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Colorado's Cold Water Fisheries: Whirling Disease Case Histories and Insights for Risk Management

R. Barry Nehring

February 2006

COLORADO DIVISION OF WILDLIFE
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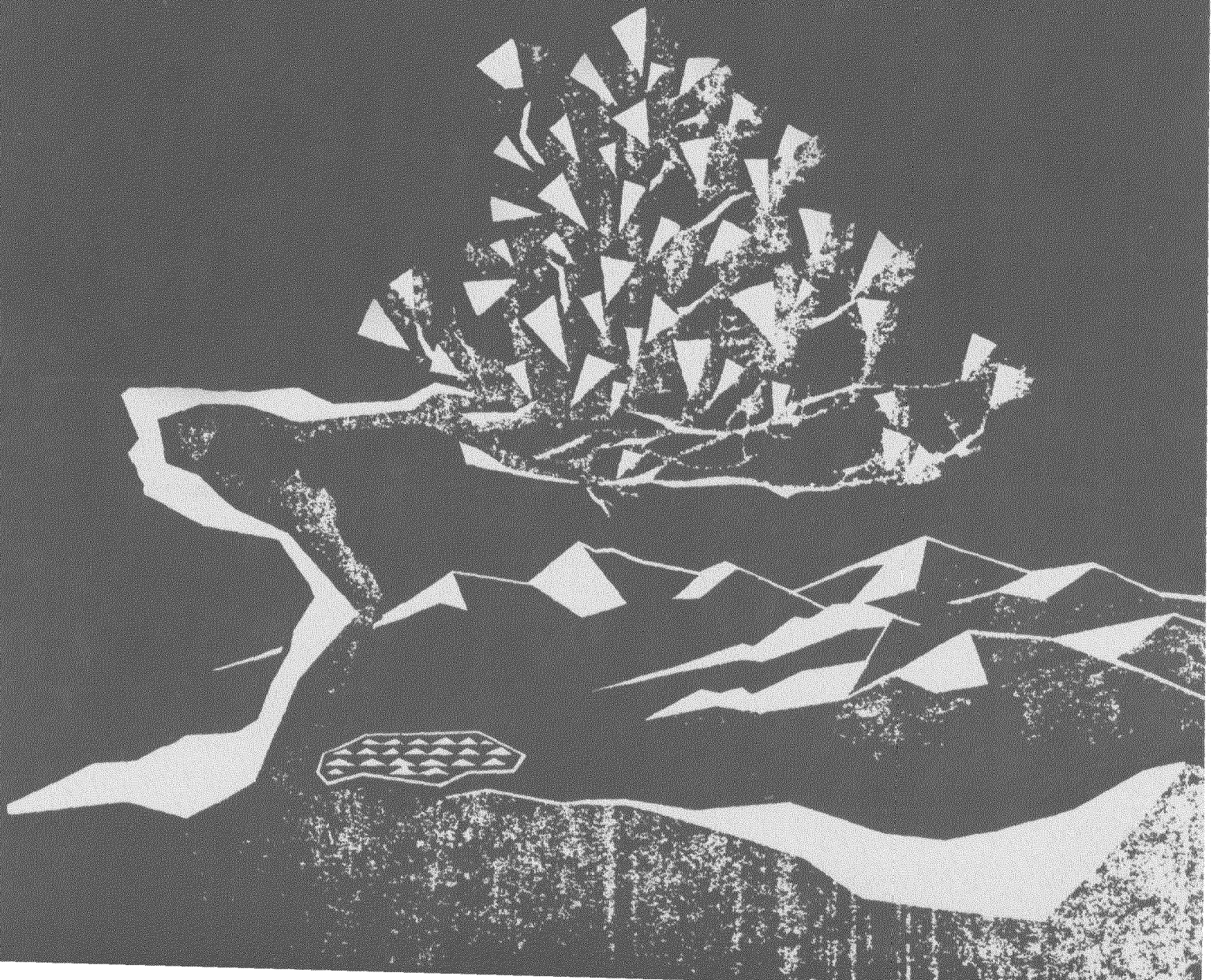

COLORADO'S COLD WATER FISHERIES: WHIRLING DISEASE CASE HISTORIES AND INSIGHTS FOR RISK MANAGEMENT

by

R. Barry Nehring

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The cover photo is of 3-mo-old Colorado River cutthroat trout (*Oncorhynchus clarki pleuriticus*) 75 days post-exposure to ambient levels of *Myxobolus cerebralis* in the Colorado River. *Myxobolus cerebralis* is the myxosporean parasite that can cause whirling disease.

FOREWORD

Myxobolus cerebralis (*Mc*), the parasite that causes whirling disease in salmonids was first discovered in Colorado in 1987. By 1997, 11 of 16 Colorado Division of Wildlife (CDOW) trout production facilities tested positive for whirling disease and the parasite was widely dispersed in salmonid waters statewide, vectored primarily through the stocking of infected fish. As a direct result, there have been significant population declines in many economically and recreationally important trout fisheries in Colorado. Whirling disease is affecting wild and hatchery fish in 23 states in the northeastern and western United States.

The Colorado Division of Wildlife and the Colorado Wildlife Commission have taken this threat to our fishery resource very seriously and have implemented a number of measures to reduce the impacts of this disease. The CDOW Aquatic Research Group is focusing on reducing and/or minimizing the impacts of this parasite on wild fish recruitment and survival as well as survival of stocked fingerlings. Some of the specific areas of research include: 1) developing techniques to detect and quantify *Mc* DNA and actinospores in samples of fish, tubifex worms and water, 2) assessing the impacts of the parasite on wild fish populations under a variety of different environmental conditions, 3) ameliorating the impacts using physical habitat alterations, 4) evaluating specific salmonid species and strains for resistance and 5) long term monitoring studies to assess *Mc* spore burden and prevalence in trout under a multitude of management scenarios. In addition, the CDOW has invested in excess of $12 million on hatchery clean up and improvements to attain *Mc* negative status. The improvements have included securing water supplies, lining dirt ponds, total disinfection, fish removal from settling ponds and implementing a variety of "best management practices" to minimize the risk of the *Mc* parasite entering production facilities. In November 2000, the Colorado Wildlife Commission approved the D-9 Policy which prevents the future stocking of whirling disease positive fish into salmonid habitats. Colorado's team approach between hatcheries, biologists and researchers has greatly improved CDOW's ability to deal with the impacts of the *Mc* parasite. A variety of tools have been developed which can be used to "manage around" whirling disease and ultimately assist biologists in developing the self sustaining populations of wild rainbow trout that once were common in Colorado.

This Special Report builds on information reported in Special Report Number 76 published in 2001 and specifically addresses the risk associated with stocking *Mc* positive fish into salmonid habitats. It also provides a thorough review of the life history of the parasite and a current literature review. It gives a clear history of the spread of whirling disease in Colorado and the various management and policy actions that have been implemented over the last 15 years in relation to this disease. Numerous case studies are cited that encompass many years of data collection to paint a clear picture for the reader of what management action was implemented and the results of that action in terms of the impacts of whirling disease in a broad spectrum of environments. Lastly, the author of this report ranks various management alternatives in terms of risk and gives recommendations for management and future research priority. This document summarizes whirling disease research and management in Colorado and will hopefully serve as a guide for fishery managers, both in Colorado as well as other Rocky Mountain States, into the next decade.

Mark S. Jones
Aquatic Research Leader

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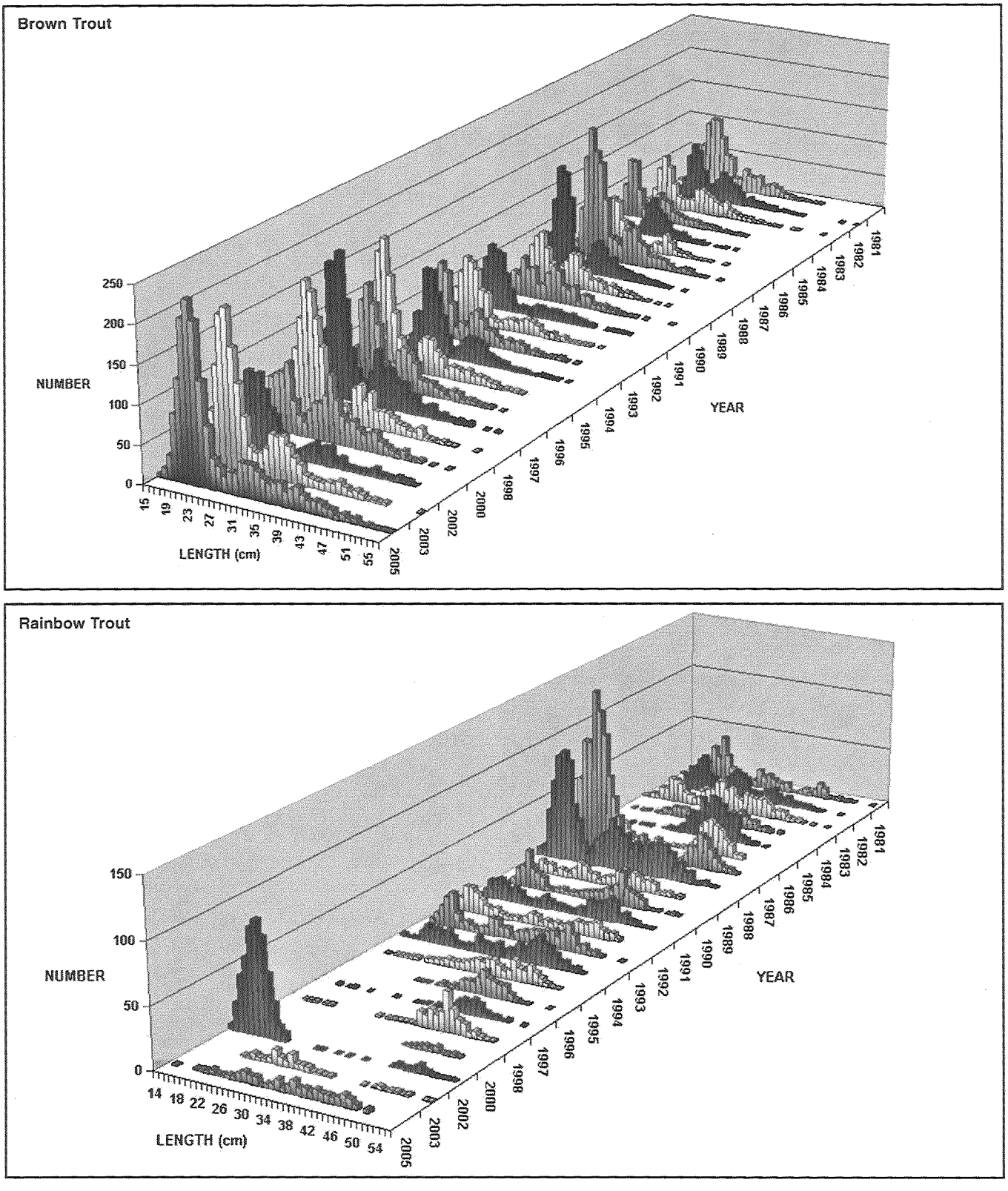

Frontispiece. Relative abundance of wild brown trout (top) and rainbow trout (bottom) ≥ age 1 captured during boat electrofishing surveys in Ute Park, a 3.2 km reach of the Gunnison River (1981-2005). Brown trout have an innate high resistance to *Myxobolus cerebralis* (the myxosporean parasite that can cause whirling disease) and when infected, do not suffer measurable mortality. Rainbow trout are very vulnerable to infection and suffer high mortality when exposed early in the first year of life. *M. cerebralis* was first detected in this reach of river in January 1994. Wild rainbow trout fry suffered near 100% mortality in this river reach every year from 1994 through 2005. Without any recruitment, density and biomass of wild rainbow trout ≥ age 1 declined rapidly. The pulse of 14 to 24 cm rainbow trout for 2002 in the bottom histogram resulted from the stocking of 7 to 12 cm fingerlings to maintain minimal densities of rainbow trout in this sport fishery. This report summarizes the impact of exposure to *M. cerebralis* on wild trout populations in Colorado.

COLORADO'S COLD WATER FISHERIES: WHIRLING DISEASE CASE HISTORIES AND INSIGHTS FOR RISK MANAGEMENT

EXECUTIVE SUMMARY

During the late 1990s the executive staff of the Colorado Division of Wildlife (CDOW) twice directed that a thorough review of the agency's efforts directed at containment and control of Whirling Disease (WD) be undertaken. The first review entitled **An Assessment of Fishery Management and Fish Production Alternatives to Reduce the Impact of Whirling Disease in Colorado** was completed in 1996. The issue was revisited again in 1998 in a second report entitled **A Review of Strategies for Fishery and Hatchery Management in Relation to Impacts of Whirling Disease.** The recommendations and conclusions in those two reports provided specific direction for fisheries research and management efforts and for the hatchery production program over the next 6 to 8 years.

In November 2000, the Colorado Wildlife Commission approved the **D-9** policy **"The Stocking and Use of Fish Tested Positive for, or Exposed to the Whirling Disease Parasite *Myxobolus cerebralis*"**. This policy set in motion a process that directed the CDOW by January 1, 2003 to "*...strive toward the objective of eliminating the stocking of WD positive fish in habitat that is capable of supporting self-sustaining salmonid populations including standing water above salmonid habitat.*" The **D-9** policy further directed the CDOW "*...to promulgate rules and regulations to prohibit private parties from stocking WD positive fish in such habitat by 2003.*" This policy has been implemented and the stocking of WD positive fish in salmonid habitats has been virtually curtailed since 2003.

This report contains a wide ranging review of most aspects of the Whirling Disease (WD) problem in Colorado, including an assessment of the degree of spread and the level of impact on the wild trout resources of the state. In 1994, the CDOW initiated a wide-ranging research effort to develop a thorough understanding of the impacts of WD on the wild trout resources of Colorado and develop management strategies to ameliorate, control, and hopefully contain the spread of *Myxobolus cerebralis*. After more than 10 years of intensive effort in the hatchery system, in fisheries management and research, strategies have been implemented to ameliorate the impacts of the parasite and minimize risk of further significant spread of WD.

In 1997, 11 of Colorado's 16 trout production facilities tested positive for *M. cerebralis*. In 1998, the CDOW entered into a multi-year capital construction investment program to make the state's coldwater hatchery system more secure from *M. cerebralis*. While the task is not yet quite complete in 2006, the 12 million dollar investment has been highly successful. Only three primary production units are still classified as positive for *M. cerebralis*. At least one more unit might be re-certified WD negative by the end of 2006. Most of the coldwater salmonid production by state rearing units is now certified WD negative. Since 2001, implementation of "best management practices" (BMPs) at many units has resulted in dramatic reductions in the level of waterborne triactinomyxon (TAM) actinospores detected in hatchery effluents being discharged back into the waters of the state. This has resulted in substantial decreases in the prevalence and severity of infection in wild brown trout in streams like the Cache la Poudre River. Test results for trout reared at units still classified as WD positive reveal that prevalence and severity of infection is much lower than it was 5 to 10 years ago. Tests of trout produced at the Watson Lake rearing unit near Laporte, Colorado rarely show evidence of infection even after the fish have been held on the unit for 18 to 24 months. The renovation and improvements at Colorado's trout production facilities (with regard to *M. cerebralis* control) has been an overwhelming success.

Beginning in 2000, CDOW fisheries managers dramatically decreased the stocking of trout produced on rearing units testing positive for *M. cerebralis* in lakes and reservoirs in salmonid habitats. Research efforts at more than a dozen study sites revealed that ambient levels of TAM spore densities began to decline exponentially within 6 months after the cessation of stocking of infected trout. In most cases, ambient densities of TAM spores in water samples decreased to undetectable levels within 24 to 30 months.

Research over an 8-year period at a number of locations provided strong evidence that there is a direct link between the stocking of trout infected with *M. cerebralis* and subsequent levels of TAM spore production. At Georgetown Lake, stocking of 167,000 highly vulnerable rainbow trout fry during the summer of 2002 was likely the cause of an explosion of TAM spore production beginning in May 2003 that has continued through December 2005. Stocking large numbers of rainbow trout into standing waters where *M. cerebralis* is established can have severe environmental consequences even when the salmonids stocked were WD negative.

Examination of CDOW stocking records associated with the lake and reservoir case studies in this report suggests that there may be a need to substantially reduce stocking rates in some habitats to help reduce levels of abundance of *M. cerebralis*. High stocking rates result in slower growth of trout, particularly among fry and fingerling rainbow trout. That can lead to more severe *M. cerebralis* infections, lower harvest rates and an increase in ambient levels of infection over time. Research results summarized in this report suggest that stocking rates for catchable trout should be adjusted so that angler harvest would remove up to 90% of the trout stocked within 6 months after stocking in small lakes and impoundments. Cranial myxospore burdens in catchable rainbow trout that remained in the waters for 1.5 to 2 years after stocking increased by more than 10 fold over the levels observed in the same group of fish at 180 – 200 days after stocking. Reduction in stocking rates would lead to more efficient use of the hatchery product, result in substantial cost savings, could eliminate the need to purchase fish from the private sector, and reduce the risk of exacerbating ambient levels of infectivity in lake and stream habitats where *M. cerebralis* is established.

Case studies of more than a dozen streams and lake or reservoir/stream ecosystems highlight the myriad and complex pathways that *M. cerebralis* can be vectored through aquatic ecosystems. Once established in an aquatic ecosystem man has few options for effective containment and control. Myxospores can be transported across aquatic ecosystems by avian, mammalian and piscine predators, fish immigration and emigration, and by "hitchhiking" on vehicles, boats, and angler's wading equipment. However, the case studies in this report indicate that the stocking of *M. cerebralis*-infected trout was the primary mechanism by which the parasite was widely disseminated in Colorado. Most importantly, the stocking of trout exposed to *M. cerebralis* in salmonid habitats was largely eliminated with the approval and implementation of the Wildlife Commission **D-9** policy beginning in January 2003.

Over a five-year period between 1995 and 1999, fry and fingerling brown, brook, rainbow and four sub-species of cutthroat trout were exposed to ambient levels of *M. cerebralis* (TAMs) in the Colorado River. The four sub-species of cutthroat trout tested were greenback, Rio Grande, Colorado River and Snake River. Greenback, Rio Grande and Colorado River cutthroat trout were at least as vulnerable or more vulnerable to the detrimental effects of *M. cerebralis* and WD and generally suffered significantly higher mortality rates than rainbow trout that were the same size or smaller at the time of initial exposure. These results demonstrated that all three sub-species of Colorado's native cutthroat trout are highly vulnerable to severe impacts from exposure to the parasite. Likewise, brook trout fry are highly susceptible and can suffer significant mortality when exposed during the first 2-3 weeks after hatching. This can lead to population level impacts among wild brook trout populations. However, emergence of brook trout fry in Colorado often occurs during March or April, at a time when ambient levels of TAM spores are very low. The end result is often a high level of infection among feral brook trout with reduced levels of acute mortality compared to that observed among wild rainbow trout.

In more than 10 years of study there is no evidence that demonstrates that *M. cerebralis* infection results in detrimental impact to wild brown trout at the population level. However, given their innate resistance to *M. cerebralis*, high-density brown trout populations tend to become natural "reservoirs" of infectivity. This increases the probability that high levels of parasite virulence will be sustained over long periods of time in lake and stream ecosystems where the necessary suite of environmental co-factors are present to complete the life cycle. For these reasons it is debatable whether or not that the lack of population level impacts resulting from exposure to *M. cerebralis* should be the sole criterion used to justify the continued stocking of *M. cerebralis*-exposed salmonids into standing waters where only wild brown trout occur downstream.

The CDOW has been at the forefront of efforts directed at containment and control of WD for more than a decade. The author recommends that the agency remain pro-active in the development of management strategies that reduce the risk of spread of the parasite in Colorado. This will require perseverance and commitment in a number of areas. **First**, research efforts to develop strains of rainbow trout with a demonstrated high-degree of resistance to *M. cerebralis* are underway and must be continued. Given an adequate level of funding and effort it is highly probable that a rainbow trout broodstock with a high degree of resistance to *M. cerebralis* could be in production in the state's hatchery system by 2010 or before. **Second**, the CDOW should explore the feasibility of using lineages of *Tubifex tubifex* that are resistant to infection by *M. cerebralis* as biological control agents to reduce ambient levels of infection in areas of high parasite virulence. Recent research findings suggest that it might be possible to utilize strains of *T. tubifex* resistant to *M. cerebralis* infection to shift the aquatic worm population structure away from highly vulnerable species or strains in habitats where the parasite is well established. **Third**, the agency should continue research efforts to evaluate the spread of *M. cerebralis* into cutthroat trout populations and recovery streams on a statewide basis. This effort began in 2003. With a sustained effort, this undertaking should be largely complete by the end of 2007. **Fourth**, the agency may need to determine the degree of risk that *M. cerebralis* poses for mountain whitefish *Prosopium williamsoni*. Anecdotal evidence from field studies in Colorado and results of laboratory exposures in Montana have demonstrated that mountain whitefish are vulnerable to infection by *M. cerebralis*. **Fifth**, a working group of six to eight people should be assembled to review the conclusions and recommendations in this report and develop a set of criteria or administrative procedures to guide fishery management and fish stocking to minimize the risks for accidental or unwarranted exposure of the cold water fishery resource to *Myxobolus cerebralis* in the 21st century.

INTRODUCTION

Cause of Whirling Disease

Whirling disease (WD) is a debilitating malady of some salmonid fishes that can result from exposure of susceptible fry or fingerlings to the waterborne triactinomyxon (TAM) form of the myxosporean parasite *Myxobolus cerebralis* (Wolf and Markiw 1984; Markiw 1991). Two hosts are required for completion of the life cycle of the parasite, a susceptible salmonid fish and the aquatic oligochaete *Tubifex tubifex*. Each host produces spores harboring sporoplasms that are infective to the alternate host. Thousands of TAMs produced in the gut lumen of *T. tubifex* are shed into the water column of lakes and streams where they are infectious to vulnerable salmonids. El-Matbouli et al. (1992) demonstrated that young trout can be infected either by coming in contact with TAMs suspended in the water or ingesting *T. tubifex* infected with the parasite. Myxospores from infected salmonids are released into the water upon death and decay of the fish carcass, and can also be shed from live salmonids infected with the parasite (Nehring et al. 2002). Susceptible aquatic oligochaetes dwelling in sediment-laden areas of lakes and streams can become infected by ingesting myxospores while feeding head down in the substrate.

Although the life cycle of the parasite was first described by Wolf and Markiw (1984), the intricate phases and details of the life cycle in each host were not completely understood and described until the 1990s (El-Matbouli et al. 1995; El-Matbouli and Hoffmann 1998; El-Matbouli et al. 1999a; El-Matbouli et al. 1999b). In young rainbow trout *Oncorhynchus mykiss* infected by *M. cerebralis*, lysis of cartilage by phagocytic vegetative stages of the parasite can compromise normal bone development and can result in skeletal deformities. An inflammatory response usually develops in peripheral tissues adjacent to sites of infection. The tissue swelling can impinge upon nerves and compromise transmission of electrical signals innervating skeletal muscle. Interruption of these signals results in erratic muscle contraction, affects the swimming ability of young salmonids, and can cause the fish to swim in tightly concentric circles. This erratic swimming behavior is known as "whirling" and hence the name whirling disease. Recent studies provide graphic histological evidence supporting the hypothesis that the inflammatory response to parasite activity in the cartilage-rich cranial region results in compression and possible damage to portions of the brain and anterior portions of the spinal cord (Rose et al. 2000). Further detailed information on the life cycle of the parasite and reviews of the state of knowledge regarding WD can be found in El-Matbouli et al. 1992, Walker and Nehring 1995, or Bartholomew and Wilson 2002.

Effects of Whirling Disease

Laboratory studies conducted over the past 30 years have demonstrated that rainbow trout, brook trout *Salvelinus fontinalis*, sockeye salmon *Oncorhynchus nerka* and Chinook salmon (*O. tshawytscha)* develop severe clinical signs of WD and can suffer significant mortality when exposed to TAMs of *M. cerebralis* during the first few weeks or month of life (O'Grodnick 1979; Markiw 1991; Hedrick et al. 1999b; Hedrick et al. 2001; Vincent 2002). Clinical signs of disease can include blacktail, skeletal deformities of the cranium, gill covers and vertebral column, exophthalmia, and erratic swimming behaviour or "whirling". Long-term exposure of young greenback (*Oncorhynchus clarki stomias)*, Rio Grande (*O. c. virginalis)* and Colorado River cutthroat (*O. c. pleuriticus)* trout and brook trout to ambient levels of TAM spores in the upper Colorado River resulted in mortalities ranging up to 90% (Thompson et al.1999). Mortality rates seen by Thompson et al. (1999) were the results of continuous exposure for the 12-18 month duration of the experiments, typical of what could be expected in the wild. These mortality rates are higher than generally reported for controlled laboratory studies, in which fish are usually subjected to a single dose exposure and then held in specific-pathogen-free (SPF) water.

History of the Parasite – Initial Identification and Spread

Myxobolus cerebralis was first detected among rainbow trout and brook trout in Germany in 1893 (Hofer 1903). Both species had been imported into Europe as eyed eggs in the late 1870s to diversify the salmonid species available for rearing in a burgeoning aquaculture industry (Hofer 1903). Plehn (1905) published the first detailed descriptions of clinical WD and the parasite that causes it, and first suggested that the resilience and longevity of the myxospore would pose difficulties for containment and control. For more than 80 years, the true life cycle of the parasite remained unknown until described by Wolf and Markiw (1984). Between initial detection in 1893 and the description and acceptance of the life cycle by fish health professionals in the late 1980s, effective methodologies for minimizing the parasite's impact on salmonids reared in aquaculture were developed. However, lack of a true understanding of the life cycle of the parasite resulted in dispersal of *M. cerebralis* across much of the world where salmonid fishes occur.

