STREAM FISHERIES INVESTIGATIONS

Federal Aid Project F-237-R5

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Federal Aid in Fish and Wildlife Restoration

Job Progress Report

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Fish Research Section

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The results of the research investigations contained in this report represent work of the authors and may or may not have been implemented as Division of Wildlife policy by the Director or Wildlife Commission.

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Study Title: Stream Fisheries Investigations

Project No.: F-237R-5

Job Title: Electrofishing Injury Investigations Job No. 1

Job Objective: Determine the effects of electrofishing with 60 cycle pulsed DC voltage (as used in Colorado's boat and walk electrofishing operations) on rainbow and brown trout populations in the Arkansas, Colorado, Dolores, Gunnison, Rio Grande, and South Platte rivers.

Period Covered: July 1, 1993 to June 30, 1998

INTRODUCTION

The field portions of this study were completed during 1993 and 1994. Two professional papers documenting the results our studies were completed, submitted, and accepted for publication in *The North American Journal of Fisheries Management*.

RESULTS

Two professional papers covering the primary results of this research project were published in the February 1997 issue of *The North American Journal of Fisheries Management*. The citations of those articles are as follows:

1. Thompson, K. G., E. P. Bergersen, and R. B. Nehring. 1997. Injuries to brown trout and rainbow trout induced by capture with pulsed direct current. North American Journal of Fisheries Management 17:141-153.

2. Thompson, K. G., E. P. Bergersen, R. B. Nehring, and D. C. Bowden. 1997. Long term effects of electrofishing on growth and body condition of brown trout and rainbow trout. North American Journal of Fisheries Management 17:154-159.

For the specific details and information on the results and findings of this study, we refer the reader to the above journal articles. The recommendations and conclusions we feel are pertinent to the Colorado Division of Wildlife are summarized below.

RECOMMENDATIONS AND CONCLUSIONS

The overall results of this study indicate that electrofishing as it is currently practiced in Colorado does not appear to have measurable detrimental population level effects on stream trout, but that it can have adverse effects on the well-being of individual fish. It is the mission of the Division of Wildlife to protect and enhance the fishery resources of Colorado; therefore, it seems reasonable to do what is possible to protect the well-being of individual animals, thereby minimizing the chance that population effects could be manifested. Accordingly, we recommend the following guidelines for the practice of electrofishing in Colorado streams:

1. Biologists with equipment capable of low pulse frequency ($\leq$ 30 pulses/s) rates should use those frequencies in electrofishing operations. We recommend using no higher pulse frequency and power output than is necessary to obtain adequate galvanotaxis of the fish.

2. As the State's inventory of VVP2C's wear out, replace them with electrofishing units that have more flexibility in terms of available waveforms, pulse rates and pulse widths.

3. Use experienced electrofishing personnel whenever possible. With new personnel, take the time to explain to them the basic guidelines for minimizing injury, e.g., removal of fish from the electrical field as quickly as possible, avoid dipping the fish back into the electrical field repeatedly, and avoid placing the electrode so close to fish (or vice versa) that tetany results.

Electrofishing remains the single most effective tool for efficiently capturing large numbers of trout from stream environments in a very short period of time. We currently employ bank electrofishing techniques to collect young-of-the-year (YOY) rainbow and brown trout fry to estimate variations in relative abundance of these fish within streams and locations between years.

For example, on a trout fry sampling trip into the Black Canyon of the Gunnison River gorge in August 1997, more than 600 YOY rainbow trout fry were collected from one 15.2 m (50 ft.) section of river bank. This is the highest number of YOY rainbow trout fry ever collected at an individual sampling station. Ten 15.2 m stations were electrofished on this three-day study. However, the combined length of these 10 sample stations represented only 0.018% of the entire stream reach being studied! This data is critically important in the assessment of the effects of the myxosporean parasite *Myxobolus cerebralis* on YOY trout recruitment. Electrofishing is stressful to healthy trout, and even more stressful to YOY trout parasitized by *M. cerebralis*. In these situations significant numbers of YOY trout can die due to the combined effects of these stressors. But what is most important for the angling public and concerned citizenry to understand is that far less than 0.5% of the total habitat (and YOY trout population therein) is subjected to electrofishing in any single year. As such, it is not logical to assume that electrofishing operations are in any way responsible for the loss of large numbers of YOY trout from the population.

Study Title: <u>Stream Fisheries Investigations</u>

Job Title: <u>Special Regulations Evaluations</u> Job No. 2

Job Objective: Determine the effects of restrictive angling regulations, altered flow regimes, and the fish pathogen *Myxobolus cerebralis* on the trout population dynamics of the Big Thompson, Cache la Poudre, Colorado, Dolores, Fryingpan, Gunnison, Rio Grande, Roaring Fork, South Platte, and Taylor rivers.

Period Covered: July 1, 1993 to June 30, 1998

INTRODUCTION

Under the original documentation, only the Colorado and Fryingpan rivers were included in this research investigation. During two previous segments (1995/1996 and 1996/9997), eight more streams were added to the job objective, bringing the total number of streams in this research study to ten.

Sport fishing is big business in Colorado. The annual economic benefit of this industry to the state is estimated at approximately 900 million dollars (Colorado Division of Wildlife, 1996). Fishing for wild trout comprises one of the major components of this recreational opportunity. Even a decade ago, the ten streams included in this study were among the most important, most heavily fished, wild trout streams in the state (Nehring 1987).

The original intent of the study was to determine if there had been any changes in the stream trout populations in the Colorado and Fryingpan rivers attributable to the effects of restrictive angling regulations, angling pressure, harvest, altered flow regimes and resultant water temperature variations.

However, during the first two study segments (1993/1994 and 1994/1995) it was determined that whirling disease was implicated as a primary factor in severe declines in wild rainbow trout recruitment in the Colorado River (Walker and Nehring 1995, Nehring and Walker 1996). Whirling disease is now considered the primary reason for severe declines in recruitment of rainbow trout across approximately 290 km of four other major trout streams in Colorado, including the Cache la Poudre, Gunnison, Rio Grande, and South Platte rivers (Nehring 1996). Since 1996, dramatic declines in recruitment of age 1+ wild rainbow trout have been observed in the Dolores, Fryingpan, and Roaring Fork rivers. At the end of 1997, wild brown and rainbow trout in the Big Thompson and Taylor rivers drainages test positive for the presence of *Myxobolus cerebralis*, the myxosporean parasite that can cause whirling disease (Markiw 1992). However, population declines in trout numbers have not been documented in these two streams.

As a result of these disturbing findings, the thrust and direction of study in this research project (F237R) has been significantly altered several times during the past five years. The job objective given above is very different from the original objective laid out in the program narrative and the first study segment (1993/1994). The original job objective read as follows:

"Determine the effects of restrictive angling regulations, angling pressure, harvest, altered flow regimes and resultant water temperature variations on the trout population dynamics of the Colorado and Fryingpan rivers."

As a result of the on-going drastic declines in the recruitment and survival of YOY rainbow trout, the Colorado Division of Wildlife (CDOW) has committed, reallocated, and redirected its fishery research and management priorities to attack this serious threat to the state's coldwater fishery resource.

METHODS

Estimation of Fry Abundance

Beginning as early as 1992 on some streams, estimates of YOY rainbow and brown trout population abundance were derived on 15.2 m (50 feet) sections of riffle habitat using bank electrofishing equipment. Multiple pass removal procedures were used to estimate YOY trout density and species composition. Estimates were completed once each month (usually from July through November) at six sites on the Colorado River from below Windy Gap Dam (near Granby) downstream to the Sunset Ranch (near Parshall, Colorado). On most other study streams, population estimates on YOY rainbow and brown trout were completed once annually during the fall field season.

Multiple pass removal procedures were used to estimate YOY trout density and species composition. The computer program CAPTURE was used to calculate the multiple pass removal estimates of trout fry abundance (White et al, 1982).

Estimation of Juvenile and Adult Trout Abundance

Juvenile (age 1+) and adult trout population estimates were derived using either the Peterson two pass mark and recapture method employed in previous studies (Nehring and Anderson 1982; Nehring 1987, 1993), or the two pass removal estimator (Seber and LeCren 1967). On smaller stream segments at low flow, walk electrofishing gear and a field crew of 10-15 people were used in the sampling process. In these instances, segments of stream up to 305 m (1000 feet) were electrofished.

On larger rivers or segments of stream at higher discharge levels, boat electrofishing gear was used to obtain trout population estimates. This methodology was used on lengthy segments of the Colorado, Dolores, Gunnison, Rio Grande, and Roaring Fork rivers. Reach lengths on individual streams varied from 3.2 km to 10.8 km. A computer software program called GOLDMEDL was used to calculate size and age-specific population and biomass estimates for these stream segments. The Peterson two-pass mark and recapture estimator was the population model used on all study sections of these streams.

Trout population estimates, density (n/ha), and biomass (kg/ha) were determined using procedures previously reported (Nehring 1980). Age and growth information was used to subdivide the trout populations estimates into age specific cohorts and construct life tables for each population estimate on the Colorado River (see Appendix Table I). With the aid of a Bausch and Lomb Microprojector to read fish scales, we conducted age and growth analyses using the computer software package DISBCAL (Missouri Department of Conservation) to back-calculate length at age.

Testing For *Myxobolus cerebralis*

During this five year study, four different methodologies have been used to test for evidence of infection by the myxosporean parasite *M. cerebralis, (MC)* the causative agent of whirling disease (Markiw 1992). During 1994 and early 1995, the plankton centrifuge method was used to test for the presence of *MC* myxospores (O'Grodnick 1975). Since late 1995, the pepsin-trypsin-digestion (PTD) method has been used to test for the presence of MC spores in near-yearling and older trout (Markiw and Wolf 1974). Spores numbers were quantified in those fish testing positive. Among YOY rainbow and brown trout fish tissues were sectioned, mounted on slides, and examined histologically for evidence of damage to cartilagenous tissues characteristic of that caused by *M. cerebralis*. Finally, the DNA-based polymerase-chain-reaction (PCR) technology has been used to detect the DNA of the parasite in very young trout during the first few months after potential exposure (Andree et al. 1997).

To characterize the relative degree of infection as determined by histological examination of YOY trout sectioned sagittally through the orbit of the eyes, we developed (in collaboration with Dr. Linda Chittum, DVM and pathologist at the Colorado Division of Wildlife (CDOW) Fish Health Laboratory) a scaled histopathology grading system. This grading system is a numeric system, ranging from zero (no evidence of infection characteristic of that caused by *M. cerebralis*) to four (significant or **marked** evidence of infection characteristic of that caused by *M. cerebralis*).

That rating system is shown below:

ZERO (0) No significant microscopic lesions (NSML)

1.0 Focal or multifocal chondrolysis with intraslesional trophozoites consistent w/ M. cerebralis, but **NO inflammatory response** noted. Lesions involve only **one tissue** or location.

1.5 Multifocal chondrolysis with intraslesional trophozoites consistent w/M. cerebralis, but **NO inflammatory response** noted. Lesions involved in **two or more** tissues or locations.

2.0 Multifocal chondrolysis with intraslesional trophozoites consistent w/M. cerebralis with **mild or moderate granulomatous inflammatory response**. Lesions usually involve only **one tissue** or location.

Possible locations can include:

a. skull cartilage
b. otoliths
c. opercle
d. gill arch cartilage
e. calvarium

2.5 Multifocal chondrolysis with **mild or moderate granulomatous inflammatory response** with intraslesional trophozoites consistent w/M. cerebralis . Lesions usually involve **two or more tissues** or locations. Intralesional trophozoites, immature and mature spores consistent with M. cerebralis noted.

3.0 Multifocal chondrolysis with **moderate granulomatous inflammatory response** with intraslesional trophozoites consistent w/ M. cerebralis. Lesions usually involve at least **two or three tissues** or locations. Intralesional trophozoites, sporoblasts, immature and mature spores consistent with M. cerebralis noted.

3.5 Multifocal chondrolysis with **moderate granulomatous inflammatory response** with intraslesional trophozoites consistent w/ M. cerebralis. Lesions usually involve at least **three or more tissues** or locations. Intralesional trophozoites, sporoblasts, immature and mature spores consistent with M. cerebralis noted.

4.0 Multifocal chondrolysis with **MARKED granulomatous inflammatory response** with intraslesional trophozoites consistent w/ M. cerebralis. Lesions usually involve **four or more tissues** or locations. Intralesional trophozoites, sporoblasts, immature and mature spores consistent with M. cerebralis noted.

Sentinel Fish Testing

As this five year study draws to a close, we are starting our fifth consecutive year of sentinel fish testing on the upper Colorado River. There have been numerous objectives for these tests. For the tests conducted during the 1994 field season, our primary objectives were to document the timing and appearance of clinical signs of whirling disease and the degree of lethal effects manifested in Tasmanian strain hatchery rainbow trout *(Oncorhynchus mykiss)*, wild Colorado River rainbow (CRR) trout, Trappers Lake strain cutthroat trout *(O. clarki pleuriticus)*, and feral brown trout *(Salmo trutta)* from the Colorado River.

There were multiple objectives for the sentinel fish studies that began in July 1995 and ended in September 1996. First, we were comparing the onset of clinical signs of disease and the degree of mortality occurring among three different treatment groups of CRR trout, three treatment groups of Colorado River cutthroat trout *(Oncorhynchus clarki pleuriticus)*, brook trout *(Salvelinus fontinalis)*, and brown trout. Second, we compared the level of *MC* myxospore production within and between species treatment groups.

Eight different treatment groups of trout were tested in the 1996/1997 sentinel fish experiments, including wild brown trout from the Colorado River, two different age groups of Snake River cutthroat trout *(Oncorhynchus clarki bouvieri)*, Colorado River cutthroat trout, Rio Grande cutthroat trout *(O. c. virginalis)*, greenback cutthroat trout *(O. c. stomias)*, and two different treatment groups of CRR trout. One group of CRR trout were progeny of wild rainbow trout parents that had hatched, survived, and matured to spawn in the Colorado river **after** the onset and establishment of whirling disease in the river. The other group consisted of progeny from the "run-of-the-river" rainbow trout spawners, the vast majority of which were fish that had been hatched and reared in the river prior to the onset of whirling disease among rainbow trout in 1991. Again, we were documenting the timing and appearance of clinical signs of whirling disease among the treatment groups, the degree of lethal effects occurring between the treatment groups, and the intensity of development of the *MC* spore burden among and between the treatment groups over time.

For the 1997/1998 sentinel fish experiments we exposed wild brown trout and three different age groups (from two different year classes) of wild CRR trout to varying levels of ambient triactinomyxon

(TAM) actinospores at four different locations in the upper Colorado River. One group of CRR trout was initially introduced into the river in October 1996. The second group had been hatched in the river and exposed to TAM spores from the time of emergence from the gravel. The third group was CRR trout hatched and reared at the Bellvue Hatchery in a specific-pathogen-free water supply and introduced to the Colorado River at an average size of 53 mm (2.1 inches). Again, the objectives of the study were to document the timing and appearance of clinical signs of whirling disease among the treatment groups, the degree of lethal effects occurring between the treatment groups, and the intensity of development of the *MC* spore burden among and between the treatment groups over time.

For the 1995/1996 and 1996/1997 sentinel fish experiments, a randomized complete block experimental design incorporating four replicates of eight species/strain treatments was used. For the 1997/1998 experiment we used a randomized complete block experimental design incorporating two replicates per treatment at each of four different exposure locations.

Daily care of the experimental fish included feeding, cleaning of the experimental cells, and daily removal and recording of mortalities. Some mortalities were preserved for subsequent histological analysis and quantification of *MC* spore burdens in individual fish. The experimental tanks were rotated 180 degrees once each week so that all groups of fish spent an equal amount of time in the upstream position, in order to equalize exposure to MC infectivity as much as possible.

Complete inventories of the experimental fish were conducted on a monthly basis, usually commencing one month after the official start of the experiments. All fish were counted, weighed in mass, and characterized for overt symptoms of whirling disease including blacktail, whirling behavior, cranial deformities, exophthalmia, and caudal deformities. Statistical analyses of differences in proportion surviving among treatments on each of the inventory dates were performed on an arc sine-squareroot transformation as well as the real proportions. Because the onset of overt clinical signs varied in time and appearance between species and treatment groups no statistical analyses were done on the symptoms. However, these clinical signs were used as empirical corroborative evidence of the presence of the parasite in the fish and considered indicative of whirling disease among the experimental fish.

Gas Supersaturation Testing

During studies conducted on the upper Colorado River in 1994 (Walker and Nehring 1995), gas supersaturation was considered a possible exacerbating factor acting in an additive or synergistic manner in concert with the *M. cerebralis* parasite that can cause whirling disease. As a result of these discoveries, gas saturation monitoring was conducted on a number of streams during the summer, fall, and early winter months of 1995 and 1996 to determine whether or not gas supersaturation phenomena were prevalent on other streams throughout the state as well. Study streams included the Arkansas, Cache la Poudre, Colorado, Dolores, East, Gunnison, South Platte, and Taylor rivers. These measurements were made using the Common Sensing Saturometer. Parameters measured at each study site on each date included the following:

BAR	= Barometeric pressure (mm Hg)
Air T	= Air temperature (degrees Celsius)
H_2O T	= Water temperature (degrees Celsius)
P_T	= Total gas pressure in water (mm Hg)
P_{O2}	= Oxygen partial pressure (mm Hg)
$P_T - P_{O2}$	= Total gas pressure minus Oxygen partial pressure (mm Hg)

Delta P	= P_T - BAR (Total gas pressure in water - barometric pressure)
P_T % SAT	= Percent saturation of total gas pressure in water
P_{O2} % Sat	= Percent saturation of Oxygen in water
% Sat N_2/Ar	= Percent saturation of Nitrogen/Argon in water

RESULTS AND DISCUSSION

By the end of 1997, trout from all of the streams under study tested positive for *Myxobolus cerebralis*. In five streams (Cache la Poudre, Colorado, Gunnison, Rio Grande, and South Platte rivers) whirling disease is strongly implicated in catastrophic loss of YOY rainbow trout. Drastic declines in rainbow trout recruitment began in the Cache la Poudre, Colorado, upper Gunnison, and South Platte rivers about 1990 or 1991, almost three years before extensive testing for *M. cerebralis* in wild trout from these streams began. In others areas (lower Gunnison River in the Gunnison Gorge, and the Rio Grande) population level declines were detected in the age 1+ rainbow trout population in 1994, the same year that presence of the *M. cerebralis* parasite was first detected in feral trout. Declines in age 1+ wild rainbow trout began to appear in the Dolores and Fryingpan rivers in 1996. Large declines in wild rainbow trout density and biomass were documented in the reach of the Roaring Fork River just downstream from Basalt, Colorado between the fall of 1995 and 1997. However, declines in wild rainbow trout in the Roaring Fork River may have begun earlier than this. In another reach of the upper Roaring Fork River at Aspen, rainbow trout density and biomass in October 1997 was much reduced from levels observed in the same reach in the late-1970s and early 1980s. At the end of 1997, the only two study streams where the effects of the parasite appear to be relatively benign are the Big Thompson and Taylor rivers.

Estimates of Trout Fry Abundance

Time constraints, an early winter, and an inadequate number of field personnel prevented us from collecting fry population estimation data on some of the study streams during the summer and fall of 1997. Estimates of rainbow and brown trout fry abundance were completed during the standard fall electrofishing procedures on the Big Thompson and Cache la Poudre rivers by the Northeast Region Fisheries crew. Their data from these streams are included in this report (see Table 1). We were able to complete our trout fry sampling protocol on the upper Colorado, Dolores, Gunnison, and South Platte rivers (Table 1). Single pass relative abundance estimates were completed at one site on Beaver Creek, tributary to the South Fork of the Rio Grande, one site on the Roaring Fork River, two sites on the Rio Grande, and three reaches of the Fryingpan River. No fry sampling was conducted on the Taylor River. However, on some of the study streams, population estimates of rainbow and brown trout fry abundance were made at a number of study sites, and on more than one occasion. These estimates are shown in Table 1. Relative abundance sampling for rainbow and brown trout fry was conducted on the remaining streams to determine the ratio of wild rainbow to brown trout fry present (Table 2).

Among the streams and stream segments where fry population estimates were conducted, wild rainbow trout fry were collected from virtually all streams and most stream segments on all sampling occasions (Table 1). In many instances, rainbow trout fry were as numerous or often far more abundant than brown trout fry collected at the same location.

Among those streams where relative abundance sampling for rainbow and brown trout fry was conducted in 1997, rainbow trout fry were collected at the majority of sites. In 1996, when fry sampling was conducted on all study streams, YOY rainbow trout were collected at 26 of 29 sites (see Table 2 in

Nehring and Thompson 1997). These data clearly indicate wild rainbow trout spawn successfully in many high quality trout streams all across Colorado. Moreover, extensive sampling during the 1980s clearly shows that this was the case in many of these streams prior to the advent of whirling disease in Colorado (see Table 1 in Nehring 1988b; Federal Aid Job Progress Report).

However, the problem in the 1990s is that in many of the high quality trout streams, the young rainbow trout are exposed to the triactinomyxon (TAM) actinospore of *Myxobolus cerebralis*, the myxosporean parasite that can cause whirling disease. Mortality rates of young rainbow trout fry are very high and few fry survive beyond the first summer of life (Tables 1, 2, and 3). This is clearly evident from the fry electrofishing data for the Colorado River in Middle Park (Table 3) and in the Gunnison River gorge (Table 4).

Rainbow and brown trout fry abundance has been monitored at six or more sites on the upper Colorado River in Middle Park approximately once a month from mid-July through mid-November each year between 1994 and 1997 (Table 3). Sampling in April of 1995, 1996, and 1997 revealed that overwinter survival of feral (stream hatched and reared) rainbow trout fry in the upper Colorado is virtually nonexistent (Table 3).

In each of the past four years the summer to fall trends in abundance of rainbow and brown trout fry have been remarkably similar (Table 3). Each year, rainbow trout fry numbers have declined in an unrelenting manner usually beginning in mid-August in synchrony with the first appearance of overt symptoms of whirling disease in the fry. These overt symptoms include, severely sloping craniums, black tail, exophthalmia (eyes bulging out of the eye socket), lordoscis, scoliosis, and whirling behavior (characterized by swimming in tightly concentric circles when frightened). The severely sloping cranium is usually the first overt symptom to appear in large numbers of rainbow trout fry each summer. Exopthalmia becomes progressively more severe as the summer season proceeds into fall and the soft tissues of the eye continue to grow but the orbit of the eye does not due to the destruction of cartilage consumed by vegetative stages of the *M. cerebralis* parasite. Whirling behavior usually first appears in some rainbow trout fry in mid-August and becomes more apparent with the passage of summer into fall. Skeletal deformities (lordoscis and scoliosis) as well as blacktail become more apparent in greater numbers of brown trout fry in the September and October. However, with the onset of winter and colder water temperatures, black tail often becomes less pronounced.

Among brown trout deformities of the cranium are not readily apparent to the untrained eye, usually consisting of a shortened and slightly sloping snout, a shortened mandible that is often twisted slightly to the side anteriorly. Blacktail is the most common easily visible overt clinical sign of WD readily apparent among brown trout fry, particularly in the upper Colorado River between Windy Gap Dam and the town of Hot Sulphur Springs. By October and November, in the upper reaches of the river immediately below Windy Gap Dam, incidence of blacktail among YOY brown trout can exceed 90%. In the fall, skeletal deformity in the caudal peduncle region usually accompanies the blacktail in brown trout fry in these reaches of the upper Colorado River.

Table 1. Estimates (n/km) of young-of-the-year rainbow and brown trout fry in streams across Colorado in 1997.

Date mo/da/yr	River	Site Location(s)	no	Rainbow Fry (numbers/km)	Range (n/km)	Brown Fry (numbers/km)	Range (n/km)
10/23/97	Big Thompson R.	Habitat Improvement Site	1	975	-----	44	-----
10/22/97	Big Thompson R.	Handicap Fishing Pier	1	194	-----	12	-----
11/05/97	Big Thompson R.	Silvendale	1	98	-----	109	-----
10/23/97	Big Thompson R.	Chuck's Place	1	261	-----	17	-----
10/22/97	Big Thompson R.	Grandpa's Place	1	193	-----	0	-----
11/05/97	Big Thompson R.	Below Waltonia	1	636	-----	11	-----
10/31/97	Big Thompson R.	Twin Pines	1	132	-----	7	-----
10/31/97	Big Thompson R.	Drake Campground	1	50	-----	22	-----
10/23/97	Cache la Poudre	Pasquinal's Cabin	1	0	-----	27	-----
11/13/97	Cache la Poudre	Upper Wild Trout Water	1	0	-----	5	-----
11/03/97	Cache la Poudre	Upper Control (Firelane)	1	6	-----	28	-----
11/04/97	Cache la Poudre	Indian Meadows	1	82	-----	33	-----
11/04/97	Cache la Poudre	Kelly Flats	1	372	-----	170	-----
09/30/97	Dolores River	various sites on river	3	218	0 - 655	175	0 - 393
07/14/97	Colorado River	various sites on river	6	416	0 - 919	3,784	787 - 10,761
08/12/97	Colorado River	various sites on river	6	656	131 - 1,444	1,422	656 - 3,150
09/17/97	Colorado River	various sites on river	6	328	0 - 919	2,756	1,181 - 4,724
10/13/97	Colorado River	various sites on river	6	219	0 - 525	2,078	787 - 4,987

Table 1 (Continued). Estimates (n/km) of young-of-the-year rainbow and brown trout fry in streams across Colorado in 1997.

Date mo/da/yr	River	Site Location(s)	no	Rainbow Fry (numbers/km)	Range (n/km)	Brown Fry (numbers/km)	Range (n/km)
08/06/97	Gunnison River	Below Chukar Trail	1	49,103	-----	21,343	-----
08/06/97	Gunnison River	Below Chukar Trail	1	4,583	-----	5,761	-----
08/06/97	Gunnison River	Below Bobcat Trail	1	19,903	-----	16,106	-----
08/06/97	Gunnison River	Below Bobcat Trail	1	4,976	-----	1,964	-----
08/07/97	Gunnison River	Duncan/Ute Trail Area	1	46,353	-----	11,785	-----
08/07/97	Gunnison River	Duncan/Ute Trail Area	1	76,076	-----	22,260	-----
08/07/97	Gunnison River	Duncan/Ute Trail Area	1	3,274	-----	2,357	-----
08/08/97	Gunnison River	Duncan/Ute Trail Area	1	13,749	-----	12,308	-----
08/08/97	Gunnison River	Below Smith Fork R.	1	12,439	-----	2,095	-----
08/08/97	Gunnison River	Below Smith Fork R.	1	8,642	-----	6,285	-----
08/97	Gunnison River	Average of 10 sites	10	23,910	3,274-76,076	10,226	1,964-22,260
09/23/97	Gunnison River	Ute Park, Upper West Side	1	2,750	-----	11,523	-----
09/23/97	Gunnison River	Ute Park, Above Riffle	1	0	-----	4,321	-----
09/23/97	Gunnison River	Ute Park, Below Riffle	1	655	-----	4,321	-----
09/24/97	Gunnison River	Ute Park, Upper East Side	1	131	-----	10,909	-----
09/24/97	Gunnison River	Ute Park, W. Side Island	1	10,868	-----	6,285	-----
09/24/97	Gunnison River	Ute Park, E. Side Island	1	1,702	-----	2,357	-----
09/24/97	Gunnison River	Ute Park, E. Below Island	1	131	-----	1,702	-----
09/25/97	Gunnison River	Top Island Pool, W. Side	1	393	-----	8,642	-----
09/25/97	Gunnison River	Bottom Is. Pool, W. Side	1	393	-----	8,642	-----
09/25/97	Gunnison River	Site w/76,000 fry 8/97	1	2,750	-----	9,297	-----
09/97	Gunnison River	Average of 10 sites	10	1,977	0 - 10,868	6,809	1,702 - 10,999
11/04/97	South Platte R.	Lower Cheesman Canyon	1	496	-----	299	-----
11/03/97	South Platte R.	Above Deckers Site	1	730	-----	2,320	-----
11/04/97	South Platte R.	Below Deckers Site	1	522	-----	2,190	-----
11/05/97	South Platte R.	Scraggy View Site	1	129	-----	2,956	-----
11/04/97	South Platte R.	Twin Cedars Site	1	222	-----	2,357	-----
11/97	South Platte R.	Average of 5 Sites	5	420	129 - 730	2,024	299 - 2,956

Table 2. Young-of-the-year rainbow and brown trout fry sampling results from across Colorado in 1997.

Date of Sample	River	Sample Site	Sample Distance (meters)	Sample Size		Percent Composition	
				Brown	Rainbow	Brown	Rainbow
09/29/97	Beaver Creek	0.81 km below Beaver Creek Reservoir	61	22	49	31	69
09/30/97	Dolores River	1.6 km below McPhee Reservoir	107	10	10	50	50
09/30/97	Dolores River	8.8 km below McPhee/Ferris Canyon	122	10	0	100	0
09/30/97	Dolores River	14.2 km below McPhee Reservoir	76	28	0	100	0
11/12/97	Fryingpan River	Taylor Creek Station	305	67	36	65	35
11/12/97	Fryingpan River	Old Faithful Station	305	77	7	95	5
11/12/97	Fryingpan River	Bridge Pool/Ruedi Dam Gage Station	305	79	4	92	8
10/06/97	Roaring Fork River	1.6 km downstream from Fryingpan R.	76	16	13	55	45
09/29/97	Rio Grande	At Marshall Park Study Reach	31	14	15	48	52
09/29/97	Rio Grande	At Ute Bluff Bridge E. of S. Fork	183	18	5	78	22

No surviving wild rainbow trout fry were collected at our standard sampling stations in the spring of 1996 or 1997 (Table 3). However, we did collect marked rainbow (adipose-clipped CRR trout fingerlings stocked in October 1994, 1995, and 1996) trout during spring electrofishing operations every year between 1995 and 1998. We believe this is strong evidence that there were very few wild rainbow trout fry that survive to reach one year of age.

