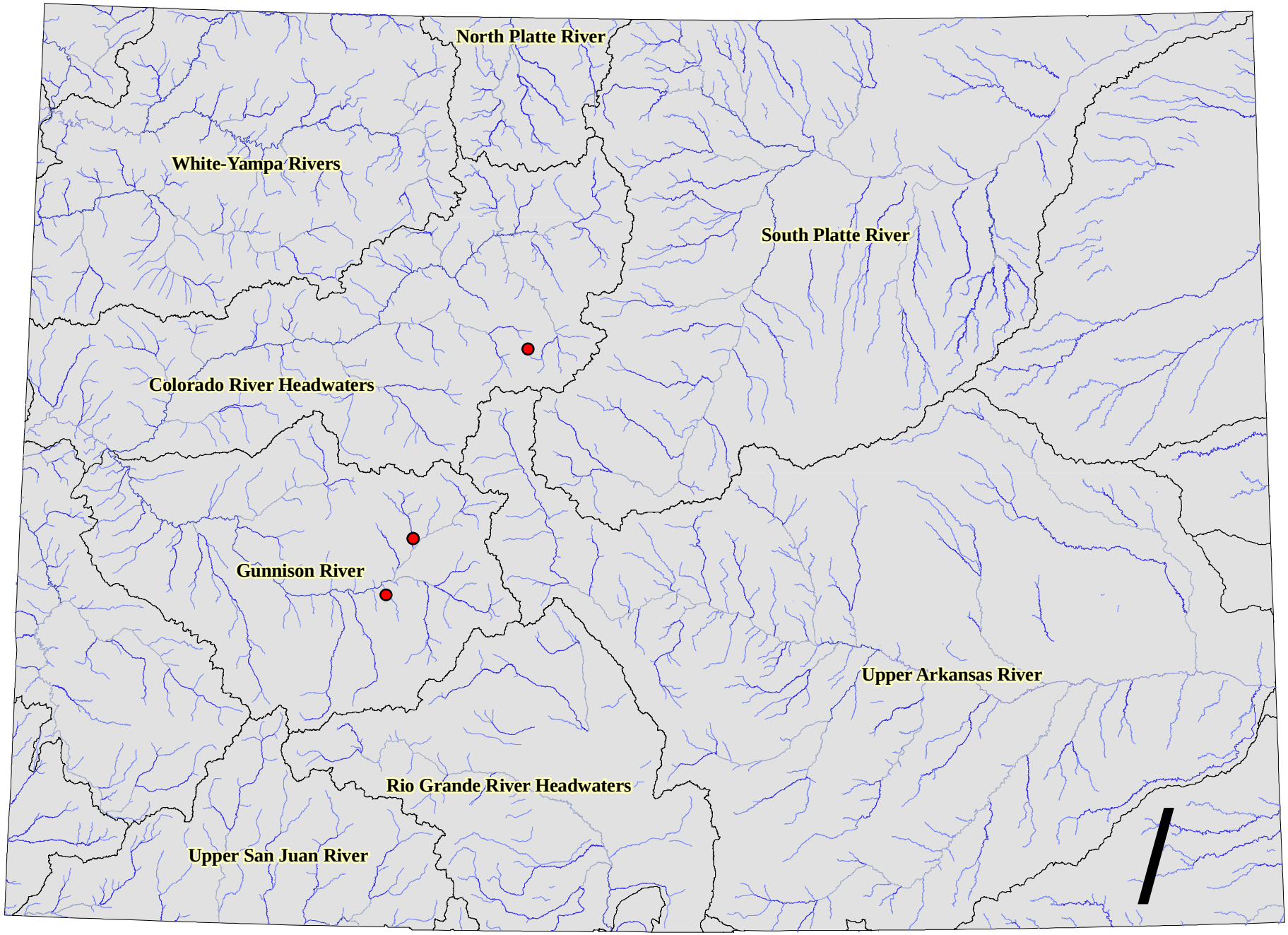
Map 6. Lineage I *Tubifex tubifex* Collection Sites for 2004 and 2005