Bartholomew and Reno (2002) present an excellent summary of the chronology of dispersal of *M. cerebralis* across Europe, Asia, New Zealand and North America during the 20th century. By the 1950s, it had spread across much of Europe and western Russia. However, with the diligent

application of control measures and implementation of better management practices, WD appeared to be under control in Europe by the 1970s (Schäperclaus 1986; Bartholomew and Reno 2002).

In North America, initial detection occurred in Pennsylvania in 1956 (Hoffman 1990). The parasite was subsequently detected in California and Nevada in the 1960s. Similar to the chronology of detection, outbreak of disease, and implementation of management strategies in Europe, by the late 1980s many fish health professionals in North America considered the parasite an aquatic nuisance species that could be managed with prophylactic measures and sound animal husbandry practices in salmonid aquaculture (Hoffman 1990). Circumstantial evidence strongly suggested that initial exposure and onset of WD in trout reared at aquaculture facilities in Pennsylvania and California resulted from the feeding of frozen rainbow trout product from Denmark (Hofmann 1990; Modin 1998). From these focal points of exposure, *M. cerebralis* spread up and down the coastlines and inland over the next two decades. However, there was no substantive evidence suggesting that the parasite was having any detectable impacts on feral salmonid populations in the eastern states or on the West Coast of North America.

The Colorado Chronology - History and Spread

Myxobolus cerebralis was first detected in Colorado at one state and three private salmonid rearing units in late 1987. Fish from all four locations displayed clinical signs of WD (Barney et al. 1988). According to Obmascik (1995), circumstantial evidence gathered by officials of the Colorado Division of Wildlife through a flurry of research and investigative activity in 1988 and 1989 strongly suggested that the parasite arrived in Colorado in private shipments of commercially reared, live trout from Idaho. Collection and testing of more than 26,000 trout by May 1989 revealed the parasite was widely dispersed in Colorado. The parasite was detected in trout from 11 public and commercial aquaculture units and in 40 wild trout populations from 11 of 15 major drainages in the state (Obmascik 1995; Walker and Nehring 1995). During this period there was a strong correlation between positive tests among the wild trout and recent stocking of fish reared at state or private aquaculture facilities where the parasite was enzootic. However, no clinical signs of disease were observed in feral trout between 1989 and 1992, which was similar to the experience in other states in the eastern and western United States in previous decades (Bartholomew and Reno 2002).

A century after the initial detection of *M. cerebralis* in Germany in 1893 and six years after initial detection in Colorado, dramatic declines in abundance of wild rainbow trout were evident in five of 13 major stream drainages that support substantial trout populations. In the fall of 1993 and in 1994, losses of young rainbow trout were observed in major reaches of the Cache la Poudre, upper Colorado, Gunnison, Rio Grande and South Platte rivers. Meanwhile brown trout recruitment levels appeared unaffected in those same reaches. Compared to rainbow trout, brown trout are known to be much less vulnerable to impacts from *M. cerebralis* (O'Grodnick 1979; Hedrick et al. 1999a; Thompson et al. 1999). During the decade between 1995 and 2004, research efforts demonstrated that the *M. cerebralis* parasite and WD were the primary factors contributing to these losses in Colorado (Walker and Nehring 1995; Nehring and Walker 1996; Nehring et al. 1998; Nehring and Thompson 2003). Nehring and Thompson (2001) estimate that *M. cerebralis* has negatively impacted recruitment and survival of wild rainbow and brook trout fry in 560 to 600 km of Colorado's premiere trout streams. This represents a very small percentage of the total stream resource since there are more than 14,000 km of stream that support salmonid populations. However, this small percentage should not be viewed as un-important because these impacted systems represent the very best of Colorado's quality stream trout fisheries. Also the majority of stream trout populations in the state have not been rigorously evaluated or tested for exposure to *M. cerebralis* and it is not currently possible to accurately quantify the actual percentage of stream miles that have been negatively impacted.

Vectors and Mechanisms of Dispersal

Myxospores of *M. cerebralis* are highly resistant to heat, cold and desiccation (Uspenskaya 1957; Hoffman and Putz 1969; Hoffman and Putz 1971; El-Matbouli et al. 1992). They have been shown to remain viable and infectious to *T. tubifex* after passage through the alimentary canals of northern pike *Esox lucius*, mallard duck *Anas platyrhynchos* (Taylor and Lott 1978; El-Matbouli et al. 1992) and black-crowned night-heron *Nycticorax nycticorax* (Taylor and Lott 1978). Clearly, there are numerous ways this parasite can be vectored between aquatic habitats and drainages across broad geographic regions. However, evidence collected over the past 100 years implicates human transport of live trout and frozen trout products as the primary method by which this parasite has been vectored across much of Europe, Scandinavia, Asia, approximately 50% of the states in the continental United States, as well as Morocco and South Africa on the African continent and in New Zealand (Hoffman 1990; Modin 1998; Bartholomew and Reno 2002). Indeed, Modin (1998) states, "The chronological appearance and distribution of *M. cerebralis* strongly implicates dispersal of live or processed state and commercially produced fish as a major factor in the spread of the parasite in California."

Bartholomew and Reno (2002) provide a detailed chronology of the spread of the parasite, taking great care to document the time frame and mechanisms of vectoring across the United States between 1956 and 2001. Public or private fish hatcheries were the initial or early sites of detection in

California (1966), Colorado (1987), Connecticut (1961), Idaho (1987), Maryland (1995), Massachusetts (1966), Michigan (1968), Nevada (1957 – 1966), New Jersey (1968), New Hampshire (1980), New York (1984), Ohio (1960s), Oregon (1986), Pennsylvania (1956), Utah (1991), Virginia (1965) and West Virginia (1969). Only in the states of Washington, Montana, Wyoming and New Mexico were the initial detections in free-ranging salmonids. However, in both Wyoming and New Mexico the initial exposure was either traced to or strongly suspected to have resulted from stocking of privately-reared, infected trout (Bartholomew and Reno 2002). In many instances containment and control of the parasite was impossible because exposed or infected fish may have been stocked into lakes and streams or moved to other facilities by the time the testing process detected myxospores.

Thompson et al. (1999) conducted numerous studies exposing wild trout fry to a free-flowing stream where *M. cerebralis* was enzootic. Those studies revealed that 1300 to 1900 degree-days (°Celsius) were required from the time of initial exposure to the time when myxospores could be reliably detected in cranial tissues using either the plankton centrifuge technique (O'Grodnick 1975) or pepsin-trypsin digest (PTD) method (Markiw and Wolf 1974). These two methodologies were the preferred diagnostic tools for detection of *M. cerebralis* between the 1970s and 2000. In many high elevation, cold water habitats in western North America, six to eight months or longer may be required after initial exposure before large numbers of myxospores develop in infected fish. Sub-clinical infections often do not result in overt clinical signs of WD. Thus, low-grade or latent infections may go undetected for months or even years. This may result in unintentional stocking of infected salmonids into highly vulnerable but previously unexposed aquatic habitats.

Stocking of infected fish greatly contributed to the spread of the parasite in Colorado. Beginning in 1988 and continuing through 2003, extensive field collections of feral salmonids have been made in stream habitats in the years subsequent to stocking of trout reared in private and public hatcheries where the parasite was enzootic. In many instances the stocking was done into lakes and reservoirs in headwater areas at elevations ≥ 3,049 m (10,000 feet). Testing for myxospores of *M. cerebralis* by plankton centrifuge (O'Grodnick 1975) or PTD (Markiw and Wolf 1974) and in later years (1998 – 2003) by polymerase chain reaction (PCR) revealed a high degree of correlation between the stocking of catchable trout exposed to *M. cerebralis* and detection of the parasite in feral salmonids downstream of the stocking sites (P. G. Walker; Colorado Division of Wildlife Senior Fisheries Pathologist, personal communication; Schisler and Bergersen 2002; Nehring 2003; Nehring 2004). As a result, the parasite became widely distributed in Colorado by the mid-1990s, primarily through stocking of millions of catchable size trout produced in state and commercial aquaculture facilities that relied on surface water supplies enzootic for *M. cerebralis*. For instance, Schisler (2001) reported that 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.

In other areas of the western United States, the rate of spread and severity of impact of *M. cerebralis* on native and introduced salmonids has been varied (Bartholomew and Wilson 2002). In Montana, the mechanisms of dispersal of *M. cerebralis* appear to be less clearly understood. However, there is no doubt that the parasite is widely distributed (Baldwin et al. 1998) and that it has had extensive and devastating impacts on several wild rainbow trout populations in the western and southwestern parts of the state (Vincent 1996a,b). In Yellowstone National Park, the Pelican Creek spawning run of Yellowstone cutthroat trout *Oncorhynchus clarki bouvieri* that once numbered in the 10,000s has been completely decimated. While the mechanism of initial introduction is unknown, it is clear that establishment of *M. cerebralis* has contributed to recent severe declines in the population of this species in Yellowstone Lake (Koel 2004).

The parasite is now enzootic throughout much of western North America including Arizona, New Mexico, Nevada, California, Oregon, Washington, Idaho, Wyoming, Montana, Colorado and Utah. However, clinical signs of disease and population level impacts among feral salmonids have been most widespread and severe in Colorado and Montana. Severe infections and overt clinical signs of disease have been observed in isolated cases among feral and native salmonids in Idaho, Wyoming and Utah (Bartholomew and Reno 2002; Hiner and Moffitt 2002).

While human transport of live trout and frozen trout products is considered the primary mechanism by which *M. cerebralis* has been vectored throughout the world (Hoffman 1990; Modin 1998; Bartholomew and Reno 2002), other factors contribute to the spread once initial introduction has occurred. Moving water is a conduit for downstream transport of TAMs and a migration route for movement of infected trout. TAM actinospores of the parasite shed into flowing or standing water by infected *T. tubifex* can be transported downstream many kilometers from the initial point of release. The viability of TAM spores can range from a few days to several weeks and is related to water temperature (El-Matbouli et al. 1999a). El-Matbouli et al. (1999a) demonstrated that more than 60% of TAM spores remain infective to rainbow trout in laboratory exposures for 15 days at 15 °C and can remain viable for even longer periods in natural systems. Myxospores *of M. cerebralis* can be dispersed upstream and downstream by migrating trout infected with the parasite. Downstream dispersal would probably only be limited by water temperatures within the thermal tolerance of the fish. Physical barriers to upstream movement by infected trout will restrict upstream dispersal by fish migration.

Habitat degradation contributes to the spread of *M. cerebralis* in the environment and can also exacerbate the severity of infection. Sediment accumulation in lakes and streams provides optimal habitat for dense colonies of tubificid worms (Zendt and Bergersen 2000; Nehring et al. 2003a). Higher densities of *T. tubifex* that are susceptible to the

parasite and more extensive areas of sedimentation within salmonid habitats increase the probability for more efficient utilization of myxospores of *M. cerebralis* by tubificid worms. An excellent case study that demonstrates the efficiency phenomenon was documented at Windy Gap Reservoir (WGR), a shallow, sediment-laden 40 ha lake in central Colorado. Although the lake is not stocked with trout, *M. cerebralis* has been enzootic in the lake and in the river upstream and downstream of the lake since the early 1990s. Zendt and Bergersen (2000) estimated the average density of *T. tubifex* in the lake exceeded 37,400/m^2 during the late 1990s. The total number of TAM spores in the effluent leaving the reservoir over a 12-month period in the late 1990s was estimated to range between 960 billion and 1.8 trillion (Nehring et al. 2002). Between 1997 and 2000, monthly water filtration studies demonstrated that TAM spore densities in the lake effluent were 1 to 3 orders of magnitude higher than in the water flowing into WGR from the Colorado and Fraser rivers (Nehring and Thompson (2003). This was the case even though *T. tubifex* known to be susceptible to *M. cerebralis* occur at densities in the river similar to those in WGR (Zendt and Bergersen 2000).

Overgrazing of riparian zones in some areas of the Intermountain West has resulted in severe streambank erosion and concomitant accumulation of sediments in the streams flowing through the riparian corridor. This type of habitat degradation may increase the rate of spread of *M. cerebralis* to upstream areas once it becomes enzootic. Wild trout in alpine and sub-alpine streams of the western U.S. tend to be highly mobile, moving both upstream and downstream, probably searching for a suitable home range (Gowan et al. 1994; Gowan and Fausch 1996ab, 2002; Peterson and Fausch 2003). Haldane (1956) hypothesized that on the whole, emigration is a density-dependent mechanism that regulates animal density in habitats favorable for population expansion. However, it is reasonable to assume that higher rates of emigration of wild trout would tend to occur from areas where habitat degradation has reduced the carrying capacity of the stream. In such a case, migrating trout infected with *M. cerebralis* would tend to increase the the rate of spread of the parasite across both time and space compared to non-degraded habitat areas. Empirical evidence from two case studies in Colorado suggest this is the case (Nehring and Thompson 2003).

Throughout much of the upper reaches of Cochetopa Creek in west-central Colorado, the riparian zone is heavily overgrazed. Extensive sloughing of stream banks has lead to sediment accumulation in the channel, providing optimal habitat for colonization by tubificid worms. Water from Dome Lake, a 30 Ha impoundment flows into Cochetopa Creek. This lake was stocked with catchable rainbow trout reared at a state fish hatchery testing positive for *M. cerebralis* in 1992, 1993 and 1994. A review of Colorado Division of Wildlife (CDOW) fish stocking records indicates there were no other sites or occasions when trout from rearing units exposed to *M. cerebralis* were stocked in the Cochetopa Creek drainage during the 1980s and 1990s. Monthly water filtrations during 1999 and 2000 revealed *M. cerebralis* TAMs were present in the creek upstream and downstream of Dome Lake. PCR and PTD testing of wild rainbow, brown and brook trout collected throughout the Cochetopa Creek drainage during the late 1990s and 2000 revealed the parasite was enzootic more than 15 km upstream of Dome Lake, the point of initial introduction. Brook trout were severely infected. Numerous specimens had clinical signs of WD, including skeletal and cranial deformities. Taken together, the empirical evidence suggests that *M. cerebralis* was vectored at least 15 km upstream between 1994 and 2000, probably by migrating trout infected with the parasite.

Beaver Creek flows through Beaver Creek Reservoir (BCR), a 46 Ha impoundment in SW Colorado. Like Dome Lake, this reservoir was stocked with catchable rainbow trout reared at a state fish hatchery testing positive for *M. cerebralis* in 1992, 1993 and 1994. In contrast to the upper reaches of the Cochetopa Creek drainage, the riparian zone of Beaver Creek upstream and downstream of BCR is very stable, has not been subjected to heavy grazing and does not have extensive areas of sediment accumulation. TAMs of *M. cerebralis* were often detected in monthly water filtrations downstream of BCR that began in 1998 and continued through 2003. PCR and PTD testing of wild brown and rainbow trout downstream of the reservoir confirmed that *M. cerebralis* was enzootic in the reservoir and in the stream below the lake. Monthly water filtrations to detect TAMs of *M. cerebralis* on Beaver Creek at two locations upstream of the reservoir began in November 1999 and continued periodically through 2003. Very low levels of TAM spores were detected at the filtration site 0.2 km upstream of the lake on 3 of 5 filtration occasions. In contrast, TAM spores were never detected during 21 filtration occasions at a filtration site 3.2 km upstream of the reservoir between December 1999 and November 2002. Results of PCR and PTD tests conducted on wild trout collected from the stream confirmed brown trout were infected with *M. cerebralis* at the site 0.2 km upstream of the lake. However, similar testing of wild brook, brown and rainbow trout collected from Beaver Creek at the study site 3.2 km upstream of the reservoir in June and September 2000 and September 2002 were all negative for infection by *M. cerebralis*. There are no barriers to migration of any kind between the upstream end of Beaver Creek Reservoir and the sampling site 3.2 km upstream. The lack of spread of *M. cerebralis* to the upstream site between 1994 and September 2002 suggests that there has been no migration of infected trout from the site 3 km downstream where both water filtration and PCR and PTD testing of trout confirmed the parasite has been enzootic at that site since at least 1999 or earlier, or, if migration of infected trout occurred, the parasite did not become established.

In the case of Beaver Creek and Beaver Creek Reservoir, a healthy riparian ecosystem may well have helped slow or inhibit the upstream spread of *M. cerebralis* in two ways. First, a healthy riparian zone and stable stream channel may provide adequate habitat to support the wild trout population thereby reducing the tendency of juvenile or adult trout to migrate

upstream. Second, the lack of sedimentation in the stream channel may severely limit the habitat requirements *for T. tubifex* that are susceptible to infection by *M. cerebralis.*

Species Vulnerability

Over the past 30 years much effort has been directed at assessing the relative vulnerability of many species of salmonids to *M. cerebralis* (O'Grodnick 1979; Hedrick et al. 1998; Hedrick et al. 1999a; Hedrick et al. 1999b; Thompson et al. 1999; Hedrick et al. 2001; Vincent 2002). Most investigators consider rainbow trout the salmonid species most vulnerable to *M. cerebralis,* particularly when exposed at a very young age (Markiw 1991; Markiw 1992a). However, relative sensitivity among salmonid species varies depending up on the metric used to assess vulnerability. The most commonly used metrics include 1) quantification of cranial myxospores 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 assess the relative amount of skeletal tissue abnormality and damage caused by the parasite 80-90 day PE (Vincent 2002).

For many studies cranial myxospore concentration has been the primary metric of effect. Using that technique, rainbow trout 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 follow as a close second (O'Grodnick 1979; Thompson et al. 1999; Vincent 2002). Bull trout *Salvelinus confluentus* and rainbow trout similarly exposed to low-dose (100-200 TAMs/fish) and high-dose (1,000 – 2,000 TAMs/fish) treatments experienced the same prevalence of infection at both exposure levels, but bull trout had significantly fewer cranial myxospores than rainbow trout five months PE (Hedrick et al. 1999b). Brown trout also show significant resistance to infection (Hedrick et al. 1999a; O'Grodnick 1979; Thompson et al. 1999) as do coho salmon *Oncorhynchus kisutch* (Hedrick et al. 2001). Sockeye salmon, including kokanee and Chinook salmon can be highly susceptible when exposed as alevins or fry (O'Grodnick 1979; Hedrick et al. 2001; Butts et al. 2000). Lake trout *S. namaycush* have been shown to be highly resistant or refractory to infection (O'Grodnick 1979; Blazer et al. 2000).

Mountain whitefish *Prosopium williamsoni* are also vulnerable to *M. cerebralis.* Prevalence of infection among wild stocks of adult whitefish collected from the Roaring Fork River in Colorado in 1997 ranged between 70 and 80% based on PTD analyses for cranial myxospores (Nehring and Thompson 2003). Similarly, field collections and results of laboratory studies in Montana demonstrated that fry and juvenile whitefish develop overt clinical signs of WD five months PE comparable to those observed in young rainbow trout that were similarly exposed (MacConnell et al. 2000). Caudal lesions of the spine were more evident in juvenile mountain whitefish compared to those seen in similarly exposed rainbow trout (MacConnell et al. 2000). These findings may suggest that mountain whitefish could experience population level impacts in habitats where *M. cerebralis* is enzootic.

Histological techniques have been used to assess the relative vulnerability of various salmonids exposed to a single dose of *M. cerebralis* TAMs and then held for 80 – 90 days in SPF water (Vincent 2002). Salmonids tested in this manner include 10 strains of rainbow trout, three subspecies of cutthroat trout, kokanee salmon, Chinook salmon, brown trout, brook trout, bull trout, and Arctic grayling *Thymallus arcticus*. Most strains of rainbow trout and eastern brook trout were the most seriously affected species, 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.

Thompson et al. (1999) used both cranial myxospore concentration and chronic mortality to assess the relative susceptibility to infection by *M. cerebralis* among seven species or subspecies of salmonids. Completed over a number of years, these tests were *in vivo* continuous field exposures to ambient levels of TAMs 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 were held in floating tanks and continuously exposed for 12 to 18 months. Mortalities were monitored on a daily basis throughout all of the experiments. When cranial myxospore concentrations were used as the metric of effect, rainbow trout were the most sensitive species across all tests. Brown trout were the most resistant while the cutthroat trout subspecies and brook trout had intermediate levels of cranial myxospore concentrations when compared to rainbow trout. In these comparisons, the findings of Thompson et al. 1999 were 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 the lack of a negative control and other pathogens can be acting concurrently with the pathogen of interest. However, if the ultimate objective of a research investigation is to determine if exposure to *M. cerebralis* in the natural environment can have lethal consequences for certain species of salmonids, then use of mortality is a valid measure of effect whether acting alone or in concert with additional stressors. 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 four 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, Colorado River cutthroat trout and

greenback cutthroat trout. The latter three sub-species are native to Colorado while Snake River cutthroat trout have been reared in the state's hatchery system and stocked into lakes, reservoirs and streams across the state since the 1970s.

As expected, in these tests brown trout suffered the least mortality in the majority of exposures. In the 1995-1996 exposures, brook trout and Colorado River cutthroat trout suffered much higher mortality than did rainbow trout exposed at the same time. During the 1996-1997 exposures, one treatment group of rainbow trout 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. Those treatment groups of cutthroat trout included the Snake River, Colorado River and Rio Grande cutthroat trout. Among the two treatment groups of cutthroat trout that survived as well as the rainbow trout treatment group, 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 rainbow trout were considered less vulnerable to chronic mortality than the three subspecies of Colorado's native cutthroat trout when exposed to ambient levels of *M. cerebralis* in the Colorado River. This is an important consideration from a species management perspective given that cutthroat trout in general are very poor competitors when occurring in sympatry with nonnative salmonids (Peterson and Fausch 2003; Kennedy et al. 2003).

ANALYTICAL TOOLS

Many analytical tools have been developed over the past 30 years to 1) document the rate of dispersal of *M. cerebralis* across geographic regions, 2) assess the prevalence and severity of infection in both hatchery-reared and feral salmonids, and 3) determine focal points of infection both in individual fish and in the environment. Depending upon the objectives of investigation, these analytical tools can be applied to three different targets: the fish, the aquatic worms, or the water.

Diagnostic Tools for Detecting *M. cerebralis* Infection in Fish

Techniques for determining prevalence and severity of *M. cerebralis* infection in salmonids focus on three different facets and levels of parasite activity in exposed fish. These include extraction and concentration of *M. cerebralis* myxospores from fish tissues, histological techniques for assessing loci of infection, tissue degradation and abnormalities at the organ and cellular level, and at the genetic or molecular level, detection and quantification of genomic DNA specific to *M. cerebralis*.

Techniques for extracting and quantifying myxospores in fish tissues include the PTD (Markiw and Wolf 1974) and the plankton centrifuge (O'Grodnick 1975) methods. The latter technique is no longer recommended as a diagnostic procedure for fish health inspections (American Fisheries Society 2003). Compared to the plankton centrifuge, the PTD method provides a higher degree of precision and accuracy because of a reduced volume of fluid in the final stages of myxospore concentration and extraction. However, the plankton centrifuge method is useful for collection of myxospores that are destined for use in laboratory experiments to expose *T. tubifex* worms to the parasite.

There are at least two distinct histological procedures used to evaluate the prevalence and severity of *M. cerebralis* infections in exposed salmonids. The most commonly used technique involves treating thin sections of target fish tissues with hematoxylin and eosin (H&E) stain and examining the sections for tissue abnormalities, inflammation and various developmental stages of the parasite that generally occur in or near fish tissues rich in cartilage (Lorz and Amandi 1994). A second histological technique is a non-radioactive *in situ* hybridization (ISH) protocol that utilizes oligonucleotide primers and labeled probes designed to bind to DNA of the *M. cerebralis* parasite. This histological procedure allows detection of DNA from *M. cerebralis* in infected tissues from either the worm or the fish host (Antonio et al. 1998). This procedure facilitates the detection of very low numbers of parasite stages within fish tissues that would probably not be detected by conventional (H&E) staining techniques. Although less sensitive than PCR, the ISH protocol is a powerful tool for evaluating the spatial and temporal orientation of the parasite in the infected host.

Finally, utilization of PCR technology is expanding at a rapid rate. The first test for genomic DNA of the *M. cerebralis* parasite was a two-stage nested PCR reaction developed and described by Andree et al. (1998). Subsequently, Baldwin and Myklebust (2002) modified the nested PCR reaction to be run as a single round test. Most recently another PCR test has been developed that targets a nucleotide sequence for the heat shock protein 70 (Hsp70) gene for detection of *M. cerebralis* (John Wood, Pisces Molecular; personal communication). The technique has been used extensively for research purposes in Colorado, Utah and Vermont since late summer 2001. As with the 18S rDNA test for *M. cerebralis* (Andree et al. 1998), the Hsp70 test works well on fish, aquatic oligochaetes and water samples.

Diagnostic Tools for Detecting *M. cerebralis* Infection in Aquatic Oligochaetes

Standard histological techniques described by Lorz and Amandi (1994) used to detect *M. cerebralis* in salmonid tissues work equally well for locating focal points of infection in *T. tubifex*. Likewise, the ISH protocols (Antonio et al. 1998), the 18S rDNA PCR reaction (Andree et al. 1998) and Hsp70 PCR test all work well for detection of infection in oligochaetes. The Hsp 70 test for *M. cerebralis* does not cross-react with DNA from *M. squamalis*, *M. neurobius* or *Henneguya insidiosis* (John Wood, personal communication, unpublished data).

Diagnostic Tools for Detecting Actinospores (TAMs) of *M. cerebralis* in Water

Currently, there are two methodologies in use for detecting and quantifying TAMs of *M. cerebralis* in water samples. They are generally described as "flat pan" and "packed-bed" filtration. The flat pan technique has been in continuous use in Colorado since April 1997. Results of an extensive field study were published in the ***Journal of Aquatic Animal Health*** (Thompson and Nehring 2000). The packed-bed filtration procedure has been in use in field studies in Montana on a limited basis since May 2002 (Lukins et al. 2004). Compared to the flat pan technique, the packed-bed filtration procedure has greater accuracy, precision and sensitivity for detection but is much less portable and generally cannot be used where road access is unavailable. Moreover, freezing temperatures preclude use of the packed-bed filtration system during the winter months. During the summer of 2004, Colorado Division of Wildlife (CDOW) aquatic researchers and field staff ran parallel tests in the field to test the sensitivity, precision and accuracy of the two techniques as well as a hybrid technique that uses flat pan in the field and packed-bed in the lab to further concentrate the filtrate. These tests revealed that the flat pan technique and the packed-bed filtration system produced equivalent results when 120 L of water were filtered (Lukins et al. 2005).