In the Colorado River, brown trout fingerling survival to age one has been very good for all four years (Table 3). The unusually low estimates of brown trout fingerlings in April 1996 may have been an artifact of unusually warm weather that caused high early spring discharge, and may have hampered electrofishing operations. The mean monthly discharge of the Colorado River below Windy Gap Dam in April 1996 was 25 m^3/s (881 ft^3/s) compared to 4.42 m^3/s (156 ft^3/s) in April 1995. Discharge of the river below Windy Gap in April 1997 was in the 8.5 to 11.3 m^3/s (300 - 400 ft^3/s) range.

Trout fry population monitoring has not been as intensive on other streams listed in Tables 1 and 2 as it has on the upper Colorado River (Table 3) over the past four years. Nonetheless, the data do show that wild rainbow and brown trout spawning, hatching, and fry production occur on a regular basis. Intensive trout fry population monitoring efforts have been on-going in the Gunnison River gorge since 1992. The results of these studies are summarized in Table 4. Dramatic declines in survival of wild rainbow trout fry between August and September began in 1994 and have been an on-going annual phenomenon ever since(Nehring and Thompson 1996, 1997; Nehring, Thompson, and Hebein 1998). This was not the case in 1992 and 1993 (Table 4). Recruitment of wild rainbow and brown trout fry to age 1+ juveniles was a normal process prior to the documentation of the presence of the parasite in YOY wild rainbow and brown trout in the river in January 1994.

The intensity of the infection among the wild rainbow trout fry in the Gunnison River gorge appears unusually severe, with massive losses of fry occurring during the six week period between early August and late September each year since 1994 (Table 4). The rate of loss of YOY rainbow trout fry here is much more rapid and severe than even in the Colorado River (Table 1 and Table 3).

Estimates of trout fry abundance for August 1996 and 1997 indicate wild rainbow trout fry are superabundant (Table 4). Indeed, August estimates of rainbow trout fry abundance during the "after whirling disease" (AWD) period (1994 - 1997) are much higher compared to the "before whirling disease" (BWD) period (1992-1993). However, estimates of wild rainbow trout fry abundance during late September for the AWD period (1994 - 1997) reveal massive mortality has occurred. In contrast, the data for the BWD years (1992 - 1993) do not reveal the same anomaly.

August to September mortality rates for brown trout fry in the Gunnison River gorge do not show the same alarming trend of massive losses between the BWD and AWD periods. Nonetheless, higher mortality rates among brown trout fry during the last three years of the AWD period may be an indication of increasing intensity of parasite virulence in this river.

Table 3. Relative abundance (numbers per kilometer - 2 bank estimates) and survivorship of fry of brown trout and rainbow trout (by year class) for 6 or more standardized (15.2 meters length) sampling stations on the upper Colorado River, July 1994 through October 1997.

Date (Mo/Da/Yr)	Rainbows Number/km	Percent Surviving[a]	Browns Number/km	Percent Surviving[a]
		1994 Year Class		
07/11/1994	4,699	100.0	3,013	100.0
08/02/1994	4,186	89.1	2,144	71.2
08/15/1994	2,256	48.0	2,760	91.6
08/31/1994	3,263	69.4	3,038	100.8
09/20/1994	2,162	46.0	2,470	82.0
10/03/1994	854	18.2	2,682	89.0
10/13/1994	1,062	22.6	2,432	80.7
11/01/1994	494	10.5	1,934	64.2
11/17/1994	262	5.58	901	29.9
04/03/1995	153	3.26	912	30.3
		1995 Year Class		
08/09/1995	2,218	100.0	3,934	100.0
09/11/1995	722	32.6	2,411	61.3
10/11/1995	481	21.7	2,578	65.5
11/15/1995	66	2.98	1,380	35.1
12/04/1995	66	2.98	1,264	32.1
04/18/1996	0	0	237	6.02
		1996 Year Class		
07/15/1996	1,877	100.0	5,173	a
08/19/1996	1,572	83.8	5,675	100.0
09/16/1996	480	25.6	4,605	81.1
10/14/1996	197	10.5	2,314	40.8
11/14/1996	109	5.81	1,702	30.0
04/14/1997	0	0	1,849	32.6
		1997 Year Class		
07/14/1997	416	a	3,784	100.0
08/12/1997	656	100.0	1,422	37.5
09/17/1997	328	50.0	2,756	72.8
10/13/1997	219	33.4	2,078	54.9

[a] Fry emergence incomplete at the time of the survey.

Table 4. Comparisons of rainbow and brown trout fry/fingerling density (numbers/km) estimates for the Gunnison River gorge for 1992 and 1993 (the pre-whirling disease period) and 1994 through 1997 (the post-whirling disease period).

Year	Brown Trout Fingerlings (n/km)			Rainbow Trout Fingerlings (n/km)		
	August	September	%Change August/Sept	August	September	% Change August/Sept
	Before Whirling Disease (BWD)					
1992	2,190	5,580	+155%	5,154	4,210	-18.1%
1993	2,089	2,881	+37.9%	2,525	2,690	+6.5%
	After Whirling Disease (AWD)					
1994	5,435	7,073	+30.1%	9,909	1,146	-88.4%
1995	7,053	2,901	-58.9%	1,392	330	-76.3%
1996	7,612	6,129	-19.5%	11,370	1,850	-83.7%
1997	10,247	6,822	-33.4%	23,957	1,981	-91.7%

Changes in Juvenile (Age 1+) Trout Abundance -Post Whirling Disease

Even though rainbow trout fry occur in abundance in the Cache la Poudre, upper Colorado, Gunnison, Rio Grande, and South Platte rivers (Tables 1, 2, 3, and 4), recruitment of those fry into the age one cohort has been unusually poor since whirling disease became established in the study reaches of these five streams (Table 5). Long-term monitoring of juvenile and adult trout populations has been an on-going effort on many streams in Colorado for almost two decades. Using these trout population data sets, we are able to determine whether or not dramatic changes in recruitment of age 1+ rainbow and brown trout occurred, and if so, when and where those changes began. Data on abundance of age 1+ rainbow and brown trout for segments of the Colorado, Cache la Poudre, Gunnison, Rio Grande, and South Platte rivers are given in Table 5.

In the previous progress report (Nehring and Thompson 1997), we stratified our data into "before whirling disease" (BWD) and "after whirling disease" (AWD) groups and applied a two-sample mean t-test to determine if there was a significant decrease in numbers of age 1+ brown trout or rainbow trout during the AWD period compared to the BWD period. We completed a natural log transformation of the data to minimize the effects of unequal variances in the data between the BWD and AWD periods. We used a one-tailed t-test statistic because we were only interested in whether or not a significant decline in age 1+ recruitment had taken place after the time when *M. cerebralis* was known to be well established in the trout population. Among rainbow trout, age 1+ recruitment was significantly lower during the AWD period for 13 of 14 comparisons. In contrast, among the brown trout there was only one of 14 instances where age 1+ brown trout numbers had declined significantly during the AWD period. This was at the Above Deckers sampling station on the South Platte River.

Table 5. Age 1 brown and rainbow trout cohort abundance in five Colorado pre- whirling disease and post-whirling disease. Post-whirling disease data are highlighted with an asterisk (*).

Spec.	1981	1982	1983	1984	1985	1986	1987	1988	1989	1990	1991	1992	1993	1994	1995	1996	1997
Cache la Poudre River - Upper Wild Trout Water (Numbers/hectare)																	
Brn	120	183	304	8	100	51	—	107	—	27*	—	18*	—	45*	22*	33*	17*
Rbw	181	196	160	29	104	130	—	108	—	51*	—	3*	—	1*	0*	42*[a]	0*
Cache la Poudre River - Indians Meadows near Hombre Ranch (Numbers/hectare)																	
Brn	56	120	162	15	68	32	—	69	—	95	—	60*	—	101*	73*	91*	71*
Rbw	226	122	111	47	146	264	—	136	—	122	—	17*	—	6*	0*	1070*[a]	4*
Cache la Poudre River - Upper Control (Firelane near Poudre Chapel) (Numbers/hectare)																	
Brn	104	116	156	0	49	88	—	155	—	73	—	35*	—	36*	34*	26*	64*
Rbw	157	258	127	21	78	296	—	387	—	238	—	39*	—	6*	0*	1237*[a]	22*
Cache la Poudre River - Kelly Flats Campground (Numbers/hectare)																	
Brn	128	158	347	21	87	80	—	171	—	322	—	149*	—	273*	83*	170*	177*
Rbw	343	300	192	72	160	193	—	446	—	228	—	61*	—	10*	0*	702*[a]	19*
Colorado River at Kemp Breeze Ranch Wildlife Area (Numbers/11.6 hectares)																	
Brn	1960	986	301	464	383	510	—	—	—	—	—	269*	1497*	2095*	5185*	2452*	1755*
Rbw	5614	708	58	232	151	1462	—	—	—	—	—	9*	14*	5*	17*	3*	11*
Gunnison River in the Black Canyon at Ute Park (Numbers/hectare)																	
Brn	641	363	242	82	36	345	982	620	330	221	492	490	443	556	770*	664*	909*
Rbw	197	212	111	4	7	61	902	512	50	121	118	129	107	97	5*	0*	4*
Gunnison River at Almont downstream from Roaring Judy Fish Hatchery (Numbers/27.5 hectares)																	
Brn						1732	853	495	2118	1265	605	1540*	1595*	851*	617*	—	
Rbw						275	220	688	495	1568	303	83*	33*	3*	6*	—	
South Platte at Lower Cheesman Canyon Station (Numbers/hectare)																	
Brn	164	158	87	117	314	165	309	368	710	228	635	119*	209*	73*	252*	121*	75*
Rbw	44	848	72	15	39	119	41	308	319	47	414	8*	19*	5*	21*	7*	157*
South Platte above Deckers Bridge (Numbers/hectare)																	
Brn	1813	1799	696	555	950	1096	566	1325	1535	1797	1937	458*	611*	606*	458*	376*	121*
Rbw	275	561	43	22	71	333	63	369	158	132	100	6*	12*	3*	18*	29*	47*
South Platte below Deckers Bridge (Numbers/hectare)																	
Brn	2062	1531	692	457	860	982	508	658	1120	1051	1455	334*	610*		756*	588*	175*
Rbw	445	603	127	3	99	454	63	219	281	280	196	17*	13*		96*	12*	69*
Upper South Platte River - Catch/Release Area Below Spinney Mountain Dam (Numbers/hectare)																	
Brn							138	18	86	1091	728*	140*					
Rbw							111	481	1534	1324	184*	64*					
Upper South Platte River - At Old Barns Sampling Site Below Spinney Mountain Reservoir (Numbers/50.4 hectares)																	
Brn							47	189	74	997	103*	14*	108*	388*			
Rbw							1112	896	482	280	17*	3*	45*	11*			
Rio Grande River at the State Bridge Section (Numbers/19.0 hectares)																	
Brn	1310	3276	2974	1966	2167	2268	5141	859	2167	2117	1109	2873		1321*	1768*	3913*	1874*
Rbw		212	62	140	466	1652	544	604	599	1276	384	1483		76*	71*	86*	62*
Rio Grande River above Marshall Park Campground (Fisherman Access Area)																	
Brn							648	57	2896	2457	705	2210	2191	915*	754*	1138*	1825*
Rbw							292	1768	203	1783	703	1185	1091	660*	232*	41*	129*

[a] More than 170,000 fingerling rainbow trout were stocked in the Cache la Poudre River in the summer of 1996. These fish were not marked to differentiate them from surviving wild rainbow trout.

With the possible exception of the Lower Cheesman Canyon, Above Deckers Bridge, and Below Deckers Bridge study sites on the South Platte River, there was no marked change in the level of recruitment of wild rainbow trout to age 1+ in any of the study streams listed in Table 5. The strong increase in age 1+ wild rainbow trout at the Lower Cheesman Canyon station in 1997 was an unexpected and pleasant surprise. Whether or not this is a one-year anomaly or the beginning of a longer term trend that could be indicative of development of resistance to the parasite remains to be seen.

Table 6. Dolores River wild brown and rainbow trout population age structure for the 17.7 km (12 mile) reach from the U.S. Forest Service Metaska Day Use Area to Ryman Draw near the Bradfield Bridge, 1991 - 1998[a].

Month/Year	Age Class Structure							
	1+	2+	3+	4+	5+	6+	7+	8+
Brown Trout								
May 1991	517	157	212	179	107	25	14	3
June 1992	381	612	124	163	27			
July 1993	951	1314	381	312	56			
June 1994	477	1128	968	158	15	6	0	
July 1995	921	1124	1588	500	7	7	7	
June 1997	463	1084	870	367	51	9	14	
June 1998	143	866	754	323	5	33	0	
Rainbow Trout								
May 1991	172	116	39	160	97	139	15	
June 1992	47	80	74	50	148	48	8	
July 1993	123	113	106	128	112	27	9	
June 1994[b]	219	903	2290	590	133	2	9	
July 1995	114	232	467	394	26	3	0	
June 1997	87	113	92	120	63	27	2	10
June 1998	0	81	70	42	24	3	0	

[a]: No sampling was conducted in 1996 by boat electrofishing due to unusually low water.

[b]: Estimates include rainbow trout from McPhee Reservoir that were released through the bottom outlet tubes in the dam during the "managed spill" from April through June 1994.

In 1997, recruitment of age 1+ wild rainbow trout declined or disappeared from segments of the Dolores, Fryingpan, and Roaring Fork rivers. Only on the Big Thompson River have wild rainbow trout fry continued to recruit to the juvenile (age 1+) year class as expected in healthy wild trout populations.

Wild rainbow trout recruitment to age 1+ declined drastically on the Dolores River below McPhee Dam between June 1997 and June 1998 (see Table 6). Boat electrofishing operations on a 17.7 km (11 mile) reach of the river from the U.S. Forest Service Metaska Day Use Area to Ryman Draw resulted in no age 1+ wild rainbow trout collected during the four day survey. This is consistent with the results of the wild trout fry collections on this river in early October 1997.

Table 7. Fryingpan River wild rainbow trout population age structure at three sampling stations, November 1994 - November 1997.

Year	Age Class Structure						
	1+	2+	3+	4+	5+	6+	7+
Ruedi Dam Site							
1994	24	21	66	128	55	4	7
1995	62	14	48	166	76	4	12
1996	41	30	72	132	51	4	5
1997	34	23	47	117	51	2	24
Old Faithful Site							
1994	8	20	39	58	23	0	1
1995	32	14	41	40	18	0	1
1996	18	20	42	46	22	1	1
1997	53	16	39	58	22	1	1
Taylor Creek Site							
1994	20	16	61	66	18	0	2
1995	14	20	63	95	32	1	1
1996	8	21	50	92	35	0	1
1997	9	8	55	82	17	0	1

No wild rainbow trout fry were collected at the two downstream survey sites. Wild rainbow fry collected near the Metaska Day Use Area possessed all of the classical overt clinical signs of whirling disease, including severe cranial deformities, black tail, severe exophthalmia, and violent whirling behavior. Based on previous experience on the Colorado and Gunnison rivers, we did not expect to see these severely affected fry survive. The boat electrofishing results for June 1998 lend credence to that expectation.

Table 8. Fryingpan River wild brown trout population age structure at three sampling stations, November 1994 - November 1997.

Year	Age Class Structure						
	1+	2+	3+	4+	5+	6+	7+
Ruedi Dam Site							
1994	45	159	470	546	91	9	3
1995	172	218	482	656	166	29	0
1996	249	265	695	521	86	13	0
1997	337	283	498	611	155	17	8
Old Faithful Site							
1994	143	328	588	316	23	1	0
1995	303	484	570	367	42	1	0
1996	276	524	531	255	24	0	0
1997	572	400	462	335	35	1	0
Taylor Creek Site							
1994	298	305	279	189	16	1	0
1995	443	471	504	301	25	1	0
1996	330	526	521	307	34	3	0
1997	202	318	500	388	45	2	0

Population age structure data for brown trout, rainbow trout, and mountain whitefish in the Roaring Fork River, from Basalt to Hook's Bridge, a 3.2 km reach of river, for the fall of 1995 and 1997 is shown in Table 9. Wild rainbow trout from this reach of the Roaring Fork River first tested positive for the *M. cerebralis* parasite in 1995. We have collected wild rainbow trout fry at a site within this reach of river every fall since 1994. It appears very few of these fry recruit to the population as the age 1+ rainbow trout cohort density was only one per hectare in 1995 and three per hectare in 1997.

Sampling on the Fryingpan River between November 1994 and November 1997 reveals that recruitment of YOY rainbow trout to the age 1+ cohort has been declining for four straight years at the Taylor Creek sampling station (Table 7). This has not been the trend at the two stations (Ruedi Dam site and Old Faithful) farther upstream where recruitment has either remained unchanged or increased over previous years. Wild trout from the Fryingpan River first tested positive for the *M. cerebralis* parasite in 1995. Subsequent testing in 1996 and 1997 indicated an increase in the percentage of fish testing positive as well as an increase in the severity of the infection. These data could indicate that a point source of higher infectivity may exist downstream of the upper two stations (Ruedi Damsite and Old Faithful sampling sites) but upstream of the Taylor Creek sampling station. Brown trout population age structure shows no apparent changes at any of the sampling sites over the past four years (Table 8).

We sampled the trout population in the upper reaches of the Roaring Fork River in Aspen in October 1997. This station was sampled several times between 1970 and 1980. Age 1+ wild rainbow trout density (n/ha) at the site was 337, 60, 143, and 56 in the late summer of 1970, 1978, 1979, and 1980. No age 1+ wild rainbow trout were collected in October 1997. The upper reaches of the Roaring Fork River were exposed to the *M. cerebralis* parasite in the late 1980s.

Table 9. Roaring Fork River salmonid population age structure of brown trout, rainbow trout, and mountain whitefish for a 3.2 km (2 mile) reach of river from Basalt to Hook's Bridge, December 1995 and October 1997.

Year	Age Class Structure								
	1+	2+	3+	4+	5+	6+	7+	8+	9+
Brown Trout									
1995	232	712	589	240	315	237	68	5	0
1997	218	554	569	239	348	310	72	0	0
Rainbow Trout									
1995	16	115	440	191	180	33	113	28	0
1997	48	35	194	131	153	33	116	8	0
Mountain Whitefish									
1995	0	48	205	602	851	690	358	157	0
1997	0	6	105	463	747	825	472	295	9

Total Trout Population Structure

Massive annual declines in survivorship of wild rainbow trout fry and recruitment to age 1+ have drastically altered the rainbow trout population density (n/ha), biomass (kg/ha), and size and age structure on many major trout streams in Colorado. These declines are clearly linked to the effects of *M. cerebralis*, the causative agent of salmonid whirling disease. Changes in the length/frequency distributions of rainbow trout through time compared to brown trout, which are resistant to the parasite, provide a graphic illustration of the devastating effects of this parasite. Length/frequency distributions of rainbow and brown trout in the Colorado River (Figure 1), in the Gunnison River gorge (Figure 2), and the South Platte River (Figure 3) portrayed in three dimensional histograms provide a quick visual assessment of the current state of reproductive success and recruitment of young fish and an indication of the dynamic changes in the trout population structure through time. We present a stream-by-stream overview of the findings of our research investigations below.

Big Thompson River

Electrofishing operations in the fall of 1995 and 1997 at multiple sites on the Big Thompson River downstream of Lake Estes indicate the wild brown and rainbow trout populations in this stream appear unaffected by the *M. cerebralis* parasite. Wild rainbow trout density (n/ha) and biomass (kg/ha), and age 1+ trout abundance remain very high at all sampling stations (Table 10). At 5 of 8 sampling stations wild rainbow trout dominate the trout population. This river was not electrofished in 1996 due to an early winter and an inadequate number of field crew members.

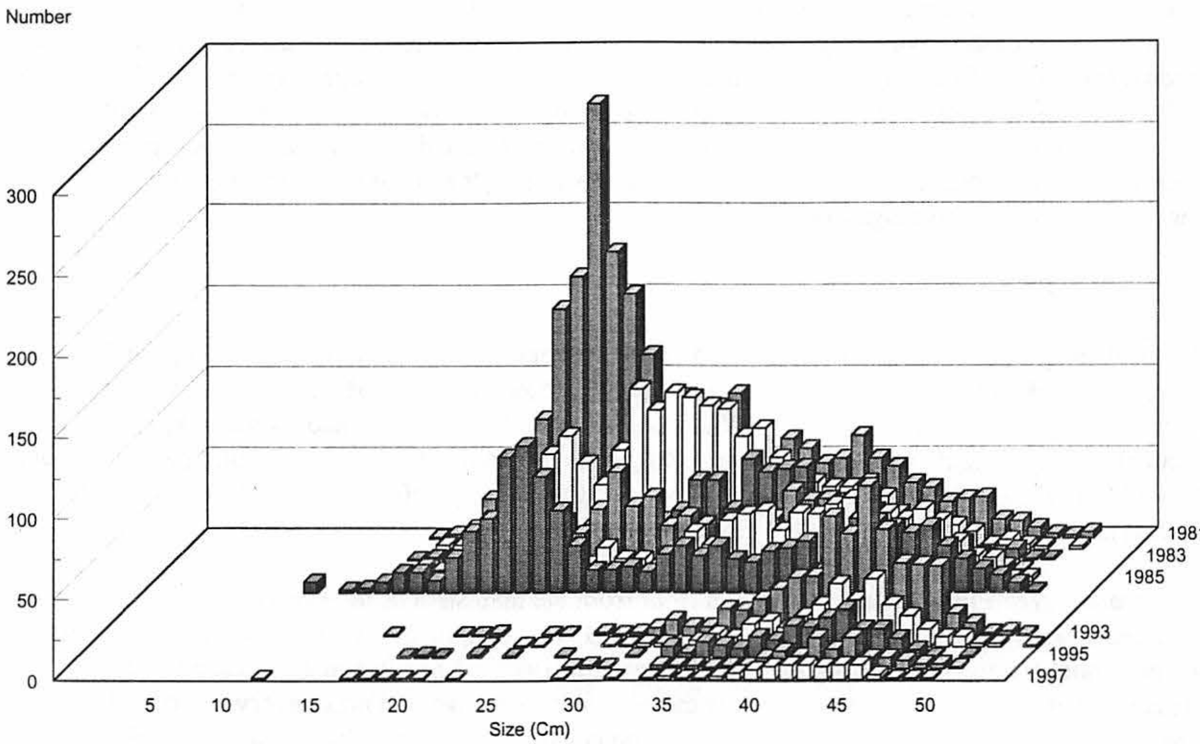
Myxobolus cerebralis was detected in wild trout from the mainstem of the Big Thompson River above the confluence with the North Fork for the first time in 1995. Despite apparent increases in infectivity among both rainbow and brown trout in 1997 compared to 1995, no population effects are being manifested as yet. If this trend continues over the next few years, we will make every effort to determine what the differences are in the dynamics of interaction between the rainbow trout and the parasite in this stream.

Cache la Poudre River

The data in Table 11 indicate dramatic declines in the rainbow trout population at four separate sites throughout some 20 km of the Cache la Poudre River. From the perspective of 20/20 hindsight, wild rainbow trout numbers were in serious decline at all study sites by the fall of 1992. However, data for age 1+ wild rainbow trout density indicate population level effects were evident at the upper wild trout (quality) water near Black Hollow Creek beginning in 1990 and at all four sites by 1992 (Table 5 and Table 11). In contrast, there are no discernible declines in population statistics for brown trout among the four sampling stations.

In 1996, supplemental stocking of high numbers of fingerling and catchable rainbow trout resulted in large increases in rainbow trout density and biomass at some sample sites. Because these fish were not marked in any discernible way, it was not possible to differentiate between truly wild juvenile rainbow trout and those originating from hatchery sources in this river. However, rainbow trout density and biomass were again at very low levels by November 1997 (Table 11) when fewer trout were available for stocking.

Rainbow trout from the Colorado Division of Wildlife's Poudre Rearing Unit, an earthen pond rearing facility, first tested positive for *M. cerebralis* in 1988. This rearing unit is 5 km upstream from the Upper Wild Trout Water sampling site listed in Table 5. Water filtration studies conducted on the Cache la Poudre River at the inflow and outflow of this rearing unit strongly indicate the effluent out of earthen settling ponds leaving the facility is the major source of infectivity for the river.



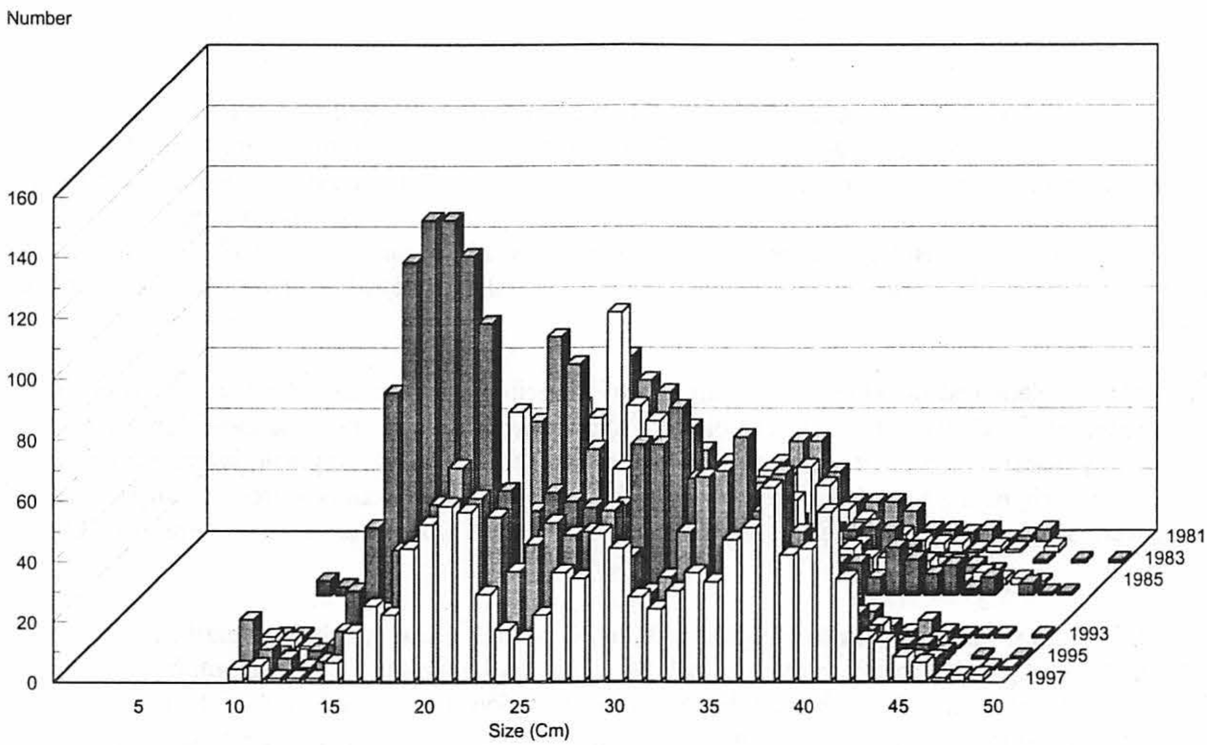
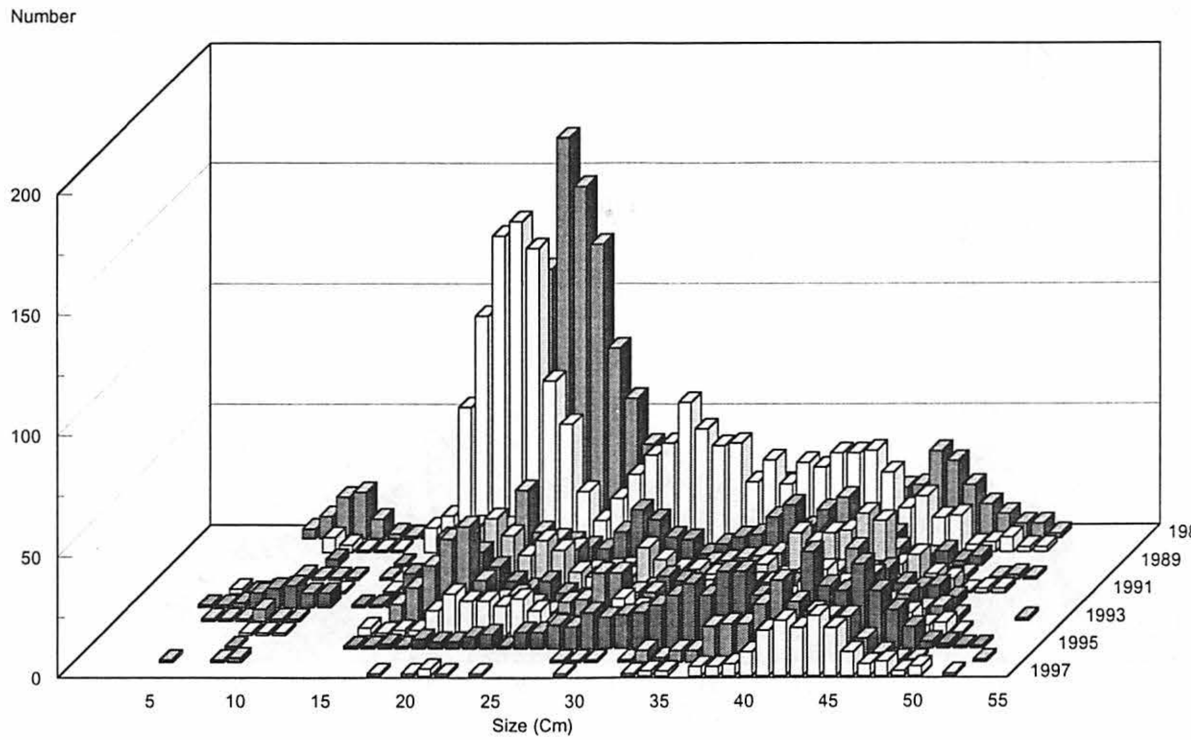


Figure 1. Length frequency distribution of juvenile and adult rainbow trout (top) and brown trout (bottom) collected by electrofishing a 3.2 km reach of the Colorado River before (October 1981 - 1986) and after whirling disease (October 1993 - 1997).