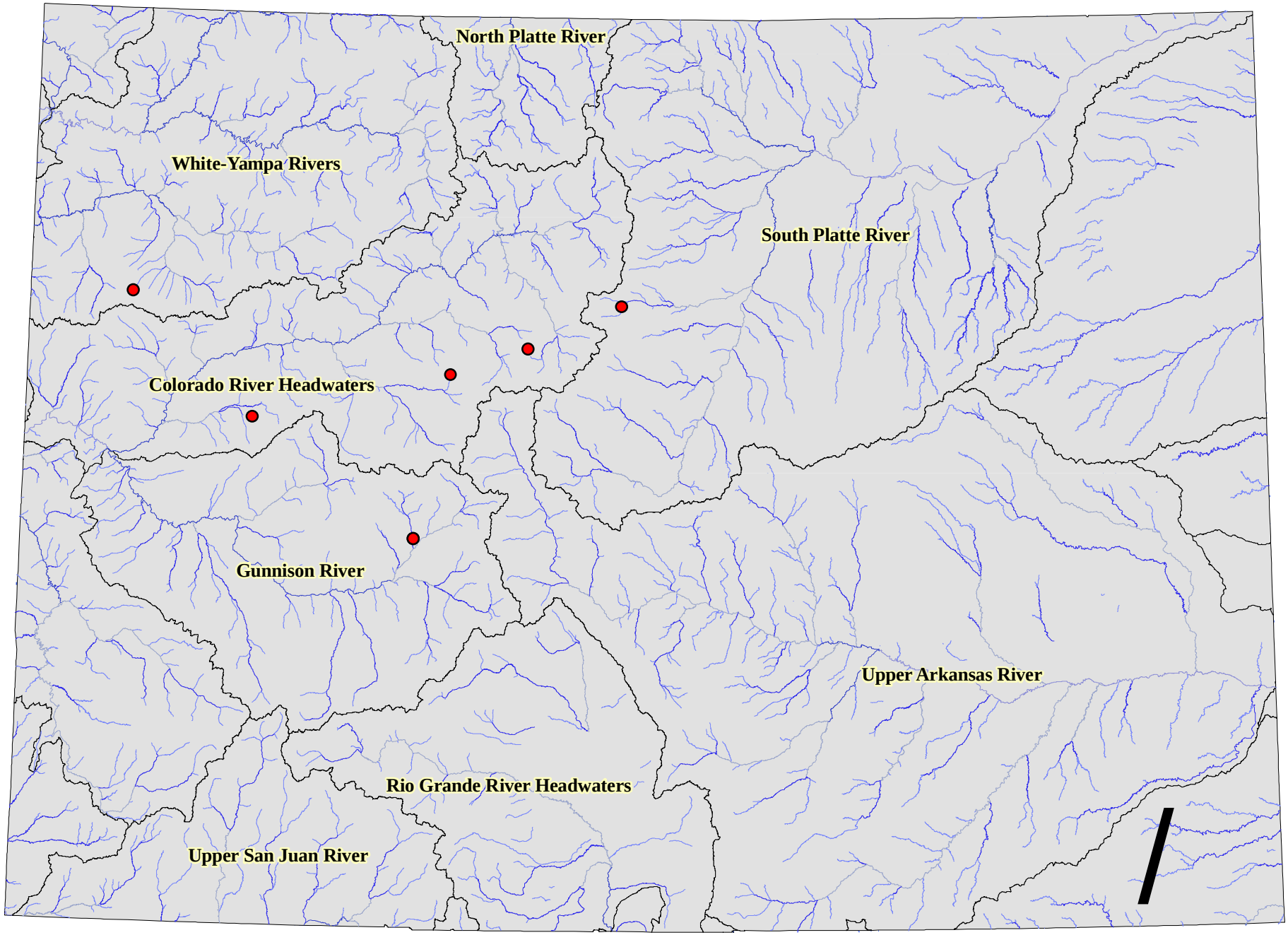
Map 7. Lineage III *Tubifex tubifex* Collection Sites for 2004 and 2005



Map 8. Lineage V *Tubifex tubifex* Collection Sites for 2004 and 2005



Map 9. Lineage VI *Tubifex tubifex* Collection Sites for 2004 and 2005



Map 10. 2004 and 2005 Collection Sites where no *Tubifex tubifex* belonging to lineages I, III, V, or VI were found