REVIEW OF RESEARCH FINDINGS

Colorado's High Lakes Studies

During 1998 and 1999, Schisler (2000) sampled trout populations from 69 high elevation waters that had been stocked with trout in 1992 or 1996 from state fish rearing units (SFRU) that had tested positive for *M. cerebralis* in 1992 (Roaring Judy SFRU) or in 1997 (Durango and Pitkin SFRUs). These inadvertent introductions of exposed fish occurred in the months prior to the time when scheduled annual inspection tests subsequently revealed the units were positive for *M. cerebralis*. In the high lakes study, the heads of the trout were tested by PCR and PTD for evidence of infection by *M. cerebralis*. The objective of the study was to ascertain the rate of establishment of *M. cerebralis* and the severity of infection in wild trout from a relatively large sub-sample of high elevation waters where *M. cerebralis* was not enzootic prior to the time of the inadvertent stocking. The study revealed that stocking history, elevation, presence of *T. tubifex* habitat and distance to waters known to be enzootic for *M. cerebralis* had strong effects on the presence of the parasite in the fish collected and tested at 69 locations across the state (Schisler 2000).

In this study, risk of establishment generally decreased as elevation increased (Schisler 2000). However, high elevation lakes and streams were vulnerable to establishment of *M. cerebralis*. The parasite could become established in these waters when other mitigating factors existed such as repeated stocking, or if they were in close proximity to other known-positive waters. For example, *M. cerebralis* was detected in trout collected from three lakes at elevations > 3,580 m. At two of the three lakes, stocking with cutthroat trout fingerlings (mean length 32 mm), potentially exposed to the parasite occurred in 1994 or 1995. Collections of trout from 44 of 69 lakes and streams sampled were from areas above 3,000 m in elevation. Among those fish collections, 22 tested positive for *M. cerebralis* and 22 tested negative.

In general, Schisler (2000) found the risk of establishment of *M. cerebralis* was greatest if trout reared at units known to test positive for *M. cerebralis* had been stocked one or more times into the body of water being tested. Indeed, *M. cerebralis* was detected by both PTD and PCR testing in trout from 8 of 10 study sites that were stocked with fish from rearing units known to be positive at the time of stocking. Detection of *M. cerebralis* was somewhat lower when there was no history of stocking with trout from rearing units either known or suspected of being positive for the parasite. PCR testing detected evidence of *M. cerebralis* infection in 66.7% (n = 24) of the bodies of water with no history of stocking with trout from rearing units either known or suspected of being positive for the parasite. Similarly, among 35 bodies of water stocked with trout from units in the year prior to detection of the parasite at the unit, evidence of infection was detected in 51.4% of the samples by PCR testing and in 25.7% of the samples by PTD testing.

Schisler (2000) found that habitat conducive for the establishment of *T. tubifex* appeared to be less abundant at

higher elevations. Relative amount of *T. tubifex* habitat was used as another factor to predict *M. cerebralis* establishment. Among 22 study sites classified as having "minimal habitat" for *T. tubifex*, evidence of infection by the parasite was found in fish collected from 9.1 % and 45.5 % of the sites as tested with PTD and PCR, respectively. In 21 waters classified as having "moderate habitat" for *T. tubifex*, the parasite was identified in 23.8 % of the waters as tested with PTD and 52.4 % of the waters as tested with PCR. The final category was waters with "abundant habitat" for *T. tubifex* such as beaver ponds. At these 26 sites, the parasite was found in 61.5 % of the waters tested by PTD and 80.4 % of the waters tested by PCR. While lack of any obvious *T. tubifex* habitat does not preclude the presence or establishment of *M. cerebralis*, greater abundance of habitat does increase the likelihood that it will become established.

All the factors studied, including stocking history, distance to waters known to be infected with *M. cerebralis*, and relative amount of available *T. tubifex* habitat contributed to the probability of establishment of *M. cerebralis* in high elevation waters. The combined influence of all of these factors contribute to this probability, and any one factor alone is often not adequate to determine the risk of establishment. It should be noted that the reduced risk identified in some of the higher elevation waters should not be taken as evidence that further stocking of infected fish would have minimal impact in these areas. Rather, the factors that have been found to contribute to the establishment of the parasite should be considered very carefully when management decisions are made to minimize the spread of the parasite into these more remote locations.

Water Filtration Studies – Lakes and Reservoirs

Presence of TAMs of *M. cerebralis* in water samples collected from free-flowing streams is *prima facie* evidence that the parasite is enzootic in the river or drainage basin at some point upstream of the sample site. Water filtration efforts to determine the relative abundance and seasonal periodicity of the TAM spores in the waters of Colorado have been on-going across the state since April 1997. The technique has been extensively applied to lakes, reservoirs and streams of various sizes and with variable stocking histories to determine whether or not there is a linkage between the stocking of trout reared in SFRUs that test positive for *M. cerebralis* and the density of TAM spores detected in the water over a number of years. A substantial amount of that data is summarized in Table 1.

In many waters capable of sustaining salmonids on a year-round basis, there has been a clear link between the stocking of catchable trout exposed to *M. cerebralis* and the relative abundance of TAMs (numbers/L) observed in monthly water samples (Table 1). This was the case for Beaver Creek, Georgetown, Montgomery, Spring Creek, Taylor Park, and Williams Fork reservoirs, and Cottonwood Lake. However, when the management protocol was changed to the stocking of catchable trout that had not been exposed to *M. cerebralis*, TAM production declined, usually beginning within a year after the cessation of the stocking of exposed trout. Twenty-four to 36 months later TAM densities usually had declined to near undetectable levels. This was the case for Montgomery, Spring Creek, Williams Fork and Taylor Park reservoirs as well as Cottonwood Lake.

In the stream immediately below Georgetown Dam, abundance of TAMs was near undetectable levels from July 2002, when monthly filtration studies were initiated, through April 2003. Even though stocking of *M. cerebralis*-exposed trout has been an on-going management strategy for more than a decade in this reservoir, TAM production remained low, probably due to rapid harvest of stocked trout. However, TAM production began to increase exponentially in May 2003 and remained at extremely high levels for more than 19 months. This large, sustained increase in TAM density was most likely the result of introduction of more than 167,000 - 32 mm fingerling rainbow trout (not previously exposed to *M. cerebralis*) from the Rifle Falls SFRU in summer 2002. The stocking of unusually large numbers of fingerling rainbow trout that were highly vulnerable to infection by *M. cerebralis* into a lake where the parasite was enzootic artificially augmented the supply of myxospores in the lake that would be available to *T. tubifex*.

There are two reservoirs in the state where high levels of TAM production have been documented that do not appear to be linked to the stocking of trout exposed to *M. cerebralis*. They are Windy Gap Reservoir on the upper Colorado River and Idylwilde Dam on the Big Thompson River near Loveland, Colorado. In these cases TAM production may be linked to the flushing of myxospores into the reservoir from upstream areas where *M. cerebralis* is enzootic (Nehring et al.2003a).

In contrast, there are a number of lower elevation lakes that have been seasonally stocked for many years with large numbers of catchable trout reared at SFRUs testing positive for *M. cerebralis* (Table 1). These bodies of water include Chatfield and Cherry Creek reservoirs in the Denver metro area as well as Chipeta and Confluence Park lakes in the Montrose/Delta area in western Colorado. All of these waters are at lower elevations and experience seasonal water temperatures $\geq$ 20°C during the summer. Exposure of *T. tubifex* infected by *M. cerebralis* to water temperatures $\geq$ 20°C have been shown to depress or inhibit production and release and TAM spores in laboratory studies (El-Matbouli et al. 1999a). Monthly water filtration efforts conducted for at least a year downstream of each of these bodies of water revealed little evidence that the parasite had become enzootic given that TAM densities were essentially below detectable levels throughout the 12-month filtration study at all four study sites.

Out of the 19 lakes and reservoirs that were extensively studied (Table 1), Clear Creek Reservoir was the only body of water in salmonid habitat where exposed trout have been

Table 1. Assessment of the relationship between probable stocking history for trout exposed or unexposed to *M. cerebralis* and the establishment, exacerbation and/or amelioration of the severity of *M. cerebralis* infection as determined by detection of triactinomyxon actinospores (TAMs) in 1900 L (500 gallons) water samples as part of the Colorado Division of Wildlife Whirling Disease Investigations research project (April 1997 – June 2004). Numbers (such as 40K) shown on the lines for a given body of water and year indicate 40,000 catchable trout stocked. Numbers in **BOLD** are fish stocked from rearing units that were diagnosed as testing positive for *M. cerebralis* in the year of the stocking. Numbers of fish not in bold letters are fish from rearing units that had not tested positive for *M. cerebralis* in the year of the stocking.

Name of Water	Elevation	Acres	History of Stocking of Trout Exposed or Unexposed to ***M. cerebralis***											
			1993	1994	1995	1996	1997	1998	1999	2000	2001	2002	2003	2004
Confluence Park Lake	4,920	18	**14K**	**20K**	**40K**	**79K**	**26K**	**32K**	**32K**	**49K**	**20K**	**20K**	39K	
Mean Tams/liter for year										0.013	0.000			
Chatfield Reservoir	5,430	1,087	159K		**425K**		**64K**	**49K**	**33K**	**76K**	**36K**	**18K**	**61K**	
Mean Tams/liter for year										0.011	0.007			
Cherry Creek Reservoir	5,551	889	**93K**	**72K**	**139K**	**249K**	**70K**	**60K**	**26K**	**166K**	**34K**	**14K**	**30K**	
Mean Tams/liter for year										0.008	0.000			
Chipeta Lake	5,900	8.5	7K	10K	7K	3K	0.1K	5K	12K	2K	2K	7K	11K	
Mean Tams/liter for year										0.000	0.000			
Idylwilde Reservoir	5,970		none	none	none	none	none	none	none	none	none	none	none	
Mean Tams/liter for year									0.505	0.670	0.282	0.118	0.172	
McPhee Reservoir[a]	6,924	4,470	360K	364K	274K	172K	83K	84K	83K	94K	83K	59K	78K	
Mean Tams/liter for year								0.030	0.020	0.002	0.000	0.001	0.002	
Crystal Reservoir	7,100	250	none	none	none	none	none	none	none	none	none	none	none	
Mean Tams/liter for year								0.010	0.083	0.082	0.020	0.036		
Estes Lake	7,468	185	30K	33K	**10K**	22K	15K	10K	7K	12K	11K	22K	13K	
Mean Tams/liter for year									0.000	0.015		0.000	0.007	
Ruedi Reservoir	7,766	1,000	29K	**19K**	2K	7K	3K	16K	24K	23K	15K	53K	40K	
Mean Tams/liter for year								0.000	0.000	0.000	0.011	0.000	0.000	0.000
Williams Fork Reservoir	7,811	1,600	51K	**42K**	20K	18K	5K	2K	**30K**	**4K**	**27K**	6K	15K	
Mean Tams/liter for year								0.032	0.054	0.138	0.035	0.039	0.003	0.014
Windy Gap Reservoir	7,833	104	none	none	none	none	none	none	none	none	none	none	none	none
Mean Tams/liter for year							3.492	6.149	6.024	6.186	1.551	0.231	0.281	0.414
Georgetown Reservoir	8,471	54	**20K**	**16K**	**42K**	**17K**	**29K**	**25K**	**13K**	**16K**	**32K**	**13K**[b]	18K	
Mean Tams/liter for year												0.050	1.155	4.338
Beaver Creek Reservoir	8,850	114	**27K**	**45K**	19K	25K	29K	21K	**12K**	31K	27K	36K	31K	
Mean Tams/liter for year							0.000	1.047	0.185	0.022	0.030	0.009	0.009	0.000
Clear Creek Reservoir	8,875	407	**42K**	**33K**	**60K**	**68K**	**21K**	**73K**	**36K**	**27K**	**31K**	**107K**	29K	
Mean Tams/liter for year												0.000	0.000	
Big Meadows Reservoir	9,200	114	**8K**	**15K**	7K	10K	11K	15K	12K	21K	21K	33K	31K	
Mean Tams/liter for year									0.000	0.000				
Taylor Park Reservoir	9,330	2,009	**40K**	**53K**	**116K**	**132K**	**128K**	**115K**	**96K**	101K	87K	58K	83K	
Mean Tams/liter for year									1.169	0.633	0.075	0.000	0.000	
Cottonwood Lake	9,552	43	**24K**	**27K**	**47K**	**30K**	**30K**	**29K**	**23K**	**13K**	**7K**	**6K**	14K	
Mean Tams/liter for year								0.282	0.212	0.165	0.017	0.006	0.004	
Spring Creek Reservoir	9,915	87	**9K**	**10K**	**22K**	**42K**	**3K**	0.2K	**10K**	6K	7K	7K	13K	
Mean Tams/liter for year							0.000	0.006	0.028	0.022	0.133	0.004	0.011	0.021
Montgomery Reservoir	10,840	97	**26K**	**27K**	**38K**	**32K**	**25K**	**36K**	**25K**	20K	10K	9K	14K	
Mean Tams/liter for year							1.232	0.677	0.142	0.304	0.069	0.069	0.039	

[a] 50,000 kokanee salmon fingerlings from Roaring Judy SFRU were stocked into McPhee Reservoir in 1992, the year the unit first tested positive for *M. cerebralis*.

[b] In 2002, 275,136 trout were stocked into this 54 acre impoundment, including 167,050 32 mm Tasmanian rainbow trout fingerling, 166 rainbow trout broodfish, 5,000 fingerling brown trout, 13,024 catchable rainbows, and 89,896 fingerling (80 – 100 mm) rainbows.

extensively stocked for many years but no TAMs were detected in 12 consecutive monthly samples immediately downstream. However, the parasite is enzootic upstream of the lake as TAMs of *M. cerebralis* were detected in Clear Creek flowing into the reservoir in April 2003. PCR testing of the water sample at that time verified the presence of DNA from *M. cerebralis*. There may be something unique about the configuration of the lake or the position of the outlet valve in the dam relative to where the TAM production might occur in the lake that would prevent TAMs from escaping from the reservoir.

Detection of TAMs in water filtered below Big Meadows, Crystal, McPhee and Ruedi reservoirs was rare to non-existent. This was not surprising since these lakes were only stocked once or twice with relatively low numbers of exposed salmonids or not at all (Table 1). No TAMs were ever detected in water flowing over the spillway of Big Meadows Reservoir, even though wild brook and rainbow trout in the streams tributary to the reservoir tested positive for *M. cerebralis*. At Ruedi Reservoir a single TAM was detected only once in 72 consecutive months of sampling. As is the case with Clear Creek, *M. cerebralis* is enzootic in the Dolores River upstream of McPhee Dam, in the Fryingpan River upstream of Ruedi Dam, and in the Gunnison and Cimarron river drainages that flow into Crystal Reservoir.

The appearance of TAMs in the effluent water of lakes and reservoirs generally occurs 12 to 24 months after the stocking of the exposed fish has occurred (Table 1). The delay in the onset of TAM spore production is the result of the complex two-host life cycle of the parasite. Myxospores from infected fish must reach the sediments and be ingested by *T. tubifex* worms that are susceptible to the parasite. Myxospores can reach the sediments after being shed from infected trout (Nehring et al. 2002; Taylor and Haber 1974) or from decomposed carcasses of infected salmonids (Baldwin et al. 1998; El-Matbouli et al. 1999b). Aquatic oligochaetes that are susceptible to the parasite begin releasing TAMs of *M. cerebralis* 75 to 150 days after initial ingestion of myxospores, depending upon the water temperature to which the worms are exposed.

Water Filtration Studies – Public and Private Hatcheries, Lakes and Settling Ponds

Many of the CDOW's coldwater hatcheries and rearing units historically have relied on surface water supplies for rearing trout. After the accidental introduction of *M. cerebralis* into Colorado in the mid-1980s, the parasite became established in most of the major coldwater stream drainages by the early 1990s. This became problematic for those SFRUs and private coldwater aquaculture units that relied on surface water; the end result being that many of the public and private coldwater rearing units tested positive for the parasite by the mid-1990s.

As part of the research effort to develop a better understanding of the dynamics of TAM production in the salmonid aquaculture arena and develop "best management practices" (BMPs) to ameliorate and hopefully contain or control the spread of *M. cerebralis*, monthly water filtration studies were instituted at a number of CDOW coldwater SFRUs and at four private facilities. Some of those studies began in 1997 and have continued through 2005. Others have been added more recently. Public sector units where water filtration studies were conducted included the Watson (WAT), Poudre Ponds Rearing Unit (PRU), Mt Shavano (MSO), Pitkin (PKN), Chalk Cliffs (CCL), and Roaring Judy (ROJ) facilities and the U. S. Fish and Wildlife Service's Leadville National Fish hatchery and rearing unit (FSL). Except for the FSL and MSO units, data for all of the public sector sites are summarized in Table 2. Private lakes and private sector facilities and sites where water filtration studies have been conducted include Hagen's Western Fisheries at Nathrop (HNU), Mt. Massive Lakes, Inc. at Leadville (MML), Blue Valley Ranch (BVR) in the Blue River drainage between Silverthorne and Kremmling, and the Cap K Ranch fishing ponds in the Fryingpan River drainage (Table 3). Mean numbers of TAMs/L/year, together with the range of variation among the positive samples in a given year derived from all of these studies are summarized in Tables 2 and 3.

In Colorado, long-term water filtration studies have provided valuable insights into whether or not changes in fish stocking policies or management strategies for lakes and reservoirs have ameliorated or controlled the *M. cerebralis* infection cycle. Those studies have also provided important insights into the dynamics of the infection cycle at several public coldwater salmonid rearing facilities that have lead to the implementation of BMPs within the hatchery system. For example, water filtration efforts at the outlets of sedimentation ponds containing large numbers of trout that were either heavily stocked for fishing recreation or escaped into the ponds (and were not periodically removed) revealed these ponds became point sources of infection that discharged TAM-laden water into the receiving streams. This was true for sedimentation ponds on the PRU, ROJ and PKN units. See Tables 2a, 2b and 2c for details. In contrast, when the fish were removed from the ponds, and/or the ponds were dried out and accumulated organic waste and sediment was removed, TAM densities detected in the effluents declined by one to two orders of magnitude or more within 12 to 24 months of implementing these simple BMPs. At the ROJ unit, removal of kokanee salmon carcasses from the sedimentation ponds and connecting channels in 2000, 2001, and 2002 helped dramatically reduce ambient levels of TAM production that occurred from March through May each year. Reductions in the number of trout stocked into the ROJ sedimentation ponds for angling recreation and stocking only trout reared from *M. cerebralis*-negative units in the summer of 2003 also appear to have contributed to continued reductions in TAM densities detected in the spring and early summer of 2004

Table 2a. Summary of water filtration results from various sampling sites on Colorado's Poudre (PRU) coldwater state fish rearing unit (SFRU) from 1997 through June 2004.

Year	Number of Samples	Positive Samples	Mean TAMs/L	Range of TAMs/L Among Positive Samples
Poudre River water above CDOW Poudre SFRU				
1997	4	2	0.034	0.037 – 0.100
1998	No data collected in 1998 at this site			
1999	4	3	0.073	0.045 – 0.170
2000	12	6	0.024	0.017 – 0.096
2001	12	7	0.050	0.017 – 0.164
2002	12	7	0.053	0.023 – 0.192
2003	12	7	0.062	0.044 – 0.210
2004	6	3	0.027	0.033 – 0.075
CDOW Poudre SFRU – Water supply lake outlet to concrete raceways/ponds				
1997	9	4	0.220	0.048 – 1.479
1998	11	8	0.506	0.045 – 2.615
1999	4	3	0.820	0.740 – 1.480
2000	No data collected at this site in 2000			
2001	8	7	1.716	0.045 – 8.96
2002	12	7	0.225	0.027 – 1.685
2003	12	9	0.387	0.088 – 0.971
2004	6	4	0.039	0.032 – 0.088
CDOW Poudre SFRU – northeast sedimentation pond effluent return to river				
1998	12	11	2.87	0.180 – 6.58
1999	12	12	6.68	0.018 – 9.36
2000	12	12	9.45	0.162 – 42.9
2001	12	10	0.564	0.094 – 3.85
2002	12	10	0.186	0.032 – 1.26
2003	12	12	0.129	0.034 – 0.299
2004	6	3	0.035	0.046 – 0.110
CDOW Poudre SFRU – southeast sedimentation pond effluent return to river				
2000	12	12	25.05	2.43 – 84.4
2001	12	11	0.768	0.036 – 3.12
2002	12	7	0.167	0.032 – 1.035
2003	6	3	0.113	0.061 – 0.528

Table 2b. Summary of water filtration results for Colorado's Roaring Judy (ROJ) coldwater state fish rearing unit (SFRU), April 1997 through June 2004.

Year	Number of Samples	Positive Samples	Mean TAMs/L	Range of TAMs/L Among Positive Samples
CDOW Roaring Judy SFRU effluent water from concrete raceways				
1997	8	6	0.233	0.050 – 1.096
1998	11	9	0.185	0.029 – 0.674
1999	11	5	0.0373	0.026 – 0.210
2000	12	4	0.0377	0.098 – 0.145
2001	12	4	0.0138	0.020 – 0.052
2002	12	0	0	-----
2003	12	0	0	-----
2004	6	0	0	-----
CDOW Roaring Judy SFRU effluent from the east sedimentation pond				
1997	6	6	1.669	0.013 – 6.104
1998	11	10	0.743	0.090 – 1.701
1999	12	11	1.065	0.088 – 5.72
2000	12	12	1.358	0.042 – 3.230
2001	10	10	0.551	0.160 – 1.089
2002	4	2	0.041	0.044 – 0.119
2003	7	6	0.162	0.035 – 0.421
2004	6	2	0.026	0.068 – 0.089
CDOW Roaring Judy SFRU effluent from the west sedimentation pond				
1997	2	2	0	----
1998	No data collected at this site in 1998			
1999	11	11	1.299	0.518 – 5.062
2000	12	11	1.737	0.122 – 4.443
2001	12	11	1.710	0.226 – 8.542
2002	12	10	0.127	0.021 – 0.307
2003	12	11	0.417	0.040 – 1.625
2004	6	5	0.257	0.033 – 0.735

Table 2c. Summary of water filtration results from Colorado's Chalk Cliff's (CCL), Pitkin (PKN) and Watson Lake (WAT) coldwater fish rearing units (SFRUs).

Year	Number of Samples	Positive Samples	Mean TAMs/L	Range of TAMs/L Among Positive Samples
Chalk Cliffs SFRU inlet water supply				
2000	2	0	0	-----
2001	12	0	0	-----
2002	6	0	0	-----
Chalk Cliffs SFRU concrete raceways outlet				
2000	2	2	0.211	0.061 – 0.360
2001	12	2	0.008	0.029 – 0.069
2002	6	0	0	-----
Chalk Cliffs SFRU sedimentation settling pond outlet				
2000	2	2	0.116	0.079 – 0.153
2001	12	5	0.037	0.033 – 0.182
2002	6	0	0	-----
Quartz Creek - 50 meters upstream of Pitkin SFRU sedimentation pond effluent				
2001	2	1	0.046	0.091
2002	14	3	0.023	0.024 – 0.201
2003	13	3	0.059	0.023 – 0.677
2004	6	2	0.028	0.069 – 0.101
Pitkin SFRU sedimentation pond effluent				
2001	2	2	3.130	3.079 – 3.181
2002	12	12	8.153	0.277 – 94.8
2003	13	7	0.856	0.055 – 5.759
2004	6	5	0.260	0.021 – 1.210
Watson Lake SFRU – inlet water supply from Poudre River to Watson Lake				
2001	7	1	0.009	0.060
2002	12	0	0	-----
2003	6	1	0.025	0.150
Outlet from Watson Lake to concrete raceways inlet				
2002	11	0	0	-----
2003	6	0	0	-----
Watson Lake SFRU concrete raceway effluent return to Poudre River				
2001	7	0	0	-----
2002	12	0	0	-----
2003	6	0	0	-----

At some other units implementation of BMPs had already taken place prior to initiation of monthly water filtration. At the CCL unit, efforts to remove trout from the sedimentation pond on a regular basis were implemented prior to the time when TAM filtration began. At this unit TAM densities in the effluent returning to Chalk Creek were already very low (see Table 2c). At the WAT unit the stocking of catchable rainbow trout into Watson Lake was terminated in September 1999 and the lake was converted to a trophy warm water fishery. Beginning in 2001, tiger musky *Esox lucius x masquinongy*, sauger *Stizostedion canadense* and smallmouth bass *Micropterus dolomieu* were stocked into the lake. Monthly water filtration efforts began in May 2001 and continued through June 2003. A single TAM was detected in samples of Poudre River water entering Watson Lake once in 2001 and once in the spring of 2003. No TAMs were ever detected in the water flowing from the lake into the intake for the raceways or in the effluent leaving the raceways and returning to the Poudre River. Moreover, it was rare for a parallel aliquot of filtrate preserved from the sample to test positive by PCR for *M. cerebralis* DNA.

Not surprisingly, since 2001 it has been rare for catchable trout reared at the WAT unit to test positive by the PTD method for *M. cerebralis*. When detected, it usually only occurred after the fish have been held in water on the unit for about 24 months. Clearly, implementation of BMPs at many SFRUs resulted in dramatic reductions in ambient levels of infectivity in the trout reared at the units and substantial reductions in TAM densities detected in the effluent water leaving the units as well.

Similarly, application of BMPs and changes in management practices at some private fishing clubs and aquaculture facilities are helping to control the rate of spread of infection and in some cases reduce it dramatically (Table 3). At the MML fishing club TAMs were never detected in the filtrates from Big Union Creek during 22 consecutive months of filtering. Big Union Creek is the source of surface water for the MML. Low numbers of TAMs were detected six times in 22 months in the effluent of Lake # 7 (Muskrat Lake), but only once in the effluent of Lake # 10 (Lower Granite Lake).