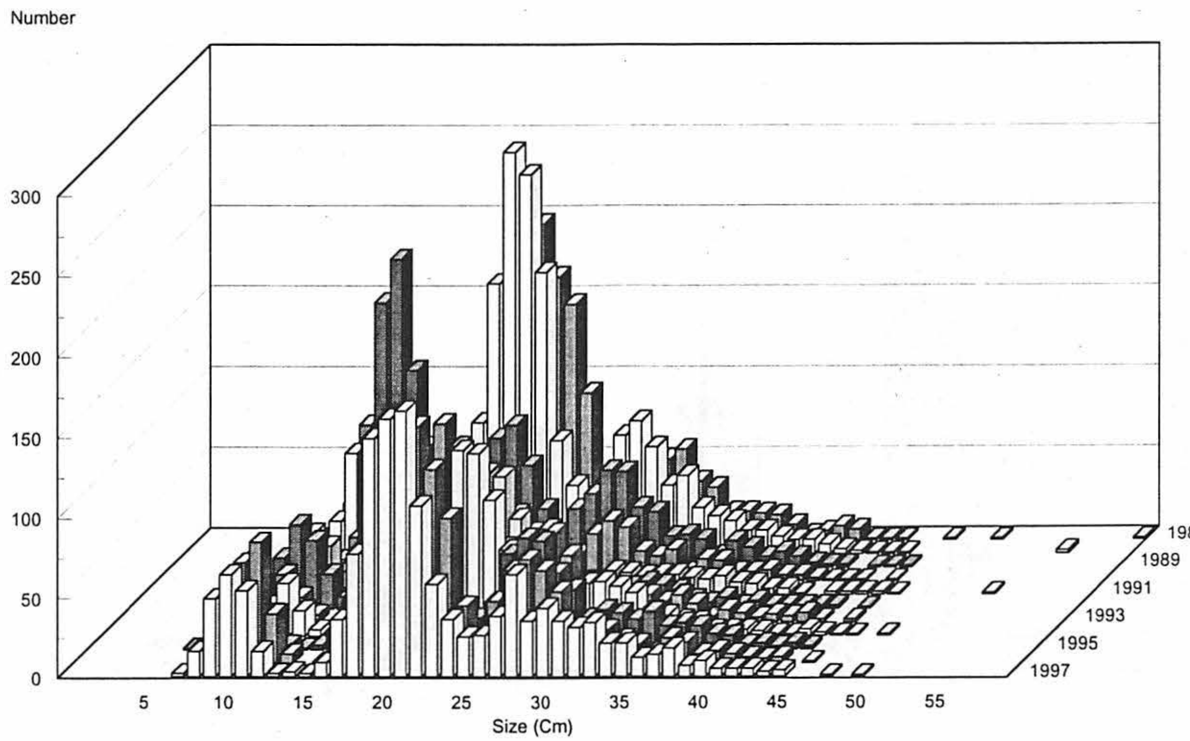
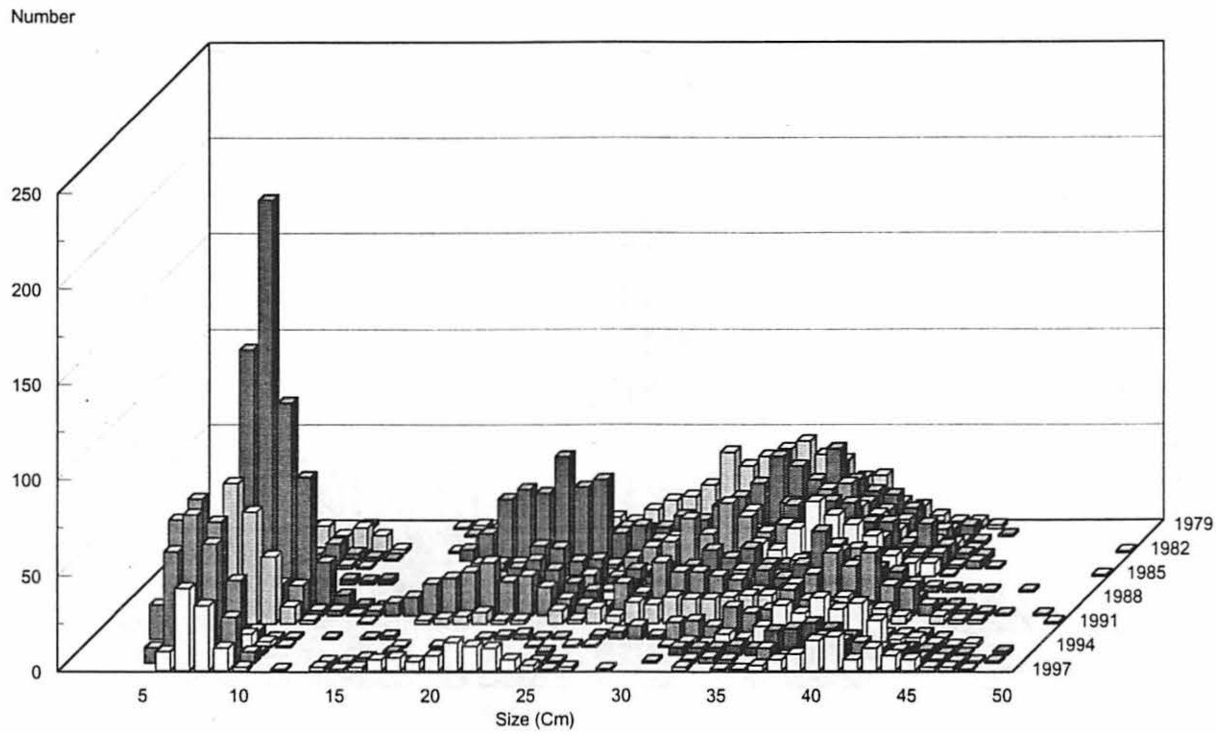


Figure 2. Length frequency distribution of juvenile and adult rainbow trout (top) and brown trout (bottom) collected by electrofishing a 3.2 km reach of the Gunnison River gorge before (September 1987 - 1993) and after whirling disease (September 1994 - 1997).



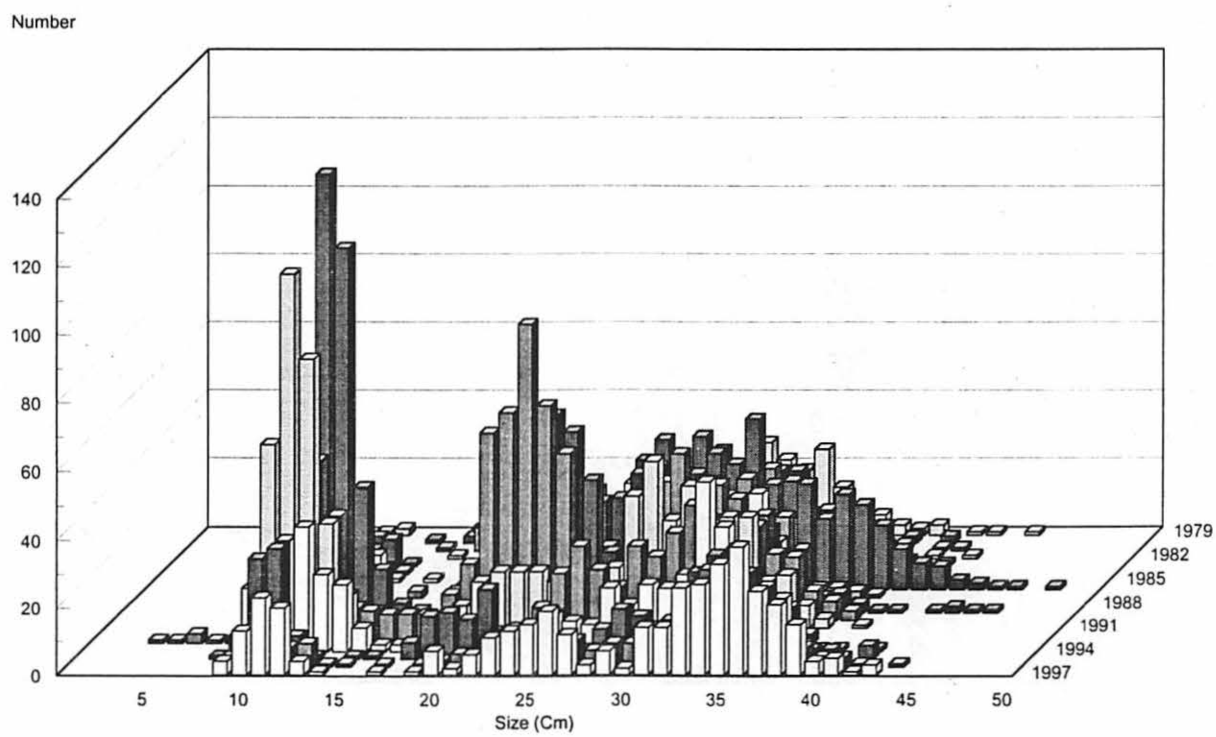


Figure 3. Length frequency distribution of juvenile and adult rainbow trout (top) and brown trout (bottom) collected by electrofishing a 229 m reach of the South Platte River before (Fall 1979 - 1988) and after whirling disease (Fall 1990 - 1997).

Table 10. Brown and rainbow trout population numbers (N with 95% confidence limits), densities (n/ha) of age 1+ fish, fish ≥ 15 cm, fish ≥ 35 cm, and biomass (kg/ha) for the Big Thompson River at various sampling sites during the fall of 1997.

Species	Population Statistics					
	N ≥ 15 cm	95% CL	Age 1+ (n/ha)	N/ha ≥ 15 cm	N/ha ≥ 35 cm	kg/ha
Habitat Improvement Area below Olympus Dam						
Brown	81	±8.7	191	437	41	80.9
Rainbow	58	±13.0	146	311	0	46.9
Chuck's Place Site						
Brown	48	±16.1	121	376	0	70.6
Rainbow	140	±12.5	252	1094	9	175.8
Twin Pines Site						
Brown	50	±4.2	79	374	0	57.9
Rainbow	90	±5.1	120	677	0	107.0
Grandpa's Place						
Brown	59	±34.6	76	253	0	42.6
Rainbow	177	±19.7	287	763	0	93.0
Handicap Fishing Pier						
Brown	124	±33.2	195	827	9	130.6
Rainbow	177	±5.2	400	1180	0	148.9
Drake Campground[a]						
Brown	30	—	141	166	0	15.7
Rainbow	30	—	141	166	0	16.6
Below Waltonia						
Brown	23	±1.3	118	289	0	43.6
Rainbow	119	±1.7	693	1526	0	170.5
Sylvandale Site						
Brown	90	±3.1	25	362	55	87.0
Rainbow	41	±3.2	13	164	55	51.7

Table 11. Trout population biostatistics at four sampling sites on the Cache la Poudre River, fall 1988 - fall 1997.

Date	Brown Trout					Rainbow Trout				
Mo/Da/Yr	N	95% CI	N/ha	Kg/ha	N/ha ≥ 30 cm	N	95% CI	N/ha	Kg/ha	N/ha ≥ 30cm
USFS Kelly Flats Campground Station										
9/28/88	126	±3.7	410	47.5	9	184	±9.5	600	58.6	1
9/25/90	172	±13.7	637	68.1	7	86	±13.4	320	36.3	2
9/4/92	122	±24.8	452	57.3	7	60	±11.1	220	31.6	2
10/4/94	174	±11.3	607	69.3	9	28	±2.9	96	17.4	4
10/31/95	112	±6.2	492	73.9	30	14	±1.3	62	11.4	7
11/4/96	82	±5.1	359	55.8	34	88	±6.9	386[a]	17.5	0
11/4/96[a]	[a] With fingerling/catchable rainbow					129[a]	±18.1	567	54.1	0
11/04/97	111	±7.1	4901	68.9	70	18	± 1.8	78	11.4	0
Indian Meadows (Near Hombre Ranch)										
9/28/88	105	±93.5	310	51.3	23	81	±30.7	238	31.9	2
9/27/90	148	±41.8	405	58.8	12	156	±37.4	427	67.7	17
9/22/92	113	±10.1	356	56.0	11	75	±15.3	234	41.5	8
10/31/94	115	±12.8	401	62.4	17	29	±1.0	103	23.6	16
10/30/95	121	±11.1	509	97.9	56	28	±4.7	117	34.3	32
10/28/96	79	±4.5	385	69.9	43	221	±728	1070[a]	70.3	0
10/28/96[a]	[a] With fingerling/catchable rainbow					729	—	3537[a]	333.4	267
11/04/97	118	±3.5	494	92.8	44	40	±52	169	37.2	54
Upper Control (Firelane @ Poudre Chapel)										
9/27/88	129	±21.4	281	32.8	9	205	±8.0	444	44.5	4
9/27/90	78	±3.2	261	39.6	13	110	±5.0	367	43.5	6
9/22/92	62	±5.7	209	35.0	12	95	±12.0	317	53.3	5
10/3/94	52	±2.5	178	27.7	10	28	±2.8	95	18.6	7
10/30/95	62	±7.4	211	41.2	20	12	±1.5	40	8.6	4
10/30/96	33	±0.9	169	36.3	25	275	±16.5	1396[a]	84.8	0
10/30/96	[a] With fingerling/catchable rainbow					563	±12.8	2859[a]	333.6	4
11/03/97	58	±5.2	198	28.6	10	31	±1.5	105	16.3	3
Upper Quality Water (Near Black Hollow Creek)										
9/26/88	140	±12.5	310	46.2	15	209	±41.5	463	87.8	36
9/24/90	113	±19.3	278	50.2	14	150	±11.8	370	72.6	33
9/3/92	94	±12.3	252	51.3	27	100	±57.3	268	69.2	48
10/4/94	129	±12.1	348	71.6	41	72	±5.3	194	50.3	30
10/20/95	107	±7.7	422	87.7	51	45	±4.9	179	50.7	40
10/30/96	99	±10.5	594	122.5	80	23	±0.6	139[a]	35.1	34
10/30/96	[a] With fingerling/catchable rainbow					79	±1.5	472[a]	69.6	34
11/13/97	54	±3.6	215	48.1	32	23	±2.3	91	24.7	13

[a] Large number of fingerling and/or catchable rainbow trout were stocked in the river in the summer of 1996.

Colorado River

Myxobolus cerebralis was first detected in rainbow trout stocked on private property at two separate locations in the upper Colorado River drainage in 1988. Annual collections of adult wild rainbow trout first tested positive for the parasite during spawn taking operations in April 1992. Rainbow and brown trout fry collected from the river in November 1993 tested positive for the presence of the parasite, after dramatic declines in numbers of wild rainbow trout fry were detected during electrofishing surveys conducted in November compared to September 1993.

During intensive research investigations on the upper Colorado during the summer and fall of 1994, overt clinical signs of whirling disease were observed in both free-ranging feral and captive brown and rainbow trout fingerlings (Walker and Nehring 1995). These clinical signs included blacktail, cranial deformities, deformities of the vertebral column (both lordoscis and scoliosis), exophthalmia, and whirling behavior.

Extensive testing of both free-ranging feral rainbow and brown trout fry from the Colorado River and *M. cerebralis*-free rainbow, brook, and cutthroat trout exposed to the river in floating test cages as sentinel fish has proven that whirling disease occurs at epidemic levels in this stream. Testing methods that have been used to document the presence of the parasite in both free-ranging feral trout and sentinel fish have included histological sectioning, staining, and evaluation of affected tissues in diseased fish, detection of very high levels of *M. cerebralis* spores in both feral and captive rainbow and brown trout using the pepsin-trypsin-digest (PTD) method (Markiw and Wolf 1974), and the PCR (polymerase chain reaction)-DNA based diagnostic test (Andree et al. 1997). During 1997, we have also used filtration of raw water of the Colorado River to detect, isolate, and quantify the density of the triactinomyxon (TAM) actinospore of the parasite that infects the trout. The PCR-DNA based diagnostic test applied to filtered water samples has confirmed the filtered actinospores are *M. cerebralis*.

The data presented in Tables 12 and 13, and in Figure 1 (top), clearly indicate that numbers of juvenile and adult rainbow trout have declined precipitously throughout the decade of the 1990s concurrent with the introduction of the *M. cerebralis* into the drainage and its subsequent infection of the wild brown and rainbow trout populations.

The data in Tables 13 and 14, and in Figure 1 (bottom) indicate that juvenile (age 1+) and adult brown trout population density and biomass at the Kemp/Breeze study area have increased significantly in since 1994. These increases are the result of large age 1+ cohorts recruiting to the population. We believe that these large cohorts of juvenile brown trout are due in large measure to the effects of heavy flushing flows released from Williams Fork Reservoir into the Williams Fork River that meets the Colorado River at Parshall, just upstream from the Kemp/Breeze study area. We have conducted monthly fry electrofishing surveys in the Colorado River downstream of the confluence with the Williams Fork River from July through October every year since 1994 (Table 3). During these surveys we have noted a bimodal length- frequency distribution among both rainbow and brown trout fry collected downstream of the Williams Fork River confluence with the Colorado River. At sampling stations upstream from this point the length frequency distributions of both rainbow and brown trout fry were always unimodal. Moreover, the bimodal length-frequency distribution downstream of the Williams Fork River was very apparent in years after high flushing flows had occurred. In some years the appearance of the bimodal length-frequency distribution was accompanied by a concomitant large increase in fry numbers compared to the previous sampling month.

The effects of high water velocities (and associated discharge) in downstream displacement of salmonid fry has been well documented by Ottaway and Clarke (1981) and Ottaway and Forrest (1983). July through September is the period when brown trout and rainbow trout fry are most susceptible to being flushed downstream by high discharge. The discharge from the Williams Fork River during this period can be as low as 0.3 to 0.9 m^3/s (10 - 30 ft^3/s) in below average water years to more than 28.3 m^3/s (1000 ft^3/s) in high run-off years. High run-off years in the Colorado River basin occurred in 1993 and 1995. Flushing flows out of Williams Fork Reservoir during June through September in 1993 reached 12.6 m^3/s (444 ft^3/s), and in 1995, 1996, and 1997 exceeded 28.3 m^3/s (1000 ft^3/s). During the 30 year period from 1964 through 1994, the only two years when the maximum discharge exceeded 28.3 m^3/s were in 1983 and 1984. Most years during that 30 year period the daily maximum discharge for the year rarely exceeded 8.5 m^3/s (300 ft^3/s). In those years when flushing flows exceeded 28.3 m^3/s, that discharge always occurred in June or July near the end of the peak discharge from melting snow.

The large statistically significant (t-test for two means) increases in brown trout density and biomass at the Kemp/Breeze Wildlife Area in the 1990s could be the result of empty ecological niches (vacated by rainbow trout) being filled by opportunistic brown trout. However, if this were a valid explanation (and not flow-induced augmentation of recruitment), then brown trout density and biomass should be increasing in a similar manner throughout the upper river. However, the data from the Lone Buck and Paul Gilbert Wildlife areas (upstream of the Williams Fork River confluence) do not lend strong credence to that hypothesis (Table 15). Brown trout density has increased, but biomass and numbers of brown trout ≥ 35 cm have not. Moreover, the magnitude of increase in brown trout density and biomass at these sites has not been nearly as great as that seen at the Kemp/Breeze study area.

Stratification of rainbow and brown trout age class numbers for the Kemp/Breeze study area into the BWD years (1981-1986) versus AWD years (1993-1997) and conducting a t-test for two means on each age class is even more revealing (Tables 12 and 14). For brown trout, every age cohort test (except age 2+ and 6+) reveals significantly greater numbers of fish in the cohort during the AWD period. On the surface this would seem to validate the empty niche hypothesis, were in not for the fact that the same t-test analysis applied to the rainbow trout also reveals that this study site has consistently supported significantly more age 6+ and 7+ rainbow trout during the AWD years as well. These data largely debunk the empty niche hypothesis. Clearly, there has been a significant increase in brown trout population parameters at this site; but it has not come as a result of a dramatic decline in numbers of large rainbow trout during the AWD years. Far and away the greatest concentration of trout density and biomass within the Kemp/Breeze study area occurred in 1981, when there was an estimated population of more than 13,700 trout ≥ 15 cm in length and total trout biomass was estimated at 313 kg/ha, more than there has been since (for the years that we have data).

Table 12. Rainbow (CRR) trout population age structure for the 3.2 km reach of the Colorado River on the Kemp/Breeze Wildlife Area, fall 1981-1986 versus fall 1993-1997.

Year	Age Class Structure							
	1+	2+	3+	4+	5+	6+	7+	8+
1981	5614	2274	1264	606	151	12	0	0
1982	708	1914	812	951	336	35	0	0
1983	58	232	661	1032	336	23	0	0
1984	232	719	552	603	267	93	0	0
1985	151	220	603	302	476	162	35	23
1986	1462	882	557	313	35	12	0	0
1993	14	9	183	466	796	275	132	6
1994	5	5	8	131	281	194	149	0
1995	17	6	18	40	168	177	102	0
1996	3	10	28	37	29	76	71	35
1997	11	18	7	14	107	112	17	11

The brown trout population density and biomass statistics for the Kemp/Breeze study area in 1996 and 1997 seem to corroborate our assertions above and do not lend credence to the empty niche hypothesis. Brown trout numbers and density at this site declined dramatically in 1996 and 1997. Total numbers and density decreased by 55.4%, and biomass decreased by 27.7% in 1997 compared to 1995 (Table 13). Of particular importance are the age-specific data for brown trout numbers in Table 14. In 1997, there were 77% fewer age 1 recruits to the brown trout population compared to 1995. Moreover, there were huge decreases in survivorship of the age 1+, 2+, and 3+ cohorts of brown trout between the fall of 1995 and 1997 (Table 14). Clearly, if there were an abundance of empty niches in this 3.2 km reach of the Colorado River (available for occupancy by brown trout) as a result of the dramatic declines in rainbow trout numbers and density for all size and age classes, it would be manifested in better survivorship of at least some of these cohorts of brown trout in the population in the fall of 1996 and 1997. But this was not the case.

Similarly, the data in Tables 16 and 17 do not show a consistent increase in numbers/km, density (n/ha), or biomass (kg/ha) for brown trout at the majority of sampling stations above the Williams Fork River confluence in the 1990s, concomitant with the massive declines in wild rainbow trout density and biomass at those same stations. We believe the primary reason for the lack of a dramatic increase in brown trout numbers, density, and biomass in the upper Colorado River (as the wild rainbow trout stocks have declined) is due to the fact that brown trout are a highly territorial species (Bachman 1984) and are also a highly object-cover oriented species (Bachman 1984; Shuler and Nehring 1994; Shuler, Nehring, and Fausch 1994). In the upper Colorado River the wild brown trout density and biomass is (and probably has been) limited by the object cover available which limits the carrying capacity of the stream for juvenile and adult brown trout.

We believe these studies over the past 4.5 years do not lend credence to any assertion that this phenomenon of massive losses of rainbow trout is the result of a multiplicity of complicating and exacerbating factors operative in an additive or even synergistic manner. Rather, we believe our data indicate that an extremely pernicious parasite, *M. cerebralis*, is rapidly reducing this once superb wild rainbow trout population to extremely low levels, compared to levels observed between 1981 and 1986 (Tables 12 and 13). According to the data in Table 12, this 3.2 km reach of the river may not have produced more than 3 to 17 age 1+ juvenile rainbow trout recruits since 1990.

Table 13. Trout population biostatistics for the 3.2 km reach of the Colorado River on the Kemp/Breeze ranches, fall 1981-1986 versus fall 1993-1997.

Year	Brown Trout					Rainbow Trout				
	N	95% CI	Kg/ha	N/ha	N/ha ≥ 35 cm	N	95% CI	Kg/ha	N/ha	N/ha ≥ 35 cm
1981	3,415	± 1335	82	294	36	10,300	±1635	231	889	185
1982	2,031	± 588	48	175	53	4,756	±739	124	410	173
1983	1,476	±710	42	127	25	2,341	±452	81	202	86
1984	1,735	±408	35	150	11	2,410	±410	78	210	78
1985	1,651	±613	55	142	34	1,976	±329	94	170	115
1986	1,230	±389	44	106	33	3,214	±538	109	277	111
Mean 81/86	1,923	----	51	166	32	4,166	----	120	360	125
1993	3,280	±1244	91	283	51	1,881	±396	128	162	154
1994	4,965	±1817	119	428	49	774	±232	53.1	67	65
1995	9,707	±2084	224	837	138	610	±219	41.2	53	50
1996	5,857	±1274	190	505	164	288	±126	19.9	25	24
1997	4,330	±926	162	373	149	293	±199	17.0	25	23

One of the unanswered questions has been whether or not brown trout population effects are being manifested on the Colorado River. Indeed, there are some indications that this may be the case at the population monitoring station 1.6 km (1 mile) downstream from Windy Gap Dam. In the fall of 1994, 35 brown trout between 14 cm and 22 cm were collected during electrofishing operations at this site, compared to only 2 (at 22 cm in length) in the fall of 1995. Brown trout in this size range are largely the age 1+ recruits from the previous year class. On two occasions during October 1994, more than 50% of the brown trout fry collected at this location were suffering from blacktail and deformities of the vertebral column. Samples taken in the same general locale during November 1994 revealed that brown trout fry numbers decreased by more than 50% in one month. At the same location in August 1995, 100% of all brown trout fry had black tails. By early fall 1995, 65% to 80% of the brown trout fry had black tails and/or skeletal deformities at this sampling locale. However, numbers of brown trout/km of various sizes (Table 16) and density (n/ha) and biomass (kg/ha) at this station (Table 17) have increased in 1996 and 1997. Indeed all brown trout population parameters for this station in 1997 are the highest they have been during the four years of study. Therefore, the data are inconclusive.

Table 14. Brown trout population age structure for a 3.2 km reach of the Colorado River on the Kemp/Breeze Wildlife Area, fall 1981-1986 versus fall 1993-1997.

Year	Age Class Structure							
	1+	2+	3+	4+	5+	6+	7+	8+
1981	1960	986	278	104	46	0	0	0
1982	986	487	464	93	0	0	0	0
1983	301	684	336	116	23	12	0	0
1984	464	731	394	81	70	0	0	0
1985	383	684	383	116	46	23	0	0
1986	510	348	244	104	12	0	0	0
1993	1497	269	777	519	181	32	6	0
1994	2095	1578	654	311	272	48	7	0
1995	5185	2239	1228	492	553	36	20	0
1996	2463	1791	1343	236	33	0	5	0
1997	1191	970	396	711	864	169	21	8

The same trends of overt symptomology of whirling disease among fry, fingerling and juvenile (age 1+) brown trout continued to be readily apparent in the upper Colorado River during the 1996 field season. Perhaps most disturbing was the dramatic increase in significant lordoscis, scoliosis, and blackened tails observed among juvenile brown trout farther downstream than had been observed in previous years. At three sites (just a few kilometers upstream from the town of Hot Sulphur Springs) we collected more than 30 age 1+ and 2+ brown trout with significant deformities of the vertebral column and caudal peduncle, in just 200 - 300 meters of river. These observations seemed to suggest that we may soon begin observing a downward spiral in brown trout density and biomass in the upper reaches of the Colorado River between Granby and Hot Sulphur Springs. However, that was not the case in the fall of 1997 (Tables 16 and 17). Adult and juvenile brown trout numbers/km, density (n/ha) and biomass (kg/ha) have continued to hold up well in the upper reaches of the river, particularly at the Hitching Post Bridge Station which is only 1.6 km downstream from Windy Gap Reservoir, a major source of high levels of TAM spores.

Our November 1996 and April 1997 electrofishing surveys indicate outstanding survival of the 79,700 CRR fingerling trout stocked in the river in October 1996. November 1996 estimates indicate a mean density of 1724 CRR fingerlings/km, ranging from 524/km to 5238/km, an estimated 64,500 fingerlings surviving in the 37.4 km reach of river stocked. Survival estimates for CRR fingerling stocked in October 1995 (for the same reach) in November and December 1995 were 30,300 and 36,700. The mean estimate of CRR fingerling survival in April 1997 was 39,600, or an amazing 49.7% survival! However, our fall 1997 electrofishing surveys throughout the river between the Hitching Post Bridge station and the Parshall to Sunset Ranch reach did not reveal the presence of nearly as many juvenile rainbow trout as we had hoped. It is quite possible that the unusually high and extended run-off during the spring and summer of 1997 may have resulted in significant numbers of the fingerlings from the October 1996 stocking being flushed downstream. Discharge records indicate the flow of the Colorado River below Windy Gap Reservoir exceeded 28.3 m^3/s (1000 ft^3/s) for 56 days and peaked at more than 113 m^3/s (4,000 ft^3/s) for seven days in early June 1997. The only period of greater discharge in the past two decades was the 1984 water year.