Table 3. Summary of water filtration results from various sampling sites on a few of Colorado's private coldwater fish rearing units and private fishing lakes.

Year	Number of Samples	Positive Samples	Mean TAMs/L	Range of TAMs/L Among Positive Samples
Mount Massive Lakes, Inc. – inlet water from Big Union Creek				
2001	8	0	0	-----
2002	12	0	0	-----
2003	2	0	0	-----
Mount Massive Lakes, Inc. – outlet of Lake # 7 to Big Union Creek/Arkansas River				
2001	8	2	0.230	0.771 – 1.07
2002	12	3	0.0193	0.023 – 0.185
2003	2	1	0.0190	0.0380
Mount Massive Lakes, Inc. – outlet of Lake # 10 to Big Union Creek/Arkansas River				
2001	8	1	0.0075	0.060
2002	12	0	0	-----
2003	2	0	0	-----
Blue River – upstream of Blue Valley Ranch intake				
2003	5	3	0.415	0.024 – 1.387
2004	6	3	0.0442	0.027 – 0.188
Blue Valley Ranch irrigation intake above headgate diversion structure				
2003	6	4	0.180	0.079 – 0.808
2004	6	4	0.062	0.029 – 0.216
Blue Valley Ranch – effluent return to Blue River				
2003	6	2	0.096	0.074 – 0.499
2004	6	4	0.073	0.027 – 0.170
Arkansas River – upstream of Hagen's Western Fisheries @ Nathrop				
2003	11	0	0	-----
2004	6	0	0	-----
Hagen's Western Fisheries effluent return to Arkansas River				
2003	11	6	0.0465	0.042 – 0.234
2004	6	0	0	-----
Fryingpan River – two km upstream of Cap K Ranch				
1999	2	1	0.015	0.030
2000	12	3	0.021	0.035 - 0.162
2001	10	1	0.0034	0.034
2002	12	2	0.0122	0.042 - 1.04
2003	12	1	0.0032	0.032
2004	6	0	0	-----
Cap K Ranch Pond # 1 outflow (to Pond # 2)				
2000	12	11	0.152	0.031 – 0.404
2001	11	4	0.038	0.043 – 0.175
2002	12	4	0.045	0.026 – 0.182
2003	12	6	0.063	0.039 – 0.337
2004	4	0	0	-----
Cap K Ranch Pond # 2 outflow (to Pond # 3)				
2000	12	12	2.830	0.617 – 15.56
2001	11	11	0.534	0.043 – 1.010
2002	12	11	0.852	0.038 – 2.588
2003	12	7	0.338	0.125 – 1.082
2004	6	6	0.177	0.028 – 0.356
Cap K Ranch Pond # 3 outflow (to Pond # 4)				
2000	6	2	0.0173	0.052
2001	11	7	0.0557	0.022 – 0.144
2002	12	5	0.0498	0.025 – 0.386
2003	7	6	0.140	0.038 – 0.333
Cap K Pond # 4 outflow (to ponds #5 and #6)				
2000	6	3	0.0213	0.027 – 0.082
2001	11	5	0.0481	0.021 – 0.206
2002	12	2	0.0077	0.017 – 0.075
2003	7	3	0.044	0.063 – 0.177
2004	2	0	0	-----
Cap K Pond # 6 effluent to Fryingpan River				
1998	5	3	0.056	0.024 – 0.195
1999	11	10	0.591	0.026 – 1.387
2000	24	23	1.405	0.028 – 6.480
2001	16	13	0.136	0.030 – 0.583
2002	10	3	0.0154	0.023 – 0.085

No TAMs were detected in the Arkansas River water just upstream of Hagen's Western Fisheries (HNU) unit near Nathrop, Colorado in 17 months of water filtration. Low numbers of TAMs were detected in the effluent return to the river from the HNU unit on six of 11 filtration occasions during 2003. None were detected during the first six months of 2004. Filtration at the Blue Valley Ranch (BVR) on the Blue River indicated densities of TAMs in the inlet channel and outflow from the lakes on the property were no higher than that observed in the river proper upstream of the property.

Myxospores of *M. cerebralis* were first detected in wild rainbow trout in the Fryingpan River in November 1996. Prevalence and severity of infection has been slowly increasing since that time (Nehring and Thompson 2003). Extensive monthly water filtration efforts were initiated at numerous sites in the Fryingpan River between Basalt, Colorado and Ruedi Dam beginning in 1998 and continued through November 2004. Ambient levels of infectivity remain very low in the river upstream of the Cap K Ranch. TAM spores were detected only once in 77 consecutive months of filtration at the outfall of Ruedi Dam between July 1998 and November 2004. Farther downstream, just two km upstream of the point where the effluent from the Cap K Ranch ponds flows into the river, TAMs have been detected on eight of 54 sampling occasions, but ambient densities have remained low. In contrast, water filtration revealed high densities of TAMs in the effluent of pond # 6 in 1999 and 2000 (Table 3).

Extensive filtration efforts at most of the lakes on the ranch beginning in 2000 revealed high levels of TAM production throughout the series of six interconnected lakes on the Cap K Ranch. No trout of any kind had been stocked into the ponds on the ranch between the late-1980s and the spring of 2002. However, brook trout spawning has been highly successful each year in pond # 2. There has been minimal spawning success of feral rainbow trout at least in pond # 1. Trap netting and electrofishing studies reveal that each lake has a small population of large brown trout (50 – 66 cm total length) that presumably prey heavily upon brook trout fry and fingerlings during the late summer and fall months, after they have become infected with *M. cerebralis*. This dynamic predator-prey relationship facilitates rapid cycling of myxospores into the sediments where they can be ingested by *T. tubifex* worms susceptible to *M. cerebralis*, resulting in TAM production from March into May each year. This dynamic is similar to the cycling of myxospores released from decaying kokanee salmon carcasses into the sedimentation ponds at the ROJ unit in September through November during those years before the carcasses were removed (1996 – 1999). In those years a 60-day pulse in TAM production in the sedimentation ponds occurred from mid-March through mid-May.

Implementation of a number of BMPs at the Cap K Ranch has reduced ambient TAM levels by more than an order of magnitude at the most severely infected sites (ponds # 2 and 6). See Table 3 for details. Removal of large numbers of juvenile and adult brook trout from the ponds was accomplished in the fall of 2002 and again in the spring and fall of 2004. In addition, several thousand newly emergent brook trout fry have been captured by electrofishing and removed from pond # 2 each spring between 2001 and 2005, thereby reducing the number of young brook trout that can 1) become infected by the *M. cerebralis* parasite and, 2) be preyed upon by large predatory brown trout. During 2003, the lower end of Pond # 6 was converted to a passive sand filter intended to remove TAMs from the discharge prior to release to the river. In April and October 2004 and 2005, attempts were made to remove most of the trout from pond # 5. The ultimate management objective is to turn ponds 5 and 6 into a "sink" for TAMs where there are no salmonid hosts to parasitize. The conveyance channel that connects the outlet of pond 4 with the inlet of pond 5 is approximately one km long. In such a case any TAMs from pond 4 transported to pond 5 would hopefully settle out before floating through ponds 5 and 6 and therefore not be discharged into the Fryingpan River.

Myxospore Case Studies

Wild Trout Myxospore Burdens - Since 1994, more than 10,000 PTD analyses of fish samples have been completed as a part of the CDOW's research effort to document the impacts of WD on wild trout populations and evaluate the dynamics of the *M. cerebralis* infection cycle in natural ecosystems. The data in Table 4 summarizes prevalence (percentage of sample testing positive) and severity of infection (as measured by cranial myxospore concentration) by species versus relative levels of TAM density as determined by water filtration. The interfacing of these data sets provides significant insight into the relationships between exposure, prevalence and severity of infection, development of clinical signs of WD and the level of exposure that leads to population level impacts. Mean levels of TAM exposure over a period of 12 months ranged from zero to more than one TAM/L. The data in Table 4 are divided into four levels of increasing TAM density, each equating to one order of magnitude.

At those streams and study sites where the average TAM density ranged from zero to 0.006/L, prevalence of infection among brown trout generally ranged from zero to 30 percent and the mean cranial myxospore level was generally less than 500. Among wild rainbow trout, prevalence of infection ranged from 45 to 100 percent, while mean cranial myxospore concentrations were < 50,000 at five of six study sites. More importantly, wild rainbow trout fry at this range of exposure were surviving to the juvenile life stage and older. No brook trout occurred at any of the study sites within this range of exposures. (See Table 4 for details).

At those times and locales where the mean TAM density ranged between 0.010 and 0.099/L prevalence of infection among brown trout increased, ranging between 10% and 70% and mean cranial myxospore concentrations ranged from 817 to 38,476. Among wild rainbow trout within this range of exposure, prevalence of infection ranged from 70 to 100% and

mean cranial myxospore concentrations were > 100,000. Prevalence of infection among brook trout ranged from 20 to 100% and mean cranial myxospore burdens ranged from 6,365 to > 100,000. Moreover, clinical signs of whirling disease were evident among both rainbow trout and brook trout in some specimens at some study sites in this range of exposure. As mean TAM exposure levels approach 0.02 TAMs/L, detectible reductions in survival and recruitment of rainbow trout fry become evident. For brook trout, detectible reductions in survival and recruitment begin to occur at mean TAM densities > 0.06/L (Table 4).

Table 4. Comparisons of mean levels of exposure to different levels of TAMs/liter for 12 months and prevalence (percentage of fish testing positive) and severity of infection as reflected in mean numbers of *M. cerebralis* myxospores in cranial tissues of brown, brook, cutthroat and rainbow trout collected from study sites across Colorado.

Stream Name and Study Site	Mean TAMs/L	Brown Trout %+	Brown Trout Mean Myxospores	Rainbow Trout %+	Rainbow Trout Mean Myxospores	Brook Trout %+	Brook Trout Mean Myxospores
Mean Levels of TAM Exposure for 12 Months Ranging from 0.0000/Liter to 0.0099/Liter							
Middle Fork S. Platte Above Montgomery Reservoir	0.0000	---	-------	0	0 [a]	---	-------
Buckeye Creek one km above Buckeye Lake	0.0000	0	0	70	9,660	---	-------
Dolores River below McPhee Dam	0.0000	70	2,274	100	339,160	---	-------
Spring Creek (SC) one km upstream of SC Reservoir	0.0000	10	111	---	-------	---	-------
Dolores River below McPhee Dam	0.0039	0	0	100	46,280	---	-------
Fryingpan River below Ruedi Dam	0.0000	10	139	100	16,420	---	-------
Big Thompson River below Lake Estes	0.0036	0	0	45	14,445	---	-------
Dolores River at CDOW Irrigation Weir	0.0055	30	444	50	14,083	---	-------
Mean Levels of TAM Exposure for 12 Months Ranging from 0.0100/Liter to 0.0999/Liter							
Spring Creek one km below Spring Creek Reservoir	0.0109	30	833	---	-------	---	-------
Fryingpan River two km upstream of Cap K Ranch	0.0150	10	38,476	100	278,444	---	-------
South Platte R. five km below Cheesman Reservoir	0.0175	45	1,420	100	107,058	---	-------
Beaver Creek one km below Beaver Creek Reservoir	0.0218	70	28,183	71	184,229 [b]	---	-------
Spring Creek one km below Spring Creek Reservoir	0.0304	70	12,448	---	-------	---	-------
S. Cottonwood Creek 3km above Cottonwood Lake	0.0413	10	817	---	-------	20	6,365
Williams Fork River @ CDOW Irrigation Weir	0.0542	70	3,580	---	-------	---	-------
Middle Fk S. Platte 0.3 km below Montgomery Rsvr.	0.0660	---	-------	---	-------	100	104,880 [b]
Cap K Ranch Pond # 1 outflow	0.0802	---	-------	---	-------	83	118,500 [b]
Mean Levels of TAM Exposure for 12 Months Ranging from 0.100/Liter to 0.999/Liter							
Cap K Ranch Pond # 3	0.107	---	-------	---	-------	78	54,800 [b]
Middle Fk S. Platte 0.3 km below Montgomery Rsvr	0.162	---	-------	---	-------	70	304,062 [b]
Gunnison River in Ute Park (7/01 – 6/02)	0.164	80	14,566 [b]	100	823,246 [bc]	---	-------
Middle Fk S. Platte 4.8 km below Montgomery Rsvr	0.192	80	47,632 [b]	---	-------	---	-------
Fraser River one km upstream Windy Gap Reservoir	0.207	100	50,700	100	355,506 [b]	---	-------
Middle Fk S. Platte 0.3 km below Montgomery Rsvr	0.266	---	-------	---	-------	90	350,667 [b]
S. Cottonwood Creek @ Cottonwood Lake outflow	0.286	50	14,739	100	589,333 [b]	---	-------
Middle Fk S. Platte 0.3 km below Montgomery Rsvr	0.353	---	-------	---	-------	100	94,440 [b]
Colorado River 26 km below Windy Gap Reservoir	0.434	76	40,881 [b]	---	-------	---	-------
Middle Fk S. Platte 4.8 km below Montgomery Rsvr	0.466	100	76,700 [b]	---	-------	---	-------
Beaver Creek 1 km below Beaver Creek Reservoir	0.567	60	20,880	100	333,008 [b]	---	-------
Spring Creek 19 km below Spring Creek Reservoir	0.614	100	62,500 [b]	---	-------	---	-------
Big Thompson River below Idylwilde Dam	0.756	50	3,311	100	333,606 [b]	---	-------
Middle Fk S. Platte 4.8 km below Montgomery Rsvr	0.925	90	26,830 [b]	---	-------	75	126,975 [b]
Mean Levels of TAM Exposure for 12 Months greater than 1.000/Liter							
Cap K Ranch Pond # 2	1.090	---	-------	---	-------	100	183,033 [b]
Colorado River 1.9 km below Windy Gap Reservoir	2.368	100	58,707 [b]	---	-------	---	-------

[a] These fish were cutthroat trout that tested negative by PCR and PTD for five consecutive years between 1998 and 2002. TAMs were never detected at any time during monthly water filtration over the same time period.
[b] One or more clinical signs of whirling disease including skeletal or cranial deformities, shortened gill covers, black tail observed in this species of trout at this study site on one or more occasions.
[c] Previously unexposed Colorado River rainbow trout (CRR) ten cm fingerlings planted in June 2002 and collected on January 20, 2003. Wild stream spawned rainbow trout fry have not survived to age 1 in this section of the Gunnison River since 1994.

When mean densities of TAMs exceed 0.100/L prevalence of infection among brown trout increases to 50 – 100% and mean cranial myxospore burdens almost always exceed 14,000, ranging up to 76,700 or greater. Mean cranial myxospore concentrations have been > 100,000 at some locations and spore burdens among individual brown trout have exceeded 1,000,000 at Spring Creek below Spring Creek Reservoir. Clinical signs of WD including cranial and skeletal deformities as well as higher incidence of blacktail become evident among brown trout fingerlings as mean TAM densities increase from 0.100/L to 0.9/L. Incidence of blacktail among brown trout fry and fingerlings usually ranges between five and 10% when mean TAM densities range between 0.10 and 0.20/L. At study sites where mean TAM densities exceed 1.0/L, incidence of blacktail among brown trout fry and fingerlings reaches 90 to 100%, often accompanied by severe spinal deformities. These severe clinical signs of WD in brown trout were a common occurrence every fall from 1994 through 2000 at study sites on the upper Colorado River in the first ten km of river below the outlet of Windy Gap Reservoir.

Wild rainbow trout fry do not survive to age 1 in any stream in the state where sampling has shown mean TAM densities are ≥ 0.10/L. Significant negative impacts on wild rainbow trout fry survival and recruitment to age 1 occur at mean TAM densities as low as 0.01 to 0.02/L. This has been well documented on numerous streams across Colorado in the 1990s (Nehring and Thompson 2001). Streams where this has been the case include Beaver Creek below Beaver Creek Reservoir in the South Fork Rio Grande drainage and South Cottonwood Creek, as well as portions of the Cache la Poudre, Colorado, Dolores, Fryingpan, Gunnison, South Fork Rio Grande, South Platte, and Williams Fork rivers.

It is noteworthy that as TAM densities declined in Beaver Creek downstream of Beaver Creek Reservoir in 2002 and 2003 (compared to 1998 and 1999) density of wild rainbow trout ≥ 15 cm increased. Numbers of age 1+ fish at this site increased dramatically in the fall of 2003 compared to all other sampling years between 1998 and 2002 (Thompson 2004). Completion of a habitat improvement project by the San Luis Valley chapter of Trout Unlimited that eliminated a sediment-laden side-channel that provided optimal habitat conditions for one or more colonies of *T. tubifex* is believed to be the primary reason for the dramatic increase in survival of juvenile rainbow trout in this stream.

For brook trout, mean TAM densities ranging between 0.10/L and 0.40/L negatively affect fry recruitment. Decreased survival and clinical signs of disease are readily apparent. Clinical signs of disease include skeletal and cranial deformities, exophthalmia, blacktail and whirling behavior. Moreover, in streams or stream reaches where monitoring has shown mean TAM densities are ≥ 0.50/L, brook trout populations generally declined to near extirpation within three to five years. This was documented on the Middle Fork of the South Platte River at the study site 4.8 km downstream from Montgomery Reservoir. In the 1980s, brook trout were abundant in South Cottonwood Creek downstream of Cottonwood Lake but were no longer present in the late 1990s. Similarly, brook trout once abundant in the lower portions of Drowsy Water Creek (tributary to the Colorado River in Middle Park) in the early 1990s have been extirpated from that stream reach as well.

The Middle Fork of the South Platte River upstream of Montgomery Reservoir was the only stream supporting a self-sustaining cutthroat trout population where monthly water filtration, PCR and PTD testing has been extensively conducted. Five years (July 1998 – June 2003) of monitoring conclusively demonstrated that *M. cerebralis* was not enzootic upstream of Montgomery Reservoir. (For more detailed information see Table 4 in this report and pages 245 and 246 in Nehring and Thompson 2003).

Myxospore Burdens Among Fry, Fingerling and Adult Trout - Comparisons of cranial myxospore concentrations for brown, brook, rainbow and the various sub-species of cutthroat trout from the exposures in the Colorado River are summarized in Table 5. These data reveal that rainbow trout produced substantially greater numbers of myxospores than any other species or sub-species of salmonid subjected to continuous flow-through exposures in the Colorado River from 1994 through 1999.

The data in Table 6 summarize the prevalence (percent positive in sample) and severity of infection (mean and range of cranial myxospores) among rainbow trout of varying sizes (mean weight in grams at time of initial exposure). The high prevalence and high densities of cranial myxospores found in large rainbow trout seem non-synchronous with the findings of Markiw (1992a). She reported cranial myxospore concentrations averaged only 6,150 among rainbow trout ranging in age from three to 3.5 years, held in water at 12.5 °C and subjected to an average of 106,350 TAMs/day for 3.5 months and sacrificed for analysis at seven months post initial exposure. However, careful scrutiny of Markiw's data suggests that cranial myxospore burdens in rainbow trout exposed to a single dose of TAMs did not plateau until approximately 1,875-2,250 degree-days (°C) after initial exposure. Thus, in Markiw's study, the cranial myxospore concentration observed at seven months after initial exposure would only be reflective of the daily TAM exposure that occurred during the first 30 to 60 days of the experiment. In two different studies (Thompson et al. 1999; Thompson et al. 2002) where various species of trout were continuously exposed to TAMs in flow-through tanks in the Colorado River for up to a year, cranial myxospore concentrations did not plateau until 2,500 – 2,600 degree-days (°C).

The data in Table 7 summarize the prevalence (percent positive) and severity of infection (mean and range of cranial myxospores) among rainbow trout of varying strains and sizes (mean weight in grams at time of initial exposure) across four different streams with varying levels of TAM density and different duration(s) of exposure. Monthly water filtration studies revealed that the Dolores River sites and the Fryingpan

Table 5. Comparisons of prevalence (percent of sample infected) and severity of infection (cranial myxospore concentrations) among species of trout, varying in age (degree-days of growth post hatch prior to initial exposure - °C) and size (mean weight - g) at the initial exposure to *M. cerebralis*, after continuous exposure as sentinel fish in the Colorado River at the bridge crossing on the Breeze State Wildlife area in the 1990s. Acronyms in the species column stand for the following trout species: CRN-Colorado River native cutthroat; GBN-greenback native cutthroat; RGN-Rio Grande native cutthroat; SRN-Snake River native cutthroat; CRR-Colorado River rainbow trout.

Species	First Exposure		Total Days Exposed	N		Myxospores in Fish Tested by PTD Methodology	
	° Days	Wt g			% +	Mean	Range Among Fish Testing Postive
CRN[a]	202	0.28	≤ 132	312		Fish suffered 87% acute/chronic mortality	
Brook[a]	112	0.18	≤ 132	312		Fish suffered 85% acute/chronic mortality	
CRN	190	0.30	≥ 267	50a	100	85,517	13,616 – 450,667
CRN	190	0.30	411	3b	100	182,672	143,372 – 248,311
CRN	190	0.15	273	40	98	185,771	2,944 – 628,288
CRN	190	0.15	387	41	100	192,475	13,578 – 1,447,600
CRN	472	0.56	≥ 267	42[a]	100	96,383	12,089 – 322,222
CRN	472	0.56	411	14[b]	100	503,903	215,889 -1,203,689
RGN	650	0.22	273	40	98	71,598	417 – 309,978
RGN	650	0.22	387	18	100	124,563	13,750 – 286,999
GBN	840	0.39	273	40	100	75,623	3,556 – 353,556
GBN	840	0.39	388	20	90	71,929	4,400 – 270,217
CRR	0	0.08	≥309	30[a]	100	249,586	41,400 – 1,011,289
CRR	0	0.08	460	22[b]	100	585,090	2,056 – 2,280,000
CRR	202	0.15	≥297	9[a]	100	213,221	55,200 – 600,178
CRR	220	0.16	≥275	21[a]	95	192,232	22,583 – 1,139,111
CRR	220	0.16	388	20[b]	100	213,570	22,583 – 429,083
CRR[c]	220	0.20	≥275	18[a]	100	1,049,183	317,055 – 2,039,111
CRR	220	0.20	388	39[b]	97	664,009	18,667 – 1,795,022
CRR	450	0.51	≥268	43[a]	100	86,519	4,500 – 292,967
CRR	1,720	5.14	334	22[b]	100	215,177	13,317 – 743,167
SRN	185	0.12	312	40	90	75,172	1,333 – 475,656
SRN	185	0.12	426	40	65	107,638	900 – 1,219,516
SRN	980	1.18	273	40	58	43,957	544 – 483,983
SRN	980	1.18	387	16	44	49,483	1,406 – 139,778
Brown	0	0.08	343	40	63	22,363	844 – 73,700
Brown	0	0.08	442	18	56	45,280	3,356 – 105,700

[a] Results for fish that died of WD before the end of the exposure experiment and prior to the time when formation of myxospores was complete.
[b] Results for fish that survived to the end of the exposure experiment.
[c] The fish in this test group were largely the progeny of a single very large seven year old female rainbow whose eggs were fertilized with milt from five or six seven-year old male rainbow trout.

River at the Ruedi Dam collection site had the lowest levels of TAM density. Similarly, water filtration studies also indicated that the Taylor Creek site on the Fryingpan River and the Deckers site on the South Platte River were areas where TAM densities would be classified as low to moderate. Density of TAMs at both sites on the Gunnison River would be considered quite high. Cranial myxospore concentrations among the two groups of Tasmanian (TAS) rainbow trout weighing 171 or 182 grams were either zero or low prevalence and very low numbers when exposed at the two Dolores River sites and the Ruedi Dam collection site for the Fryingpan River. The same groups of fish exposed at the Taylor Creek site on the Fryingpan River and the Deckers site on the South Platte River experienced 57% to 90% prevalence of infection with mean cranial myxospore concentrations ranging from 16,700 to 54,900 (Table 7). In contrast, the same groups of rainbow trout exposed to the Gunnison River in the Smith Fork to North Fork reach experienced 100% prevalence of infection. Mean cranial myxospore concentrations were 484,457 and 582,696 and maximum myxospore concentrations ranged up to almost two million in both exposure groups.

Colorado River rainbow (CRR) trout weighing only 1.8 grams at initial exposure stocked into the Dolores River at the Metaska site experienced 100% infection but had an average of only 42,680 cranial myxospores even after 715 days in the river. In contrast, CRR rainbow trout that weighed 11.8 grams at initial exposure when stocked into the Gunnison River at the

Table 6. Cranial myxospore burdens in Colorado River rainbow trout (CRR) initially exposed to TAMs of *M. cerebralis* at different sizes and ages at various sites within the upper Colorado River downstream of Windy Gap (WG) Reservoir between 1995 and 2000.

Site	Exposure		First Exposed	N	Cranial Myxospores in Fish Tested by PTD Methodology		
	Size (g)	Days	mmddyy		% +	Mean	Range Among Fish Testing Postive
Below Windy Gap	5.15	326	10/19/95	10	80	42,160	1,060 – 126,500
Breeze Bridge	5.15	326	10/19/95	10	90	57,240	3,500 – 173,100
Breeze Bridge	5.15	420	07/15/97	17	82	35,620	4,440 – 173,300
Breeze Bridge	16.3	421	07/22/00	8	100	1,216,700	385,467 – 2,540,033
Williams Fork	21.6	421	07/22/00	3	67	88,105	77,333 – 186,983
Hitching Post	24.9	421	07/22/00	5	80	255,663	235,400 – 402,183
Below Windy Gap	24.9	365	06/01/00	60	92	159,926	3,372 – 773,6121
Paul Gilbert SWA	460	133	06/01/00	4	100	239,219	2,933 – 866,433
Below Windy Gap	500	686	06/01/00	10	90	101,278	1,667 – 300,000

Table 7. Comparisons of cranial myxospore burdens among various strains of rainbow trout exposed to *M. cerebralis* TAMs at different sizes and ages at various sites across four different streams with different levels of TAM density and different lengths of time between stocking (initial exposure) and collection for PTD analysis. All trout that averaged 171 grams in weight at each stocking site were TAS rainbow trout from the Rifle Falls State Fish Rearing Unit (SFRU). All trout that averaged 182 grams weight at each stocking site were from the Crystal River SFRU.

Site	Exposure		First Exposed	N	Cranial Myxospores in Fish Tested by PTD Methodology		
	Size (g)	Days	mmddyy		% +	Mean	Range Among Fish Testing Postive
Dolores River below McPhee Dam							
Metaska[a]	1.8	715	10/04/00	12	100	46,280	2,989 – 105,622
Metaska	171	164	04/08/02	25	0	0	--------
Metaska	182	164	04/08/02	24	13	742	4,300 – 6,811
Metaska	171	1,082	04/08/02	1	100	153,033	153,033
Metaska	182	1,082	04/08/02	4	25	39,000	156,000
Metaska	unk	730+	unknwn	9	100	219,302	25,000 – 731,928
Ferris Canyon	171	164	04/08/02	25	0	0	--------
Ferris Canyon	182	164	04/08/02	24	25	2,400	4,267 – 21,011
Fryingpan River below Ruedi Dam							
Ruedi Dam	171	200	04/12/02	20	0	0	--------
Ruedi Dam	171	565	04/12/02	10	90	284,158	20,511 – 1,179,383
Ruedi Dam	182	200	04/12/02	20	0	0	--------
Ruedi Dam	182	565	04/12/02	10	80	102,179	75,328 – 279,194
Taylor Creek	171	200	04/12/02	20	90	54,888	6,344 – 149,750
Taylor Creek	171	565	04/12/02	15	100	1,399,169	25,889 – 11,270,922
Taylor Creek	182	200	04/12/02	20	85	38,414	3,922 – 213,150
Taylor Creek	182	565	04/12/02	15	100	1,087,742	25,311 – 2,090,256
Gunnison River downstream of Black Canyon of the Gunnison Gorge							
Smith Fork	171	204	04/11/02	30	100	582,696	90,400 – 2,101,111
Smith Fork	182	204	04/11/02	27	100	484,457	23,767 – 1,955,733
Smth Fork[c]	119	145	06/23/03	31	100	311,828	20,000 – 1,013,333
Smth Fork[d]	51.8	145	06/23/03	31	94	173,217	33,333 – 844,444
Smth Fork[e]	61.9	145	06/23/03	31	97	262,115	2,778 – 1,561,000
Ute Park[b]	11.8	216	06/18/02	10	100	823,246	50,933 – 2,338,661
Ute Park[b]	11.8	310	06/18/02	11	100	502,571	151,800 – 1,106,700
South Platte River below Cheesman Dam							
Deckers	171	210	04/08/02	21	76	37,308	4,239 – 292,444
Deckers	182	210	04/08/02	21	57	16,734	5,561 – 1,740,000

[a] Colorado River strain rainbow (CRR) stocked in the river at an average size of 1.8 grams almost two years before collection for PTD analysis. Collected from same site on same days as the fish from the Rifle Falls and Crystal River SFRUs.

[b] CRR strain fingerling rainbow trout stocked at an average size of 11.8 g.

[c] CRR strain rainbow; [d] Arlee strain rainbow; [e] Bellaire strain rainbow

Ute Park collection site experienced 100% infection and cranial myxospore concentrations averaging over 802,000 after 216 days in the river (Table 7).

During 2003, three different strains of rainbow trout of approximately the same size were marked and stocked into the Smith Fork to North Fork reach of the Gunnison River to determine their relative sensitivity to *M. cerebralis*. The CRR strain experienced the greatest level of infection (as determined by cranial myxospore concentrations). Average cranial myxospore concentration in this strain was over 311,800 compared to 262,000 and 173,000 for the Bellaire and Arlee strain rainbow trout, respectively. Given that the Arlee strain produced the fewest myxospores but was more than 50% smaller than the CRR strain at the time of initial exposure, the data are suggestive that there may be differences in susceptibility among these three different strains (Table 7).

Similar exposure experiments were also completed on some small lakes using catchable rainbow trout from the Rifle Falls and Crystal River SFRUs during 2002. Results of those studies are summarized in Table 8. The mean cranial myxospore concentrations detected in the TAS rainbow trout in the Cap K ponds vary widely among the five ponds. However, rainbow trout in ponds 2 and 5 had much higher spore counts than those trout collected from ponds 1, 3 and 4. Not surprisingly, ponds 2 and 5 historically had very high mean TAMs/L compared to the other ponds (see Table 3 for details). In contrast, in ponds 1, 3 and 4 that had much lower estimated densities of TAMs, mean cranial myxospore counts from the marked rainbow trout collected from those ponds in the fall of 2002 were much lower.

In another experiment at the Roaring Judy ponds, *M. cerebralis*-negative catchable size rainbow trout were stocked in June to determine what the prevalence and severity of infection would be after approximately five months exposure. Among two groups of fish held in the pond for 158 days, prevalence of infection averaged 29% and 44% and average myxospore burdens were 5,083 and 17,240 (Table 8). In contrast, among two groups of rainbow trout known to have been exposed to ambient levels of TAMs in the Roaring Judy ponds and/or the water conveyance channel flowing into the ponds for 720 and 523 days, respectively, prevalence of infection averaged 65% and 94% and mean cranial myxospore concentrations were 214,917 and 365,735. Maximum cranial myxospore concentrations among individual trout in each group exceeded 1.38 million (Table 8).

The data in Tables 7 and 8 suggest that maximizing angler harvest of catchable rainbow trout within six months of the time of stocking into aquatic habitats where *M. cerebralis* is enzootic should reduce the risk of exacerbating TAM production. Conversely, as time of exposure begins to exceed 200 days, prevalence of infection usually approaches 95 to 100% and average cranial myxospore concentrations range from 100,000 to almost 500,000 with myxospore concentrations among individual fish ranging as high as one

Table 8. Comparisons of cranial myxospore burdens in Tasmanian (TAS) strain rainbow trout exposed to *M. cerebralis* TAMs at different sizes and ages in various small ponds with different levels of TAM density and for different lengths of time between stocking (initial exposure) and collection for PTD analysis. All trout that averaged 171 grams in weight at each stocking site were TAS rainbow trout from the Rifle Falls State Fish Rearing Unit (SFRU). All trout that averaged 182 grams weight at each stocking site were from the Crystal River SFRU.

Site	Exposure		First Exposed	N	Cranial Myxospores in Fish Tested by PTD Methodology		
	Size (g)	Days	mmddyy		% +	Mean	Range Among Fish Testing Postive
Cap K Ranch Ponds on the Fryingpan River							
Pond # 1	171	216	03/24/02	20	95	99,047	15,917 – 311,500
Pond # 1	182	216	03/24/02	20	95	61,520	6,456 – 203,600
Pond # 2	171	216	03/24/02	20	100	432,369	32,167 – 1,234,833
Pond # 2	182	216	03/24/02	17	100	368,441	43,700 – 1,403,733
Pond # 3	171	216	03/24/02	20	100	110,391	4,272 – 529,306
Pond # 3	182	216	03/24/02	24	92	64,667	5,828 – 279,933
Pond # 4	171	216	03/24/02	20	85	41,010	5,494 – 230,289
Pond # 4	182	216	03/24/02	20	95	59,744	5,556 – 250,600
Pond # 5	171	228	03/24/02	19	100	473,430	80,750 – 1,045,161
Pond # 5	182	228	03/24/02	21	100	336,860	6,933 – 843,922
Roaring Judy (RJ) West Ponds							
West Pond	134	158	06/20/03	23	44	17,240[a]	3,306 – 136,461
West Pond	195	158	06/20/03	28	29	5,083	3,344 – 40,000
West Pond	160	523	06/20/02	16	94	365,735[b]	7,189 – 1,387,422
RJ Channel	Unk	unk	2002?	21	100	367,442	3,683 – 2,242,483
RJ Channel	Unk	720	Unknwn	20	65	214,917	2,222 – 1,711,111

[a] This test group was Bellaire strain rainbow trout.

[b] Unmarked holdover rainbow trout from stocking in June 2002 or earlier.

million or more (Table 8). This is the case for catchable size fish stocked into streams as well as lakes where moderate infections exist. The data for the Fryingpan River in Table 7 for the Ruedi Dam and Taylor Creek study sites for each group of fish at 200 days PE versus 565 days PE are particularly illustrative of this point. Two of 5 marked TAS rainbow trout collected from the Dolores River in March 2005, that had been in the in the river for 1,082 days had cranial myxospore concentrations that exceeded 150,000 (Table 7).

All of the foregoing suggests that stocking of fry, fingerling or catchable rainbow trout not previously exposed to *M. cerebralis* into aquatic habitats with moderate to high levels of *M. cerebralis* infectivity may exacerbate ambient levels of infection in a lake or reservoir. Waters with high levels of infection are of concern because of the implications for potential spread of the parasite and development of population-level impacts for wild salmonids upstream as well as in downstream receiving waters.

Clinical Whirling Disease in Fry and Fingerling Trout - In Colorado, the number of streams and stream mileage impacted by the loss of wild rainbow trout has increased since 1994 (Nehring et al. 1998; Nehring and Thompson 2001; Nehring 2003). Annual stocking of wild-strain fingerling rainbow trout has been one management approach directed towards offsetting the loss of natural reproduction. Much of the information compiled on vulnerability of fry and fingerling trout to *M. cerebralis* since the 1970s suggests that rainbow trout fingerlings do not suffer substantial mortality even when exposed to relatively large doses of TAM spores once they reach about five cm in length or three to four months of age (O'Grodnick 1979; Markiw 1991, 1992a,b). Ryce et al. (2004) reported that clinical evidence of WD and mortality among young rainbow trout exposed to 100, 1000 or 10,000 TAMs/fish was substantially reduced when initial exposure to *M. cerebralis* did not occur until the fish had reached an age of nine to 11 weeks post-hatch or older. At nine to 11 weeks of age (756 – 924 degree-days at 12 ° C) the average weight of the exposed fingerlings ranged from 0.86 to 1.30 g. Mortality rates among rainbow trout exposed to 100, 1000 and 10,000 TAMs/fish at one week post-hatch ranged from 50% to 70% (Ryce et al. 2004). In the same study, cranial myxospores among rainbow trout fry exposed to 100 TAMs/fish at one week post-hatch averaged almost 600,000 and ranged up to about 760,000.

However, it is unlikely that the results of single dose exposures of trout to varying concentrations of TAMs in a laboratory setting accurately reflect the epidemiology of *M. cerebralis* in fry or fingerling trout of any species continuously exposed to the parasite in the natural environment. Thompson et al. (1999) conducted long-term continuous flow-through exposures of various sizes and species of salmonids to ambient levels of *M. cerebralis* TAMs in the Colorado River over a five-year period from 1994 through 1999 in an effort to address this issue. These studies reaffirmed some of the previous findings, e.g., brown trout are highly resistant to the parasite (O'Grodnick 1979) in comparison to rainbow trout (O'Grodnick 1979; Hedrick et al. 1999a). However, the study (Thompson et al. 1999) also demonstrated that brook trout and Colorado River cutthroat trout suffered high chronic mortality when exposed to continuous doses early in life. Brook and cutthroat trout first exposed at three weeks post-hatch suffered 87% and 85% cumulative mortality within 132 days. In comparison, rainbow trout fry concurrently first exposed at hatch and three weeks post-hatch in the same study experienced only 40% and 30% mortality in 132 days. Each species treatment group had four replicate exposures. Even greenback, Rio Grande and Colorado River cutthroat exposed at a much larger size than rainbow trout did not survive as well. Heavy mortality occurred among all sub-species of cutthroat trout native to Colorado during the spring and summer months (May through mid-August) of the second year in the river.

Trout Population Response to *Myxobolus cerebralis* Infection in Streams

In the 1970s and 1980s, before the introduction and establishment of *M. cerebralis* in Colorado, wild rainbow trout populations had been established and were thriving in extended reaches of many major coldwater streams in the state. These streams included Beaver Creek in the South Fork of the Rio Grande drainage, the Big Thompson, Cache la Poudre, Colorado, Dolores, Fryingpan, Gunnison, North Platte, Rio Grande, Roaring Fork, South Platte and Williams Fork rivers. By the end of the 20th century (with the exception of the Big Thompson and perhaps the North Platte rivers), wild rainbow trout populations in long reaches of all of the aforementioned streams were in a state of decline or near complete collapse due to WD (Nehring and Thompson 2001).

Detailed information on the suspected mode of introduction of the *M. cerebralis* parasite, the chronology of the outbreak of WD, the dynamics of the infection cycle and the collapse of the wild rainbow trout populations in each of these streams have been chronicled in great detail (Nehring and Thompson 2003). However, a brief review of the unique sets of factors that led to the epizootic outbreaks of *M. cerebralis* in each of these streams is instructive in several ways. First, it provides insight into the broad adaptability of this parasite that has negatively impacted self-sustaining salmonid populations across a wide range of aquatic ecosystems in Colorado. Second, a thorough understanding of the effects of WD in Colorado over the past 15 years provides insight for evaluation of risk related to fishery management programs and for taking appropriate action to minimize or avoid future unwanted consequences. Third, a thorough understanding of the case histories is foundational for development of wise public policy for containment and control of the continuing threat that *M. cerebralis* poses for salmonid fisheries in Colorado and across the western U.S.

Beaver Creek (South Fork Rio Grande drainage) – By the late 1980s a thriving wild rainbow trout population had been established throughout most of a 120 km reach of the Rio Grande drainage upstream of Del Norte, Colorado. Wild rainbow trout resident to the 35 km reach of river between South Fork and Del Norte migrated up the four km reach of Beaver Creek downstream of Beaver Creek Reservoir (BCR) and the South Fork of the Rio Grande (SFRG) to spawn each spring. Sampling surveys conducted each fall between 1988 and 1992 revealed that Beaver Creek accounted for 48 - 68% of the total rainbow trout fry production in the SFRG drainage. Estimates of rainbow trout fry production ranged between 51,000 and 67,000 for three years of the five-year study. In contrast, brown trout fry production throughout the SFRG basin averaged 45,300 during the same five-year period. By the fall of 1992, Nehring (1993b) concluded a robust wild rainbow trout population was thriving throughout much of the Rio Grande basin as evidenced by the abundance of wild rainbow trout fry production and sustained increases in juvenile and adult wild rainbow trout density and biomass observed between 1988 and 1992.

However, PTD testing of trout in Beaver Creek below BCR and the SFRG drainage between 1994 and 1998 revealed prevalence of *M. cerebralis* infection was usually 100% among wild rainbow trout fingerlings. Prevalence and severity of infection among young-of-the-year (YOY) and age 1+ brown trout was increasing. Clinical signs of WD among rainbow trout fingerlings included exophthalmia, blacktail, skeletal deformities and whirling behavior. Concomitant with these observations, estimated abundance of YOY rainbow trout had declined to 7,500 in September 1998 compared to an average of 27,500 for the five-year period from 1988 through 1992, prior to the time when *M. cerebralis* is thought to have become enzootic in the drainage.

Review of CDOW records reveal 26,000 to more than 44,000 catchable rainbow trout exposed to *M. cerebralis* were stocked annually in BCR between 1992 and 1994. Stocking of *M. cerebralis*-exposed catchable rainbow trout in Big Meadows Reservoir in the headwaters of the SFRG also occurred between 1992 and 1994. The introductions in these reservoirs were most likely the cause of establishment of the parasite and the resulting infection in both Beaver Creek and the SFRG drainage by April 1994. Concurrent with the annual loss of rainbow trout fry production in this system, the wild rainbow trout population in the Rio Grande began to decline. By 1999, density and biomass of wild rainbow trout had declined by more than 90% in an 11-km study reach downstream of the confluence with the SFRG, falling to levels only observed between 1981 and 1984, prior to the time when research and management efforts to establish wild rainbow trout in the drainage were initiated (Nehring and Thompson 2003).

Testing of filtered water and PTD testing of brown trout collected from Beaver Creek 0.5 km upstream of Beaver Creek Reservoir in late 1999 and early 2000 revealed *M. cerebralis* was enzootic in the stream at that point. However, population estimates and testing of wild brook, brown and rainbow trout in Beaver Creek three km upstream of Beaver Creek Reservoir conducted in 2000 and 2002 revealed no evidence of infection in the fish when tested by PTD and PCR. Monthly water filtration studies were carried out at this site from November 1999 through March 2001, through most of 2002 and again in the spring of 2003. No TAM spores were ever detected in the water filtrates collected at this site. Upstream of the reservoir, the stream meanders in a sweeping oxbow pattern through a low gradient meadow. There are no barriers to free movement of fish between the inlet to the reservoir and the upstream sampling site. It is both surprising and encouraging that the parasite has not been vectored 3 km upstream of the reservoir by fish-eating birds, mammals, anglers or migration of infected fish during 10 years since the parasite was introduced into the reservoir in 1992.

Big Thompson River – Myxospores of *M. cerebralis* were first detected in wild rainbow trout in this river at four sampling sites downstream of Lake Estes in March and April 1997. Based on empirical evidence, observations and chronology of experiences in similar streams across Colorado there was great concern that this wild rainbow trout fishery was in danger of collapse as well. Intensive fish population monitoring from 1997 through 2002 revealed that wild juvenile and adult rainbow trout largely dominated the trout population at most sampling sites on the river between Lake Estes and Idylwilde Dam downstream. Water filtration efforts and PTD testing of trout between 1998 and 2003 revealed the parasite is enzootic in the Big Thompson drainage upstream and downstream of Lake Estes but not at levels sufficient to precipitate an acute epizootic that would be lethal to fry and juvenile rainbow trout (Nehring and Thompson 2003).

Conditions unique to the Big Thompson River may have prevented the collapse of the rainbow trout fishery. First, and perhaps most important, CDOW stocking records indicate Lake Estes and the North Fork of the Big Thompson River from Drake to Glen Haven were only stocked once with 11,250 catchable-size rainbow trout exposed to *M. cerebralis* in the spring of 1995. Given the heavy fishing pressure all along Colorado's Front Range, it is likely that most of these fish were quickly removed by anglers. All subsequent stocking of rainbow trout into Lake Estes has been from rearing units testing negative for *M. cerebralis*. High angler use results in rapid harvest of these catchable rainbow trout that insures removal from the system before they can significantly augment the input of *M. cerebralis* myxospores into the drainage. Second, because much of the Big Thompson River drainage lies inside Rocky Mountain National Park where there is no stocking of rainbow trout, the drainage as a whole has not been infused with hatchery-reared trout potentially exposed to *M. cerebralis*. Third, the Big Thompson River drainage downstream of Lake Estes is moderately high in gradient with a dearth of low-gradient, sediment-laden sections of stream that provide optimal habitat conditions for *T. tubifex*, the obligate alternate host necessary for TAM

production and completion of the life cycle. Fourth, there are no public or private trout rearing facilities in the drainage with associated sediment retention ponds that can provide optimal habitat conditions for aquatic oligochaetes such as *T. tubifex*. In the absence of adequate habitat for tubificid worms and no supplemental stocking of large numbers of catchable trout reared in facilities testing positive for *M. cerebralis*, this wild rainbow trout fishery has continued to thrive.

Anglers have speculated that the Big Thompson River wild rainbow trout are just resistant to *M. cerebralis*. To evaluate this theory, wild rainbow trout eggs collected by spawning wild fish in the Big Thompson River were hatched and the fry were exposed to controlled doses of *M. cerebralis* actinospores to assess their relative vulnerability to the parasite. The exposed fish were highly vulnerable (Schisler 2004), disproving the hypothesis that this population of rainbow trout had high resistance to the parasite.

Cache la Poudre River - Like the Big Thompson River, this stream lies within a deeply incised canyon and has a moderately high gradient throughout most of its reach. Except for a short section through the glacial moraine area approximately 80 km west of Fort Collins, this stream has very little habitat conducive to sustaining *T. tubifex* worms (Allen and Bergersen 2002). The Cache la Poudre River supported thriving wild rainbow and brown trout populations for 30 years or more. Extensively studied between the 1960s and the 1980s (Klein 1974; Nehring and Anderson 1985; Nehring 1987) prior to the establishment of *M. cerebralis*, monitoring of the wild rainbow and brown trout populations in this stream has continued (Schisler 2001; Nehring and Thompson 2003; Thompson 2004).

Unlike the trout population response in the Big Thompson River, collapse of the wild rainbow trout population in the Cache la Poudre River was well underway by the early 1990s (Nehring and Thompson 2001; Nehring and Thompson 2003). Several factors contributed to the development of a severe epizootic in this river that began in the late 1980s. First, the Poudre Rearing Unit (PRU), a large catchable rainbow trout production facility with ten or more earthen ponds is located on the upper reaches of the river approximately 80 km west of Fort Collins. Rainbow trout produced at the PRU facility first tested positive for *M. cerebralis* in 1988. Second, studies have shown that the earthen ponds at this unit supported dense populations of *T. tubifex* worms (Allen and Bergersen 2002) that produced high densities of TAMs of the *M. cerebralis* parasite (Nehring and Thompson 2001, 2003). Throughout all of the 1990s the TAM-laden effluent from earthen ponds was discharged into the river. Third, catchable rainbow trout produced in the earthen ponds were often heavily infected with *M. cerebralis*. Prevalence of infection was often 100 percent with mean cranial myxospore concentrations $\geq$ 470,000 and ranging as high as 1.63 million for individual trout (Nehring and Thompson 2003). Stocking of relatively small numbers of these fish in high elevation lakes or reservoirs was shown to exacerbate *M. cerebralis* infections in both standing waters where the fish were stocked and in wild trout populations in downstream waters (Nehring and Thompson 2003). Fourth, Schisler (2001) reported that more than one million trout from *M. cerebralis*-infected hatcheries and rearing units were stocked into the Cache la Poudre River and lakes and reservoirs tributary to the drainage between 1990 and 2001. Many of these fish were actually reared at the PRU facility.

All of these factors contributed to the loss of the thriving wild rainbow trout population in the river. However, sentinel fish studies (Thompson et al. 1999; Thompson et al. 2002) and water filtration studies conducted on the upper Colorado River (Thompson and Nehring 2000) and Cache la Poudre River (Nehring and Thompson 2003) demonstrated that TAM densities produced in the PRU sedimentation ponds and discharged to the river from 1997 through the spring of 2001 were sufficient to cause the complete loss of rainbow trout fry in the river downstream of the PRU facility. This would have been the case even without stocking of exposed catchable rainbow trout anywhere in the drainage.

Colorado River (Grand County) - Privately-reared rainbow trout exposed to *M. cerebralis* were stocked into at least three locations in private lakes or ponds in Grand County in the upper Colorado River basin in the mid-1980s. Rainbow trout collected from these sites in the fall of 1988 tested positive for myxospores of *M. cerebralis* according to archived records maintained by the CDOW Aquatic Animal Health Laboratory. Two of the three sites are located in the headwaters of the Colorado River upstream of Windy Gap Reservoir (WGR).

Studies that began in the fall of 1993 (Nehring 1993a) and continued through the 1990s demonstrated that WD was the primary factor implicated in the collapse of the wild rainbow trout population in the Colorado River in Grand County downstream from WGR (Nehring and Thompson 2001). The 1991 year class was the first to be impacted. Studies conducted during the summer of 1994 eliminated heavy metal pollution, avian and piscine predation, emigration, short or long term fluctuations in stream discharge, and thermal stress as possible co-factors implicated in the demise of the YOY rainbow trout (Walker and Nehring 1995). Subsequent research also revealed that gas supersaturation (Schisler and Bergersen 1999; Schisler et al. 1999a; Schisler et al. 2000) and ectoparasites (Schisler et al. 1999b) were not major exacerbating co-factors contributing to the loss of wild rainbow trout fry in this river.

Water filtration studies initiated in 1997 (Thompson et al. 1999) and long-term sentinel fish exposures conducted in 1998 and 1999 on the upper Colorado River (Thompson et al. 2002) clearly revealed WGR was a major source of *M. cerebralis* TAMs . Two separate studies conducted in 1998 demonstrated that WGR supported very high densities (37,000/m^2) of *T. tubifex* (Zendt and Bergersen 2000) that produced very large numbers of TAMs that passed through the reservoir (Nehring et al. 2003a). Nehring et al. (2003a) hypothesized that myxospores originating in salmonids in the Fraser and Colorado river drainages upstream of WGR were

transported into the reservoir during the spring run-off. WGR is not open to public fishing, has never been stocked with trout, and does not support a substantial trout population (Nehring and Thompson 2003). Four different gill net surveys between 1992 and 2004 repeatedly demonstrated very high densities of catostomid fishes consistently comprised approximately 90% of the fish fauna in WGR.

In an effort to restore the superb rainbow trout sport fishery in the upper Colorado River, CDOW fisheries research and management biologists have stocked tens of thousands of wild fingerling Colorado River rainbow trout (CRR) annually over the past decade, beginning in 1994. In recent years larger fingerling rainbow trout have been stocked in an attempt to increase survival. However, the effort had limited success according to trout population surveys conducted annually at numerous sites downstream of WGR (Nehring and Thompson 2003). Rainbow trout density and biomass at many locations remain approximately 90% below the levels observed between 1979 and 1986, prior to the time when *M. cerebralis* became established in the drainage.

Two or three factors operating in concert may tend to negate the stocking effort. First, high densities of large brown trout in the river undoubtedly exert heavy predation on rainbow trout fingerlings stocked into the stream in the first few weeks after stocking. Brown trout biomass in the Colorado River at many monitoring sites ranges from 200 to more than 400 kg/ha. Second, because brown trout are more than 100 times as resistant to *M. cerebralis* compared to rainbow trout (Hedrick et al. 1999a) they act as a source of myxospores that continually re-infect *T. tubifex* in the river. Third, continual exposure to heavy doses of *M. cerebralis* in the river may overcome the limited resistance to the parasite that Ryce et al. (2004) suggests occurs after rainbow trout have reached nine to 11 weeks of age and a mean weight of 1.30 g.