During the wild rainbow trout spawning operations on the upper Colorado River in April 1998, we held all rainbow trout collected in holding pens and examined every fish for an adipose fin-clip, since a significant number of the fingerling rainbow trout stocked in 1994, 1995, and 1996 were clipped for easy differentiation from wild rainbow trout that would have survived the WD epidemic in the upper Colorado River. Details on the stocking history of Colorado River rainbow (CRR) trout fingerlings in the upper river are shown in Table 18. While it appeared we had not seen the numbers of adipose-fin clipped (AFC) rainbow trout in our fall electrofishing surveys in 1996 and 1997 that we had hoped for, the results of our spring 1998 electrofishing were far more encouraging. Of 336 wild rainbow trout captured during spring electrofishing operations, 40 (11.9%) were AFC rainbow trout. On the surface this would not seem to be very good survival.

However, the majority of the AFC trout are smaller than most rainbows in the spawning population. Eight-eight percent of the unmarked (non-AFC) rainbow trout were ≥ 37 cm in length, ranging from 37 cm to 55 cm long. Forty-five percent of the wild rainbow trout that ranged in size between 27 cm (10.6 inches) and 36 cm (14.2 inches) collected during the spawning operation were AFC rainbow trout. Twenty-nine of 64 wild rainbow trout ≤ 36 cm in length were AFC rainbow trout. This is a conservative estimate of the contribution of AFC rainbow trout to this segment of the population, since some AFCs do regenerate. Thus, in all likelihood, it is safe to conclude that 50% of the rainbow trout population in the upper Colorado River (≤ 36 cm in length) in the spring of 1998 are derived from the stocking operations between 1994 and 1996. The fingerlings stocked in November 1997 were too small to be vulnerable to our electrofishing gear in the spring of 1998.

Table 15. Density (n/ha), biomass (kg/ha), and numbers per hectare of rainbow and brown trout ≥ 35 cm at the Lone Buck and Paul Gilbert Wildlife Units on the Colorado River during September and October for various years from 1979 through 1997.

Year	Brown Trout			Rainbow Trout		
	N/Ha	Kg/Ha	N/Ha ≥ 35 cm	N/Ha	Kg/Ha	N/Ha ≥ 35 cm
Lone Buck Wildlife Area						
1979	30	15	—	230	148	—
1981	23	14	10	98	31	20
1982	27	17	17	88	32	33
1983	63	39	13	80	45	21
1984	57	22	14	180	54	36
1985	22	8	2	107	44	42
1986	53	23	20	184	73	87
1988	51	33	31	543	217	250
1994	234	46	12	94	51	59
1995	41	14	12	53	24	32
1996	213	59	40	33	17	21
1997	210	36	16	33	8.6	10
Paul Gilbert Wildlife Area						
1981	—	—	—	29	4	1
1982	39	17	15	16	4	2
1983	137	29	13	236	30	10
1984	83	23	11	110	29	11
1985	64	28	9	129	45	39
1986	83	41	38	267	71	125
1987	91	33	27	729	188	158
1988	94	31	16	223	57	35
1994	146	32	12	61	25	21
1995	325	86	43	103	27	34
1996	598	120	38	17	7	7
1997	396	92	47	51	13	10

Table 16. Numbers of brown and rainbow trout ≥ 15 cm, ≥ 35 cm, and ≥ 40 cm per kilometer for various sample locations and years on the upper Colorado River.

	Brown Trout per Kilometer			Rainbow Trout per Kilometer		
Mo/Year	≥ 15 cm	≥ 35 cm	≥ 40 cm	≥ 15 cm	≥ 35 cm	≥ 40 cm
Hitching Post Bridge Station						
09/1994	517	138	46	184	150	59
10/1995	163	95	69	43	40	30
09/1996	380	138	61	125	66	40
09/1997	834	217	140	47	33	29
Chimney Rock Ranch HQ above Drowsy Water Creek						
10/1995	199	108	59	284	264	170
09/1996	140	40	17	97	70	53
09/1997	231	72	53	122	111	86
John Sheriff Ranch Station						
10/1995	56	43	33	62	62	36
09/1996	304	37	12	98	38	27
09/1997	266	54	36	41	24	20
Thompson/Doucette Ranch Station						
09/1994	442	74	33	384	254	49
10/1996	290	8	0	188	59	42
09/1997	598	20	12	61	43	22
Paul Gilbert Wildlife Area						
09/1994	404	33	0	169	60	11
10/1995	952	124	33	289	96	24
09/1996	1,476	95	32	87	17	12
09/1997	1,109	131	24	99	29	9
Lone Buck Wildlife Area						
09/1994	655	33	0	254	172	41
10/1995	115	33	11	136	77	9
09/1996	450	84	23	101	46	20
09/1997	588	46	10	57	29	11

Table 16 (continued). Numbers of brown and rainbow trout ≥ 15 cm, ≥ 35 cm, and ≥ 40 cm per kilometer for various sample locations and years on the upper Colorado River.

	Brown Trout per Kilometer			Rainbow Trout per Kilometer		
Mo/Year	≥ 15 cm	≥ 35 cm	≥ 40 cm	≥ 15 cm	≥ 35 cm	≥ 40 cm
John Stark Ranch						
09/1996	374	75	17	104	62	25
09/1997	185	68	25	16	12	8
Jack Horn Ranch						
09/1994	242	59	13	35	35	28
10/1995	105	36	13	10	10	10
09/1996	218	80	55	7	4	4

Table 17. Density (n/ha) and biomass (kg/ha) of brown and rainbow trout ≥ 15 cm for various locations and years at sampling sites on the upper Colorado River.

Mo/Year	Brown Trout		Rainbow Trout	
	n/ha	kg/ha	n/ha	kg/ha
Horn Ranch Above Windy Gap				
09/1994	97	30.1	11	9.1
10/1995	111	37.0	10	11.7
09/1996	179	84.2	6	6.6
Hitching Post Bridge Station				
09/1994	156	51.2	53	35.0
10/1995	96	60.2	21	19.2
09/1996	136	54.4	44	20.9
09/1997	269	85.0	16	11.1
Chimney Rock Ranch HQ Above Drowsy Water Creek				
10/1995	72	40.1	102	65.7
09/1996	51	18.6	43	30.6
09/1997	74	30.3	39	34.0
Sheriff Ranch				
10/1995	20	15.6	23	15.0
09/1996	98	17.9	31	12.3
09/1997	95	27.9	18	6.9
Thompson/Doucette Ranch				
09/1994	155	42.2	135	73.2
09/1996	103	11.0	28	20.4
09/1997	214	23.8	22	10.7
Paul Gilbert Wildlife Area				
10/1988	94	31.0	223	57.3
09/1994	289	60.2	121	42.2
10/1995	325	85.9	103	27.3
09/1996	523	105.2	31	7.1
09/1997	396	91.9	35	10.0

Table 17 (continued). Density (n/ha) and biomass (kg/ha) of brown and rainbow trout ≥ 15 cm for various locations and years at sampling sites on the upper Colorado River.

Mo/Year	Brown Trout		Rainbow Trout	
	n/ha	kg/ha	n/ha	kg/ha
Lone Buck Wildlife Area				
10/1988	51	33.2	543	216.9
09/1994	312	58.1	121	55.1
10/1995	55	19.0	53	24.0
09/1996	159	44.3	36	13.9
09/1997	210	36.3	20	8.6
John Stark Ranch Station				
09/1996	133	33.8	37	19.1
09/1997	66	25.0	6	3.0

Table 18. Details of the stocking history of the upper Colorado River with Colorado River rainbow (CRR) trout fingerlings from spawn collected from the river, 1993 - 1998.

Hatchery Source	Date Stocked (mo/da/yr)	Number per Pound	Average Size mm (in)	Number Stocked	Number Marked	Location of Stocking
Rifle Falls	10/19/94	81.8	78.7 (3.1)	6,000	6,000	Granby Rsrvr/Williams Fork R.
Riffle Falls	10/19/94	81.8	78.7 (3.1)	7,030	7,030	Parshall to Sunset Ranch
Rifle Falls	10/19/95	88.1	76.2 (3.0)	45,000	45,000	Granby Rsrvr/Williams Fork R.
Rifle Falls	10/19/95	88.1	76.2 (3.0)	45,000	45,000	Parshall to Troublesome Creek
Glenwood Springs	10/18/96	168.8	61.0 (2.4)	42,031	42,031	Windy Gap/Hot Sulphur Springs
Glenwood Springs	10/18/96	314.2	50.8 (2.0)	37,704	0	Parshall to Troublesome Creek
Bellvue	11/25/97	91.0	76.2 (3.0)	30,030	0	Granby Rsrvr/Williams Fork R.
Bellvue	11/25/97	91.0	76.2 (3.0)	62,153	0	Parshall to Troublesome Creek
Glenwood Springs	07/15/98	96.6	73.7 (2.9)	24,285	0	Parshall to Troublesome Creek
Glenwood Springs	07/15/98	96.6	73.7 (2.9)	25,860	0	Windy Gap/Hot Sulphur Springs

Table 19. Creel census statistics on angler use (trips), angler hours, total catch, brown catch, rainbow catch, total catch/hour, brown catch/hour, and rainbow catch/hour for the Colorado River public assess points at the Breeze Unit Wildlife Area (Parshall Hole downstream through the Breeze Ranch Access), BLM Access to Sunset Ranch property, Williams Fork River below Williams Fork Reservoir, and the Beaver Creek/Paul Gilbert/Lone Buck Wildlife Areas, 1996 and 1997.

Statistics	Kemp/Breeze Wildlife Area	BLM/Sunset Access	Williams Fk below Reservoir	Lonebuck/Paul Gilbert Wildlife Area	Total All Areas	Total w/o Williams Fk.
			1996			
Angler Trips	3,541	548	N	3,362	7,441	
Angler Hours	20,636	2,691	O	14,233	37,560	
Total Catch	26,986	2,281	C	14,867	44,134	
Brown Catch	23,524	1,679	E	10,003	35,206	
Rainbow Catch	3,113	463	N	4,579	8,155	
Total Catch/Hour	1.31	0.85	S	1.04	1.17	
Brown Catch/Hr	1.14	0.62	U	0.70	0.94	
Rainbow Catch/Hr	0.15	0.17	S	0.32	0.22	
			1997			
Angler Trips	7,682	1,828	7,427	2,426	19,363	11,936
Angler Hours	36,878	9,872	42,497	11,779	101,026	58,529
Total Catch	64,431	9,657	52,483	12,624	139,195	86,712
Brown Catch	52,642	8,356	43,539	10,586	115,123	71,584
Rainbow Catch	10,985	1,302	8,488	1,827	22,602	14,114
Total Catch/Hour	1.75	0.98	1.23	1.07	1.38	1.48
Brown Catch/Hr	1.43	0.85	1.02	0.90	1.14	1.22
Rainbow Catch/Hr	0.30	0.13	0.20	0.16	0.22	0.24

One of the objectives of this study was to document the effect of restrictive angling regulations on wild trout populations as well as the level of angler use on restricted regulation waters, particularly on public waters acquired through purchase of stream access. The CDOW acquired the Kemp and Breeze Ranches that sit astride prime portions of the Williams Fork River and Colorado River in Middle Park, near Parshall, Colorado. These pieces of property are known as the Kemp and Breeze Wildlife Management Units. Approximately 3.2 km (2 miles) of public stream access exists on the Williams Fork River (Kemp Unit) and another 3.2 km on the Colorado River (Breeze Unit). In addition, public angling access on the Colorado River exists on the Beaver Creek, Paul Gilbert, and Lone Buck Wildlife Areas, just a few kilometers upstream from the Kemp/Breeze Management Units.

From January 1996 through December 1997, we conducted an intensive creel census on all of these public access angling areas together with the BLM Sunset angler access area, downstream and immediately adjacent to the Breeze Wildlife Unit. During the winter months of both years, on the open water areas on the Williams Fork and Colorado rivers within the Kemp/Breeze and BLM access areas were censused. We used the vehicle count/postcard method of creel census developed and used extensively in the 1980s on streams subject to restrictive angling regulations. The methodology of the vehicle count/postcard questionnaire creel census is summarized in Nehring (1987) and will not be reiterated here. Two week days and one weekend day were randomly selected as census days. On census days, vehicles were counted twice each day at all parking access areas and stamped-mailback creel census postcards were placed on the windshields of all vehicles.

Over the two years of the survey, we recieved 1217 responses. The data for those angler use and catch surveys are summarized in Table 19. For all access areas (exclusive of the Williams Fork River below Williams Fork Reservoir) in 1996, an estimated 7,441 anglers fished an estimated 37,560 hours. The estimated catch was more than 44,000 trout, with more than 35,200 brown trout and 8,100 rainbow trout. Total catch per hour was estimated at 1.17 trout/hour, 0.94 brown trout/hour and 0.22 rainbow trout/hour.

In 1997 we added the Williams Fork River into the census survey. For the year we estimate that more than 19,300 anglers fished an estimated 101,026 hours, caught an estimated 139,195 trout, with the brown catch estimated at over 115,100 and the rainbow catch estimated at more than 22,600! The remainder of the catch was comprised of brook trout and other species. Total catch per hour was estimated at 1.38 trout/hour. The brown catch rate was estimated at 1.14 and the rainbow catch rate at 0.22/hour. Using the October 1996 trout population estimates for the Parshall reach of the Colorado River as the baseline trout population available for angling in 1997 within the Kemp/Breeze Management Unit and BLM/Sunset Access areas (Table 13) we can estimate the number of times the anglers caught each trout within this reach of river in 1997. We estimate the brown trout population was turned over 10.4 times and and the rainbow population was turned over an estimated 42.7 times!!

These turnover rates are much higher than those observed on restrictive angling regulation stream segments in the early to mid-1980s (Nehring 1987). However, with the huge increase in the literature available on fly fishing, "how-to-do-it" books, and vast improvements in fly fishing equipment, neoprene waders that make angling in cold and inclement weather almost pleasurable, it is not surprising that angling success and catch has increased greatly in the past decade.

Dolores River

We have been able to boat electrofish a 17.7 - 19.4 km (11 - 12 mile) reach of the Dolores River from the USFS Metaska Day Use area below McPhee Dam during the spring/early summer period between 1991 and 1998 every year except for 1996. The results of those surveys are shown in Table 20.

During some of those years the salmonid population parameters were unusually high because of large amounts of water spilling out of McPhee Reservoir, either over the surface spillway, or through the outlet valves in the bottom of the dam. The 1993 population and biomass estimates for rainbow trout were artificially high since McPhee Lake source rainbow trout spilled over the dam from early March through June 28, 1993. Silver coloration and significant scale abrasion resulting from contact with the concrete spillway facilitated easy differentiation of these fish from those reared in the river below the dam. The 1993 total trout population and biomass estimates include rainbow trout and kokanee salmon flushed into the Dolores River from McPhee Lake.

The 1994 rainbow trout population and biomass estimates and the total trout estimates were artificially inflated due to the influx of rainbow trout flushed through the bottom release outlet tubes of McPhee Reservoir during the managed spill from April 1-June 15, 1994. Lake-source and stream-reared rainbow trout were not differentiated during the estimation process.

Snake River cutthroat trout (*Oncorhynchus clarki bouvieri*) were abundant in the river throughout much of the 1980s, prior to the time when this study was undertaken (Mike Japhet, personal communication; unpublished data). However, poor survival of stocked fingerlings in the late 1980s, perhaps due to warmer water temperatures resulting from reduced flows between 1987 and 1991, lead to the cessation of stocking of these fish in 1991. Without stocking the abundance of this species in the river dwindled rapidly. Colorado River cutthroat trout (*O. c. pleuriticus*) fry and fingerlings were stocked in the river below McPhee Dam in the early to mid-1990s. Population data in the Snake River cutthroat trout section of Table 20 for the 1997 and 1998 sampling years are actually for Colorado River cutthroat trout. The largest of these fish were approaching 40 cm total length during the surveys conducted in 1998.

We had been encouraged by the survival of some of the Colorado River cutthroat trout fingerlings and the spawning, hatch, and recruitment of wild rainbow trout to the population between 1993 and the fall of 1996. *Myxobolus cerebralis* was first detected in the rainbow and brown trout population in the river below McPhee Dam in the fall of 1995. Wild rainbow trout fry were found at all sampling sites in the fall of 1995 and electrofishing operations in October 1996 revealed many of these fry recruited to the juvenile (age 1+) cohort in 1996.

We were dismayed in October 1997 however, when electrofishing surveys revealed no rainbow trout fry could be found at any sampling stations more than about 2 km downstream from the dam. Even more discouraging was the discovery that most rainbow trout fry collected within the 2 km reach below the dam were exhibiting all the overt clinical signs of severe whirling disease, including exophthalmia, severe cranial deformities, blacktail, and violent whirling behavior. Testing confirmed these fish were severely infected and that many of the age

Table 20. Population estimates (N) with 95% confidence limits (CL), density (n/ha), and biomass (kg/ha) for trout ≥ 15 cm, and density (n/ha) of trout ≥ 35 cm, for the Dolores River from McPhee Dam to Bradfield Bridge, 1991 - 1998.

Year	N	95% CL	n/ha	kg/ha	n/ha ≥ 35 cm
Brown trout					
1991	1,006	± 391	33	14.1	16
1992	1,320	± 454	38	12.8	13
1993	2,618	± 846	84	25.1	17
1994	2,522	± 1,167	73	18.5	15
1995	3,965	± 758	131	38.1	15
1997	2,652	±804	88	24.2	24
1998	2,006	± 902	58	19.3	20
"Wild" Rainbow Trout					
1991	586	± 326	19	10.2	12
1992	459	± 169	13	9.0	9
1993	553	± 389	18	12.5	12
1993	6,037	± 975	194	61.0	28
1994	4,008	± 2,227	116	46.8	45
1995	1,188	± 239	39	17.5	23
1997	444	± 247	15	6.5	7
1998	219	± 183	6	2.7	3
Snake River Cutthroat Trout					
1991	433	± 419	14	0.4	4
1992	52	± 36	1	0.9	1
1993	38	± 14	1	1.2	1
1994	15	± 13	0.5	0.3	0.5
1995	3	± 3	0	0.1	0
1997	101	± 104	3	0.5	0
1998	20	± 17	1	0.2	0
Kokanee Salmon					
1993	1,209	± 350	39	11.3	21
1994	2	± 2	0	<0.1	0
1995	18	± 12	1	0.1	0
1997	0	—	-	—	-
1998	0	—	-	—	-
Total Trout					
1991	2,039	± 645	67	28.8	35
1992	1,771	± 435	51	23.7	23
1993	2,878	± 56	93	38.9	28
1993	10,165	± 1,272	213	95.3	80
1994	6,191	± 2,165	179	65.6	54
1995	4,967	± 692	164	56.6	54
1997	3,232	± 852	107	31.2	32
1998	2,294	± 929	66	22.3	24

1+ wild rainbow and brown trout collected in October 1997 were carrying heavy burdens of MC myxospores. Not surprisingly, no age 1+ wild rainbow trout were collected during boat electrofishing operations in June 1998. All rainbow trout collected were age 2+ and older.

Early Spanish explorers named this river *Rio Dolorosa*. Literally translated into English, *Rio Dolorosa* means "river of sorrow", an apt discriptor for a beautiful trout stream that now is severely infected by salmonid whirling disease.

Fryingpan River

Trout population parameters for the Fryingpan River have been intensely studied since the early 1970s. Estimates of density (n/ha), biomass (kg/ha), and abundance of fish ≥ 35 cm/ha for brown, rainbow, and cutthroat trout at three sampling sites from 1972 through 1997 are shown in Tables 21, 22, and 23. Density, biomass, and numbers of trout ≥ 35 cm changed very little for rainbow and brown trout at the Ruedi Dam (RD), Old Faithful (OF), and Taylor Creek (TC) study sites in 1997 compared to 1995 and 1996. Similarly, density and biomass of brook trout at the RD and OF study sites changed very little in 1997 either.

Rainbow and brown trout fry collected at all sample locations showed few if any significant overt clinical signs of whirling disease that have been obvious in other study streams such as the Colorado, Dolores, Rio Grande, and Gunnison rivers. Length/frequency data indicate that YOY and age 1+ brown and rainbow trout were present at the RD, OF, and TC sample areas in 1997. However, fewer age 1+ rainbow trout have recruited to the population at the Taylor Creek study site in 1996 and 1997 compared to the earlier years (Table 7).

Malfunction of equipment during our November 1997 survey caused a half-day delay in beginning electrofishing operations making it impossible to complete the sampling at"Quallude Corner". This station (called the Big Pullout site in previous reports) is so named because of the very short radius of the curve in the Fryingpan River Road at that juncture.

Evidence of the *MC* parasite was first detected in feral trout from this river in November 1995. While there still is no significant evidence of a population level effect at the end of 1997 when density (n/ha), biomass (kg/ha), and numbers of rainbow trout/ha ≥ 35 cm are the metrics of evaluation, the steady decline in age 1+ rainbow trout at the TC site is disturbing evidence that population level impacts are likely in the near future.

Inasmuch as brown trout density and biomass are increasing at all three (RD, OF, and TC) of the most upstream study sites, it is apparent the two brown trout ≤ 14 inches bag limit is not having any measurable effect in reducing brown trout density and biomass. Nonetheless, it is probable the increases in rainbow trout density and biomass are the result of the catch and release restriction in effect on rainbow trout.

It is unfortunate the brook trout population continues to decline in density and biomass. It now seems almost inconceivable that brook trout biomass at the RD study section ranged from 109 to 143 kg/ha between the fall of 1984 and 1986, but has declined to only 3 kg/ha in November 1996 (Table 23). It is also hard to conceive of the possibility that brown

Table 21. Density (n/ha) and biomass (kg/ha) of brown trout ≥ 15 cm and abundance (n/ha) of fish ≥ 35 cm on the Fryingpan River, 1972 - 1997.

Season/year	Ruedi Dam			Old Faithful			Taylor Creek		
	n/ha	kg/ha	n/ha ≥ 35 cm	n/ha	kg/ha	n/ha ≥ 35 cm	n/ha	kg/ha	n/ha ≥ 35 cm
Fall 1972	161	48	—	—	—	—	704	172	—
Fall 1973	180	44	—	—	—	—	432	110	—
Spring 1977	170	40	14	—	—	—	80	20	6
Fall 1977	340	50	12	—	—	—	320	—	11
Fall 1978	366	87	24	—	—	—	272	43	28
Fall 1979	466	101	44	742	104	10	724	75	22
Spring 1980	251	66	31	48 3	64	0	469	67	8
Fall 1980	431	87	11	952	131	0	504	78	24
Spring 1981	349	79	17	689	107	27	871	138	30
Fall 1981	461	70	10	873	147	21	591	91	15
Spring 1982	511	83	22	712	114	19	703	131	18
Fall 1982	495	86	23	1049	169	14	724	157	44
Fall 1983	672	146	54	962	150	7	539	122	47
Fall 1984	582	140	24	1177	217	17	427	102	37
Fall 1985	693	198	62	1062	233	24	567	186	125
Fall 1986	999	254	92	1295	240	11	723	221	135
Spring 1989	1716	508	215	2057	456	52	632	194	80
Spring 1992	1458	542	350	1515	422	51	547	170	92
Fall 1993	1666	719	488	1705	435	131	873	195	139
Fall 1994	1302	558	369	1323	351	105	928	188	103
Fall 1995	1614	736	610	1614	407	140	1483	305	135
Fall 1996	1633	604	323	1435	329	82	1536	323	142
Fall 1997	1699	699	486	1389	355	138	1371	341	173

Table 22. Density (n/ha), biomass (kg/ha) of rainbow trout ≥ 15 cm and abundance (n/ha) of fish ≥ 35 cm on the Fryingpan River, 1972 - 1997.

Season/year	Ruedi Dam			Old Faithful			Taylor Creek		
	n/ha	kg/ha	n/ha ≥ 35 cm	n/ha	kg/ha	n/ha ≥ 35 cm	n/ha	kg/ha	n/ha ≥ 35 cm
Fall 1972	368	45	—	—	—	—	891	181	–
Fall 1973	358	82	—	—	—	—	889	186	—
Spring 1977	510	230	71	—	—	—	390	130	55
Fall 1977	680	220	82	—	—	—	320	—	38
Fall 1978	847	208	47	—	—	—	762	95	4
Fall 1979	220	88	49	324	104	48	635	51	25
Spring 1980	297	116	88	263	99	44	422	59	24
Fall 1980	241	73	64	344	83	46	280	30	5
Spring 1981	261	114	105	205	72	43	442	46	11
Fall 1981[a]	138	15	9	93	26	18	349	31	0
Spring 1982	466	126	125	137	45	20	379	34	10
Fall 1982	464	113	53	145	44	20	181	29	23
Fall 1983	574	120	44	746	137	17	101	28	35
Fall 1984	762	280	163	479	163	64	116	28	26
Fall 1985	1509	465	435	769	225	174	263	23	10
Fall 1986	1481	707	477	657	187	109	223	60	50
Spring 1989	723	503	476	475	214	154	296	70	55
Spring 1992	368	202	156	236	99	73	233	70	40
Fall 1993	207	112	106	132	56	39	263	88	68
Fall 1994	290	166	151	144	158	140	176	54	33
Fall 1995	337	239	212	125	47	32	216	73	56
Fall 1996	301	170	143	144	51	35	207	73	55
Fall 1997	270	210	167	144	64	47	165	61	31

[a] Stocking of fingerling rainbow trout began in October 1981, one month after the fall 1981 population estimates were completed.

Table 23. Brook trout density (n/ha) and biomass (kg/ha) for the Ruedi Dam and Old Faithful study sites, 1978 - 1997.

Season/Year	Ruedi Dam Site		Old Faithful Site	
	n/ha	kg/ha	n/ha	kg/ha
Fall 1978	241	41	—	—
Fall 1979	569	53	124	10
Spring 1980	281	24	62	3
Fall 1980	468	56	278	326
Spring 1981	366	55	68	18
Fall 1981	297	32	74	11
Spring 1982	483	86	23	2
Fall 1982	532	72	54	12
Fall 1983	615	75	36	6
Fall 1984	806	109	85	5
Fall 1985	676	143	78	13
Fall 1986	562	134	110	16
Spring 1989	269	96	75	15
Spring 1992	139	30	32	6
Fall 1993	37	9	17	5
Fall 1994	36	8	9	4
Fall 1995	21	5	6	2
Fall 1996	22	3	5	1
Fall 1997	29	3	17	3

trout predation could have that significant of an effect on another salmonid existing in sympatry. Although no stomach samples have ever been taken from brown trout in the Fryingpan River to prove or disprove this hypothesis, brown trout piscivory remains the most plausible explanation. In a classic 10-year study of the sympatric interactions of brown and brook trout in the Ausable River in Michigan, Alexander (1977) found that small fish comprised up to 82% of the diet of brown trout ≥ 30 cm in length in both summer and winter. Moreover, brook trout comprised up to 61% of the total diet, while brown trout fry comprised only 2% to 5% of the diet during the same time period.

The fact that brown trout density and biomass were exploding in the Fryingpan River beginning in the mid-1980s and continuing into the early 1990s, concurrent with the decline in brook trout makes the brown trout predation hypothesis highly plausible. This all occurred concomitant with the onset of the flushing of opossum shrimp (*Mysis relicta*) out of Ruedi Reservoir, that dramatically altered the food supply for the rainbow and brown trout in the river immediately downstream (Nehring 1991). As a result, rainbow and brown trout in the RD and OF sites often exceed 4.5 kg (10 pounds) in weight, with some very large specimens occasionally attaining 10 kg (22 pounds) in weight. These very large fish may become highly piscivorous, particularly during periods when opposum shrimp abundance may decline due to decreased discharges from Ruedi Reservoir during below average water years. Previous studies indicated reductions in entrainment of opposum shrimp in discharge from the reservoir occurred in concert with lower volumes of water flowing through the outlet tubes (Nehring 1991).

As was the case for the Dolores River, Colorado River cutthroat trout fingerlings were stocked in the Fryingpan River in the early 1990s in an effort to establish a quality native cutthroat trout population in this stream. However, due to disease problems, loss of brood stocks, and severe shortages of WD-free trout in the CDOW hatchery system, the stocking of cutthroat trout in this river was eliminated. In 1997, a few native cutthroat trout were collected during electrofishing operations. As was the case in the Dolores River, a few specimens ranged from 35 cm (14 inches) and 40 cm (16 inches) in length. When the CDOW hatchery system begins to produce significantly more numbers of cutthroat trout, hopefully early in the 21st century, supplemental stocking of native cutthroat trout fingerlings might be a worthy management consideration.

We will monitor the Fryingpan River closely on an annual basis for the next five years to determine whether or not *M. cerebralis* begins to cause population levels effects on the rainbow trout population in this river.