All of these factors operating in concert manifest in an extremely severe epizootic of *M. cerebralis* throughout much of the upper Colorado River and many tributary streams in Grand County. In the 25 km reach of the main river below WGR, fry sampling surveys conducted between 1994 and 1999 revealed that most wild rainbow trout fry died from acute exposure to *M. cerebralis* by early October. Overt clinical signs of WD were evident in YOY brown trout each year as early as July and occurred with increasing prevalence at the study sites in closer proximity to WGR (Nehring and Thompson 2001, 2003). In contrast, in the Williams Fork River (tributary to the Colorado River at Parshall) ambient levels of infection were lower. Water temperatures in this tailrace fishery below Williams Fork Reservoir were much colder, rarely exceeding 10 ° C at any time during the year. Clinical signs of WD were rare in brown trout and signs of disease in YOY rainbow trout did not begin to appear until December and January. In this river in the 1990s rainbow trout fry survived into March and April but died in May and June.

Dolores River (below McPhee Reservoir) – Wild CRR trout fingerlings were stocked into the Dolores River beginning in the early 1990s. Annual sampling of this river at three locations in the upper 18-km reach has occurred almost every year since the mid-1980s. Spring boat electrofishing operations were conducted every year between 1991 and 2000 with the exception of 1996. These studies revealed natural reproduction of rainbow trout occurred in the river as early as 1993 and the rainbow trout population appeared to be capable of sustaining itself by the mid-1990s. However, low numbers of *M. cerebralis* myxospores were first detected in five of ten rainbow trout from the river below McPhee Dam in October 1995. By October 1997, YOY wild rainbow trout could only be found at the sampling site two km below the dam. All of those rainbow trout fry were heavily infected and displaying all of the classical signs of WD, including exophthalmia, cranial and skeletal deformities, blacktail and violent whirling behavior (Nehring and Thompson 2003). Prevalence of infection was 100% among juvenile rainbow trout and mean cranial myxospore concentration was 348,000. By the summer of 2000, rainbow population numbers had declined more than 90% from levels observed in 1994 and 1995.

A review of CDOW fish stocking records revealed that kokanee salmon from the Roaring Judy SFRU were stocked into McPhee Reservoir in both 1991 and 1992 at a time when *M. cerebralis* was enzootic in that fish rearing facility. Kokanee salmon were collected from the river below the dam during boat electrofishing surveys in 1993, 1994 and 1995, having reached the river during extended periods of surface spills from the lake during spring run-off. The largest spill of kokanee salmon occurred in 1993. A boat electrofishing survey conducted on a 48 km reach of the river below the dam in late June and early July that year revealed an estimated 1,200 salmon were in the river. No cranial myxospores were detected in rainbow trout collected from the river in January 1994. The chronology of fish sampling and testing strongly suggests that initial introduction *M. cerebralis* into this river occurred with the unintended escape of kokanee salmon from McPhee Reservoir.

Fryingpan River - Over the past 30 years, the Fryingpan River below Ruedi Reservoir has been one of the most intensively studied trout streams in Colorado (Nehring 1979; Nehring and Anderson 1981, 1982, 1985; Nehring 1987; Thompson et al. 1997; Nehring and Thompson 2001, 2003). Throughout most of the 1970s, 1980s and 1990s this stream supported a thriving, world-class sport fishery for rainbow and brown trout. By the mid-1980s, opossum shrimp *Mysis relicta* had become well established in the reservoir and were being entrained in water drawn into the intake structure of the hydropower plant retrofitted into Ruedi Dam. Entrained mysid shrimp discharged into the river below the lake augmented the forage base for the trout fishery (Nehring 1991). Rainbow and brown trout ranging in size from three to five kg were common in the river in the first km below the lake, with a few rainbow trout attaining a weight of 10 kg.

Wild brown and rainbow trout collected from the Fryingpan River below Ruedi Dam in January 1994 and

November 1995 tested negative for *M. cerebralis*. Continued PTD testing revealed rainbow trout collected in November 1996 were positive for the parasite and in the fall of 1997 both rainbow and brown trout tested positive. Continued PTD testing since then has revealed that the prevalence and severity of infection among both species has increased each year and has continued to spread both upstream and downstream from the initial site of detection in 1996 (Nehring and Thompson 2003). Testing in the early years indicated that the infection appeared to originate in the middle reaches of the river approximately 8 km upstream of Basalt, Colorado, near the confluence with Taylor Creek. The density of age 1+ wild rainbow trout at the Taylor Creek study site declined 90% between 1994 and 1998. Intensive water filtration efforts were initiated in 1998 and continued through the fall of 2002. The effluent from a series of six interconnected, off-channel, spring-fed private ponds located approximately 12 km upstream of the confluence with the Roaring Fork River was the only site where high levels of TAMs were being discharged into the river. Monthly water filtrations initiated in January 2000 quickly revealed very high levels of TAMs were being produced in a few of the ponds. Moreover, brook trout in these ponds were heavily infected. One-month old rainbow trout fry were continuously exposed to the water flowing from the pond producing the greatest numbers of TAMs for a two-week period in mid-March 2001. These trout suffered 67% mortality due to WD over a 6-month period after removal to SPF water at the CDOW Aquatic Toxicology Wet Lab in Fort Collins. Testing of the exposed fry and juvenile trout by both PCR and PTD methodologies revealed 100% of the fish were severely infected by *M. cerebralis* (Nehring et al. 2003b).

It is noteworthy that after 77 consecutive months (July 1998 – November 2004) of water filtration sampling, TAM spores were detected only once in the water flowing out of Ruedi Reservoir, even though *M. cerebralis* was enzootic in brown and brook trout in the Fryingpan River upstream of this large, deep oligotrophic reservoir. However, there is no extended history of stocking catchable trout reared in SFRUs testing positive for *M. cerebralis* in this large impoundment. It will be shown subsequently that this was not the case for another large deep oligotrophic reservoir in the Taylor River basin.

Gunnison River – Wild rainbow and brown trout in this river collected from two widely separated locations in early 1994 tested positive for *M. cerebralis*. In the upper portion of the river near Almont, 96% of age-1 brown trout tested positive with a mean cranial myxospore concentration of 28,750, ranging between 1,750 and 155,500. In this reach of the river, the wild rainbow trout population that had been established in the 1980s began to collapse in the early 1990s (Nehring and Thompson 2001, 2003) at approximately the same time that trout reared at the Roaring Judy SFRU first tested positive for *M. cerebralis*. A review of CDOW records from the 1980s reveals rainbow trout collected from Meridian Lake in June 1988 tested positive for *M. cerebralis*. This lake is located in the East River drainage approximately 30 km upstream of the Roaring Judy SFRU. The East River is tributary to the Gunnison River at Almont. The lake had been stocked with catchable rainbow trout produced at a private commercial aquaculture facility that first tested positive for *M. cerebralis* in December 1987 or March 1988. There was a three to four year delay between the time when initial introduction of exposed trout occurred in the drainage and detection of the parasite in trout at the Roaring Judy SFRU and in the Gunnison River near Almont.

In the lower Gunnison River, *M. cerebralis* was first detected in catchable-size rainbow trout collected from the stilling basin below Crystal Reservoir in January 1994. Additional sampling 27 km downstream of Crystal Reservoir in Ute Park revealed 40% and 90% of seven and 11-month old wild rainbow trout of the 1993 year class collected from the river in January and May 1994 were positive for myxospores of *M. cerebralis*. The river downstream of Crystal Reservoir had not been stocked with trout of any kind since the late-1970s according to CDOW fish stocking records. A thorough review of CDOW fish stocking records and evaluation of stream discharge indicated the initial introduction of the parasite to the lower river likely occurred during June and July 1993. At that time Crystal Reservoir was experiencing an uncontrolled surface spill of water ranging from 113 to 150 m^3/s (4000 to 5000 ft^3/s, Nehring 1998). State fish stocking records indicated that catchable rainbow trout exposed to the *M. cerebralis* parasite reared at the Roaring Judy SFRU were stocked into the Cimarron River a few km upstream of Crystal Dam in late June or early July 1993, during the time when the uncontrolled spill was occurring.

Data from electrofishing studies in this same reach of river revealed that there was no appreciable decline in the density of the 1993 year class of wild rainbow trout in the fall of 1994 compared to the abundance of the 1992 year class observed during the fall 1993. However, abundance of all year classes of age-1 wild rainbow trout in this reach of river between 1995 and 2003 were reduced by 90 to 100% compared to densities seen in the fall of 1993 and 1994. This tends to support the hypothesis that initial exposure of the 1993 year class of wild rainbow trout occurred during the late summer or fall of 1993. At that time the fish would have been large enough to sustain a chronic infection that did not result in substantial acute or chronic mortality (Ryce et al. 2004).

The consequences of whirling disease on this world class sport fishery have been catastrophic. Except for the years 1999 and 2001, trout population estimates were completed on the 3.2 km reach of the Gunnison River gorge known as Ute Park every year between 1981 and 2003. Estimates of peak abundance of wild rainbow trout $\geq$ 15 cm in this reach during the pre-WD period were 11,100 and 10,540 in 1987 and 1988, respectively. After failure of four successive year classes, estimated abundance of wild rainbow trout in the same reach of river had declined to 1,700 in 1998. By the fall of 2003,

after ten consecutive years of near complete loss of wild rainbow fry, the estimated abundance of wild rainbow trout ≥ 15 cm had fallen to 86 fish in the 3.2 km reach.

Middle Fork of the South Platte River – This five-year (1998-2003) study provided stark contrasts in the dynamics and vectoring of *M. cerebralis* in an alpine stream, upstream and downstream of a heavily stocked reservoir. Sampling sites on this stream were located upstream and downstream of Montgomery Reservoir, a 39-ha (97 acres) lake situated at an elevation of 3,305 meters (10,840 feet). An allopatric cutthroat trout population thrives in the river just 300 m upstream of the lake. A series of cascading waterfalls ranging in height from one to three m with a combined vertical drop of 68 m prevent immigration of trout from the lake into this study reach. This section of stream has not been stocked in more than two decades.

Two study sites downstream of the reservoir were located 0.3 and 4.8 km below the dam spillway and outlet works. Wild brook and brown trout existed in sympatry in this study reach between 1998 and 2003. Between 1988 and 1999 the reservoir was stocked annually with catchable rainbow trout reared at CDOW SFRUs testing positive for *M. cerebralis*. Numbers of catchable trout stocked annually into the lake during that 12-year period ranged from 9,000 to 36,000. In addition, the lake was often stocked with fingerling cutthroat or rainbow trout ranging from 3 to 10 cm in size. Beginning in 2000, the lake was stocked with reduced numbers of catchable trout from SFRUs testing negative for the parasite.

Filtration efforts initiated in September 1997 revealed high densities of *M. cerebralis* TAMs in the water samples at the two study sites 0.3 km and 4.8 km below the reservoir, indicating *M. cerebralis* was enzootic in the lake and in the stream below the lake. In contrast, TAMs were never detected in filtrates from the river 0.3 km upstream of Montgomery Reservoir. Over the entire five-year study period all PCR tests of water filtrates from that site were negative. Upon the cessation of stocking catchable rainbow trout reared in SFRUs testing positive for *M. cerebralis* after 1999, the relative density of TAMs detected in monthly water filtrations declined dramatically between January 2001 and June 2003. These data show a direct link between the stocking of *M. cerebralis*-exposed rainbow trout and the subsequent intensity of the *M. cerebralis* epizootic in that body of water.

Annual PCR and PTD testing of brown and brook trout collected from the two sampling sites downstream of the lake between 1998 and 2002 indicated a severe epizootic of the parasite in both species of trout. Similar tests conducted on rainbow trout collected from the lake in 1997, 1999 and 2001 revealed a high prevalence and severity of infection as well. Clinical signs of WD were evident among YOY brook and brown trout fingerlings at sampling sites downstream of the reservoir during electrofishing surveys each fall. Clinical signs of disease included blacktail, exophthalmia, skeletal deformities and whirling behavior among brook trout and blacktail and some skeletal deformities among brown trout fingerlings. In contrast, results of annual PCR and PTD tests of YOY cutthroat and age one cutthroat trout from the reach upstream of the lake were negative for *M. cerebralis* every year between 1998 and 2002 and again in 2004.

Over the five-year study, dynamic differences were observed in the trout populations among the three study sites. At the sampling site upstream of the lake, where *M. cerebralis* was not enzootic, a thriving cutthroat trout population was evident throughout the five-year study period. Scale age analysis, evaluation of annual growth increments and length-frequency size distribution indicated substantial numbers of trout up to six years old were present in the population. Substantial numbers of YOY fry and age 1+ juveniles were present each year and recruiting to the older age cohorts (Nehring and Thompson 2003). In contrast, the brook trout population at the sampling site 4.8 km downstream of the reservoir at the beginning of the study in 1998 was probably in a severe state of decline from the impact of WD. Although brook trout fry were more abundant than same age brown trout fry at this study site in 1998 and 1999, they were not recruiting to the population because of greater vulnerability to *M. cerebralis* (O'Grodnick 1979; Thompson et al. 1999). Brook trout fry abundance declined precipitously every year from 1999 through 2002. Without natural recruitment, biomass of juvenile and adult brook trout declined 94% between 1998 and 2002.

It was very encouraging that densities of TAMs detected in the monthly water filtrations declined substantially at both downstream sampling sites between January 2001 and June 2003 after cessation of stocking of catchable trout reared in facilities testing positive for *M. cerebralis* (Nehring and Thompson 2003). However, it may never result in a substantial recovery of the brook trout population in the river downstream of Montgomery Reservoir due to the vulnerability of brook trout to *M. cerebralis* in comparison to brown trout (O'Grodnick 1979; Hedrick et al. 1999a, Hedrick et al. 1999b, Thompson et al. 1999). Numerous sediment-laden beaver ponds in the drainage below the reservoir provide abundant habitat for tubificid worms. That, together with a high density of brown trout, may provide the requisite parameters needed to maintain a reservoir of infectivity sufficient to depress or eliminate brook trout from much of the river between that lake and the village of Alma approximately 8 km downstream.

North Platte River – The North Platte River in North Park, Colorado, downstream through the Northgate Canyon to the Wyoming state line was included in a multi-year research investigation to determine whether *M. cerebralis* was firmly established in the basin (Schisler 2001, 2002). Periodic electrofishing studies conducted on the reach of river from the upstream entrance to Northgate Canyon downstream through the Ginger Quill Ranch during the 1980s (Nehring 1987) and 1990s (NE Region fisheries biologist Ken Kehmeier, personal communication) indicated that this reach of river supported a self-sustaining wild rainbow trout population that was not impacted by *M. cerebralis*.

Schisler (2002) presented substantial evidence that aquatic oligochaetes were abundant throughout most reaches of the river. Extensive collections of wild rainbow and brown trout from numerous sites throughout the North Platte River basin tested by both the PCR and PTD methodologies revealed *M. cerebralis* infection was detected in one or both species of fish at most sampling sites. The relative infection score (as determined by PCR ratings) and the prevalence and severity of infection (as measured by cranial myxospore concentrations with the PTD methodology) indicated that infectivity levels were high enough to be causing population-level impacts on the wild rainbow trout population. However, estimates of rainbow trout density and biomass in the Northgate Canyon reach for 1996 and 2000 were well within the range of six estimates completed for the same reach of river between 1982 and 1988, prior to the time when *M. cerebralis* most likely became enzootic in the drainage (Schisler 2001; Nehring 1987). Extensive fry sampling efforts conducted during 2000 and 2001 failed to reveal any evidence of natural reproduction of wild rainbow trout either in Northgate Canyon nor any major spawning areas for rainbow trout in the basin upstream (Schisler 2002). Given these findings, Schisler (2002) concluded that recruitment of wild rainbow trout in the Northgate Canyon reach of the basin was most likely the result of immigration by wild juvenile rainbow trout produced in downstream tributaries in Wyoming.

Rio Grande – Between 1981 and 1993, various reaches of the Rio Grande (RG) were the target of intensive research and management efforts. Those studies included evaluation of the effects of restrictive angling regulations on four different reaches of the river (Nehring 1987), introduction and establishment of wild rainbow trout in the river and tributary streams (Nehring 1993a), evaluation of habitat improvement projects and instream flow modeling efforts (Nehring 1988; Shuler and Nehring 1994; Shuler et al. 1994). Research and management efforts to establish a self-sustaining, wild rainbow trout population were initiated when wild strain rainbow trout fry were stocked in three separate reaches of the river in October 1984. Fingerling stocking efforts were terminated after 1987. Electrofishing surveys during the late summer and fall of 1988 revealed wild rainbow trout fry at many locations throughout the RG, the SFRG and its tributaries. By the fall of 1992, a thriving wild rainbow trout population was well established in more than 100 km of stream in the Rio Grande basin (Nehring 1993b).

Brown and rainbow trout fingerlings were collected from numerous sites in the RG and SFRG drainages during February and April 1994 and tested by PTD for evidence of *M. cerebralis* infection. These tests revealed evidence of infection in rainbow trout at all sampling sites (Walker and Nehring 1995). Tens of thousands of catchable rainbow trout reared at CDOW SFRUs testing positive for *M. cerebralis* were stocked annually during 1992, 1993 and 1994 into Beaver Creek, Big Meadows and Rio Grande reservoirs. Rio Grande Reservoir is located in the headwater reaches of the RG basin at the boundary of the Weminuche Wilderness. Introduction of these fish most likely led to the rapid establishment and spread of the parasite throughout the upper RG basin during the 1990s.

Continued PCR and PTD testing and annual electrofishing surveys throughout the basin during the 1990s clearly indicated that the robust wild rainbow trout population established in the late 1980s and early 1990s began to decline in 1993 and 1994 and had largely collapsed by the end of the 20th century (Nehring and Thompson 2003). In the 11-km study reach known as State Bridge or Twin Mountain Bridge downstream of the confluence with the SFRG, density and biomass of wild rainbow trout had declined by more than 90% by the fall of 1999, falling to levels only observed between 1981 and 1984, prior to the time when efforts to establish wild rainbow trout in the drainage were initiated. Similarly, in the 6-km reach of the river between the CDOW RG Fisherman Area and the U. S. Forest Service (USFS) Marshall Park Campground 11 km upstream of Creede, rainbow trout density and biomass declined by more than 90% between 1991 and 2001. It is noteworthy that there was no compensatory increase in brown trout density or biomass at either study site on the Rio Grande after the collapse of the wild rainbow trout population.

South Platte River – *Myxobolus cerebralis* is enzootic throughout most of the South Platte River basin, from headwater reaches of the North Fork, Middle Fork and South Fork to the confluence of the North Fork and main South Platte River approximately 25 km downstream of Cheesman Reservoir. The dynamics of infection and assessment of impacts on wild rainbow trout populations have been extensively studied on three separate reaches of the river. These include the reach between Spinney Mountain and 11 Mile reservoirs, from 11-Mile Reservoir downstream to Lake George, and the mainstem reach from Cheesman Dam to the confluence with the North Fork of the South Platte River.

Schisler (2003) reported that *M. cerebralis* infection in the reach of river between Spinney Mountain and 11-Mile reservoirs remains critically high and that total year class failure for rainbow trout continues despite the cessation of stocking of infected fish into Spinney Mountain Reservoir. The presence of a number of risk factors in this river reach may preclude redevelopment of a self-sustaining wild rainbow trout fishery. These include an abundance of infection-tolerant brown trout, a low gradient stream, and accumulation of sediments that provide optimal habitat conditions for development of extensive colonies of *T. tubifex*, the alternate host for the parasite.

In the river below 11-Mile Reservoir, prevalence of infection among wild rainbow trout was only 8.3% as determined by PCR testing immediately downstream of the dam but increased to 90% among rainbow trout 12.8 km downstream (Schisler 2001). Not surprisingly, in the immediate vicinity of the dam survival and recruitment of wild rainbow trout has continued. However, at the downstream site where prevalence of infection among rainbow trout was

higher, recruitment and survival of this species was depressed, numbers and biomass were lower, and clinical signs of WD were evident among wild brown trout fry and juveniles (George Schisler, personal communication). However, the wild brown trout population continues to thrive, showing no impact at the population level despite the presence of clinical signs of WD in individual brown trout. This suggests that large, deep oligotrophic reservoirs where the parasite is enzootic may pass lower numbers of TAM spores (as suggested by the very low prevalence of infection among rainbow trout near the dam outfall), but prevalence and severity of infection increase in downstream reaches where the gradient decreases and sediment accumulation increases, possibly exacerbated by the presence of greater numbers of infection-tolerant brown trout.

In the reach downstream of Cheesman Reservoir, CDOW disease testing records indicate the parasite was already enzootic in 1988. This reach of river has been the object of annual trout population monitoring since the fall of 1979 (Nehring and Thompson 2003). These trout population estimates indicate that rainbow trout density and biomass peaked at an all-time high at both the Deckers and Lower Cheesman Canyon monitoring sites in the fall of 1989 or 1990 and then began declining precipitously. By the fall of 2000 at the Lower Cheesman Canyon site, density had declined by 85% and biomass by 73%. Survival and recruitment of reduced numbers of wild rainbow trout fry in some years have served to maintain a minimal wild rainbow trout fishery in the 5-km reach of river immediately downstream of Cheesman Dam. At the Deckers monitoring site, by the fall of 1998 density and biomass of rainbow trout had decreased by 95% and 97%, respectively. Ancillary water filtration studies conducted in the late 1990s suggested ambient levels of infection were being augmented with TAM spores entering the stream from Wigwam Creek, tributary to the South Platte River approximately 2 km upstream of the Deckers study site. The rainbow trout fishery in this reach of river is being maintained in large part through the stocking of fingerling rainbow trout from CDOW SFRUs testing negative for *M. cerebralis*.

As was the case for the Rio Grande basin study streams, there has been no concomitant compensatory increase in brown trout density and biomass at either the Cheesman Canyon or Deckers area study reaches with the decline in the wild rainbow trout population.

Spring Creek – Beginning in July 1998 and continuing through June 2003, Spring Creek in the Taylor River drainage was the focus of an intensive research project to determine whether or not a definitive linkage could be established between the stocking of catchable rainbow trout exposed to *M. cerebralis* and subsequent increases in ambient TAM levels in the water and increases in cranial myxospore concentrations in age 1 brown trout as determined by PTD testing.

Spring Creek is formed by melting snows at an elevation 3,600 m (11,800 feet) in the Gunnison National Forest and flows into Spring Creek Reservoir (SCR) located at an elevation of 3,023 m (9,915 feet). According to CDOW records for 1992 through 1996, this 35 ha (87 acre) impoundment was stocked annually with 10,000 to 42,000 catchable rainbow trout from the Roaring Judy SFRU. Rainbow trout reared at this unit first tested positive for *M. cerebralis* in 1992. The unit remained positive for *M. cerebralis* until late fall 2004.

Initial collection and PTD testing of brown trout from Spring Creek 0.8 km downstream of SCR in October 1996 revealed 96% of age-1 brown trout were infected with *M. cerebralis*. Mean cranial myxospore concentrations among the fish tested by PTD averaged 141,900, ranging from 17,500 to 1.13 million (Nehring and Thompson 2003). This was the highest cranial myxospore burden ever detected among age 1 brown trout anywhere in Colorado between 1994 and 2004. In contrast, only one of ten age-1 brown trout collected one km upstream of SCR tested positive by PTD on the same date with a cranial myxospore concentration of 794. Moreover, on five of eight sampling occasions conducted at the site upstream of SCR between October 1998 and September 2002 all fish collected tested negative for *M. cerebralis* infection. On the other three sampling occasions when evidence of *M. cerebralis* infection was detected by PTD testing, the prevalence and severity of infection was very low. These data inferred that the *M. cerebralis* TAMs detected in Spring Creek below SCR were emanating from the reservoir and not the stream above the lake.

During 1997 and 1998 there was no stocking of catchable trout in SCR from SFRUs testing positive for *M. cerebralis* infection. Among age-1 brown trout collected 0.8 km downstream of SCR in October 1998, prevalence of infection was 90% but the mean cranial myxospore concentration had declined to 36,700. Among age-1 brown trout collected at the same site in September 1999, prevalence of infection had declined to 30% and mean cranial myxospore concentrations decreased to 833, a decrease of 99.4% from levels observed in October 1996. All age-1 brown trout collected at the site 1 km upstream of SCR in October 1998 tested negative for *M. cerebralis*. Among age-1 brown trout collected at this site in September 1999 prevalence of infection was 10% and the mean cranial myxospore concentration was 111. These data strongly infer that ambient levels of infection emanating from SCR between 1997 and September 1999 had declined dramatically in absence of the stocking of fish infected with *M. cerebralis*.

In July 1999, 10,000 catchable rainbow trout reared at the CDOW Poudre SFRU were marked with a fin clip and stocked into SCR. A sub-sample of 20 fish from this stocking tested by PTD indicated the prevalence of infection with *M. cerebralis* was 95% and the estimated mean cranial myxospore burden was 470,000. Individual cranial myxospore concentrations ranged from 14,000 to 1,625,000 among the fish testing positive for the parasite. Over the next three years (2000 through June 2002) prevalence of infection among age-1 brown trout collected from the site 0.8 km

downstream of SCR ranged between 60% and 80% and mean cranial myxospores concentrations ranged between 12,448 and 33,685 (Nehring and Thompson 2003). During the same time period, prevalence of infection among age-1 brown trout collected concurrently from the sampling station one km upstream of SCR ranged between zero and 10% on most sampling occasions. On the two concurrent sampling dates in August and September 2001 when PTD testing revealed evidence of *M. cerebralis* infection among brown trout at the sampling sites upstream and downstream of SCR, the mean cranial myxospore concentration among the brown trout from the site downstream of SCR was four times and 11 times higher than that for the trout from the site upstream of SCR.

Concurrent water filtration samples were collected 46 times across 44 consecutive months at sampling sites one km upstream and 0.8 km downstream of SCR from November 1999 through June 2003. The concentrated filtrates from each of these 1,900-L (500-gallon) water samples were screened by stereozoom microscopy for detection of TAMs of *M. cerebralis* and also tested by PCR for DNA of *M. cerebralis*. No TAMs were ever detected among the water samples collected from the study site one km upstream of SCR and all PCR tests were negative for DNA of *M. cerebralis*.