Gunnison River

Commercially-reared rainbow trout infected with the *MC* parasite were privately stocked into the East River drainage near Crested Butte, Colorado, in 1988. The parasite was first detected at the CDOW Roaring Judy State Fish Rearing Unit (RJSFRU) located on the East River downstream from Crested Butte in late 1991. The Gunnison River begins at the confluence of the East and Taylor rivers at Almont, Colorado, approximately 5 km downstream from the RJSFRU.

The *MC* parasite was detected in rainbow trout at two sites in the lower Gunnison River in January 1994. The first site was in the stilling basin below the Crystal Dam spillway. Half of the rainbow trout samples (8 pooled samples of 4 to 5 fish per pool) at this location were heavily infected with *MC* spores (Pete Walker, CDOW Fisheries Pathologist; personal communication; unpublished data). All of the fish were in the 25 cm to 37 cm size range. At the second site (in Ute Park approximately 27 km downstream from Crystal Reservoir), the 1993 year class of feral rainbow trout tested positive in January and May 1994, but no measurable population level effects were manifested in that year class. However, overt clinical symptoms of whirling disease, including exophthalmia, black tail, and whirling behavior were observed in YOY rainbow trout fry in August 1994. Massive declines in rainbow fry abundance were documented in September and November 1994, compared to that observed in August.

Based upon the above information, we assume that exposure of the lower Gunnison River to the *MC* parasite probably resulted from the passage of *MC*-infected rainbow trout over the spillway of Crystal Dam in late June or early July 1993 when 113 to 150 m^3/s (4000 to 5300 ft^3/s) of water were bypassed through the outlet tubes and over the spillway. The Cimarron River, which flows into Crystal Reservoir, was stocked with catchable size rainbow trout infected with the *MC* parasite from the RJSFRU during that time period. Previous to that time, the three reservoirs (Blue Mesa, Morrow Point, and Crystal dams) had not sustained a surface spill since July 1986.

Tagged fish studies conducted on the South Platte River in 1979 and 1980 revealed that hatchery-reared stocked rainbow trout can be rapidly flushed downstream under conditions of high discharge. Jaw-tagged rainbow trout stocked in the South Platte at the Wigwam Club on June 12, 1979 were caught by anglers many kilometers downstream just a few days after stream discharge was increased from 0.7 m^3/s (25 ft^3/s) to more than 23 m^3/s (800 ft^3/s). Some fish were caught up to 50 km downstream from the point of release (R. Barry Nehring, unpublished data).

Intensive YOY trout fry sampling has been an on-going annual process in the lower Gunnison River gorge since 1992. Thus, we have two years (1992 and 1993) of fry abundance estimates before the advent of population level effects of whirling disease (WD), and four years (1994, 1995, 1996, and 1997) of post-WD fry abundance estimates. The data from these fry sampling efforts are summarized in Table 24. The dramatic declines in rainbow trout fry abundance between the August and September sampling periods for 1994, 1995, 1996, and 1997 are evident.

The massive losses of wild rainbow trout fry over a period of 50 days are particularly startling. This is the only study stream where the losses occur so rapidly, in such severity, and over such a short period of time. This suggests very high levels of infectivity, high parasite virulence, and a very early exposure in the life of the rainbow trout fry. This is puzzling because after more than a year of water filtration efforts to identify and quantify TAM spore densities in the river, we have yet to document high levels of parasite density in this river. We have not (yet) found anywhere near the densities of TAM spores in the Gunnison River gorge that we see in other streams where the rate of loss of young rainbow trout fry occurs much more slowly, over many months.

Table 24. Comparisons of rainbow and brown trout fry/fingerling density (numbers/km) estimates for the Gunnison River gorge for 1992 and 1993 (the before whirling disease period) and 1994 through 1997 (the sfter whirling disease period).

Year	**Brown Trout Fingerlings (n/km)**			**Rainbow Trout Fingerlings (n/km)**		
	August	September	%Change August to September	August	September	% Change August to September
	Before Whirling Disease (BWD)					
1992	2,190	5,580	+155%	5,154	4,210	-18.1%
1993	2,089	2,881	+37.9%	2,525	2,690	+6.5%
	After Whirling Disease (AWD)					
1994	5,435	7,073	+30.1%	9,909	1,146	-88.4%
1995	7,053	2,901	-58.9%	1,392	330	-76.3%
1996	7,612	6,129	-19.5%	11,370	1,850	-83.7%
1997	10,247	6,822	-33.4%	23,957	1,981	-91.7%

Age-specific estimates of juvenile and adult rainbow and brown trout density for all fish ≥ age 1+ for the 3.2 km Duncan/Ute study section are shown in Appendix Table I. These data indicate that the age 1+ cohort of rainbow trout (1994 year class) sampled in September 1995 was the the second smallest in 15 years of sampling. This was not unexpected, given the catastrophic losses that were documented among the YOY rainbow trout fry between August 10 and September 26, 1994 (Table 24).

Even more disconcerting, the lower levels of rainbow trout fry estimates in August and September 1995 resulted in no detectible recruitment of age 1+ rainbow trout into the population in the 3.2 km reach of the Gunnison River gorge between the Duncan Trail and the north end of Ute Park when electrofishing surveys were conducted over a four day period in September 1996. During that four day study we captured 227 rainbow trout ≥ 15 cm, but only two of them were less than 30 cm (28 and 29 cm). Age and growth analysis revealed they were age 2+, recruits from the 1994 year class. In contrast, we captured 637 brown trout that were age 1+ during the same four day period. Total brown trout ≥ 15 cm captured during the four day survey was 1060. The reason for the unusually low levels of rainbow trout fry occurring in 1995 (compared to 1992, 1993, 1994, 1996, and 1997) was that discharge levels in the Gunnison River gorge in July 1995 averaged 206 m^3/s (7254 ft^3/s) and peaked at 261 m^3/s (9210 ft^3/s) during the period of rainbow trout fry emergence. Excessively high discharge levels are known to be extremely detrimental to survival of 2-4 week old rainbow and brown trout fry (Nehring and Anderson 1993). Extended periods of very high flows in the Gunnison River gorge during 1983 and 1984 produced the two poorest year classes of both rainbow and brown trout fry in the river between 1981 and 1993, resulting in the lowest levels of age 1+ cohorts for both species in 1984 and 1985 (see Table I in the Appendix).

However, discharge level fluctuations can not be blamed for the poor survival and recruitment of YOY rainbow trout to age 1+ for 1994, 1996, or 1997. Densities of rainbow trout fry observed during August 1994, 1996, and 1997 were the highest for the six years of study (Table 24). Unfortunately, all three year classes suffered losses in excess of 80% during the 50 day period between the August and September samples (Table 24). August 1997 rainbow trout fry estimates were more than double those seen in 1996. At one sample site rainbow trout fry densities were more than 73,000/km (118,000/mile) and more than 49,000/km (79,000/mile) at another site. Stream discharge in the Gunnison River gorge in June and July 1997 were generally in the 85 - 113 m^3/s (3000 - 4000 ft^3/s) range. It will be interesting to see how many rainbow fry from this huge year class in 1997 survive to recruit as age 1 juveniles in the fall of 1998.

Rainbow trout density (n/ha), biomass (kg/ha) and abundance of fish ≥ 35 cm (14 inches) in length are at an all-time low for the Ute Park section of the Gunnison River gorge (Table 25). Brown trout population parameters are the highest ever observed. Some might conclude this is the result of the "empty niche" vacated by the disappearing rainbow trout. In contrast, we believe it is largely the result of seven years of good recruitment of YOY brown trout fry into the age 1+ and older cohorts of the population (Appendix Table I). Numbers of age 1+ brown trout recruiting to the population have been above 400/ha every year since 1991 and above 660/ha for 1995, 1996, and 1997. Nonetheless, total trout density, biomass, and abundance of trout ≥ 35 cm were far greater in 1987 and 1988 than 1997 (Table 25). We do not believe the brown trout population in the Gunnison River gorge will ever equal or compensate for the loss of the rainbow trout population because of habitat limitations, the territorial nature, and object-cover-oriented behavioral traits of brown trout (Bachman 1984; Shuler and Nehring 1994; Shuler, Nehring and Fausch 1994).

Except for 1996, trout population estimates have been completed on an 8 km reach of the upper Gunnison River from 1985 through 1997. While there have been large fluctuations in brown trout density (n/ha), biomass (kg/ha), and abundance of trout/ha ≥ 35 cm over the 11-year sampling period, there is no discernible pattern (Tables 26). In contrast, dramatic declines in abundance of age 1+ rainbow trout began in 1992, the year after the state fish rearing unit some 5 km upstream from the study area became *MC* positive, suffering a severe outbreak of whirling disease (Table 5). Moreover, after four consecutive years of increase that began in 1988, rainbow trout density and biomass suffered the greatest single year decline between 1991 and 1992 seen up to that time (Table 26). Those statistics fell to the lowest levels for 11 years of study in 1993 and have largely remained at that level since (Table 26). The slight increase in rainbow trout population parameters observed in the 1997 survey was undoubtedly the result of the stocking of hatchery rainbow trout into the study reach carried out by the Trout Unlimited (TU) based in Gunnison, Almont, and Crested Butte. These fish were not marked and therefore were indistinguishable from the wild stream-reared rainbow trout.

Table 25. Estimates of brown and rainbow trout population density (n/ha ≥ 15 cm), biomass (kg/ha), and numbers of trout ≥ 35 cm (14 inches) per hectare (ha), for the Duncan/Ute Trail reach of the Gunnison River gorge, fall 1981 through fall 1997.

Year	Brown Trout			Rainbow Trout		
	n/ha	kg/ha	n/ha ≥ 35 cm	n/ha	kg/ha	n/ha ≥ 35 cm
1981	869	201.1	71	339	110.7	84
1982	603	143.8	43	392	110.3	97
1983	586	134.5	39	427	149.8	146
1984[a]	541	54.6	18	217	84.5	110
1985[a]	330	53.6	13	346	164.5	261
1986	469	69.8	31	275	132.8	190
1987	1,236	170.2	72	1,110	236.9	194
1988	936	117.7	44	1,054	243.0	245
1989[b]	672	98.8	51	249	84.8	112
1990	565	103.5	62	308	108.9	146
1991	632	96.2	66	310	114.2	139
1992	692	90.8	44	278	92.7	104
1993	751	101.9	56	370	135.3	163
1994[c]	802	125.8	58	334	146.8	174
1995	1,075	130.2	75	220	117.1	152
1996	953	141.0	72	138	89.4	131
1997	1,468	209.8	124	86	61.9	80

[a] Peaks flows during late June 1983 and 1984 were between 283 and 340 m^3/s (10,000 - 12,000 ft^3/s), leading to very poor survival of rainbow and brown trout fry those two years.

[b] A devastating flash flood occurred in the Gunnison river gorge in August 1989, one month prior to our population estimation surveys, killing 1000s of fish, mostly juvenile rainbow trout; this is the explanation for the dramatic decline in density of rainbow trout between 1988 and 1989.

[c] Whirling disease has devastating effects on survival of YOY rainbow trout fry beginning in 1994.

After dividing the data set into BWD (1985-1992) and AWD (1993-1995) time periods, a t-test for two means applied to the data set revealed that rainbow trout density (n/ha) and biomass (kg/ha) experienced a significant ($\rho \leq 0.05$) decline in the AWD time period. There were no significant changes in the brown trout population parameters when that data set was divided into the same BWD and AWD time periods. We believe it is unlikely that these significant declines in age 1+ recruitment, density, and biomass of rainbow trout that began in 1992 in synchrony with the documented presence of *M. cerebralis* just upstream of the study reach in November 1991 are merely the result of cosmic happenstance. This is the result of whirling disease. The disappearance of wild rainbow trout fry is occurring in this stream (as it is on the Cache la Poudre, Colorado, Dolores, lower Gunnison, Rio Grande, and South Platte rivers) across all types of water years, i.e., below average, average, and above average discharge regimes.

We did not include the 1997 trout population data in the statistical analysis because of the effect of stocking of hatchery-reared rainbow trout into the study reach in 1997.

Table 26. Trout population density (n/ha), biomass (kg/ha) and density of quality size (≥ 35 cm) trout for the Gunnison River from Almont to Rocky River Resort, 1985-1997.

	Brown Trout			Rainbow Trout		
Year	n/ha	kg/ha	≥ 35 cm	n/ha	kg/ha	≥ 35 cm
1985	250	50.5	28	22	5.3	6
1986	284	74.8	26	42	11.8	11
1987	271	84.6	32	74	19.4	10
1988	145	37.9	15	50	10.3	7
1989	400	111.6	42	81	19.5	12
1990	363	93.8	35	84	19.6	14
1991	196	59.0	25	122	37.9	44
1992	224	64.5	30	53	20.3	22
1993	413	120.2	63	7	3.1	4
1994	185	59.9	38	11	6.3	8
1995	112	43.1	31	7	4.8	6
1997	143	51.5	32	46	16.8	10

Rio Grande

Although trout taken from private catch-out ponds and a fish-rearing facility in the Rio Grande drainage tested positive for the presence of *MC* in 1988, the parasite was not detected in feral rainbow and brown trout in the river drainage until April 1994 (Nehring and Thompson 1996). Extensive collection and testing of feral trout from the Rio Grande River was done in 1988. Annual inspections of fish from private trout-rearing facilities (in the drainage) from 1988 through 1993 did not result in detection of the parasite at any other location. However, the stocking of *MC*-positive rainbow trout from the RJSFRU into the Rio Grande drainage began in 1992 and continued in 1993.

This river has been the subject of intensive study for more than 15 years. Except for 1993 (when equipment failure prevented sampling), electrofishing operations have been conducted on a 10.8-km reach of the river known as State Bridge (SB) west of Del Norte, Colorado every year since 1981. Similarly, a 6-km reach of river upstream from the Marshall Park U.S. Forest Service Campground near Creede, Colorado, has been sampled annually by electrofishing since 1987. Over the years we have referred to this section of river as the Marshall Park or Rio Grande Fisherman Area (RGFA) study reach. Rainbow and brown trout density and biomass estimates for these two study reaches are shown in Tables 27 and 28.

During the decade of the 1980s, rainbow trout fingerlings were stocked in the three study sections in an attempt to establish wild, self-sustaining rainbow trout populations (Nehring 1991, 1992). Colorado River rainbow trout fry/fingerlings were stocked for five years in the SB section between 1984 and 1989. The segment of the Rio Grande within the Coller Wildlife Area was stocked for 4 years between 1984 and 1988. The segment of the river within the RGFA was stocked for three years from 1986 through 1988. No rainbow trout fry or stocking of any kind took place within the three study areas between 1989 and 1995.

The presence of wild rainbow trout fry ≤ 50 mm was documented in the river and the South Fork of the Rio Grande (SFRG) beginning in 1988 and every year after that through 1992 (Nehring 1993). Indeed, for the five year period from 1988 through 1992, estimates of wild rainbow trout fry production exceeded that of brown trout for two of the five years and averaged 45,700 over the five year period, compared to an average of 45,300 brown trout fry for the same period (see Table 16 in Nehring 1993).

The data in Table 27 and Table 28 do not indicate any detectible decline in brown trout population parameters. While there have been major fluctuations between years, there are no established trends. However, there have been large declines in all rainbow trout population parameters for both the RGFA and SB study reaches. These declines began concomitant with the detection of the MC parasite in both wild rainbow and brown trout throughout the drainage in April 1994. Wild rainbow trout numbers in the SB study reach declined 87% between 1992 and 1997 (Table 27). Wild rainbow trout numbers declined 91% within the RGFA study reach between 1995 and 1997 (Table 28). Age 1+ rainbow trout numbers within this study reach are 96% and 89% lower than what was estimated for this reach in September 1992 (see Appendix Table I).

Table 27. State Bridge trout population estimates for the Rio Grande between 1981 and 1997. Study reach length is 10.8 km (6.7 miles) and the surface area is 50 ha.[a]

Year	Brown trout			Rainbow trout		
	N	kg/ha	N/ha ≥35 cm	N	kg/ha	N/ha ≥35 cm
Pre-rainbow fingerling stocking						
1981	5,168	39.3	29	295	2.8	4
1982	6,753	38.9	35	143	0.8	1
1983	8,948	45.4	31	285	1.9	2
1984	6,597	32.9	15	325	1.7	2
Post-rainbow fingerling stocking						
1985	6,372	30.9	28	896	3.5	3
1986	6,373	32.0	24	2,077	5.2	2
1987	7,483	35.8	35	1,791	4.1	3
1988	7,844	36.7	21	3,644	9.4	15
1989	5,286	27.0	25	1,496	7.2	9
1990	5,747	23.7	24	2,840	14.6	22
1991	3,935	21.0	19	1,039	6.9	8
1992	7,429	35.7	33	2,713	21.2	28
1994[b]	4,965	25.8	21	919	7.4	8
1995[c]	5,105	27.3	26	1,960	16.3	21
1996	6,661	33.9	30	643	5.1	6
1997	6,485	34.5	34	362	3.7	5

[a] No sampling was conducted in 1993 due to equipment malfunctions.
[b] Significant numbers of *M. cerebralis* spores found in feral brown and rainbow trout in the river in April 1994.
[c] 25,000 CRR fingerlings were stocked in this reach of river in October 1995.

Table 28. Rio Grande River Fisherman Access Area above Marshall Park Campground; trout density (n/ha) and biomass (kg/ha), 1987-1997.

Year	Brown Trout				Rainbow Trout			
	N	N/Ha	Kg/Ha	N/Ha ≥ 35 cm	N	N/Ha	Kg/Ha	N/Ha ≥ 35 cm
1987	2,862	151	31.3	18	1,630	86	11.7	5
1988	2,050	108	31.1	17	3,630	192	25.7	6
1989	5,061	266	43.1	22	4,325	228	23.5	3
1990	6,523	343	57.1	30	2,859	150	20.7	6
1991	5,775	304	59.1	25	4,360	231	45.1	22
1992	6,214	327	61.6	30	1,994	105	17.3	8
1993	3,913	206	43.0	24	1,532	81	17.7	13
1994	3,562	187	36.6	17	1,262	66	13.4	7
1995	6,214	327	61.6	30	1,994	105	17.3	8
1996	3,167	167	35.3	20	419	22	6.3	6
1997	4,219	222	42.0	24	184	10	2.2	2

Similarly, since the fall of 1994, age 1+ rainbow trout numbers for the 10.8-km SB section have consistently been lower than at any time since September 1984, prior to the time when CRR fingerlings were stocked in an attempt to establish a wild rainbow trout population (see Appendix Table I). Not surprisingly, after four consecutive years of minimal to nonexistent recruitment of wild rainbow trout fingerlings, rainbow trout density and biomass at both the SB and RGFA study reaches have declined dramatically (Tables 27 and 28). In all likelihood, rainbow trout density and biomass within the SB study section may well have been even lower than shown in Table 27, were in not for the stocking of 25,000 CRR fingerlings in October 1995.

Roaring Fork River

Due to an early winter and an inadequate number of summer temporaries, we were unable to electrofish the Roaring Fork River during the 1996/1997 segment. We did have adequate personnel, time, equipment, and good weather to conduct electrofishing surveys at two locations on the Roaring Fork River during the 1997/1998 segment. Data from the November 1995 study were presented in a previous progress report (Nehring and Thompson 1996), but are included again for continuity and comparison with data from surveys conducted 18 to 28 years ago. That data for the Roaring Fork River, a reach of river in the town of Aspen immediately behind the Aspen Institute and near the Aspen Center for Environmental Studies (ACES) was included in the ***Stream Fisheries Investigations*** research project in 1979 and 1980 (Nehring 1980; Nehring and Anderson 1981). The data from those studies together with that collected in October 1997 are summarized in Table 29 and discussed below.

Table 29. Population estimates (N), density (n/ha), biomass (kg/ha),and numbers of fish ≥ 35 cm/ha for the Roaring Fork River, at the Aspen Institute, Aspen Colorado; 1970, 1978, 1979, 1980, and 1997.

Date	N	95%CI	n/ha	kg/ha	n/ha ≥ 35 cm
Brown Trout					
08/25/70	64	±53	288	80.3	36
09/1978	58[a]	----	190[a]	35.8	23
09/28/79	181	±57	388	110.1	58
09/18/80	121	±23	260	75.2	37
10/08/97	119	±4	532	127.5	11
Rainbow Trout					
08/25/70	222	±71	995	270.0	64
09/1978	120[a]		393[a]	71.2	0
09/28/79	350	±76	752	164.4	7
09/18/80	226	±38	487	99.5	3
10/08/97	43	± 1	190	41.7	4
Brook Trout					
08/25/70	69	±91	309	49.0	0
09/1978	7[a]		23[a]	2.9	0
09/28/79	22	±12	46	5.6	0
09/18/80	20	±7	43	5.2	0
10/08/97	0		0	0	0

[a] Estimates from a single electrofishing pass only.

In late fall 1995, a 4-km reach, just downstream from Basalt and the confluence with the Fryingpan River, was sampled with boat electrofishing equipment for the first time. Population estimates were obtained on three salmonid species, including brown trout, rainbow trout, and mountain whitefish (*Prosopium williamsoni)*. Whitefish were the most abundant species with a population estimate of 2,910 fish (95% confidence limits ± 1031) and an estimated biomass of 130.9 kg/ha. The brown trout population was estimated at 2,398 (± 960) with an estimated biomass of 53.0 kg/ha. The rainbow trout population was estimated at 1,115 (± 582) with an estimated biomass of 35.2 kg/ha.

The same reach of river was electrofished again in early October 1997. Again, population estimates were obtained on three salmonid species, including brown trout, rainbow trout, and mountain whitefish. Whitefish were the most abundant species with a population estimate of 3,059 fish (95% confidence limits ± 1754) and an estimated biomass of 471.6 kg/ha. The brown trout population was estimated at 2,312 (± 1125). Brown trout biomass was estimated at 44.3 kg/ha. The rainbow trout population was estimated at 711 (95%

confidence limits ± 467) with an estimated biomass of 18.2 kg/ha. The large increase in whitefish biomass between 1995 and 1997 is most likely the result of the difference in the time of the survey, i.e., early October 1997 versus late November-early December 1995. The whitefish is a fall-spawning salmonid and runs up the Roaring Fork and Fryingpan rivers to spawn. In late-November 1995, it was likely that the spawning run was over, and larger adult whitefish had moved downstream for the winter months.

As was the case in 1995, the whitefish and brown trout populations were relatively well balanced with both small (≤ 29 cm) and larger (≥ 30 cm) fish represented in the population. Maximum size of brown trout was 45 cm. The largest whitefish were 53 cm in length. Thirty-one percent of the brown trout population were ≤ 30 cm in length. In contrast, the rainbow trout population was dominated by fish ≥ 30 cm in length. Approximately 90% of the population was ≥ 30 cm in length. During the 1995 population estimation, of 238 rainbow trout handled, only five were ≤ 24 cm, compared to 50 brown trout in that size range. Similarly, in October 1997, only 9 of 142 rainbow trout handled were ≤ 24 cm. Fish of this length would comprise the age 1+ and age 2+ cohorts.

Wild rainbow trout were abundant in the upper Roaring Fork River from 1970 through 1980, according to the data in Table 29. Age one rainbow trout ranged in size from 10 to 18 cm throughout that time period. In 1997 at the station in Aspen, there were no rainbow trout in the sample less than 22 cm. Rainbow trout population parameters at this station in October 1997 are greatly reduced compared to the same parameters observed between 1970 and 1980 (Table 29). Indeed, during the 11 year period between 1970 and 1980, rainbow trout dominated virtually every trout population category. In 1997, brown trout are the dominant salmonid species in all categories.

This trout population profile is remarkably similar to that seen among the rainbow trout populations in the Cache la Poudre, Colorado, Dolores, Gunnison, Rio Grande, and South Platte rivers, where whirling disease is affecting wild rainbow trout density at the population level. In all likelihood this is the case for the Roaring Fork River as well.

Rainbow trout from the Roaring Fork River drainage first tested positive for *M. cerebralis* in 1988. During 1995, both YOY fingerling rainbow trout and older fish collected from the Basalt to Hook's Bridge study reach tested positive for the parasite. We have collected wild rainbow trout fry from the Roaring Fork River downstream from Basalt every year since 1994. However, few if any of these YOY trout seem to be recruiting to the population. If they were, they would be found in the age 1+ cohort during electrofishing operations. For the 3.2 km study reach between Basalt and Hook's Bridge, rainbow trout density and biomass declined dramatically between 1995 and 1997. We conclude that the wild rainbow trout population of the Roaring Fork River is experiencing population-level impacts similar to those observed on the aforementioned rivers.

South Platte River

Feral rainbow trout collected from the South Platte River in the Deckers area in the fall of 1988 and 1989 tested positive for *M. cerebralis*. Trout population estimates have been completed on several segments of this river almost every year since 1979. Moreover, trout population estimates on this river are the most precise and accurate of any produced by fish research and management personnel for any river in the state. Population estimates (N), density (n/ha), biomass (kg/ha), and abundance (n/ha) of trout ≥ 35 cm for the Lower Cheesman Canyon and Above Deckers monitoring sites are shown in Tables 30 and 31.

During the period from 1979 through 1990 all population parameters fluctuated up and down at both the Lower Cheesman Canyon and Above Deckers sampling sites. Since 1990, the data indicate that rainbow and brown trout population parameters at both study sites have been declining for seven straight years. Moreover, with one or two exceptions, these declines have been large enough that the upper 95% confidence limit in year *n* does not overlap with the lower 95% confidence limit in year *n-1*, a precedent never seen on this river (Tables 30 and 31) or in the experience of the authors on any stream in Colorado in more than 18 years of sampling.

No changes in angling regulations have occurred on either of these two study sections since 1985. Therefore, we stratified the data and applied a two-sample mean t-test to determine if rainbow trout density and biomass declined from 1992 through 1996 compared to 1985-1991. All population parameters were natural logarithm transformed to minimize the effect of unequal variances. We used a one-tailed t-test statistic because we were only interested in whether or not there was a decline in the trout population after the time *M. cerebralis* was well established in the drainage. Rainbow trout density and biomass declined significantly ($p \leq 0.05$) at both study sites during the 1991-1996 period. Density of rainbow trout ≥ 35 cm also declined significantly ($p \leq 0.01$) at the lower Cheesman Canyon site, but there were no significant declines in any of the brown trout population parameters at either site.

Relative abundance of YOY rainbow and brown trout through the first six months of life have not declined during the "after whirling disease" (AWD) period (Table 32). However, very few YOY rainbow trout have survived to the end of the second summer of life (Table 5). Length-frequency distributions for rainbow and brown trout at the lower Cheesman Canyon sampling station is shown in Figure 3. The data in Figure 3 indicate that the 1990 year class of rainbow trout was the last one to experience near normal levels of recruitment from age 0 to age 1. To determine if there has been a significant decline in the numbers of YOY rainbow and brown trout recruiting to the population as age 1 juveniles, we again stratified the data sets (Table 5) into the BWD (1985-1991) and AWD (1992-1996) periods, and conducted two-sample mean t-tests on each data set. Age 1 rainbow and brown trout densities at all three sites declined significantly ($p \leq 0.05$) during the AWD period (Table 33).

Table 30. South Platte River trout population (N), density (N/Ha), biomass (Kg/Ha), and abundance of trout ≥ 35 cm (N/Ha) at the Lower Cheesman Canyon monitoring site (1979 - 1997).

	Brown Trout				Rainbow Trout			
Year[a]	N	N/Ha[b]	Kg/Ha	N/Ha ≥ 35 cm	N	N/Ha[b]	Kg/Ha	N/Ha ≥ 35 cm
F1979	327	791 (±29)	192	84	512	1238 (±60)	401	342
S1980	329	795 (±130)	176	79	514	1243 (±72)	422	404
F1980	333	805 (±22)	165	44	384	929 (±27)	336	355
S1981	259	766 (±27)	170	52	496	1467 (±33)	566	673
F1981	221	534 (±53)	135	62	264	638 (±85)	259	394
S1982	305	738 (±48)	164	34	344	832 (±46)	344	526
F1982	231	559 (±58)	121	33	232	561 (±77)	234	384
F1983	427	1032 (±41)	244	99	570	1378 (±31)	359	476
F1984	261	631 (±82)	168	61	373	802 (±85)	322	381
F1985	186	449 (±29)	120	83	247	597 (±12)	262	474
F1986	251	607 (±75)	143	69	262	634 (±80)	315	463
F1987	258	822 (±76)	186	47	230	735 (±108)	320	436
F1989	463	896 (±23)	204	108	384	743 (±15)	280	362
F1990	716	1435 (±36)	292	120	538	1078 (±18)	376	391
F1991	445	860 (±27)	216	83	238	461 (±17)	191	194
F1993	396	766 (±29)	200	110	199	385 (±14)	184	248
F1994	323	735 (±34)	210	121	176	400 (±14)	240	358
F1995	286	802 (±36)	219	192	121	339 (±20)	201	283
F1996	276	583 (±45)	201	245	101	212 (±18)	137	183
F1997	335	639 (±17)	218	285	182	348 (±15)	150	181

[a] F prefix preceding the year denotes fall sample; S denotes a spring sample.

[b] 95 % confidence limits in parentheses.