At the sampling site 0.8 km downstream of SCR, TAMs were detected on one of 14 filtration occasions between July 1999 and August 2000. However, TAMs were detected in filtrates on 10 of 21 occasions over the 16-month period between September 2000 and December 2001. No TAMs were detected at this sampling site on six sampling occasions between January and June 2002. TAM production had apparently stopped. Beginning in September 2000 and continuing through June 2003 additional monthly water filtrations were collected at the outlet of SCR to verify that the TAMs were emanating from SCR rather than habitat within the 0.8 km reach of stream below the reservoir outlet and above the standard sampling site. During the 16-month period between September 2000 and October 2001, TAMs of *M. cerebralis* were detected at the outlet of SCR on 12 of 15 sampling occasions. In contrast, TAMs were detected at that sampling site on 2 of 20 sampling occasions between November 2001 and June 2003, indicating that the 14-month long "pulse" of TAM production resulting from the stocking of the 10,000 infected rainbow trout in July 1999 ended in the fall of 2001.

Prevalence of infection was 75% among age 1+ brown trout at the sampling site 0.8 km downstream of SCR in August 2001 and the cranial myxospore concentrations among the infected fish averaged 26,500. The maximum cranial spore level was 82,300. In contrast, in September 1999, prevalence of infection among age 1+ brown trout at the same site was 30%, mean cranial myxospore concentration was 833, and the maximum spore level was only 5,000.

All of the foregoing is strong corroborating evidence that there is a direct linkage between the stocking of catchable trout infected with *M. cerebralis* into lakes and reservoirs and subsequent production of TAMs of *M. cerebralis* that exacerbate the prevalence and severity of infection among susceptible salmonids in downstream waters.

Taylor River – The Taylor River originates in a cirque basin near Crystal Peak in the Gunnison National Forest at an elevation of 3,600 m (11,800 feet). This river became part of the WD research effort because it flows into a large reservoir with a unique stocking history. Taylor Park Reservoir (TPR) is a large deep oligotrophic reservoir approximately 810 ha (2,009 acres) at full pool with a storage capacity of 131 million m^3 (106,200+ acre-feet). It is a bottom outlet reservoir located at an elevation of 2,844 m (9,330 feet). From 1992 through September 1999, TPR was stocked annually with 40,000 to 143,000 catchable trout reared at CDOW SFRUs testing positive for *M. cerebralis*. This case study stands in stark contrast to that of Ruedi Reservoir on the Fryingpan River where there was no history of stocking of catchable trout reared at SFRUs testing positive for *M. cerebralis*.

Water filtration sampling in the Taylor River at a site 0.1 km downstream of the bottom outlet valves of TPR began in December 1999 and continued through June 2003. This unique situation offered an opportunity to determine whether or not large, deep oligotrophic reservoirs acted as a "sink" where *M. cerebralis* infection could be contained. Or alternatively, could they become a major source of TAMs that could infect susceptible salmonid species downstream?

Between December 1999 and June 2000, large numbers of TAMs of *M. cerebralis* were detected on 11 of 13 (84.5%) water sampling occasions. Mean TAM density in the river leaving TPR during this 7-month period was 0.944/L (3.57/gallon). Maximum TAM density was estimated at 2.71/L (10.3/gallon). These data demonstrate that when enough *M. cerebralis*-positive salmonids are stocked into a body of water with the complement of environmental parameters that will support a viable population of the susceptible oligochaete host, large size and great depth of the impoundment cannot be relied upon to preclude production and escapement of TAM spores.

Beginning in 2000 and continuing through 2003, only salmonids produced at rearing units testing negative for *M. cerebralis* were stocked into TPR. This change in the stocking regime afforded an opportunity to determine the length of time needed for an extreme epizootic of TAM production to begin to ameliorate and hopefully subside to undetectable levels.

Water filtration at this location continued on a monthly basis from July 2000 through June 2001. TAM spores were detected in samples on nine of 14 (64%) occasions. Mean TAM density was 0.164/L for all sampling occasions, ranging from 0.0198 to 1.17/L among the samples when TAM spores were detected. For the sampling occasions between December 2000 and June 2001, mean TAM density was 0.0772/L, a decrease of 91.8% in mean TAM density when compared to the same time period one year earlier. TAM spores of *M. cerebralis* were detected on five of 12 (41.7%) sampling occasions at this site between July 2001 and June 2002. TAM densities dropped below levels detectible by stereozoom

microscopy from May 2002 through June 2003. However, PCR testing of the water samples from this site during this period revealed DNA of *M. cerebralis* was present in the water on three of 14 filtration occasions.

Once again, these results support the hypothesis that the stocking of *M. cerebralis*-infected trout can exacerbate ambient levels of infection observed in downstream waters. Moreover, it appears to take 24 to 30 months for TAM production in standing waters to decrease to undetectable levels using flat pan water filtration after the stocking of *M. cerebralis*-positive trout ceases. The results of the water filtration sampling downstream of TPR stand in stark comparison to those below Ruedi Dam. Both dams impound the water from high elevation tributaries and form large, deep oligotrophic reservoirs prior to discharge through bottom release outlets. *M. cerebralis* is enzootic among brown and brook trout populations in tributary streams above both lakes. During 77 consecutive months (from July 1998 through November 2004) of water filtration sampling below the outfall of Ruedi Dam, TAM spores were detected in the water only once.

There were two streams in Colorado where long-term electrofishing data sets collected by regional and area fisheries management personnel provide significant insights into the dynamic relationships between wild trout populations and *M. cerebralis*. Those streams were Clear Creek in the South Platte River basin near Georgetown and the South Arkansas River west of Salida. Trout population data for these streams were examined and interfaced with data on the prevalence and severity of infection by *M. cerebralis* as determined from cranial myxospore concentrations, CDOW historical fish stocking records, and (in the case of Clear Creek near Georgetown) with information on TAM densities developed from monthly water filtration studies.

Georgetown Lake and Clear Creek – Clear Creek in Clear Creek County is formed by melting snow and precipitation run-off from the east side of the Continental Divide near the Eisenhower Tunnel. Beginning at an elevation of 3,600 m (11,800 feet), the first 23 km (14.5 miles) of the stream has an average gradient of 4.4% as its cascades downhill before flowing into Georgetown Lake at an elevation of 2,583 m (8,470 feet). Review of CDOW fish stocking records indicate catchable-size and fingerling trout reared on SFRUs testing positive for *M. cerebralis* were first stocked into Georgetown Lake and Clear Creek upstream and downstream of the lake in 1989. Annual stocking of trout exposed to the parasite in the stream above and below the lake continued through 1995. Annual stocking of fry/fingerlings as well as sub-catchables and catchable-size trout exposed to *M. cerebralis* into the lake continued through the summer of 2002. The stocking history for Clear Creek and Georgetown Lake are summarized in Table 9.

Monthly water filtration sampling upstream and downstream of Georgetown Lake was initiated beginning in July 2002 and has continued through September 2005. The

Table 9. Approximate numbers of fry/fingerling, sub-catchable and catchable size trout stocked into Georgetown Lake and Clear Creek #1 (downstream of the lake) from the confluence with the West Fork Clear Creek two km east of Empire to the confluence with Chicago Creek at Idaho Springs and Clear Creek #3 (upstream of the lake) from the confluence with the South Fork Clear Creek at Georgetown to the headwaters near the Eisenhower Tunnel. Numbers highlighted in **bold** print are fish reared on SFRUs testing positive for *M. cerebralis*.

Year	Clear Creek #1	Georgetown Lake		Clear Creek # 3
	Fingerling/Catchables	Catchables	Fry/Fingerlings	Fingerling/Catchables
1987	4,064	23,300	0	27,664
1988	2,697	21,100	0	4,902
1989	**35,604**	**22,000**	**5,400**	**17,699**
1990	**34,462**	**15,700**	15,377	**17,700**
1991	**34,939**	**20,300**	5,400	**16,980**
1992	**20,098**	**21,000**	5,400	**17,737**
1993	**19,784**	**20,000**	5,400	**17,196**
1994	**20,082**	**15,900**	0	**15,199**
1995	**21,992**	**41,600**	0	**18,710**
1996	1,750	**17,400**	0	1000
1997	0	**34,000**	0	0
1998	10,000	**25,600**	**5,000**	16,544
1999	0	**16,400**	**5,000**	0
2000	0	**16,000**	0	0
2001	10,000	**32,500**	5,000	6,006
2002	25,000	**102,900**[a]	167,100[b]	13,600
2003	10,000	17,800	3,300	6,000
2004	25,500	14,700	5,000	16,511

[a] 102,900 fish includes 13,000 catchable rainbow trout, 89,900 advanced fingerling (11.9 – 12.7 cm) TAS rainbow trout from the Rifle Falls Rearing Unit that was not re-certified negative for *M. cerebralis* until April 2003.

[b] TAS strain rainbow trout. PCR testing indicated these fish were negative for *M. cerebralis*. These fish were reared in the hatchery spring water supply which was negative for *M. cerebralis*.

study objective was to evaluate the effect of the continued stocking of *M. cerebralis*-positive trout into an impoundment and a downstream ecosystem dominated by brown trout where *M. cerebralis* was already well established. The water filtration effort revealed the ambient density of TAMs estimated to be in Clear Creek flowing into Georgetown Lake were very near or below detectible levels for all samples (Table 10). At the filtration site upstream of the lake TAMs of *M. cerebralis* were detected in only six of 39 samples. This is not surprising given that Clear Creek upstream of the lake was last stocked with trout reared at SFRUs testing positive for *M. cerebralis* in 1995, seven years prior to the initiation of monthly water filtration efforts.

Similarly, numbers and density of TAMs detected in water flowing out of Georgetown Lake were very low from July 2002 through April 2003 (Table 11). However, numbers and density of TAMs detected in the effluent of the lake began to increase exponentially in May 2003 and remained at high levels during most months through September 2005. The sudden increase in TAM production in the lake that was

Table 10. Results of water filtrations to identify, enumerate, and quantify TAM spores in 1900 L (500 gallons) of water drawn from Clear Creek 0.2 km upstream of Georgetown Lake from July 2002 through September 2005. Samples collected after July 1, 2004 were 114 L (30 gallon) samples.

Date Mo/da/yr	Time Military	Temp. (° F)	Tams Counted	No. Tams	95% C.L.	Tams/ Gallon	Tams/ Liter
07/14/02	1355	63.0	2	114	±153	0.228	0.060
08/17/02	1330	59.5	0	0	±	0	0
09/09/02	1545	52.1	0	0	±	0	0
10/17/02	1320	43.7	0	0	±	0	0
11/20/02	1245	38.1	0	0	±	0	0
12/11/02	0930	31.1	0	0	±	0	0
01/30/03	1245	34.8	0	0	±	0	0
02/24/03	1145	32.6	0	0	±	0	0
03/25/03	1330	40.9	0	0	±	0	0
04/15/03	0940	35.9	0	0	±	0	0
05/21/03	1000	38.6	0	0	±	0	0
06/25/03	1045	43.1	0	0	±	0	0
07/14/03	1010	47.4	0	0	±	0	0
08/11/03	1440	54.1	0	0	±	0	0
08/25/03	1220	51.7	0	0	±	0	0
09/03/03	1420	51.3	0	0	±	0	0
10/21/03	1030	42.3	2	183	±246	0.365	0.096
11/10/03	1330	39.6	2	72	±98	0.145	0.038
12/18/03	1000	30.7	2	123	±165	0.245	0.065
01/19/04	1410	30.7	0	0	±	0	0
02/23/04	1510	34.1	0	0	±	0	0
03/16/04	0945	30.8	0	0	±	0	0
04/12/04	1245	38.6	0	0	±	0	0
05/19/04	1240	46.2	1	83	±162	0.165	0.044
07/06/04	1310	51.0	0	0	±	0	0
08/04/04	1125	52.1	0	0	±	0	0
09/01/04	1910	51.0	0	0	±	0	0
10/10/04	1220	42.0	0	0	±	0	0
11/08/04	0930	36.0	0	0	±	0	0
12/06/04	1355	31.0	0	0	±	0	0
01/10/05	1525	34.3	0	0	±	0	0
02/01/05	1550	32.7	0	0	±	0	0
03/06/05	1400	42.8	0	0	±	0	0
04/11/05	1348	42.0	0	0	±	0	0
05/18/05	1235	46.7	0	0	±	0	0
06/01/05	1445	48.7	0	0	±	0	0
07/11/05	1050	53.1	0	0	±	0	0
08/08/05	1237	54.3	0	0	±	0	0
09/16/05	0750	49.2	1	22	±43	0.708	0.187

sustained for 29 consecutive months appears to be directly linked to the more than 10-fold increase in stocking of trout into this 21.9 ha (54 acre) shallow lake in 2002 compared to the previous years (Table 9). It is highly probable the 167,000 TAS rainbow trout fry (3.1 cm – 1.24 inches) and 89,900 fingerling TAS rainbow trout (11.9 – 12.7 cm) that were unexposed to *M. cerebralis* when stocked in 2002, became heavily infected with *M. cerebralis* due to their small size at the time of stocking. Although TAM production declined during the winter months between December 2004 and March 2005, it increased exponentially again beginning in April 2005 and continued through September, 3 years after the infusion of trout in 2002 (Table 11). The empirical evidence illustrates the potential consequences of excessive stocking of trout, particularly fry or fingerling rainbow trout (that are highly vulnerable to *M. cerebralis*) into ecosystems where the parasite is already well established.

Table 11. .Results of water filtrations to identify, enumerate, and quantify TAM spores in 1900 L (500 gallons) of water drawn from Clear Creek 0.1 km downstream of Georgetown Lake from July 2002 through September 2005. Samples collected after July 1, 2004 were 114 L (30 gallon) samples.

Date Mo/da/yr	Time Military	Temp. (° F)	Tams Counted	No. Tams	95% C.L.	Tams/ Gallon	Tams/ Liter
07/14/02	1505	62.4	4	218	±196	0.435	0.115
08/17/02	1445	60.2	2	166	±224	0.333	0.088
09/09/02	1500	55.9	0	0	±	0	0
10/17/02	1340	45.5	0	0	±	0	0
11/20/02	1220	37.5	2	48	±64	0.095	0.025
12/11/02	0945	37.7	4	85	±76	0.170	0.045
01/30/03	1320	42.6	0	0	±	0	0
02/24/03	1215	41.9	0	0	±	0	0
03/25/03	1410	38.8	0	0	±	0	0
04/15/03	1015	45.6	2	115	±225	0.230	0.061
05/21/03	1030	44.3	12	653	±391	1.31	0.345
06/25/03	1125	44.1	77	2,791	±621	5.58	1.475
07/14/03	1040	52.6	93	4,301	±635	8.60	2.273
08/11/03	1440	54.1	15	769	±382	1.54	0.406
08/25/03	1300	54.9	10	519	±346	1.04	0.274
09/03/03	1515	52.6	34	1,998	±557	4.00	1.06
10/21/03	1120	44.9	192	8,640	±2,333	17.3	4.57
11/10/03	1350	36.0	166	7,470	±1,679	14.9	3.95
12/18/03	1035	35.4	94	3,349	±1,560	6.70	1.77
01/19/04	1445	36.1	44	2,090	±585	4.18	1.10
02/23/04	1545	33.7	59	2,323	±507	4.65	1.23
03/16/04	1015	39.9	258	9,191	±3,036	18.4	4.86
04/12/04	1320	40.1	102	5,483	±1,602	11.0	2.90
05/19/04	1300	49.3	353	16,326	±2,361	32.7	8.63
06/14/04	1615	50.8	168	13,860	±4,564	27.7	7.32
07/06/04	1330	51.0	364	3,610	±1,012	120.1	31.7
08/04/04	1125	52.1	117	2,377	±764	80.2	21.2
09/01/04	1910	51.0	29	1,466	±647	39.7	10.5
10/10/04	1200	46.8	20	312	±127	10.6	2.75
11/08/04	1000	39.3	116	979	±139	32.6	8.62
12/06/04	1415	34.3	1	21	±41	0.688	0.182
01/10/05	1535	35.5	0	0	±	0	0
02/01/05	1550	33.0	5	115	±131	3.85	1.02
03/06/05	1400	42.8	0	0	±	0	0
04/11/05	1325	41.1	26	630	±369	21.0	5.55
05/18/05	1300	46.7	65	4,779	±1,330	159.3	42.1
06/01/05	1450	48.7	53	3,033	±766	101.1	26.7
07/11/05	1030	53.1	60	619	±256	20.6	5.45
08/08/05	1252	57.2	53	837	±313	27.9	7.37
09/16/05	0815	50.7	24	571	±338	19.0	5.03

Trout collected from Clear Creek and the North Fork of Clear Creek in 1997 and tested by PTD revealed the parasite was enzootic in the drainage downstream of Georgetown Lake. This would be expected given the high level of stocking of exposed trout in the lake and the drainage basin between 1989 and 1996 (Table 9). Subsequent collection and PTD testing of trout at various sites from the headwater reaches near the Eisenhower Tunnel downstream throughout the Clear Creek watershed in 2004 revealed substantial *M. cerebralis* infections among five different species of salmonids (Table 12). These results are of significant concern given that two pure populations of greenback native (GBN) cutthroat trout were discovered in two small tributary streams (Dry Gulch and Herman Gulch) flowing into Clear Creek just east of the Eisenhower Tunnel during 2003 and 2004 . The cutthroat trout that tested positive for *M. cerebralis* in Clear Creek at exit 218 on I-70 just a few kilometers east of the Eisenhower Tunnel occur within 100 to 200 meters of the recently discovered pure greenback cutthroat trout population in Dry Gulch and Herman Gulch.

Fisheries management personnel for the Northeast and Central regions obtained fall and spring trout population estimates for five sampling locations on Clear Creek between Georgetown Lake and Idaho Springs almost every year between 1995 and 2004. These estimates comprise a superb data base to determine whether or not the exponential increase in TAM production in the lake that began in the spring of 2003 had any measurable impact on the trout population downstream of the lake in 2003 and 2004. Across all five sampling stations, brown trout fry abundance in October 2003 declined by 96% compared to the fall of 2002. It is possible some YOY brown trout might have been lost through acute exposure to *M. cerebralis*. However, the dramatic difference in relative abundance of fry between 2002 and 2003 was more likely the result of extremely high recruitment in 2002 due to the severe drought that created ideal habitat conditions for survival of brown trout fry. In contrast, the June 2003 mean monthly discharge was approximately 114% of normal. Above normal spring discharge levels are known to reduce survival of brown and rainbow trout fry during the first month post emergence from the gravel (Nehring and Anderson 1993). The negative effects of above normal discharge levels on fry during the month of emergence would be exacerbated in high gradient streams such as Clear Creek. Linear regression analysis revealed total numbers of brown trout fry captured at the five study sites downstream of Georgetown Lake during fall electrofishing operations from 1995 through 2003 were negatively correlated with mean monthly discharge for June for the nine years of study ($r = -0.771$; $p < 0.01$).

Taken together, the foregoing results suggest there has been no detrimental impact on the brown trout population downstream of Georgetown Lake due to the stocking of trout reared in SFRUs testing positive for *M. cerebralis*. However, the stocking of trout testing positive for *M. cerebralis* into the drainage upstream of Georgetown Lake between 1989 and 1995 resulted in the establishment of the parasite in the uppermost headwater areas. This puts the relict populations of pure greenback cutthroat trout discovered in 2003 and 2004 in Dry Gulch and Herman Gulch at risk of exposure to *M. cerebralis* given that the parasite is enzootic in cutthroat trout in Clear Creek at Exit 218 on I-70 east of the Eisenhower Tunnel. Little more than 100 meters of stream separate the exposed and unexposed populations.

Clear Creek and Clear Creek Reservoir – The headwaters of the North, South and Lake Forks of Clear Creek in the Arkansas River drainage flow east from the Continental

Table 12. Summary of PTD testing of various species of trout collected from the Clear Creek watershed upstream and downstream of Georgetown Lake during 2004.

Date Mo/da/yr	Species	Age.	Sample Size		Overall Mean Myxospore	Myxospores in Positive Fish	
			No.	No.+	Burden	Mean	Range
Dry Gulch one km upstream of confluence with Clear Creek one km east of the Eisenhower Tunnel							
07/28/2004	GBNCut	1+	10	0	0	---	-----
Clear Creek # 3 @ I-70 Exit 218 - two km east of the Eisenhower Tunnel							
07/28/2004	GBNCut	1+	10	5	1,806	3,611	556 – 10,000
Clear Creek # 3 approximately one km upstream of Silver Plume							
03/29/2004	Brook	1+	2	2	580,714	580,714	411,761 – 749,667
03/29/2004	SRN-Cut	1+	4	1	9,389	37,556	37,556
03/29/2004	Rainbow	1+	14	8	408,352	714,617	9,500 – 4,972,000
Clear Creek #2 just downstream from Georgetown Lake							
03/30/2004	Brown	1+	18	4	7,319	32,935	5,950 – 87,389
03/30/2004	Rainbow	1+	15	12	617,992	772,490	5,911 – 2,448,983
07/28/2004	Brown	1+	17	6	35,948	101,851	1,111 – 324,444
Clear Creek # 1 upstream of the Spring Gulch confluence							
03/24/2004	SRN-Cut	1+	1	0	0	---	-----
03/24/2004	Rainbow	1+	1	0	0	---	-----
03/24/2004	Brown	1+	27	16	10,489	17,700	2,344 – 69,600

Divide into Clear Creek Reservoir. Located at an elevation 2,705 m (8,875 feet), this 164 ha (407 acre) impoundment was annually stocked with an average of 40,000 catchable rainbow trout that had been reared at SFRUs testing positive for *M. cerebralis* from 1989 through 2002 (Table 13). Numbers of catchable rainbow trout stocked annually over that 14-year period ranged from 15,900 to almost 113,000. Fry and fingerling brown trout and kokanee salmon together with sub-catchable or catchable- size Snake River cutthroat were also stocked during many years between 1989 and 2004.

Similar to Georgetown Lake, this reservoir was also selected as a study site for evaluating the effect of the continued stocking of *M. cerebralis*-positive trout into an impoundment and a downstream ecosystem dominated by brown trout where *M. cerebralis* was already well established. Monthly water filtration sampling in Clear Creek upstream and downstream of the reservoir began in July 2002 and continued through June 2003. Throughout the year-long study, *M. cerebralis* TAMs were detected only once in Clear Creek upstream of the lake. None were ever detected in the outflow below the reservoir. This is an enigma given the unusually large numbers of catchable trout stocked into the lake annually from SFRUs testing positive for *M. cerebralis*. However, there are a number of plausible explanations.

First, it is possible that there are no *T. tubifex* worms dwelling in the sediments at the bottom of the reservoir. This seems unlikely given all of the other study areas where this has not been the case. Second, if the lake does support a *T. tubifex* population it may be dominated by strains of worms that are not susceptible to the parasite (Beauchamp et al. 2001, 2002; Beauchamp et al. 2005). Third, there may be something unusual about the location of the outlet valve that precludes TAMs produced in the lake from being entrained and discharged through the outlet works of the dam. This would seem to be a very distinct possibility. Finally, given that the 135,000 GBN cutthroat trout fry were not stocked into the lake until October 2003 and stocking of large numbers of fingerling rainbow trout did not occur until October and November 2003, it is quite possible that the monthly water filtration efforts were initiated and terminated before an adequate period of time had allowed for the infection cycle to proceed to the point of TAM production.

South Arkansas River – The South Arkansas River arises from the east side of the Continental Divide near the summit of Monarch Pass and flows into the Arkansas River at Salida, Colorado. Brown trout dominate the trout fishery in the river throughout the low gradient meadow reaches west of Salida and Maysville to the confluence with Como Creek. Wild rainbow trout begin to appear in the fish population at Maysville, becoming increasingly abundant with increasing elevation. In the stream near the village of Garfield rainbow trout are the dominant salmonid. Brook trout occur incidentally in the river near Maysville, increase in abundance near Garfield, and are allopatric in the South Fork of the South Arkansas River two km downstream from the USFS Monarch Park Campground. Southeast Region fisheries management personnel began conducting annual trout population estimates at numerous sites on this stream in 2001. Estimates of density (n/ha) and biomass (kg/ha) for brown trout, rainbow trout and brook trout from those studies for eight electrofishing stations from 2001 through 2004 are summarized in Table 14. At those stations where brown trout are the dominant salmonid species there is some fluctuation

Table 13. Numbers, species and size ranges of fish stocked annually into Clear Creek Reservoir in the Arkansas River drainage between 1989 and 2004.

Year	Rainbow Trout			Brook	Brown	Koke	GBN	SRN	Mack[b]/	Totals
	WD- Catchable	WD+ Catchable	Fry Fing.	Fry	Fry	Fry[a]	Fry		Tiger Muskie	
1989	16,160	26,960		15,069	20,140	25,017		3,539		106,885
1990	19,661	33,572		15,038	13,002	25,254				106,527
1991	21,921	32,170		18,276	25,090	25,410	4,572			127,619
1992	22,762	38,000			25,000	25,051				110,813
1993	61	41,920			20,040	24,971		15,169		102,161
1994		33,086			19,992	25,035		15,766		93,879
1995		65,369			20,060	60,030		15,052		160,511
1996		52,991	20,000		18,079	57,368		15,122	27,516	191,076
1997		21,446			20,001					41,447
1998		37,641		4,592	89,260	29,984		34,999		196,476
1999	15,001	36,300			40,099					91,400
2000		15,878			12,503	30,000		20,920		79,301
2001	36,478	18,854				57,228		11,997		124,557
2002	10,075	102,679			20,006		135,192	84,068		352,020
2003	29,721	-----	72,438		20,000	50,966				173,125
2004	26,539	-----	49,840		20,020	57,062			4,000	157,461

[a] "Koke" stand for kokanee fry . [b] "Mack" stands for Mackinaw or Lake trout

in density and biomass among years at the various sampling sites; however, there is no consistent upward or downward trend. In contrast, a definite downward trend in density and biomass for wild rainbow trout is readily apparent at most sampling sites between 2001 and 2004.

It is very likely that this consistent downward trend in density and biomass for the wild rainbow trout is the result of exposure to *M. cerebralis* as the parasite is well established in the drainage. Among brown trout collected approximately 6 km downstream of the Fooses Creek confluence in 2000, 24 of 30 brown trout were infected with *M. cerebralis* when tested by PTD. The average cranial myxospore concentration was 26,380. The most severely infected brown trout had more than 261,000 cranial myxospores. Among brook trout collected in the South Arkansas River near Monarch Campground in 2003 and tested by PTD, prevalence of infection was 90% and the mean cranial myxospore concentration was 156,087. Cranial myxospore concentrations ranged from 50,900 up to 420,500 among the *M. cerebralis*-infected brook trout. Among brook trout and rainbow trout collected in the North Fork of the South Arkansas River in 2000, prevalence of infection was 72% and 91%, respectively. The mean cranial myxospore concentrations for brook trout and rainbow trout in this

Table 14. Estimates of biomass (kg/ka) and density (numbers/ha) of brown, rainbow and brook trout for eight study sites on the South Arkansas River from 2001 through 2004. Study sites are listed in the table from a downstream to upstream direction.

Year	Brown Trout		Rainbow Trout		Brook Trout	
	Biomass	Density	Biomass	Density	Biomass	Density
Below # 2 Powerhouse (C1)						
2001	119.7	1,453	----	----	----	----
2002	92.1	969	----	----	----	----
2003	96.2	1,104	----	----	----	----
2004	123.1	1,384	----	----	----	----
At Maysville (B1)						
2001	168.5	2,005	3.3	27	----	----
2002	90.7	1,157	----	----	----	----
2003	109.4	1,660	----	----	----	----
2004	155.4	1,548	----	----	----	----
Below Lost Creek (B2)						
2001	224.6	2,997	6.9	51	----	----
2002	228.2	2,870	7.5	51	----	----
2003	233.9	3,106	1.6	51	----	----
2004	323.1	2,462	2.3	25	----	----
Above Lost Creek (B3)						
2001	141.1	1,482	27.2	206	----	----
2002	114.7	1,082	21.9	184	1.3	22
2003	151.4	1,623	4.5	45	----	----
2004	256.7	2,201	3.1	23	----	----
Above Cree Creek (B4)						
2001	102.9	1,333	11.1	184	3.5	104
2002	91.5	1,066	17.3	184	----	----
2003	144.3	1,919	9.0	144	----	----
2004	237.2	2,223	15.3	192	7.7	69
Above Como Creek (B5)						
2001	105.2	1,396	30.1	239	9.8	209
2002	84.6	1,464	16.1	209	----	----
2003	79.4	1,079	12.3	118	3.3	30
2004	106.8	1,128	11.7	85	4.5	38
Below Garfield Forebay (B6)						
2001	7.6	57	52.4	399	79.5	1,334
2002	12.3	95	24.0	208	35.9	959
2003	12.4	114	44.3	323	73.7	960
2004	23.0	152	35.0	192	36.5	491
Above Garfield Forebay (C2)						
2001	---	---	121.1	1,636	32.4	585
2002	---	---	85.4	1,089	18.9	407
2003	---	---	77.7	919	35.5	776
2004	---	---	97.8	895	15.6	529

tributary were 90,300 and 149,962, respectively. Compared to biomass, the downward trend in density for both brook and rainbow trout is evident between 2001 and 2004. This is often the case for trout populations impacted by *M. cerebralis*. Densities decline more rapidly than biomass because the fry and fingerling life stages are the most vulnerable to the effects of the parasite.

Whirling Disease Risk Management in Colorado – 1994 to 2004.

Whirling disease has been an overarching area of concern for the CDOW since late 1993 when an intensive research investigation first implicated *M. cerebralis* in the disappearance of wild rainbow trout fry in the upper Colorado River (Nehring 1993a; Walker and Nehring 1995). Subsequent studies clearly indicated that WD was linked to severe declines in wild rainbow trout in several major streams in Colorado (Nehring 1996; Nehring and Walker 1996; Nehring et al. 1998; Nehring and Thompson 2001) in Montana (Vincent 1996ab; Baldwin et al. 1998) and in other places throughout the western U.S. (Bartholomew and Reno 2002). Across the western U.S., once thriving wild trout fisheries on a region-wide scale were being threatened as never before (Hedrick 1998; Hedrick et al. 1998).

Faced with a rising threat of unknown magnitude and consequences, in April 1996 the Director of the CDOW assembled a review panel and assigned the tasks of 1) assessing the impact of WD on all aspects of the coldwater fisheries resource of Colorado, and 2) making recommendations to reduce the impact of WD, and 3) determining what changes in fisheries management activities would lower the risk of spread. The end product of that assignment was a report entitled ***An Assessment of Fishery Management and Fish Production Alternatives to Reduce the Impact of Whirling Disease in Colorado*** (Bennett et al. 1996). Another panel revisited the issue again in 1998 that resulted in a second report entitled ***A Review of Strategies for Fishery and Hatchery Management in Relation to Impacts of Whirling Disease*** (Krieger et al. 1998). The recommendations and conclusions in those two reports have provided specific direction for fisheries research and management efforts and for the hatchery production program since 1999.

In November 2000, the Colorado Wildlife Commission approved the **D-9** policy **"The Stocking and Use of Fish Tested Positive for, or Exposed to the Whirling Disease Parasite *Myxobolus cerebralis*"**. This policy set in motion a process that directed the CDOW by January 1, 2003 to "*...strive toward the objective of eliminating the stocking of WD positive fish in habitat that is capable of supporting self-sustaining salmonid populations including standing water above salmonid habitat.*" The **D-9** policy further directed the CDOW "*...to promulgate rules and regulations to prohibit private parties from stocking WD positive fish in such habitat by 2003.*" Finally, this policy also stipulates that the Director of the CDOW has the authority to review and rule upon requests for exemption(s) that would permit 1) the stocking of WD positive fish in salmonid habitats under certain circumstances, and 2) the continued operation of public or private fish culture facilities testing positive for *M. cerebralis* within salmonid habitats. This policy was implemented in 2003.

On November 4, 2004, the CDOW set forth the most recent revision of Administrative Directive **W-6: Fish Management and Stocking.** This directive mandates revised guidelines for the classification and categorization of waters as well as the management and stocking of those waters on a statewide basis.

Often times goals outlined in "blue ribbon" panel reports, the guidelines in commission policies, and rules and procedures in administrative directives get lost in the day-to-day operations at the lowest echelons of government. However, a review of all of these documents and the guidance provided within them reveals that with rare exception the CDOW has been remarkably faithful in the implementation of the direction given. As a result of that faithful adherence to the direction given and perseverance through the difficult times, tremendous progress has been made in containment and control of the spread of this parasite in Colorado.

Through an all-out research effort on national and international fronts, tremendous strides have been made in understanding the epidemiology of the parasite. Molecular-based diagnostic tools such as PCR (Andree et al. 1998) and ISH protocols (Antonio et al. 1998) have dramatically increased the probability for detection and diagnosis of an infection very early in the post-exposure period. Since the late 1990s, this has virtually eliminated the possibility of accidental stocking of potentially exposed fingerling trout into as yet unexposed high country habitats in Colorado. That happened on a large scale basis twice in the 1990s. The first was in 1992, two years before there was even a hint that *M. cerebralis* had the capacity to devastate wild trout populations. The second was in 1996, two years prior to the development of the PCR diagnostic test. In both instances, stockings occurred from two rearing units that were thought to be negative for *M. cerebralis* but were subsequently shown to be positive.

Since 1997, the CDOW has invested millions of dollars in capital construction to modernize the CDOW's fish hatchery production system and reclaim an *M. cerebralis*-negative certification for most of the coldwater trout production units. Although not yet complete, the effort has been highly successful in that there has been no reversion to an *M. cerebralis*-positive status among any of the units that have received re-certification as an *M. cerebralis*-negative facility. Hopefully, the negative status of these units can be maintained over the long term. It appears that if a facility becomes exposed it should be possible to eliminate the parasite in each of the units that have been modernized and

no longer use surface water for production. Production of *M. cerebralis* negative trout in 2003 and 2004 increased dramatically compared to the late 1990s. Even more capability for production of *M. cerebralis*-negative salmonids became available in 2005 since the Roaring Judy production unit was re-certified negative in late 2004. The implementation of BMPs, together with the capital investments in the hatchery system, has been an on-going success story over the past 8 years. Moreover, it is of great importance in the agency's overall ability to effectively and efficiently manage around the WD problem.

CONCLUSIONS

The information in this report encompasses a thorough review of most of the research activities undertaken by the CDOW between January 1994 and December 2004. A clear understanding of the past impacts of WD on the Colorado's wild trout resources is the foundation for development of effective management strategies to ameliorate, control, and hopefully contain the spread of *M. cerebralis* in the 21st century. After more than 10 years of intensive effort, the CDOW is in a far better position to implement management strategies to contain and control the spread of the parasite and minimize risk of further significant spread than it was in the early 1990s.

In 1997, 11 of 16 of Colorado's trout production facilities tested positive for *M. cerebralis*. In 1998, the CDOW entered into a multi-year, multi-million dollar capital construction investment program to modernize the state's coldwater hatchery system. While the task was not quite complete in 2005, there can be no doubt that the 12 million dollar investment has been highly successful. Only 3 of Colorado's catchable trout primary production facilities remained positive for *M. cerebralis* as of December 2005. One more unit might be recertified *M. cerebralis*-negative by the end of 2006. Most of the coldwater salmonid production by state rearing units is now certified negative for *M. cerebralis*. Implementation of "best management practices" (BMPs) at many units has resulted in dramatic reductions in the level of TAM spores detected in the effluents being discharged back into the waters of the state. Test results for trout reared at units still classified as *M. cerebralis*-positive reveal the prevalence and severity of infection is much lower than it was 5 to 10 years ago. Prevalence and severity of infection among trout produced at the Watson Lake SFRU are so low that testing by PTD rarely detects any evidence of infection even after the fish have been held on the unit for 18 to 24 months. The hatchery story is one of overwhelming success.

Beginning in 2000, the CDOW dramatically reduced the stocking of trout produced on rearing units testing positive for *M. cerebralis* in lakes and reservoirs in salmonid habitats. More than a dozen long-term research investigations that began in the mid-1990s had suggested a link between the stocking of *M. cerebralis*-infected fish and the ambient levels of infection detected in both the water flowing out of the lakes and reservoirs and in the wild trout populations downstream. Wild brook trout and rainbow trout populations were dramatically impacted. In those cases where population-level impacts occurred, biomass and density among vulnerable species were reduced by 80% to 90% compared to levels seen prior to exposure to *M. cerebralis*. At study locations where the stocking of trout testing positive for *M. cerebralis* was stopped, ambient levels of TAM spore densities began to decline exponentially within six months. In most cases, densities of TAM spores occurring in water samples decreased to undetectable levels within 24 to 30 months after the cessation of stocking of trout infected by *M. cerebralis*.

At Spring Creek Reservoir, ambient levels of TAM production increased substantially 14 months after 10,000 highly infected catchable rainbow trout were stocked in the reservoir. The pulse in TAM spore production continued unabated for another 16 months and then subsided to levels largely below the limits of detection using water filtration. This study provided evidence that there was a direct link between the stocking of trout infected with *M. cerebralis* and subsequent levels of TAM spore production.

Between 1989 and 2002, Georgetown Lake was annually stocked with substantial numbers of trout produced at SFRUs testing positive for *M. cerebralis*. Monthly water filtration sampling upstream and downstream of the lake from July 2002 through March 2003 revealed a low level of TAM production was occurring in the lake. However, continued sampling revealed that TAM production began to increase exponentially in May 2003 and remained at high levels through September 2005. A review of CDOW stocking records indicated that this 21 ha (54 acre) impoundment had been stocked with more than 276,000 trout of various sizes and species in 2002, including more than 167,000 rainbow trout fingerling. It is suspected that this large number of vulnerable young trout became severely infected by *M. cerebralis* and died of acute infection or succumbed to predation by larger fish due to reduced fitness (Snieszko 1974). This would have provided an enormous number of myxospores for ingestion by *T. tubifex* that led to the massive pulse in TAM production that continued for 29 months. Analysis of a sample of 100 tubificid oligochaetes from Georgetown Lake in the summer of 2004 using qPCR

technology revealed 100% of the detected DNA was for the lineage III *T. tubifex,* the type of worm that is most vulnerable to *M. cerebralis* (Beauchamp et al. 2002).

In any large fish production system, there are times when fish become available that (for a number of reasons) could not be stocked in the waters they were originally reared for. The untimely draining of an irrigation reservoir is one example. When this happens, alternate waters are selected for these fish to be stocked into. However, the Spring Creek, Georgetown Lake and Taylor Park Reservoir case studies are examples of the possible environmental consequences that can occur when "surplus" trout that are vulnerable to *M. cerebralis* are stocked into standing waters where the *M. cerebralis* parasite is enzootic. Two simple changes in fish stocking guidelines could help avoid a repeat of the examples listed above. First, the numbers of trout stocked into lakes or streams should be kept within the proscribed guidelines for management of recreational fisheries as set forth in the Wildlife Commission's D-9 policy and the CDOW Administrative Directive W-6: (Fish Management and Stocking). Second, "surplus" fry or fingerling trout production (fish over and above the number needed to manage within the guidelines) should be stocked into terminal water supplies that are not directly connected to salmonid waters.

A substantial body of evidence contained in this report indicates that catchable size rainbow trout that are not infected by *M. cerebralis* when stocked into aquatic habitats where the parasite is enzootic have a high probability of developing a substantial infection. This can occur even in areas where the ambient level of TAM density (as determined by water filtration) is low to moderate. This has the potential to substantially augment ambient myxospore levels in the aquatic habitat when substantial numbers of the stocked fish remain in the lake, reservoir or stream for more than 200 days. The situation is exacerbated when the fish remain in the ecosystem for 1 or 2 years longer. (See data in Tables 5, 6 and 7 for details). Management of put-and-take recreational fisheries for high harvest rates within 6 months after stocking would help to minimize the problem in these types of habitats.

It is evident from several case studies highlighted in this report that the stocking of excess production of both *M. cerebralis*-negative and *M. cerebralis*-positive trout into cold water lakes and reservoirs resulted in augmentation of TAM production. This led to significant increases in prevalence and severity of *M. cerebralis* infections among wild salmonid populations downstream. In other cases the stocking rates were far above what was necessary to meet management objectives. In some cases these actions resulted in localized extirpation of self-sustaining wild brook trout or rainbow trout populations. In other instances, the prevalence and severity of infection among wild brown trout downstream was increased 5 to 10 fold. In the future, excess production of both *M. cerebralis*-negative and *M. cerebralis*-positive fry or fingerling trout should be stocked in non-salmonid standing waters where they can be a forage base for warm water fishes. Surplus production of catchable-size trout can also be stocked into standing waters along Colorado's Front Range and other urban settings capable of sustaining cold water species on a seasonal basis. Lakes and reservoirs close to urban areas typically sustain high angler use.

Over the past decade it has often been suggested that it shouldn't matter whether salmonids are negative for *M. cerebralis* or not when destined to be stocked into habitat(s) where the parasite is already established. However, the data contained in Tables 5, 6 and 7 reveal that there are at least three reasons why stocking of trout not previously exposed to *M. cerebralis* pose less risk from a fish management perspective. First, when trout that are uninfected by *M. cerebralis* are stocked into habitats where the parasite is enzootic and infection is immediate, it will take a minimum of 180 to 200 days for maturation of myxospores. If the majority of those fish are harvested within the six months after stocking, there will be very little augmentation of the myxospore burden in that ecosystem. Second, if trout infected by *M. cerebralis* are stocked into an aquatic ecosystem where the parasite is already established, augmentation of the myxospore burden in that environment could begin right after stocking. The data in Table 7 also demonstrate that the myxospore burden in a group of trout can increase by orders of magnitude between 200 and 565 days post-stocking. Therefore, when trout already carrying myxospores are stocked into an environment where *M. cerebralis* is enzootic the result will be an immediate and more rapid augmentation of the myxospore burden in that ecosystem. Third, catchable trout infected with *M. cerebralis* at the time of stocking enter a hostile environment suffering from reduced fitness and are likely to suffer higher mortality per unit time than uninfected trout stocked into the same habitat (Snieszko 1974). This too will exacerbate the rate of myxospore input into the ecosystem. For all these reasons, the stocking of trout unexposed to *M. cerebralis* in lakes and streams capable of supporting salmonids on a year round basis significantly reduces the risk of augmentation of the myxospore burden for a minimum period of six months in habitats where the parasite is enzootic.

More than a dozen case studies for streams and lake or reservoir/stream ecosystems summarized in this report highlight the myriad and complex pathways that *M. cerebralis* can be vectored through aquatic ecosystems. Once established in an aquatic ecosystem, there are numerous ways that *M. cerebralis* can spread over which man has very little control. Myxospores can be transported vertically and horizontally across aquatic ecosystems by avian, mammalian and piscine predators, fish immigration and emigration, and by "hitchhiking" on vehicles, boats, and angler's wading equipment. However, all of the case studies and evidence reviewed in this report indicate that the stocking of *M. cerebralis*-infected trout is the primary mechanism by which the parasite has been spread in Colorado. Investigators in

other states have reached the same conclusion (Modin 1998). With the approval and implementation of the Wildlife Commission **D-9** policy whereby stocking of the *M. cerebralis*-infected trout into salmonid habitats was largely eliminated beginning in January 2003, this completely controllable source of infection has been greatly reduced.

The case studies summarized in this report and others reported previously (Nehring and Thompson 2003) reveal the complexity and unpredictability of this parasite once established in aquatic environments. It is very difficult to predict with any degree of certainty where, when and under what circumstances the impact of *M. cerebralis* might be devastating and where it would be benign. In situations where there is a high degree of uncertainty and risk involved with certain management activities, scientists often resort to multi-dimensional modeling analyses to reduce uncertainty and minimize risk. In the 1980s, CDOW researchers developed and tested a model (FISHREGS) for evaluating the effects of different angling regulations on trout populations in Colorado streams (Espegren et al. 1990). That effort produced reasonably accurate results because the five or six parameters needed for model input were either readily available from field data or could be reliably estimated or gleaned from previous studies.

Modeling efforts can be utilitarian in a general sense to gain a better understanding of the dynamic interactions between a parasite, its hosts and the interactions with the physical environment. Indeed, three different modeling approaches to evaluate impacts and assessment of risk with *M. cerebralis* were presented at the WD Symposium in Denver, Colorado, in February 2005 (Budy et al. 2005; Arsan et al. 2005; Kaeser and Reno 2005). However, when the life cycle of the parasite in question has two hosts, involves two distinct spore stages and can require up to two years for completion of the life cycle, the modeling effort becomes enormously complex and unwieldy compared to the FISHREGS model (Espegren et al. 1990). Kaeser and Reno (2005) used 13 different parameters in their model just to address the dynamics of TAM spore production on the worm side of the life cycle. Any attempt to model the augmentation of myxospores that would result from stocking of trout infected with *M. cerebralis* into an ecosystem where the parasite is already established with any degree of accuracy would require reasonable approximations of the following parameters:

1. number of myxospores per stocked fish
2. number of fish stocked
3. size of fish stocked
4. species of fish stocked
5. rate of angler harvest
6. average density of TAM spores of *M. cerebralis* in the environment
7. water temperature (seasonal range and variation)
8. rate of development of myxospores post stocking
9. fish mortality/survival if not removed
10. length of time in water post stocking
11. density of wild trout in the target environment
12. prevalence of infection in the wild salmonid population
13. mean cranial myxospore concentrations among wild salmonids
14. mortality rates among wild salmonids
15. density of *T. tubifex* aquatic oligochaetes
16. relative abundance of strains of *T. tubifex*
17. relative rates of TAM production for susceptible strains of *T. tubifex.*

The parameterization of a mathematical model with 17 variables would be an enormous task. Only information for parameters 1 through 4 would or could be known with great precision and accuracy at the outset. The information needed for parameters 5 through 17 would be more difficult to obtain, would require considerable investment of personnel time and costs, and would require days, weeks or even months to obtain in many instances. There would be a wide range of unpredictable variation for many of the parameters and almost all of the information needed to parameterize the model would be specific to a single ecosystem. Very little of the required information is going to be available on a water by water basis. The modeling effort would be an impossibly complex process. In the final analysis, the investments of time, human resources and cost for such a modeling effort would be prohibitive.

In more than 10 years of study there is virtually no evidence that demonstrates that *M. cerebralis* infection results in detrimental impacts among wild brown trout that can be detected at the population level. However, due to their innate high level of resistance to the parasite, high-density brown trout populations tend to become natural reservoirs of infectivity. This increases the probability that a high level of parasite virulence will be sustained over a very long period of time in lake and stream ecosystems where the necessary suite of environmental co-factors are present to complete the life cycle of *M. cerebralis*. For these reasons it is debatable whether or not the lack of detrimental impacts resulting from exposure to *M. cerebralis* should be the sole criterion used for justification for continued stocking of *M. cerebralis*-exposed salmonids into standing waters where only allopatric populations of wild brown trout occur downstream.

RECOMMENDATIONS

There are a number of areas where the CDOW needs to continue efforts in the on-going battle to contain and control the spread of WD and remain pro-active in the development of management strategies that reduce the risk of exacerbating the spread of the parasite in Colorado.

First, the agency should pursue efforts to develop strains of rainbow trout with a demonstrated high degree of resistance to *M. cerebralis*. This work has begun in the aquatic research section and should be continued. Given an adequate level of funding and effort it is highly probable that a rainbow trout broodstock with a very high degree of resistance to *M. cerebralis* could be in production in the state's hatchery system before 2010. This effort is of paramount importance.

Second, the author recommends that the CDOW begin exploring the use of lineages of *Tubifex tubifex* that are resistant to *M. cerebralis* as biological control agents to shift the aquatic oligochaete community population structure away from strains that are highly susceptible to *M. cerebralis*. This should be explored in aquatic ecosystems with a high degree of parasite virulence. Researchers at the University of California-Davis identified four different lineages of *T. tubifex* with varying levels of susceptibility to infection by *M. cerebralis*. Beauchamp et al. (2001, 2002) reported that lineages I, III and VI were susceptible to infection by *M. cerebralis* while lineage V is known to be non-susceptible. Lineage III oligochaetes were shown to be the most vulnerable to infection by *M. cerebralis*. In another study, DuBey and Caldwell (2004) detected no evidence of *M. cerebralis* infection among lineage I and VI *T. tubifex* in the San Juan River in New Mexico while evidence of infection was apparent in lineage III oligochaetes. Results of a subsequent laboratory exposure of *T. tubifex* to *M. cerebralis* corroborated the results of the field study in the San Juan River. Lineage III *T. tubifex* were shown to be susceptible to infection by the parasite, while no infection was detected among lineage VI oligochaetes exposed to the parasite (DuBey et al. 2005). Extensive testing of tens of thousands of *T. tubifex* from Windy Gap Reservoir (WGR) on the upper Colorado River during 2004 and 2005 suggest that lineage I, V and VI *T. tubifex* are the dominant strains of oligochaetes across wide sections of habitat in WGR. This may in large part explain the 90 – 95% reduction in TAM production emanating from WGR that began in the summer of 2001 and has continued through December 2005. All of the foregoing suggests that lineage V and VI *T. tubifex* may be effective as biological control agents to shift the aquatic oligochaete community population structure towards strains that are less susceptible to infection by *M. cerebralis*.

Third, the author recommends that the CDOW continue a systematic evaluation of the spread of *M. cerebralis* into cutthroat trout populations and recovery streams on a statewide basis. This effort began in the summer of 2003 and is projected to continue through at least 2007. This is a critical component in the agency's efforts to 1) assess the level of exposure that has already occurred, and 2) determine the degree of risk for establishment of *M. cerebralis* in high elevation habitats designated as native cutthroat trout recovery areas.

Fourth, there may be a need to determine whether or not recent declines in populations of mountain whitefish in the White and Yampa rivers (Tom Nesler, personal communication) are possibly due to *M. cerebralis* infection or some other factor(s). Anecdotal evidence from field studies in Colorado from the 1990s (Nehring 2003) together with results of laboratory exposures (MacConnell et al. 2000) have demonstrated that mountain whitefish *Prosopium williamsoni* are vulnerable to infection by *M. cerebralis*. Results of controlled laboratory exposures in Montana revealed mountain whitefish developed overt clinical signs of WD five months PE comparable to those observed in young rainbow trout that were similarly exposed (MacConnell et al. 2000). Caudal lesions of the spine were especially evident in juvenile mountain whitefish compared to those seen in similarly exposed rainbow trout. These findings may suggest that mountain whitefish could experience population level impacts in habitats where to *M. cerebralis* is enzootic. Mountain whitefish, like the greenback, Rio Grande and Colorado River cutthroat trout, are native to Colorado.

Finally, the CDOW should explore the formation a working group of six to eight individuals to develop risk assessment guidelines and alternatives to better manage around *M. cerebralis* and minimize the potential for future epizootics of whirling disease. Potential members of the working group might be comprised of three fisheries researchers involved in WD research over the past 10 years and the three senior fisheries managers. The tasks of the working group would at a minimum be as follows:

Thoroughly review the recommendations made in this report and add to, modify, or adjust those recommendations as deemed necessary by the group.

Consider undertaking a detailed review of the current cold water fish stocking plan as developed in the CDOW's computerized TRANS 5 fish stocking data base. The purpose of that review would be to determine whether or not there are a significant number of instances where over stocking of fry, fingerling or catchable trout might pose a threat for exacerbating ambient levels of *M. cerebralis* infectivity in habitats where the parasite is already enzootic.

Develop a list of non-salmonid waters that could be destinations for the safe disposal of excess trout production when such an occasion might arise.

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