Table 31. South Platte River trout population (N), density (N/Ha), biomass (Kg/Ha), and abundance of trout ≥ 35 cm (N/Ha) at the Above Deckers monitoring site (1979 - 1997).[a]

	Brown Trout				Rainbow Trout			
Year	N	N/Ha[c]	Kg/Ha	N/Ha ≥ 35 cm	N	N/Ha[c]	Kg/Ha	N/Ha ≥ 35 cm
F1979[b]	416	1097 (±179)	141	14	140	369 (±53)	61	0
S1980	409	1079 (±34)	137	0	58	152 (±18)	28	7
F1980	545	1318 (±293)	164	5	130	314 (±203)	44	11
S1981	303	733 (±41)	103	7	37	89 (±10)	15	13
F1981	396	957 (±421)	185	46	88	213 (±324)	40	7
S1982	205	496 (±48)	77	4	17	41 (±5)	6	0
F1982	696	1683 (±210)	190	8	117	285 (±36)	32	8
F1983	973	2352 (±48)	270	20	313	757 (±19)	106	23
F1984	393	951 (±82)	145	3	132	319 (±15)	77	34
F1985	405	979 (±29)	158	3	244	590 (±10)	173	81
F1986	487	1179 (±34)	202	8	199	482 (±10)	165	156
F1987	641	2049 (±42)	306	14	224	716 (±80)	188	128
F1989	959	2140 (±16)	328	36	379	847 (±2)	238	193
F1990	1092	2643 (±58)	460	56	310	751 (±15)	247	199
F1991	1204	2686 (±49)	407	63	242	539 (±7)	171	145
F1993	806	1798 (±31)	398	37	162	361 (±9)	156	184
F1994	520	1325 (±23)	266	55	66	167 (±3)	89	126
F1995	419	934 (±85)	199	99	52	117 (±7)	58	80
F1996	334	745 (±66)	130	82	23	50 (±3)	20	30
F1997	274	605 (±19)	179	169	35	76 (±22)	19	20

[a] An 8 trout/day bag limit was in effect on this section up through December 1982. Catch and release and/or a 2 trout ≥ 16 inches bag limit with fly and lure only terminal tackle went into effect beginning in 1983.

[b] F prefix preceding the year denotes fall sample; S denotes a spring sample.

[c] 95% confidence limits in parentheses

Table 32. Young-of-the-year (YOY) rainbow and brown trout numbers captured during two-pass removal fall (September - December) electrofishing operations at three sites on the South Platte River, from 1980 through 1997.

Station	Cheesman Canyon		Above Deckers		Below Deckers	
Year	Brown	Rainbow	Brown	Rainbow	Brown	Rainbow
1980	7	2	15	2	[a]	[a]
1981	2	5	268	22	326	15
1982	4	48	188	69	181	46
1983	15	28	19	8	16	8
1984	10	3	19	1	28	6
1985	16	2	34	2	13	3
1986	24	8	78	56	118	63
1987	11	1	12	12	25	16
1989	369	586	493	75	484	133
1990	137	92	747	125	789	138
1991	292	199	573	45	433	68
1993	22	252	308	128	150	37
1994	139	21	368	64	a	a
1995	80	14	215	16	199	49
1996	38	235	273	148	228	184
1997	65	102	406	117	365	96

[a]: This station was not sampled this year.

Table 33. Summary of two sample mean t-tests for differences in densities of age 1+ brown and rainbow trout for three sites on the South Platte River between the before whirling disease (BWD) and after whirling disease (AWD) period.

	Pre-Whirling Disease				Post-Whirling Disease				
Species	Years	n	Mean	Range	Years	n	Mean	Range	t-value
South Platte River In Cheesman Canyon									
Browns	85-91	7	390	165-710	92-96	5	151	73 - 252	+2.4623***
Rainbows	85-91	7	184	39-414	92-96	5	14	5 - 21	+2.3565***
South Platte River above Deckers Bridge									
Browns	85-91	7	1,315	950-1,937	92-96	5	495	342 - 611	+3.6634*****
Rainbows	85-91	7	189	22-561	92-96	5	11	3 - 18	+2.9004****
South Platte River below Deckers									
Browns	85-91	7	948	508 - 1,455	92, 94-96	4	571	334 - 756	+2.1946**
Rainbows	85-91	7	227	63 - 454	92, 94-96	4	36	13 - 96	+2.8125***

[a] Significance at the 90% level = *; 95% level = **; 97.5% level = ***; 99% level = ****; 99.5% level = *****.

The decreases in rainbow trout density are truly alarming, having declined more than 80% at the Lower Cheesman Canyon site since 1990 and more than 94% at the Above Deckers station since 1989. In 1996, all rainbow trout population parameters at the Lower Cheesman Canyon site dropped to all time low levels since monitoring began in 1979. At the Above Deckers site, rainbow trout density, biomass, and numbers of trout ≥ 35 cm peaked in 1989 and 1990, just one or two years after *M. cerebralis* was detected in the wild trout at this site. All rainbow population parameters have been in an unrelenting decline ever since.

Similarly, brown trout density (n/ha) and biomass (kg/ha) at the Above Deckers site also reached the highest levels in 1990 - 1991, and also have been in unrelenting decline between 1991 and 1996. Only numbers of brown trout ≥ 35 cm in length have increased during the AWD period. This has occurred at both the Lower Cheesman Canyon and Above Deckers stations.

However, in 1997 there was a significant turn around in population parameters for both brown and rainbow trout at the Lower Cheesman Canyon station. All brown trout population parameters increased at this site as did most of the rainbow trout population parameters (Table 30). Of particular importance was a dramatic increase in the recruitment of age 1+ wild rainbow trout in 1997 from the 1996 year class. The fall 1997 electrofishing survey revealed an estimated 157 age 1+ wild rainbow trout had recruited to the population at this site, the largest age 1+ rainbow trout cohort since 1991 (see Table 5 for details). Whether this is the beginning of a lasting reversal of the disturbing and unrelenting downward trends in population parameters or a one-time occurrence remains to be seen. We will be monitoring this situation with great attention during the next five year study on this reach of river.

Taylor River

Electrofishing studies to categorize the adult brown trout population of the Taylor River have not been conducted regularly since the mid-1980s (Nehring and Anderson 1983). However, YOY trout fry collections have been made regularly during the late 1980s and 1990s (Nehring 1988; Nehring and Shuler 1992; Nehring 1993). Wild rainbow trout fry were first collected from the lower reaches of the Taylor River in the early 1990s (Nehring 1993). Wild rainbow were never found in the river during the 1970s (Burkhard 1977) or the early 1980s (Nehring and Anderson 1983). Documentation of wild rainbow trout fry in this stream in the 1990s is believed to be the result of establishment of the wild rainbow trout population in the Gunnison River, a few kilometers downstream, that run up the Taylor River and its tributaries to spawn. Wild rainbow trout fry were collected at several sites on the river as well as from Lottis Creek, a tributary stream during October 1996. Sadly, histological evaluation revealed even these fish were significantly parasitized by *M. cerebralis*. It is unknown whether or not these young rainbow trout are recruiting to the Taylor River trout population because no electrofishing surveys have been conducted on the lower reaches of the river since the mid-1980s. Due to inadequate numbers of field personnel, extra job assignments, and a lack of time to accomplish the task, no extensive electrofishing operations were conducted on this river during the 1996/1997 or 1997/1998 segments.

It was reported in a previous progress report (Nehring and Thompson 1996) that histological sections of rainbow and brown trout fry collected during October 1995 had tissue damage consistent with an *M. cerebralis* infection. One of three rainbow trout fry appeared affected and 8 of 10 brown trout fry from the same location were affected. The brown trout possessed intralesional organisms consistent with *M. cerebralis* (Dr. David Getzy, DVM, Colorado State University, personal communication).

Evidence of Whirling Disease in Wild Trout

Research efforts over that past four and a half years have demonstrated whirling disease is having severe effects on wild rainbow trout populations in at least six or seven major streams in Colorado. Population level effects are devastating wild rainbow trout in a 40 km reach of the upper Colorado River (Walker and Nehring 1995; Nehring and Walker 1996). Since then additional evidence has been amassed that strongly supports the conclusion that whirling disease-induced population level effects are occurring in the Cache la Poudre, Gunnison, Rio Grande, and South Platte rivers (Nehring 1996; Nehring and Thompson 1996; Nehring et al, 1997). The latest additions of streams suffering population level effects for WD include the Dolores and Roaring Fork rivers. As of the spring of 1998, it is too early to conclusively determine whether or not population level effects are occurring on the Fryingpan River. However, declines in age 1+ rainbow trout density over four consecutive years at the Taylor Creek site suggest population level effects are beginning.

During sentinel fish experiments on the upper Colorado River in 1995 and 1996, our studies demonstrated that brook trout and A+ Colorado River (Nanita Lake strain) cutthroat trout were as sensitive or more sensitive to the debilitating effects of the ambient levels of the *M. cerebralis* parasite than were wild rainbow trout (Thompson, et al 1997).

During our 1996/1997 sentinel fish tests, we exposed wild brown trout, CRR (Colorado River rainbow) trout, greenback cutthroat trout, and Snake River, Rio Grande, and Colorado River cutthroat trout to ambient levels of the *M. cerebralis* parasite in the Colorado River. With the exception of the Snake River cutthroat trout, all three subspecies of Colorado's native cutthroat trout were as vulnerable or more vulnerable to whirling disease than CRR trout. We completed the data analysis (species-specific histology, species-specific *MC* spore concentrations with increasing time of exposure, and species and species-specific monthly mortality and survivorship data) for all treatment groups. A draft manuscript has been submitted to the editors of the ***Journal of Aquatic Animal Health*** for publication in the professional literature.

Histological evidence of Myxobolus cerebralis parasitism

A very large number of feral, free-ranging rainbow and brown trout were collected for histological analysis during the 1995/1996 and 1996/1997 study segments. Much of that data analysis has been completed. However, some reports (particularly for collections made during the fall of 1997) for some streams are still not completed. What has been completed and supplied to us by the CDOW Brush Fish Health Laboratory is summarized and reported in Table 34.

These data reveal a number of important points regarding the impacts of whirling disease on wild trout. First, at a given location and date, in most cases fewer brown trout are infected than rainbow trout and they are generally less severely affected. Second, among those streams where the rainbow trout population is collapsing due to lack of survivorship of fry to the juvenile (age 1) cohort, the average severity rating is usually ≥ 2.0 and in many cases ≥ 3.0. Third, at a given location, the average severity rating usually increases with increasing exposure time, such as is shown for the Cache la Poudre at the Black Hollow Creek and USFS Kelly Flats Campground sample sites (Table 34). Fourth, when the average severity rating for brown trout fry is ≥ 1.5, it is quite likely all of the rainbow trout fry at that site will have already died. Such was the case in October 1996 on the Colorado River at the John Sheriff Ranch, Chimney Rock Ranch, and Hitching Post Bridge monitoring sites.

A summary of some of the data from histologic analyses conducted on salmonids used as sentinel fish in the upper Colorado River and exposed to ambient levels of the TAM spores of *M. cerebralis* beginning at different times and for varying lengths of exposure are shown in Table 35. The average severity ratings for the histological damage caused by the parasite in these sentinel fish do not differ greatly from that seen in feral free-ranging brown and rainbow trout collected from this river or other stream trout populations affected by the parasite (compare with data from Table 34). Perhaps the only surprising result was the complete lack of evidence of histological damage in the CRR trout initially exposed on 10/19/95 that were cropped after 189 days. Subsequent testing of other fish from this treatment group later in the summer of 1996 revealed they did develop clinical infections caused by *M. cerebralis* and carried heavy burdens of MC myxospores later in the summer of 1996.

Table 34. Summary of histopathology evaluations of wild brown and rainbow trout for evidence of parasitism by the myxosporean parasite *Myxobolus cerebralis* for various streams in Colorado.

Date (mo/da/yr)	Brown Trout			Rainbow Trout		
	N+/N	Severity Rating	Range	N+/N	Severity Rating	Range
Cache la Poudre at Big Bend Campground						
09/19/96	2+/10	0.40	0 - 2.0	4+/10	0.40	0 - 1.0
Cache la Poudre at Black Hollow Creek						
09/19/96				5+/10	0.70	0 - 2.0
11/21/96				10+/10	1.75	1.0 - 3.0
Cache la Poudre at USFS Kelly Flats Campground						
09/19/96	3+/10	0.55	0 - 2.0	9+/10	1.40	0 - 2.0
11/21/96				9+/9	2.89	2.0 - 3.5
Colorado River at the Breeze Bridge Wildlife Area						
10/14/96	4+/10	0.80	0 - 2.5	8+/10	1.40	0 - 2.5
Colorado River at Lone Buck Wildlife Area						
10/14/96	6+/10	0.90	0 - 2.0	10+/10	3.30	2.0 - 4.0
Colorado River at John Sheriff Ranch						
10/14/96	4+/4	2.38	2.0 - 3.0	No rainbow fry collected or seen		
Colorado River at Chimney Rock Ranch above Drowsy Water Creek						
10/14/96	3+/4	2.38	0 - 3.5	No rainbow trout collected or seen		
Colorado River at Hitching Post Bridge						
10/14/96	7+/9	1.56	0 - 3.0	No rainbow trout collected or seen		
Dolores River at USFS Metaska Day Use Area 1.6 km below McPhee Dam						
10/01/96	0+/10	0	——	0+/10	0	——
01/09/97	3+/5	1.2	0 - 2.0	4+/7	1.00	0 - 3.0
Dolores River at USFS Ferris Canyon Campground 8.9 km below McPhee Dam						
10/01/96				4+/6	1.33	0 - 3.0
11/19/96				2+/9	0.44	0 - 2.0
Dolores River above Cabin Canyon Campground 14.2 km below McPhee Dam						
10/01/96				9+/10	2.05	0 - 4.0

Table 34 (continued). Summary of histopathology evaluations of wild brown and rainbow trout for evidence of parasitism by the myxosporean parasite *Myxobolus cerebralis* for various streams in Colorado.

Date (mo/da/yr)	Brown Trout			Rainbow Trout		
	N+/N	Severity Rating	Range	N+/N	Severity Rating	Range
East River above CDOW Roaring Judy Fish Hatchery						
10/02/96	0+/10	0	——	8+/10	1.40	0 - 2.5
East River at Almont at Taylor River confluence						
09/18/95				0+/3	0	——
10/02/96	7+/10	1.05	0 - 2.0	13+/19	1.45	0 - 2.5
Fryingpan River between Basalt and Ruedi Reservoir						
11/11/96	3+/10	0.40	0 - 2.0	2+/12	0.33	0 - 2.0
Gunnison River at Ute Park						
09/24/96	2+/10	0.30	0 - 2.0	10+/10	2.20	1.0 - 2.5
Gunnison River below Gunnison Tunnel Weir 1.6 km below Crystal Dam						
10/04/96	0+/7	0	——	3+/10	0.30	0 - 1.0
12/12/96	0+/4	0	——	12+/13	2.58	1.0 - 3.5
Beaver Creek - 1.6 km below Beaver Creek Reservoir, South Fork of the Rio Grande						
10/10/96	0+/10	0	——	8+/9	1.83	0 - 2.5
South Fork of the Rio Grande at Beaver Creek Bridge access						
10/10/96	3+/10	0.50	0 - 2.0	10+/10	3.15	2.0 - 3.5
Park Creek, 1.6 km upstream of confluence with South Fork of the Rio Grande						
10/11/96	1+/10	0.10	0 - 1.0	9+/10	1.15	0 - 2.0
South Fork of the Rio Grande, 0.4 km above Beaver Creek confluence						
10/10/96	4+/10	0.75	0 - 2.5	10+/10	2.55	2.0 - 3.0
South Fork of the Rio Grande, 1 km below Columbine Falls at USFS Columbine Picnic Area						
10/10/96	0+/10	0	——	8+/10	2.10	0 - 3.5
South Fork of the Rio Grande above Columbine Falls[a]						
10/10/96	0+/10	0	——	0+/10	0	——
South Platte River 0.2 km below Spinney Mountain Reservoir						
08/14/96				7+/7	3.43	2.5 - 4.0

[a] Ten brook trout also tested showed no evidence of *M. cerebralis* parasitism.

Table 34 (continued). Summary of histopathology evaluations of wild brown and rainbow trout for evidence of parasitism by the myxosporean parasite *Myxobolus cerebralis* for various streams in Colorado.

Date (mo/da/yr)	Brown Trout			Rainbow Trout		
	N+/N	Severity Rating	Range	N+/N	Severity Rating	Range
South Platte above the Wigwam Creek						
11/04/96	3+/10	0.65	0 - 2.5	9+/10	2.85	0 - 4.0
South Platte above Deckers, Colorado						
11/04/96	2+/10	0.30	0 - 2.0	9+/10	2.86	1.0 - 4.0
South Platte below Deckers, Colorado						
11/20/96				9+/10	2.45	0 - 3.5
Taylor River 1.6 km upstream from East River confluence						
10/02/96	4+/8	1.12	0 - 4.0	4+/7	0.43	0 - 1.0
Taylor River at Canyon Campground						
10/31/96	6+/10	0.90	0 - 2.0	5+/6	1.08	0 - 2.0
Lottis Creek above Taylor River confluence						
10/21/96	3+/11	0.27	0 - 1.0	6+/10	0.75	0 - 2.5

Table 35. Summary of histopathology evaluations in salmonids exposed as sentinel fish to the myxosporean parasite *Myxobolus cerebralis* in the Colorado River.

Species	Days Post Exposure	N+/N	Average Severity Rating	Range
Colorado R. Rainbow (CRR) - exposed 8/1/95; cropped 4/9/96	253	7+/7	2.86	1.5 - 3.5
CRR trout-exposed 8/1/95; cropped 4/25/96	269	6+/6	3.25	2.5 - 4.0
CRR trout-exposed 7/1/95; died 10/95 - 3/96	92 - 244	10+/10	3.00	2.5 - 3.5
CRR trout-exposed 7/1/95; died 12/95 through 3/96	153 -275	10+/10	3.45	2.5 - 4.0
Brown trout exposed from hatch 6/1/95; cropped 4/25/96	314	3+/8	0.75	0 - 2.0
CRR trout exposed 10/19/95; cropped 4/25/96	189	0+/10	0	——
Brook trout-exposed 7/1/95; mortalities March/April 96	244-269	9+/9	3.83	3.0 - 4.0
Colorado River cutthroat-exposed 8/1/95;died 11/96 & 12/96	92 - 153	9+/10	2.25	0 - 2.5
Colorado River cutthroat-exposed 8/1/95; died 3/96	213 - 244	9+/9	2.72	1.0 - 3.5
Colorado R. cutthroat; exposed 7/1/95; died 12/95-3/96	153 - 275	10+/10	3.40	2.0 - 4.0
Snake River cutthroat; exposed 5/1/96; cropped 9/9/96	132	3+/3	3.67	3.0 - 4.0
Snake River cutthroat; exposed 5/1/96; cropped 10/7/96	160	4+/4	4.0	——
Snake River cutthroat; exposed 7/10/96; cropped 8/2/96	23	7+/10	1.70	0 - 3.0

Myxobolus cerebralis spore concentrations in feral trout

Over the past four years, thousands of feral, free-ranging brown, brook, cutthroat, and rainbow trout have been sent to the CDOW Brush Aquatic Animal Health Laboratory for determination of the concentration of *MC* myxospores in the cranium of the fish. Similarly, thousands more brown, brook, rainbow, and Snake River, Rio Grande, Colorado River, and greenback cutthroat trout tested as sentinel fish in the Colorado River have been submitted to the Brush Lab for quantification of *MC* myxospore burden by the pepsin-trypsin digest (PTD) method described by Markiw and Wolf (1974). Much of that data was summarized and reported by Walker and Nehring (1995) and Nehring and Thompson (1996).

In comparing *MC* myxospore burdens occurring in wild rainbow trout with those observed in wild rainbow trout held as sentinel fish in the Colorado River, an interesting phenomenon has occurred over and over again. In wild rainbow trout surviving from hatch to 18 months of age, we rarely find *MC* myxospore burdens exceeding 50,000 spores. In contrast, among wild rainbow trout collected as fry and held as sentinel fish in flow-through live cages in the Colorado River, *MC* spore burdens can range between 100,000 and 2.8 million, usually averaging more than 500,000 spores after 18 months exposure. We believe that very few YOY rainbow trout with *MC* spore burdens ≥ 50,000 survive beyond the first year of life. We believe the data summarized in Table 36, for the South Platte River is strong empirical evidence that this is the case. Apparently most YOY rainbow trout with cranial *MC* spore concentrations ≥ 50,000 die about the time they reach age 1 or shortly thereafter.

Table 36. Incidence of infection and concentrations of *Myxobolus cerebralis* (MC) spores among various ages groups of wild brown and rainbow trout from the South Platte River downstream from Cheesman Reservoir between January 1994 and February 1997.

Date Mo/Da/Yr	Age	Sample Size		MC Spores for Positive Fish	
		Nº	Nº+	Mean	Spore Range in Positive Fish
Rainbow Trout					
01/19/94[af]	7 mo	10	7	139,000	25,000 - 550,000
01/23/97[cd]	7 mo	46	unk	100,000	unknown - a pooled sample
02/11/97[ce]	8 mo	10	7	152,130	10,390 - 387,390
02/18/97[ce]	8 mo	11	11	235,358	6,222 - 523,611
07/12/95[ae]	1 yr	1	0	0	--------
11/21/95[ae]	17 mo	7	3	5,250	1,917 - 9,667
11/21/95[be]	17 mo	10	7	16,631	1,500 - 36,667
07/12/95[ae]	2 yr	0	0	------	--------
07/12/95[ae]	3 yr	5	2	27,584	5,167 - 50,000
07/12/95[ae]	4 yr	5	0	0	--------
07/12/95[ae]	5 yr	6	0	0	--------
07/12/95[ae]	6 yr	1	0	0	--------
07/12/95[ae]	7 yr	2	1	28,000	--------
Brown trout					
02/11/97[ce]	9 mo	20	16	32,750	944 - 301,000
02/18/97[ce]	9 mo	19	8	7,028	1,333 - 19,500
11/21/95[cg]	18 mo	10	2	3,333	2,167 - 4,500
11/21/95[be]	18 mo	10	0	0	--------

[a] Samples collected 0.5 km upstream from Horse Creek confluence at Deckers
[b] Samples collected at Twin Cedars sample site approximately 16 km downstream from Horse Creek confluence.
[c] Samples collected 0.5 km downstream from Horse Creek confluence at Deckers
[d] Sample analyzed by Terry McDowell, plankton centrifuge method at U. CA - Davis
[e] Sample analyzed by CDOW Brush Fish Health Laboratory by PTD method
[f] Sample analyzed by CDOW Brush Fish Health Laboratory by plankton centrifuge method
[g] Sample collected 2.5 km downstream from Cheesman Dam

We have observed this age vs *MC* spore load pattern among rainbow trout of different ages from the Rio Grande, Gunnison, and Colorado rivers as well. For example, among age 2 rainbow trout collected from the Gunnison River below Crystal Dam in August 1996, two of three fish tested negative, and the one that tested positive had about 20,000 to 25,000 spores in the head. Among age 1+ rainbow trout collected at the same time and location, 16 of 20 tested positive for *MC* spores, but only 3 of 20 had cranial spore burdens ≥ 50,000. These data seem to suggest that when the majority of wild rainbow trout ≤ age 1 are carrying an *MC* spore burden ≥ 50,000 spores/head, that population is most likely going to decline in density and biomass quite rapidly.

Sentinel Fish Testing

In July 1995, we began an elaborate sentinel fish experiment on the upper Colorado River. Initially, we exposed eight different treatment groups of young trout to ambient levels of the triactinomyxon (TAM) form spore of *M. cerebralis* to ascertain differences in susceptibility to this parasite. Another treatment group was added in October 1995. This experiment was terminated in September 1996. Those treatment groups were as follows:

1. Wild brown trout - collected from the river and exposed from hatch (May/June) - BNTW.

2. Wild Colorado River rainbow (CRR) trout - collected from river and exposed from hatch - CRRW.

3. CRR trout - Glenwood Springs Hatchery (GSH) and exposed 7/7/95 after one week on feed - CRR71W.

4. CRR trout - hatched at GSH and exposed 8/3/95 after four weeks on feed - CRR84W.

5. Brook trout - from Trappers Lake in NW Colorado, exposed 7/1/95 after one week on feed - BKT71W.

6. A+ Colorado R. cutthroat (CRN) trout-hatched at GSH; exposed 7/7/95; one week on feed - CRN71W.

7. A+ CRN cutthroat trout - hatched at GSH and exposed 8/3/95 after one week on feed - CRN81W.

8. A+ CRN cutthroat trout - hatched at GSH and exposed 8/3/95 after four weeks on feed - CRN84W.

9. Adipose-clipped CRR trout - hatched at GSH; exposed 10/19/95; 16 weeks on feed - CRRAD16W.

A nineth treatment group (CRR trout 16 weeks on feed) were introduced into the Colorado River on October 19, 1995, and added to the experiment beginning in December 1995. Comparisons of survivability through time among the various treatment groups are shown in Table 37. Details on the experimental design, care and feeding of the fish, and other methodologies, are given in Nehring and Thompson (1996).

During the July/August to November exposure, the treatment groups exposed in August suffered less mortality than those exposed in July or earlier (Table 37). We believe the high mortality in the CRN84W treatment group was due to rancid feed problem that originated in the fish while at the Glenwood Springs Hatchery. Brook trout (BKT71W) and Colorado River native cutthroat trout exposed in July (CRN71W) suffered significantly higher mortality than any other treatment groups, making them far more vulnerable to the effects of the *M. cerebralis* parasite than either brown trout or Colorado River rainbow trout (CRRW and CRR71W treatment groups) exposed at an equivalent size, age and time (Table 37). By November 16, 1995, there were too few CRN71W treatment fish remaining to complete a single treatment block for the continuation of the experiment during the winter months, and only enough for a single block of 25 fish in the BKT71W treatment group.

For the winter to spring segment of the experiment, the surviving fish within each treatment group were pooled into a single lot, then re-randomized into replicate blocks, and the mortality clock was restarted on December 6, 1995. During this period the brown trout had the best survival, followed by the October-exposed CRR trout (CRRAD-16W) (Table 37). These were followed by the August-exposed cutthroat and rainbow trout four weeks (4W) on feed, then the wild rainbows exposed from hatch and July exposed brook trout and rainbow trout - one week (1W) on feed. The poorest survival was among the cutthroat trout exposed in July - one week on feed.

Table 37. Overall percentages of sentinel fish surviving on November 16, 1995 and April 9, 1996, and statistically significant ($p \leq 0.05$) differences between treatment groups as determined by Least Squares Differences (LSD) pair-wise comparisons of arc sine square root transformed data. Treatment groups that do not share a common letter in the column labeled "LSD" (within each time period) are significantly different from one another.

Treatment Code*a*	Date of Exposure	% Surviving 11/16/95	LSD					% Surviving 04/09/96	LSD			
CRR84W	August 3	93.3	a					88.0		b		
CRN81W	August 3	80.1		b				46.4				d
BNTW	May/June	79.2		b				100.0	a			
CRR71W	July 7	70.5		b	c			48.0			c	d
CRRW	June/July	60.9			c	d		72.0			c	
CRN84W	August 3	48.1				d		92.0		b		
CRN71W	July 7	15.4					e	*na1*				
BKT71W	July 1	13.1					e	68.0			c	d
CRRAD16W	October 19	*na2*						94.4		b		

a Treatment codes are given above at the end of each descriptive phrase for the nine treatment groups.
na1 Too few CR1-71W treatment group fish survived for one block after the winter exposure.
na2 The CRR-AD16W treatment group were not exposed to the river until mid-October 1995.

Those fish surviving beyond April 9, 1996 (the end of the winter experiment) were again pooled into one group within a single treatment, and up to 100 fish of each group were held through the spring-summer period in 1996. Daily mortalities were preserved and sent to the CDOW Brush Fish Health Lab for analysis for *MC* spore counts. These results are summarized in Table 38 through Table 42.

Table 38. *Myxobolus cerebralis* (*MC*) spore concentrations in fish from the CRR71W treatment group (Colorado River rainbow fry, hatched at Glenwood Springs Hatchery, and introduced into the Colorado River on 7/7/1995, after one week on feed) that died between 4/27/1996 and 9/18/1996. Brush Fish Health Case Number is 96-361.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*MC* Spores in Positive Fish	
				Mean	Range
36	4/30/96	1	1	202,322	
85	5/2/96	1	1	136,694	
99	5/11/96	1	1	55,200	
29	5/19/96	1	1	289,333	
9	6/11/96	1	1	87,433	
64	6/16/96	1	1	266,367	
43	6/26/96	1	1	217,678	
39	6/28/96	1	1	63,783	
4	8/2/96	1	1	600,178	

Comparisons of *MC* spore concentrations between free-ranging rainbow and brown trout collected from the Colorado River during fall 1996 electrofishing operations with spore concentrations found in surviving wild CRR trout, exposed to the river since hatching in June 1995 are shown in Table 43. These data further corroborate our hypothesis that very few wild rainbow trout survive beyond age one if they are carrying an *MC* spore burden ≥ 50,000 spores. While it might be argued that the rainbow trout are somehow either shedding the spores or destroying them internally, the data on *MC* spore burdens among three different test groups of CRR trout (Tables 36, 37, and 38) do not support that suggestion. On the contrary, the *MC* spore burden among the 22 CRR trout that survived to the end of the experiment on 9/18/1996 was as high or higher than those levels that were found in CRR trout that died during the weeks and months prior to the end of the experiment. These fish were of the exact same age and genetic background.

Table 39. *Myxobolus cerebralis* (*MC*) spore concentrations in fish from the CRRW treatment group (Colorado River rainbow fry collected from the river by electrofishing in June/July 1995, exposed from hatch) that died between 4/27/1996 and 9/18/1996. Brush Fish Health Case Number is 96-361.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº+	*MC* Spores in Positive Fish	
				Mean	Range
95	4/29/96	2	2	224,600	68,200 - 381,000
100	5/14/96	1	1	63,889	
103	5/16/96	1	1	84,972	
31	5/19/96	2	2	592,178	173,067 - 1,011,289
34	5/29/96	1	1	344,322	
14	6/05/96	2	2	279,845	234,000 - 325,689
7	6/11/96	1	1	253,500	
70	6/12/96	1	1	480,000	
76	6/13/96	2	2	251,439	96,778 - 406,100
71	6/14/96	2	2	301,689	288,889 - 314,489
66	6/16/96	2	2	222,245	168,489 - 276,000
87	6/17/96	3	3	358,022	253,800 - 494,400
52	6/19/96	5	5	128,997	65,017 - 230,267
41	6/25/96	2	2	180,250	77,000 - 283,500
44	7/03/96	1	1	41,400	
46	7/04/96	1	1	182,089	
38	7/08/96	1	1	107,139	
Comparative Fish from Other Exposure Times, Places, & Treatments					
a	9/18/96	22	22	585,090	2,056 - 2,280,833
b	9/09/96	5	5	26,652	1,044 - 94,600
c	9/09/96	10	10	52,742	1,056 - 126,472
d	9/09/96	10	10	63,590	3,500 - 173,056
e	6/10/96	1	1	0	

a These fish were still alive in the sentinel fish tanks on 9/18/96.
b Free-ranging surviving wild CRR trout collected from the Colorado River.
c Live, free-ranging CRRAD trout (stocked 10/19/95) collected from the Colorado River above the Williams Fork River confluence.
d Live, free-ranging CRRAD trout (stocked 10/19/95) collected from the Colorado River below the Williams Fork River confluence.
e CRRAD mortality (put into sentinel fish tanks on 10/19/95).

Table 40. *Myxobolus cerebralis* (*MC*) spore concentrations in fish from the CRR84W treatment group (Colorado River rainbow fry, hatched at Glenwood Springs Hatchery, and introduced into the Colorado River on 8/3/1995, after four weeks on feed) that died between 4/27/1996 and 9/18/1996. Brush Fish Health Case Number is 96-361.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº+	*MC* Spores in Positive Fish	
				Mean	Range
82	4/28/96	1	1	34,467	
56	4/30/96	1	1	4,500	
84	5/03/96	1	1	98,222	
98	5/13/96	1	1	16,100	
33	5/23/96	1	1	146,544	
16	5/28/96	2	2	32,317	14,933 - 49,700
15	5/30/96	2	2	40,489	16,267 - 64,711
24	6/2/96	1	1	96,056	
17	6/4/96	1	1	34,722	
6	6/7/96	1	1	434,133	
20	6/10/96	3	3	32,317	22,167 - 116,444
8	6/11/96	1	1	102,200	
79	6/12/96	1	1	254,178	
81	6/13/96	3	3	100,289	46,000 - 171,500
74	6/14/96	3	3	199,763	85,878 - 292,967
61	6/16/96	4	4	94,035	48,750 - 165,467
67	6/17/96	1	1	34,572	
93	6/18/96	1	1	71,789	
55	6/19/96	1	1	31,033	
42	6/21/96	2	2	172,622	86,633 - 258,611
47	6/25/96	3	3	42,050	21,750 - 70,194
53	7/1/96	1	1	20,044	
45	7/2/96	2	2	18,337	17,506 - 19,167
54	7/3/96	1	1	20,067	
48	7/5/96	1	1	32,500	
37	7/8/96	3	3	87,420	7,578 - 182,861

Table 41. *Myxobolus cerebralis* (*MC*) spore concentrations in fish from the CR181W treatment group (Colorado River cutthroat trout fry, hatched at the Glenwood Springs Hatchery and exposed to the river on August 3, 1995) that died between 4/27/1996 and 9/18/1996. Brush Fish Health Case Number is 96-361.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº+	*MC* Spores in Positive Fish	
				Mean	Range
102	4/27-29/96	6	6	60,259	29,600 - 84,972
58	4/30/96	1	1	14,672	
2	5/02/96	1	1	102,122	
83	5/03/96	1	1	25,967	
91	5/04/96	3	3	69,946	45,333 - 99,706
104	5/05/96	2	2	106,603	19,650 - 193,556
101	5/07/96	1	1	170,806	
110	5/10/96	3	3	58,300	
57	5/11/96	3	3	45,417	27,611 - 59,167
89	5/12/96	1	1	63,700	
109	5/13/96	4	4	42,592	13,616 - 59,972
88	5/14/96	1	1	108,100	
97	5/16/96	2	2	50,555	30,333 - 70,778
30	5/19/96	1	1	71,261	
26	5/22/96	3	3	93,915	28,244 - 221,833
28	5/23/96	2	2	46,039	34,222 - 57,856
12/108	6/04/96	2	2	109,833	32,433 - 187,233
1	6/07/96	1	1	70,267	
72	6/11/96	2	2	60,453	58,556 - 62,350
18/77	6/13/96	2	2	104,798	63,067 - 146,528
65	6/16/96	1	1	165,600	
69/107	6/17/96	2	2	251,564	52,461 - 450,667
90	6/18/96	1	1	64,500	
49	7/06/96	1	1	82,500	
86*a*	9/18/96	3	3	182,672	143,372 - 248,311

a These three fish were the only survivors left at the end of the experiment, from a starting sample size of 400 fish.

Table 42. *Myxobolus cerebralis* (*MC*) spore concentrations in fish from the CR184W treatment group (Colorado River cutthroat trout fry, hatched at the Glenwood Springs Hatchery and exposed to the river on August 3, 1995 after 4 weeks on feed) that died between 4/27/1996 and 9/18/1996. Brush Fish Health Case Number is 96-361.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº+	*MC* Spores in Positive Fish	
				Mean	Range
92	5/05/96	1	1	61,533	
94	5/06/96	1	1	26,033	
106	5/11/96	1	1	168,778	
96	5/12/96	1	1	322,222	
21	5/19/96	1	1	96,350	
25	5/22/96	2	2	62,889	46,944 - 78,833
32	5/23/96	3	3	86,994	65,167 - 100,833
23	5/28/96	1	1	61,328	
27	5/31/96	1	1	125,733	
19	6/01/96	1	1	153,750	
11	6/02/96	1	1	60,900	
3	6/04/96	2	2	104,450	84,028 - 124,872
13	6/05/96	1	1	101,600	
22	6/06/96	1	1	63,000	
5	6/07/96	3	3	115,280	80,267 - 153,750
10	6/10/96	4	4	78,961	19,067 - 193,200
73	6/11/96	5	5	116,538	16,133 - 193,556
75	6/12/96	2	2	85,875	37,839 - 133,911
80	6/13/96	2	2	102,856	88,833 - 116,878
68	6/17/96	1	1	41,400	
105	6/18/96	2	2	127,936	92,094 - 163,778
63	6/16/96	1	1	47,233	
51	6/19/96	1	1	34,711	
50	6/24/96	3	3	70,043	12,089 - 102,706

Table 43. *Myxobolus cerebralis* (*MC*) spore concentrations in various groups of rainbow trout collected during electrofishing surveys of the upper Colorado River during September and October 1996 compared with surviving sentinel Colorado River rainbow trout (CRRW treatment group).

Case Nº	Date of Death (Mo/Da/Yr)	Species Treatment Group	Nº	Nº+	*MC* Spores in Positive Fish	
					Mean	Range
96-372-42	9/18/96	CRRW*a*	22	22	585,090	2,056 - 2,280,833
96-338-13	9/09/96	CRRAD*b*	10	8	52,742	1,056 - 126,472
96-338-14	9/09/96	CRRW*c*	5	5	26,652	1,044 - 94,600
96-338-15	10/14/96	CRRAD*d*	10	9	63,590	3,500 - 173,056

a **Surviving CRRW sentinel trout at end of 1995/1996 experiment.**

b **Live, free-ranging CRRAD trout collected from above the Williams Fork River confluence.**

c **Live, free-ranging CRRW trout collected from above the Williams Fork River confluence.**

d **Live, free-ranging CRRAD trout collected from below the Williams Fork River confluence.**

The data contained in Tables 35 through 43 shed a great deal of light on the dynamics of species differences, size differences, time of first exposure, and differences in spore concentrations among captive and free-ranging fish of the exact same species, age, and genetic stock. There were vast differences in mortality among the four species groups (brown, brook, rainbow, and Colorado River cutthroat trout). Not surprisingly, over the entire experiment brown trout suffered the least mortality. O'Grodnick (1979) found brown trout to be among the most resistant to the debilitating effects of *M. cerebralis*. Our test demonstrated that Colorado River rainbow trout are slightly more resistant to the effects of the ambient levels of *MC* and whirling disease than are brook trout and Colorado River native cutthroat trout.

Among the CRR treatment groups, those exposed at the earliest age (CRRW and CRR71W) suffered the highest mortality over the course of the experiment (Table 37). They also demonstrated the highest *MC* spore burdens (Tables 38 and 39) compared to the CRR84W treatment group that was introduced into the river at a larger older size (4 weeks on feed) approximately one month later (August 3rd versus early July for the CRRW and CRR71W groups). Mean *MC* spore burden among the 22 CRRW treatment group survivors on September 18, 1996 was 585,090 with the highest spore concentration at 2,280,833 (Table 39).

Among the Colorado River cutthroat trout treatments groups (CR171W, CR181W, and CR184W), those exposed earliest at the smallest size (CR171W) suffered very severe mortalities during the first fall and over the winter (Table 37). Too few survived to be included in the second summer (1996) spore concentration tests. Among the CR181W treatment group, only three trout (of 400 at the start of the experiment) survived until September 18, 1996, when the experiment was terminated. Scrutiny of the *MC* spore concentration data (Tables 41 and 42) reveal there was very little difference between the two August treatment groups of cutthroat trout. This would seem to indicate that the additional size and age (for the CR184W treatment group) did not make them more resistant to the effects of the parasite (if spore burden is a reliable metric of parasite damage). Additional size (and age) prior to exposure conferred an advantage as 14 fish from the CR184W treatment group survived to the end of the experiment.

We found minimal differences in *MC* spore concentrations among mortalities of rainbow trout (CRR84W) and cutthroat trout (CR184W) exposed at a similar size and age in August 1995 that died during the spring and summer of 1996 (Tables 40 and 42). Among the cutthroat group there were 42 mortalities between May 5th and June 24, 1996, compared to 43 mortalities among the rainbow group between April 28th and July 8th, again very little difference. Likewise, there was no significant difference in survivorship between the two groups during the overwinter stress period (Table 37).

Perhaps the most significant differences to be gleaned from all this data occur between *MC* spore concentrations seen in the fish held in the sentinel cages compared to those found in surviving free-ranging rainbow trout captured during the fall 1996 electrofishing operations (Table 43). Mean *MC* spore concentration among 22 CRRW sentinel fish surviving until September 18, 1996, was 585,090 spores, compared to an average of 26,652 spores among five free-ranging CRR trout from the 1995 year class. These two groups are of the same year class, parentage, and genetic stock; yet the spore burden in the captive fish was 22 times greater than that seen among the surviving free-ranging fish! Similarly, the average *MC* spore burden among two groups of adipose-clipped CRR rainbow trout fingerlings (CRRAD) stocked in October 1995 from wild spawn collected in the spring of 1995 and hatched and reared at the Glenwood Springs Hatchery was vastly different from that seen in the CRRW treatment group from the sentinel fish experiment (Table 43). The average *MC* spore burden among the two groups of 10 CRRAD fish (captured in September 1996) was 52,742 and 63,590, 9.0% and 10.9% of that seen in the captive fish! The extremely low spore burden among the free-ranging rainbow exposed from the hatch and surviving until September 1996 is strongly suggestive that very few free-ranging rainbow trout survive to the end of the second summer of life if their spore burden is ≥ 40,000 - 50,000.

There are three possible explanations for the low *MC* spore burden among the free-ranging CRRAD groups. First, it is possible that they are highly resistant (an acquired immunity) to the effects of the parasite by virtue of exposure to very low levels of TAM form *M. cerebralis* spores during the fall to spring months in the river. Second, they may be the few survivors (like the free-ranging CRR trout) that are carrying a low spore burden. Third, because they did not get exposed to a high density of TAM spores until July-August 1996, they may not have developed their full spore burden because of an inadequate number of degree days required for maturation of the vegetative stages of the parasite into *MC* spores. One objective of another sentinel fish experiment that began during August 1997 scheduled for completion in October 1998 is to determine which possibility is the real explanation.

Sentinel Fish Testing 1996 - 1997

In sentinel fish tests conducted during the 1996/1997 study segment, we examined the relative susceptibility of eight different treatment groups of salmonids to ambient levels of *MC* actinospores in the upper Colorado River. The fish were exposed, held, and maintained in flow-through floating live tanks in the river at the Breeze Wildlife Unit. The exposure groups included wild brown trout (BNTW) exposed from the hatch, two groups of Colorado River rainbow trout (CRR-P4 and CRR-P6) of different parentage, two groups of Snake River cutthroat trout (SRN-May and SRN-July) exposed at different times and ages, and one treatment group each of Colorado's three subspecies of native trout. The native trout treatment groups were the Rio Grande (RGN), Colorado River (CRN), and greenback (GBN) native cutthroat trout. Each species group started with 100 fish per replicate and four replicates per treatment. All treatment groups were held in the tanks and exposed for a minimum of three weeks before the fish in each replicate were randomized and placed in their randomly selected cells. This allowed for adequate time for initial exposure to ambient levels of actinospores and acclimation to food and environmental conditions in the river.

Testing by PCR revealed that samples from all groups except SRN-July were 90-100% positive for the presence of the parasite at the time the fish were randomized into their respective cells. Only 3 of 10 SRN-July sample fish tested positive. Overt clinical signs of whirling disease were evident in varying degrees among all treatment groups by mid-September 1996. These clinical signs included black tail, whirling behavior, skeletal deformities, cranial deformities, and exophthalmia. Whirling behavior was prevalent among RGN, GBN, CRN, RBT-P4 and RBT-P6 treatment groups by November 1996.

Table 44. Percent of each treatment group surviving on April 7, 1997, and results of least squares means multiple comparisons (LS means differences). Treatments not having a letter in common are significantly different ($p<0.05$).

Treatment	Date of Exposure	Percent Surviving	LS Means Differences		
BNTW	5/1/96	95.5	a		
RBT-P6	7/9/96	88.8	a	b	
GBN	7/10/96	88.6	a	b	
SRN-Jul	7/10/96	88.8		b	
RBT-P4	7/9/96	87.0		b	
RGN	7/10/96	72.5			c
CRN	7/9/96	70.5			c
SRN-May	5/2/96	63.3			c

We used survivorship from the time of initial exposure during the spring/summer of 1996 through April 8, 1997 as one metric to compare susceptibilty to the parasite (Table 44). Brown trout, known to be highly resistant to the effects of the parasite again survived better than any of the other treatment groups. Rio Grande, Colorado River, and Snake River cutthroat trout (exposed in May 1996) suffered significantly greater mortality than the other treatment groups. There was no significant difference in the survival among the greenback cutthroat, Snake River cutthroat (exposed in July), and the two treatment groups of rainbow trout.

By February 1997, virtually all histological sections of mortalities examined contained lesions, tissue damage, vegetative stages, immature, and mature myxospores characteristic of severe infection by *Myxobolus cerebralis*. At the end of the experiment only two of 10 brown trout examined by histology exhibited lesions and intralesional organisms characteristic of an *MC* infection, despite the fact that 100% of all brown trout tested (10 fish) at the beginning of the experiment were positive for the parasite's DNA in August 1996.

A second metric used to assess differences in response to infection by the *MC* parasite was the burden of *MC* myxospores occurring in treatment fish that died after April 9, 1997 prior to the end of the experiment, and that occurring in treatment survivors sacrificed at termination of the experiment on July 30, 1997. These data are summarized in Table 45.

At the end of the mortality portion of the experiment (April 9, 1997) necropsies of five fish from each treatment group showed a variety of gill and skin ectoparasites, but only one of 40 fish examined was deemed to be potentially terminally infected with ectoparasites. Additional testing of these fish revealed they were free of all viral or bacterial diseases normally tested for in fish culture according to the Blue Book protocol of the American Fisheries Society.

Table 45. *Myxobolus cerebralis* (MC) myxospore burden in the heads of trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996. Each treatment group began with 100 surviving trout on April 8, 1997. Class "Killed" indicates fish remaining alive on July 30, 1997 while class "Morts" indicates mortalities in the treatment group that died between April 9, 1997 and July 30, 1997.

Treatment Group	Date of Exposure	Class	Nº	Nº +	MC Spores per Head in Fish	
					Mean	Range
BNTW	05/1996	Killed	3	3	5,937	3,511 - 9,044
BNTW	05/1996	Morts	3	1	6,444	0 - 19,333
SRN-May	5/2/96	Killed	40	26	42,526	0 - 441,278
SRN-May	5/2/96	Morts	11	11	220,619	2,889 - 555,378
SRN-July	7/10/96	Killed	16	7	21,649	0 - 139,778
SRN-July	7/10/96	Morts	15	10	30,138	0 - 121,133
CRR-P6	7/9/96	Killed	39	38	664,009	0 - 1,795,022
CRR-P6	7/9/96	Morts	18	18	1,049,183	317,055 - 2,039,111
CRR-P4	7/9/96	Killed	20	20	213,570	22,583 - 429,083
CRR-P4	7/9/96	Morts	21	20	192,232	0 - 1,139,111
CRN	7/9/96	Killed	59	59	171,759	13,578 - 1,447,600
CRN	7/9/96	Morts	46	46	219,938	35,833 - 803,933
GBN	7/10/96	Killed	20	18	64,736	0 - 270,217
GBN	7/10/96	Morts	73	69	60,247	0 - 821,767
RGN	7/10/9	Killed	0		------	---------
RGN	7/10/96	Morts	82	80	71,424	0 - 274,689

Myxobolus cerebralis myxospore burdens were lowest among brown trout, followed by the levels found in Snake River cutthroat trout (in both treatment groups) that remained alive at the end of the experiment. Myxospore burdens were highest among the RBT-P6 treatment group. In general, mean spore burden among the RGN and GBN treatment groups were lower than that documented among the CRN and RBT-P4 treatment groups. Although comparisons are not statistically significant, nonetheless, it may be biologically significant that within most treatment groups, the mean myxospore burden was higher among the fish that died prior to the end of the experiment compared to those that were alive on July 30, 1997. Once again, this is empirical evidence supporting our conclusion that more severely infected fish tend to die, while those less infected (as shown by *MC* myxospore burden) have a greater probability of survival.

Perhaps the most intriguing result of this study is the consistent, statistically significant difference in myxospore burden between the P4 and P6 treatment groups of rainbow trout. Statistical comparisons of MC myxospore loads between these two treatment groups were made in April and July 1997. The P4 treatment group were progeny of mature fish that were spawned, hatched, and reared in the Colorado River AFTER whirling disease reached epidemic levels in 1991. The P6 treatment groups were progeny of mature fish recruited from year classes that pre-date the onset of population-level effects of whirling disease in the upper Colorado River. While there was no difference in survivorship of these two treatment groups over the course of the experiment, the differences in MC spore burden may be an indication of developing resistance to the parasite. Another sentinel fish experiment was initiated on the upper Colorado River in July 1998 to investigate this phenomenon in greater depth. That study is scheduled to be completed in late 1999 or early 2000.

Detailed data on *MC* spore burdens occurring in the heads of sentinel fish from the eight treatment groups that died between April 9 and July 30, 1997 are presented in Table 46 through Table 53. Data within a treatment group are arranged in chronological order. Perusal of the data in these tables do not show any trends that would lead to the conclusion that the most highly infected fish (as measured by myxospore burden) die more quickly than those with a lower spore burden. This is not surprising given the very high degree of variation in myxospore burden among fish within each treatment group.

Myxobolus cerebralis Myxospore Burden in Feral Trout

Data on *MC* myxospore burdens quantified from the heads of feral trout collected from streams across Colorado between 1994 and 1998 are summarized in Table 54 through Table 76. These data are presented here primarily to pull together all of the pertinent data on myxospore burdens in the craniums of feral trout in one report, and in a singular format. Careful scrutiny of the data yields some intriguing insights. One insight is that among streams with wild rainbow trout, the highest levels of *MC* spore loads usually occur in streams where there are no detectible levels of population impact, or where population impact is perhaps just beginning to occur. Such data are found in wild rainbow trout from the Big Thompson River (Table 56), the Dolores River (Table 64), and the Fryingpan River (Table 66). Average *MC* myxospore burdens in feral rainbow trout in these streams in 1997 were in excess of 100,000. This stands in sharp contrast to the myxospore loads observed in the few surviving age 1 feral rainbow trout in streams such as the Cache la Poudre (Table 58), Colorado (Tables 62 and 63), Gunnison (Table 68), Rio Grande (Tables 71 and 73), and South Platte rivers (Table 75). Average cranial spore loads in rainbow trout surviving to 18 months of age in these streams were much less than 100,000 spores. Rainbow trout populations in these streams began suffering severe declines between 1988 and 1993.

It will be interesting to determine if the wild rainbow trout populations in the Big Thompson and Fryingpan rivers will remain self-sustaining with the apparent increase in levels of infection that has occurred in these rainbow trout populations between 1995, when evidence of the presence of the parasite was first detected in these streams and those documented in 1997. If the wild rainbow trout populations in these streams continue to sustain themselves, it will be very important to ascertain the mechanisms by which that occurs.

Table 46. *Myxobolus cerebralis (Mc)* spore burden in the head of Colorado River cutthroat trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996, that died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº+	*Mc* Spores per Head in Fish	
				Mean	Range
50	4/17/97	1	1	135,778	------
50	4/19/97	2	2	309,467	258,667 - 360,267
50	4/21/97	1	1	235,222	------
50	4/22/97	3	3	313,267	191,689 - 523,444
50	4/23/97	2	2	150,667	147,578 - 153,756
50	4/25/97	1	1	284,378	------
50	5/1/97	3	3	391,444	133,000 - 644,700
50	5/4/97	1	1	223,256	------
50	5/7/97	3	3	199,548	152,800 - 259,667
50	5/8/97	3	3	150,222	93,167 - 248,111
50	5/11/97	1	1	296,111	------
50	5/15/97	1	1	125,600	------
50	5/19/97	1	1	220,622	------
50	5/23/97	3	3	230,615	63,644 - 425,533
50	5/24/97	5	5	299,015	67,200 - 830,933
50	5/29/97	2	2	41,695	41,500 - 41,889
50	5/30/97	1	1	327,278	------
50	6/4-6/97	3	3	120,813	46,961 - 241,278
50	6/10/97	1	1	63,200	------
50	6/15-17/97	2	2	316,187	214,317 - 418,056
50	7/1/97	1	1	72,622	------
50	7/4/97	1	1	235,167	------
50	7/7-8/97	3	3	111,378	74,256 - 185,378
50	7/16/97	1	1	206,822	------
Totals		**46**	**46**	**219,938**	**35,833 - 803,933**

Table 47. *Myxobolus cerebralis (Mc)* spore burden in the head of Snake River cutthroat trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996, that died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*Mc* Spores per Head in Fish	
				Mean	Range
51	4/23/97	1	1	2,800	-------
51	4/23/97	1	0	0	-------
51	4/29/97	1	1	6,844	-------
51	5/7/97	1	1	16,528	-------
51	6/19/97	1	0	0	-------
51	6/23/97	1	0	0	-------
51	6/26/97	1	1	822	-------
51	6/30	1	1	41,611	-------
51	7/1/97	1	1	38,622	-------
51	7/1/97	1	0	0	-------
33	7/10/97	2	2	1,597	861 - 2,333
34	7/18/97	1	1	121,333	-------
35	7/20/97	1	0	0	
36	7/21/97	1	0	0	
37	7/30/97	1	1	112,517	-------
TOTALS		**15**	**10**	**30,138*a***	**0 - 121,333**

a The mean Mc spore loading in these fish was significantly lower ($P \leq 0.01$) than that for the Snake River cutthroat trout exposed to the Colorado River beginning on May 2, 1996 (as 1-2 week old fry). Those fish are in lot 52 of Case # 97-253.

Table 48. *Myxobolus cerebralis (Mc)* spore burden in the head of Snake River cutthroat trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge beginning on May 2, 1996, that died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*Mc* Spores per Head in Fish	
				Mean	Range
52	4/22/97	1	1	113,333	-------
52	5/4/97	1	1	555,378	------
52	5/11/97	2	2	214,511	38,889 - 390,133
52	5/23/97	1	1	554,588	-------
52	6/9/97	1	1	2,889	-------
52	6/20/97	1	1	284,667	-------
52	6/26/97	1	1	344,133	-------
52	6/28/97	1	1	3,200	-------
52	7/6/97	2	2	69,797	10,833 - 128,761
Totals		**11**	**11**	**220,619**	**2,889 - 555,378**

Table 49. *Myxobolus cerebralis (Mc)* spore burden in the head of Colorado River rainbow trout that were progeny of parental spawners that hatched in the Colorado River AFTER population level effects were being manifested on rainbow trout beginning in 1991. These fish were exposed as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996, and died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*Mc* Spores per Head in Fish	
				Mean	Range
53	5/18/97	2	2	106,561	47,289 - 165,833
53	5/27/97	2	1	87,400	0 - 174,800
53	6/1/97	1	1	254,622	-------
53	6/3/97	2	2	323,672	249,011 - 398,333
53	6/4/97	1	1	259,722	-------
53	6/10/97	1	1	322,144	-------
53	6/18/97	2	2	71,584	24,800 - 118,367
53	6/24/97	1	1	122,544	-------
53	6/26/97	1	1	10,450	-------
53	6/28/97	2	2	617,845	96,578 - 1,139,111
53	6/29/97	1	1	108,722	-------
53	6/30/97	1	1	66,000	-------
53	7/9/97	2	2	55,500	-------
40	7/12/97	1	1	360,967	-------
39	7/16/97	1	1	6,578	-------
Totals		**21**	**20**	**192,232*b***	**0 - 1,139,111**

b The mean Mc spore burden is these fish is very significantly ($p << 0.00001$) lower than that in the Colorado River rainbow trout in Lot 54, Case Number 97-253, that are largely progeny of parent spawning rainbow trout from the Colorado River that were hatched in the river before *M. cerebralis* became established.

Table 50. *Myxobolus cerebralis (Mc)* spore burden in the head of brown trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge from hatch in May 1996, that died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*Mc* Spores per Head in Fish	
				Mean	Range
55	4/21/97	1	1	19,333	-------
55	4/22/97	1	0	0	
55	7/2/97	1	0	0	
Totals		**3**	**1**	**6,444**	**0 - 19,333**

Table 51. *Myxobolus cerebralis (Mc)* spore burden in the head of Colorado River rainbow trout that were progeny of parental spawners that hatched in the Colorado River BEFORE population level effects were being manifested on rainbow trout beginning in 1991. These fish were exposed as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996, and died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*Mc* Spores per Head in Fish	
				Mean	Range
54	4/27/97	3	3	531,143	459,167 - 587,600
54	5/7/97	1	1	573,844	-------
54	5/9/97	2	2	623,861	-------
54	5/11/97	3	3	841,935	433,333 - 1,498,167
54	5/26/97	1	1	1,728,611	-------
54	5/31/97	1	1	667,500	-------
54	6/3/97	1	1	1,157,222	-------
54	6/9/97	1	1	587,211	-------
54	6/10/97	1	1	1,206,444	-------
54	6/12/97	2	2	1,875,056	1,711,000 - 2,039,111
54	6/20/97	1	1	1,883,956	-------
39	7/20/97	1	1	2,003,556	-------
Totals		**18**	**18**	**1,049,183*c***	**317,055 - 2,039,111**

c **The mean spore burden in the heads of these fish is very significantly greater (p> >0.00001) than that found in the heads of fish in lot 53, case number 97-253, in Table 4.**

Table 52. *Myxobolus cerebralis (Mc)* spore burden in the head of greenback cutthroat trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996, that died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº+	*Mc* Spores per Head in Fish	
				Mean	Range
56	4/14/97	2	2	30,528	11,556 - 49,500
56	4/21/97	4	4	152,718	67,083 - 324,133
56	4/23/97	1	1	64,956	——
56	4/25/97	1	1	141,944	-------
56	4/30/97	6	5	49,111	0 - 168,000
56	5/04/97	1	1	3,344	——
56	5/06/97	3	3	306,937	45,500 - 821,767
56	5/07/97	3	3	72,239	9,217 - 169,922
56	5/08/97	1	1	53,900	-------
56	5/11/97	2	2	24,056	23,044 - 25,067
56	5/13/97	3	3	57,967	34,167 - 73,733
56	5/14-16/97	3	3	49,224	13,806 - 66,733
56	5/18/97	4	4	41,114	3,378 - 133,644
56	5/20/97	3	3	62,819	13,789 - 131,967
56	5/23/97	6	6	8,683	1,567 - 26,133
56	5/24-25/97	3	3	11,209	756 - 22,400
56	5/27-31/97	3	3	24,526	8,333 - 36,644
58	6/1-2/97	3	3	38,894	17,911 - 52,622
58	6/4-10/97	4	4	39,633	11,589 - 66,861
58	6/15-16/97	7	7	56,320	0 - 255,500
58	6/19-21/97	2	2	48,714	3,811 - 93,617
58	6/23-26/97	2	2	1,034	0 - 2,067
58	6/30/97	1	1	683	——
56	7/4/97	2	0	0	
56	7/8-10/97	2	2	85,711	63,378 - 108,044
56	7/16/97	1	1	206,822	——
Totals		**73**	**69**	**60,247**	**0 - 821,767**

Table 53. *Myxobolus cerebralis (Mc)* spore burden in the head of Rio Grande cutthroat trout exposed to the Colorado River as sentinel fish at the Breeze Wildlife Area Bridge beginning in July 1996, that died between April 9 and July 30, 1997. Brush Fish Health Lab Case # 97-253.

Lot Nº	Date of Death (Mo/Da/Yr)	Nº	Nº +	*Mc* Spores per Head in Fish	
				Mean	Range
57	4/17/97	1	1	1,333	-------
57	4/22-23/97	2	2	12,524	1,967 - 23,100
57	4/25-30/97	2	2	142,034	126,578 - 157,489
57	5/4-6/97	4	4	69,515	37,333 - 118,750
57	5/8-13/97	5	5	28,087	1,522 - 104,267
57	5/14/97	2	2	81,375	65,617 - 97,133
57	5/16-20/97	3	3	64,309	2,889 - 186,400
57	5/27/97	3	3	131,472	99,733 - 172,206
57	6/3-4/97	2	2	62,761	39,722 - 85,800
57	6/5-6/97	10	9	67,424	0 - 194,844
57	6/8-10/97	8	7	124,598	0 - 245,694
57	6/12-14/97	6	6	95,368	14,056 - 198,722
57	6/15-16/97	9	9	73,280	789 - 274,689
57	6/17-18/97	8	8	61,093	3,800 - 200,356
57	6/19-21/97	6	6	47,816	13,800 - 85,333
57	6/23-24/97	7	7	60,121	21,106 - 102,756
57	6/29/97	2	2	32,858	2,267 - 63,450
57	7/6/97	1	1	41,667	-------
57	7/7/97	1	1	36,389	-------
Totals		**82**	**80**	**71,424**	**0 - 274,689**

Table 54. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Arkansas River in 1996 just downstream from Salida, Colorado. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
96-405	4/05/96	RBT	12	9	12,127	2,778-30,250
96-405	4/05/96	BNT	136	45	8,457	544-30,311

Table 55. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Big Thompson River in 1994-1995 and 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that that positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
94-171	4/21/94	BNT	1	0.00	0.00	0.00
94-172	5/11/94	BNT	2	0.00	0.00	0.00
94-173	5/11/94	BNT	3	0.00	0.00	0.00
94-174	5/11/94	BNT	3	0.00	0.00	0.00
94-175	5/12/94	BNT	3	0.00	0.00	0.00
94-176	5/12/94	BNT	1	0.00	0.00	0.00
95-249A	10/24/95	BNT	9	0.00	0.00	0.00
95-249B	10/24/95	BNT	16	0.00	0.00	0.00
95-301	11/06/95	BNT	12	0.00	0.00	0.00
95-302	11/07/95	BNT	10	0.00	0.00	0.00
95-241	11/07/95	BNT	3	0.00	0.00	0.00
95-242	11/07/95	BNT	4	0.00	0.00	0.00
97-040	3/24/97	BNT	1	0.00	0.00	0.00
97-064	4/11/97	BNT	5	1	10,833	10,833
97-072	4/21/97	BNT	4	4	6,522	1,478-16,889

Table 56. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Big Thompson River in 1994-1995 and 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
94-177	4/12/94	RBT	6	0.00	0.00	0.00
94-171	4/21/94	RBT	2	0.00	0.00	0.00
94-172	5/11/94	RBT	21	0.00	0.00	0.00
94-173	5/11/94	RBT	6	0.00	0.00	0.00
94-174	5/11/94	RBT	12	0.00	0.00	0.00
94-175	5/12/94	RBT	5	1	25,000	25,000
94-176	5/12/94	RBT	2	0.00	0.00	0.00
95-301	11/06/95	RBT	10	0.00	0.00	0.00
95-302	11/07/95	RBT	15	0.00	0.00	0.00
95-241	11/07/95	RBT	17	1	833	833
95-242	11/07/95	RBT	14	4	6,792	833-17,500
97-040	3/24/97	RBT	31	8	50,007	2,500-118,056
97-064	4/11/97	RBT	25	20	40,950	2,978-312,889
97-072	4/21/97	RBT	26	15	114,946	3,378-389,550

Table 57. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Cache la Poudre River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Cache la Poudre River near Black Hollow Creek						
94-179	4/27/94	BNT	5	0.00	0.00	0.00
95-248B	10/25/95	BNT	9	0.00	0.00	0.00
97-312	11/21/96	BNT	9	6	38,692	778-80,400
Cache la Poudre River at USFS Kelly Flats Campground						
94-182	4/27/94	BNT	3	0.00	0.00	0.00
94-183	5/09/94	BNT	13	0.00	0.00	0.00
95-248A	10/25/95	BNT	6	0.00	0.00	0.00
97-313	11/21/96	BNT	10	7	32,402	6,133-96,444
Cache la Poudre River at USFS Big Bend Campground						
94-180	5/09/94	BNT	9	0.00	0.00	0.00
95-248C	10/25/95	BNT	10	0.00	0.00	0.00
Cache la Poudre River #4						
97-025	3/07/97	BNT	14	9	12,864	1,222-45,500
97-047	3/31/97	BNT	12	7	17,718	1,611-42,667

Table 58. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Cache la Poudre River in 1994-1996 and 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Cache la Poudre River near Black Hollow Creek						
94-016	1/20/94	RBT	1	1	475,000	475,000
94-179	4/27/94	RBT	5	0.00	0.00	0.00
94-178	5/09/94	RBT	4	0.00	0.00	0.00
97-312	11/21/96	RBT	10	9	24,854	1,444-56,800
Cache la Poudre River at USFS Kelly Flats Campground						
94-183	5/09/94	RBT	4	2	3,584	1,917-5,250
97-313	11/21/96	RBT	9	2	3,916	3,000-4,833
Cache la Poudre River at USFS Big Bend Campground						
94-180	5/09/94	RBT	6	0.00	0.00	0.00
CDOW Poudre Fish Rearing Unit						
94-136	7/11/94	RBT	34	31	257,505	12,500-2,362,500
Cache la Poudre River #4						
97-025	3/07/97	RBT	14	13	26,581	1,667-134,000
97-047	3/31/97	RBT	19	19	50,728	8,667-113,556

Table 59. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Colorado River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*. Case Nº marked with an *a* indicate a collection above the site and *b* indicates a collection below the site.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Williams Fork River						
94-034B	3/10/94	BNT	50	12	60,417	25,000-100,000
94-068	4/07/94	BNT	17	1	25,000	25,000
94-163	4/26/94	BNT	21	6	66,667	25,000-150,000
95-062	2/24/95	BNT	32	15	8,555	500-54,000
95-063	4/05/95	BNT	25	14	4,358	278-15,000
95-183*a*	10/04/95	BNT	10	0.00	0.00	0.00
97-063	4/14/97	BNT	29	17	49,882	739-244,689
Colorado River at Hot Sulphur Springs						
94-051	4/14/94	BNT	18	1	25,000	25,000
94-264	11/18/94	BNT	16	0.00	0.00	0.00
95-061	2/23/95	BNT	2	2	3,622	3,077-4,167
96-338*a*	9/09/96	BNT	12	10	11,565	1,867-34,667
Colorado River - Downstream of Granby Dam						
94-060	4/15/94	BNT	66	32	80,781	25,000-675,000
95-067	4/21/95	BNT	50	41	12,826	417-36,667
96-120	4/29/96	BNT	40	0.00	0.00	0.00
97-071	4/21/97	BNT	30	28	29,599	2,333-115,833
Colorado River at Drowsy Water Creek						
94-266	11/17/94	BNT	8	6	592	192-1,667
95-066	4/04/95	BNT	8	7	27,429	5,000-55,000
95-184*a*	10/04/95	BNT	3	0.00	0.00	0.00

Table 60. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Colorado River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Colorado River at John Sheriff's Ranch						
94-261	11/17/94	BNT	4	2	1,459	1,250-1,667
95-068	4/04/95	BNT	3	0.00	0.00	0.00
Colorado River at Chimney Rock Ranch						
94-262	11/17/94	BNT	9	4	208	208
Colorado River at Kemp/Breeze Ranch						
94-263	11/17/94	BNT	10	5	537	362-634
97-359	10/15/97	BNT	10	4	2,853	544-5,989
Colorado River below Windy Gap Reservoir						
95-153	9/11/95	BNT	3	2	2,750	2,000-3,500
Beaver Creek, tributary to the Colorado River below Byers Canyon Bridge						
94-161	4/20/94	BNT	1	0.00	0.00	0.00
94-161	4/20/94	BKT	26	5	60,000	25,000-100,000
Corral Creek, tributary to the Colorado River 2 km downstream from Williams Fork River						
94-162	4/20/94	BKT	31	2	25,000	25,000
Willow Creek, tributary to the Colorado River below Granby Dam						
94-165	4/21/94	BNT	18	4	43,750	25,000-75,000
Fraser River, tributary to the Colorado River at Granby						
94-185	4/20/94	BNT	1	0.00	0.00	0.00
94-185	4/20/94	BKT	6	1	3,333	3,333
94-166	4/21/94	BNT	3	0.00	0.00	0.00

Table 61. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Colorado River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Colorado River at BLM/Con Ritschards Ranch						
94-168	4/25/94	BNT	34	8	56,250	25,000-125,000
Colorado River at John Horn Ranch						
94-055	4/15/94	BNT	26	8	34,375	25,000-75,000
Colorado River at Paul Gilbert Ranch						
94-058	4/13/94	BNT	50	5	35,000	25,000-75,000
94-058	4/13/94	BKT	1	1	75,000	75,000
Colorado River at Lone Buck Wildlife Area						
94-059	4/14/94	BNT	8	2	85,416	37,500-133,333
94-059	4/14/94	BKT	1	1	100,000	100,000

Table 62. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Colorado River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*. Case Nº marked with an *a* indicate a collection above the site and *b* indicates a collection below the site.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Williams Fork River, tributary to the Colorado River at Parshall, Colorado						
94-034A	3/10/94	RBT	27	18	148,512	10,714-550,000
94-068	4/07/94	RBT	13	6	120,833	50,000-200,000
94-163	4/26/94	RBT	6	2	75,000	50,000-100,000
95-062	2/24/95	RBT	17	9	3,333	1,000-6,000
95-063	4/05/95	RBT	5	3	8,611	6,667-10,000
95-183*a*	10/04/95	RBT	10	0.00	0.00	0.00
95-183*b*	10/04/95	RBT	10	0.00	0.00	0.00
96-338*a*	9/09/96	RBT	15	13	42,707	1,044-126,472
96-338*b*	9/09/96	RBT	10	9	63,590	3,500-173,056
97-063	4/14/97	RBT	4	4	68,882	15,900-143,650
Colorado River at Hot Sulphur Springs						
94-051	4/14/94	RBT	2	2	50,000	50,000
95-061	2/23/95	RBT	3	3	187,222	50,000-317,917
95-059	4/04/95	RBT	2	2	13,750	3,333-24,167
95-059	4/04/95	RBT	8	8	67,844	2,917-379,833
96-106	4/17/96	RBT	60	20	18,096	833-77,000
Colorado River at Parshall, Colorado						
94-170	4/24/94	RBT	1	1	400,000	400,000
95-068	2/23/95	RBT	1	1	205,000	205,000

Table 63. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Colorado River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Colorado River from Windy Gap Dam to the Williams Fork River						
97-249	9/09/97	RBT	11	11	87,065	12,033-228,000
Beaver Creek, tributary to the Colorado River below Byers Canyon Bridge						
94-161	4/20/94	RBT	2	0.00	0.00	0.00
Corral Creek, tributary to the Colorado River downstream from the Williams Fork River						
94-162	4/20/94	RBT	2	1	25,000	25,000
Fraser River, tributary to the Colorado River at Granby						
94-185	4/20/94	RBT	6	1	3,333	3,333
94-166	4/21/94	RBT	2	2	112,500	25,000-200,000
Colorado River at BLM/Con Ritschards Ranch						
94-168	4/25/94	RBT	1	1	50,000	50,000
Colorado River at Kemp/Breeze Ranch						
95-255	7/11/95	RBT	20	8	26,896	917-63,333
Colorado River at Paul Gilbert Ranch						
94-058	4/13/94	RBT	1	1	125,000	125,000
94-252	7/29/94	RBT	1	0.00	0.00	0.00
94-265	11/17/94	RBT	5	3	902	169-1,479
95-255	7/11/95	RBT	20	8	28,396	1,333-63,333
Colorado River at Lone Buck Wildlife Area						
94-059	4/14/94	RBT	4	3	93,750	56,250-125,000
95-060	4/03/95	RBT	3	3	23,194	2,083-38,333

Table 64. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Dolores River below McPhee Dam from 1994 through 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
94-014	1/17/94	RBT	11	0.00	0.00	0.00
95-190	10/03/95	RBT	10	5	10,333	6,250-12,250
95-190	10/03/95	BNT	10	0.00	0.00	0.00
96-337	10/01/96	RBT	8	0.00	0.00	0.00
96-337	10/01/96	BNT	10	0.00	0.00	0.00
97-245	10/01/97	RBT	5	5	347,956	205,111-453,200
97-245	10/01/97	BNT	10	1	34,667	34,667

Table 65. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the East River in 1995. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
East River at Roaring Judy Hatchery Bridge						
95-208	10/30/95	RBT	1	0.00	0.00	0.00
95-254B	10/18/95	BNT	10	1	2,500	2,500
East River at Almont						
95-254A	10/18/95	BNT	10	0.00	0.00	0.00

Table 66. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Fryingpan River between 1994 and 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
94-015	1/21/94	RBT	4	0.00	0.00	0.00
95-253	11/14/95	RBT	10	0.00	0.00	0.00
95-253	11/14/95	BNT	10	0.00	0.00	0.00
97-272	11/12/96	RBT	20	9	128,576	806-692,756
Old Faithful Site						
97-362	11/12/97	RBT	10	6	26,930	2,083-135,644
97-362	11/12/97	BNT	10	2	2,100	1,450-2,750
97-362	11/12/97	BKT	6	0.00	0.00	0.00
Taylor Creek Site						
97-362	11/12/97	RBT	2	2	115,419	96,050-135,789
97-362	11/12/97	BNT	10	1	4,311	4,311
97-362	11/12/97	BKT	1	1	4,550	4,550
97-362	11/12/97	SCULPIN	10	0.00	0.00	0.00

Table 67. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Gunnison River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Gunnison River in Black Canyon at Ute Park						
95-189	9/28/95	BNT	10	0.00	0.00	0.00
97-280	9/26/97	BNT	10	6	27,539	3,417-87,889
Gunnison River near Almont						
94-050B[a]	4/11/94	BNT	24	23	30,445	1,750-115,500
94-050[b]	4/11/94	BNT	24	23	29,227	1,235-115,500
95-070	5/01/95	BNT	18	0.00	0.00	0.00
95-246	11/07/95	BNT	10	0.00	0.00	0.00
95-246	11/07/95	CUT	1	0.00	0.00	0.00
97-042	3/24/97	BNT	29	21	63,143	6,611-307,556
97-054	4/07/97	BNT	30	27	19,854	1,389-170,444
Gunnison River in Black Canyon						
94-102A	5/10/94	BNT	26	19	1,750	40-10,000
Gunnison River below Crystal Dam						
96-335	8/08/96	BNT	10	0.00	0.00	0.00

[a] These fish were analyzed at the CDOW Brush Fish Health Laboratory using the plankton centrifuge methodology.

[b] These fish were analyzed at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC, under the direction of Dr. Greg Lewbart, using the pepsin-trypsin digest (PTD) method.

Table 68. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Gunnison River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Gunnison River in Black Canyon at Ute Park						
94-011	1/10/94	RBT	10	4	87,500	25,000-150,000
95-189	9/28/95	RBT	11	10	14,242	667-57,000
97-280	9/26/97	RBT	1	1	139,300	139,300
Gunnison River near Almont						
94-050A[a]	4/11/94	RBT	3	1	87	87
94-050[b]	4/11/94	RBT	3	1	926	926
95-070	5/01/95	RBT	34	2	10,209	3,000-17,417
95-246	11/07/95	RBT	1	0.00	0.00	0.00
97-042	3/24/97	RBT	4	4	85,306	22,222-140,444
97-054	4/07/97	RBT	1	1	286,722	286,722
Gunnison River in Black Canyon						
94-102B	5/10/94	RBT	31	28	21,572	333-180,333
95-185	9/20/95	RBT	1	1	36,667	36,667
Gunnison River below Crystal Dam						
95-185	9/20/95	RBT	1	1	36,667	36,667
96-335	8/08/96	RBT	23	16	31,031	1,978-123,750

[a] These fish were analyzed at the CDOW Brush Fish Health Laboratory using the plankton centrifuge methodology.

[b] These fish were analyzed at the College of Veterinary Medicine, North Carolina State University, Raleigh, NC, under the direction of Dr. Greg Lewbart, using the pepsin-trypsin digest (PTD) method.

Table 69. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Roaring Fork River in 1994-1995 and 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
94-013	1/21/94	RBT	10	0.00	0.00	0.00
95-252	11/14/95	BNT	10	0.00	0.00	0.00
95-263	11/30/95	RBT	11	3	7,472	1,167-11,250
97-242	10/07/97	RBT	3	2	2,600	900-4,300
97-242	10/07/97	BNT	10	7	2,265	400-5,989
97-232	10/07/97	WFH	60	43	6,799	903-26,700
97-231	10/08/97	RBT	10	4	66,235	450-229,017
97-231	10/08/97	BNT	9	1	1,667	1,667

Table 70. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Rio Grande River in 1994-1995 and 1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Rio Grande River at State Bridge between South Fork and Del Norte, Colorado						
94-053	4/13/94	BNT	40	2	160	160
95-187	8/30/95	BNT	10	0.00	0.00	0.00
97-243	9/05/97	BNT	10	7	27,857	4,444-140,767
Rio Grande River between USFS Marshall Park Campground and Fisherman Access Area						
94-057	4/12/94	BNT	34	1	25,000	25,000
95-188	8/30/95	BNT	10	0.00	0.00	0.00
97-056	4/07/97	BNT	9	4	15,840	2,167-46,944
97-081	4/28/97	BNT	30	5	6,657	739-17,478
97-081	4/28/97	BKT	1	1	2,956	2,956
97-244	9/04/97	BNT	20	8	4,779	633-17,378
Rio Grande River at Coller Wildlife Area						
94-061	4/12/94	BNT	18	1	25,000	25,000
Rio Grande River at Wason Ranch						
94-062	4/12/94	BNT	45	22	1,381	46-4,583
95-251B	10/17/95	BNT	10	0.00	0.00	0.00

Table 71. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Rio Grande River in 1994-1997. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
Rio Grande River at State Bridge						
94-053	4/13/94	RBT	5	0.00	0.00	0.00
95-187	8/30/95	RBT	10	6	13,472	3,750-48,750
96-334	9/09/96	RBT	9	9	277,523	62,089-916,378
97-243	9/05/97	RBT	4	4	349,372	86,667-678,922
Rio Grande River at Marshall Park Campground						
94-057	4/12/94	RBT	16	9	94,444	25,000-275,000
95-188	8/30/95	RBT	10	6	12,138	1,167-20,583
95-251A	11/30/95	RBT	4	1	14,000	14,000
96-336	9/05/96	RBT	10	10	148,259	3,767-439,200
97-056	4/07/97	RBT	2	2	404,222	77,000-731,000
97-081	4/28/97	RBT	23	17	215,726	744-765,844
97-244	9/04/97	RBT	11	11	28,668	6,378-53,333
Rio Grande River at Wason Ranch						
94-062	4/12/94	RBT	4	3	28,056	4,583-25,417

Table 72. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from Spring Creek in 1996. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*. Case Nº marked with an *a* indicate a collection above the site and *b* indicates a collection below the site.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
97-203	10/31/96	BNT	10	9	77,293	1,644-422,222
Spring Creek above and below Spring Creek Reservoir						
97-355*a*	10/31/96	RBT	1	0.00	0.00	0.00
97-355*a*	10/31/96	BNT	10	1	794	794
97-271*b*	10/31/96	BNT	36	25	146,830	17,500-1,128,989
97-355*a*	10/31/96	CUT	1	0.00	0.00	0.00

Table 75. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the South Platte River in 1994-1997. Nº is the number of fish tested for Mc and Nº+ is the number of fish that tested positive for *Mc*. Case Nº marked with an *a* indicate a collection above the site and *b* indicates a collection below the site.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
South Platte River 100 meters downstream of Spinney Mountain Reservoir						
94-012	1/19/94	RBT	7	3	108,333	25,000-175,000
South Platte River at Deckers Bridge						
94-017	1/19/94	RBT	10	7	139,286	25,000-550,000
95-245C*a*	7/12/95	RBT	7	3	5,250	1,917-9,667
95-245A	7/12/95	RBT	23	5	19,900	4,667-50,000
97-356*a*	11/4/96	RBT	11	11	151,888	44,906-339,111
97-305*b*	1/23/97	RBT	10	9	155,880	17,122-394,389
97-012*b*	2/11/97	RBT	10	7	140,770	10,389-387,389
97-018*b*	2/18/97	RBT	11	11	235,358	6,222-523,611
97-360*a*	11/3-4/97	RBT	10	10	69,331	4,700-164,811
South Platte River in Elevenmile Canyon						
95-205	11/04/95	RBT	32	11	23,181	1,500-139,333
South Platte in Cheeseman Canyon						
97-357	11/05/96	RBT	8	6	49,146	3,089-145,689
97-360	11/3-4/97	RBT	10	10	141,716	9,183-579,367
South Platte River at Twin Cedars						
95-245D	7/15/95	RBT	10	7	16,631	1,500-36,667
97-361	11/3-4/97	RBT	7	6	154,320	1,127-383,689

Table 76. *Myxobolus cerebralis* (*Mc*) spore density in the heads of fish collected from the Taylor River in 1995-1996. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
95-247	10/18/95	BNT	10	0.00	0.00	0.00
96-341	10/02/96	BNT	5	5	11,878	2,444-39,111
97-354	10/31/96	BNT	10	4	10,025	2,350-20,078
Lottis Creek						
97-352	10/31/96	RBT	1	1	2,011	2,011
97-352	10/31/96	BNT	10	1	1,744	1,744
97-352	10/31/96	BKT	1	0.00	0.00	0.00
Taylor River above Taylor Reservoir						
97-353	10/31/96	BNT	10	1	9,956	9,956
97-353	10/31/96	BKT	1	1	163,033	163,033

Another interesting insight is the dramatic decrease in the average *MC* spore burden among rainbow trout in streams experiencing recurrent year class failures among the wild rainbow trout population. Data in Table 75 reveal that the average cranial spore load among rainbow trout for the 1996 year class of rainbow trout always averaged between 140,770 and 235,358 at four distinct sampling dates between November 4, 1996 and February 18, 1997. However, the average spore burden was 69,331 among survivors of this year class collected and analyzed on November 4, 1997. The same phenomenon was observed among three separate collections of rainbow trout from the 1996 year class taken from the reach of the Rio Grande upstream of the USFS Marshall Park Campground (Table 71). Average spore burdens among rainbow trout collected on April 7, 1997 and April 28, 1997 (when this cohort was about nine months old) were 404,222 and 215,726. In contrast, surviving rainbow trout from this same year class collected on September 4, 1997 had an average spore burden of only 28,668. These data are convincing empirical evidence that very few YOY rainbow trout carrying *MC* spore burdens ≥ 50,000 survive to the end of the second summer of life..

Yet another intriguing paradox that we have observed time and time again over the past four years of this study is the dramatic decline in the *MC* myxospore burden that occurs in brown trout between 10 months and 16 months of age in many streams all across Colorado. Among age 1+ brown trout collected from streams all across Colorado in the fall of 1995, only 18 of 243 (7.4%) tested positive for the presence of *M. cerebralis* spores. This is remarkable considering that histological tests conducted on YOY brown trout fry collected at the same sites and streams reveal that up to 50% - 70% of these fish show significant evidence of intralesional organisms and/or mature spores of *M. cerebralis*. This suggests that there may be an immune response mechanism whereby brown trout either destroy the trophozoites, pre-spore, or even mature spore forms of the *M. cerebralis* parasite or else shed them through excetory processes. Given the apparent paradox that there are not very many places where we can detect a measurable decline in age 1+ brown trout populations, yet a high percentage of YOY brown trout are testing positive (by histology) for this parasite, the immune-response hypothesis seems to have merit.

We have documented this same phenomenon in brown trout in separate sentinel fish experiments two years apart in the upper Colorado River. These fish were held as sentinel fish from approximately two weeks post emergence from the gravel for 12 to 18 months. In these sentinel fish experiments it was a rare occurrence that we suffered more than an occasional mortality among the brown trout after they reached one year of age. Thus, we know that the decline in cranial *MC* myxospore burden among brown trout is real and not due to the death (and loss) of highly infected individual trout from the experiment. Data on mean cranial myxospore burdens in brown trout cropped at four different times during the 1995/1996 experiment are summarized in Table 77. Similar results are shown for brown trout treated similarly in another sentinel fish experiment that began in August 1997 and continues through October 1998. Thus, the data in Table 78 summarize the results from the first two of four scheduled croppings, each approximately 60 days apart.

Table 77. *Myxobolus cerebralis* (*Mc*) spore density in the brown trout exposed to ambient levels of *Mc* actinospore infectivity in the upper Colorado River held as sentinel fish beginning in July 1995 and sacrificed for determination of *Mc* myxospore burden at periodic sampling intervals. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Case Nº	Date of collection (Mo/Da/Yr)	Species	Nº	Nº+	*Mc* Spores in Positive Fish	
					Mean	Range
1995/1995 Sentinel Fish Experiment						
96-371	06/17/96	BNT	20	10	6,540	1,567-20,850
96-371	07/16/96	BNT	18	10	45,280	3,356-105,700
96-371	08/19/96	BNT	31	20	28,925	1,611-174,044
96-371	09/18/96	BNT	6	5	16,695	4,633-38,928

Table 78. *Myxobolus cerebralis* (*Mc*) spore density in the brown trout exposed to ambient levels of *Mc* actinospore infectivity in the upper Colorado while being held as sentinel fish beginning in June 1997 and cropped in April and June 1998 for determination of *Mc* myxospore burden at the periodic sampling intervals. Nº is the number of fish tested for *Mc* and Nº+ is the number of fish that tested positive for *Mc*.

Cell Nº	April 1998 Sample			June 1998 Sample		
	Nº	Nº+	Average *Mc* myxospores	Nº	Nº+	Average *Mc* myxospores
1	5	5	195,747	15	6	8,207
8	5	5	35,578	15	9	5,268
10	5	1	28,667	10	7	8,877
14	5	5	78,519	15	6	1,038
17	5	5	64,633	15	7	4,482
23	5	3	62,063	15	8	1,702
28	5	5	64,461	15	9	9,441
30	5	5	226,355	15	13	27,099

As can be seen from the data in Tables 77 and 78, the *Mc* myxospore burden in feral brown trout held as sentinel fish in tanks appear to lose these spores from the cranium at a very high rate commencing early during the second summer of life. Whether these spores are somehow shed or excreted from the trout in viable form or destroyed within the fish by some as yet undescribed immune mechanism remains to be determined. We intend to investigate this phenomenon in greater depth with additional experimentation in the next year or two.

Other Biotic, Abiotic, and Anthropenic Factors

Some people within the aquatic ecology profession as well as the general public continue to suggest that there is no problem with whirling disease at all, that this issue is all a hoax conjured up by "the government". Or alternatively, some suggest other factors (biotic, abiotic, or anthropogenic) are the real cause and the *M. cerebralis* parasite is only a minor factor in the equation. The first assertion is without a doubt patently false. Hubert (1996) rightly suggests that the second possibility is plausible and worthy of serious consideration. We couldn't agree more.

However, an enormous effort has been directed at investigation of many of these other possibilities over the past four and a half years, both in the Colorado River, and on other streams throughout the Colorado (Nehring and Thompson 1996. 1997). Indeed, a large technical report titled ***Analysis of the Possible Roles of Thermal Stress, Variations and Fluctuations in Stream Discharge, Gas Supersaturation, and Whirling Disease in the Decline of Wild Rainbow Trout Recruitment in the Cache la Poudre, Colorado, Gunnison, Rio Grande, and South Platte Rivers in Colorado*** has been written that summarizes the possible roles that the most plausible factors might play in this equation. This technical report has been reviewed by professional staff within the CDOW and several independent reviewers outside the agency. The comments and criticisms of these reviewers will be considered and as necessary addressed in the final document. Our objective is to complete the final draft of the report and submit it for publication before the end of 1998.

Gas supersaturation was perhaps the most plausible exacerbating co-factor that we studied over the past four years, particularly on the upper Colorado River. This factor was addressed in depth in two previous progress reports (Nehring and Thompson 1996, 1997).

In the 1994 research investigation on the Colorado River (Walker and Nehring 1995) chronic low level gas supersaturation was documented at some sites in the upper Colorado River. Evidence of possible gas bubble trauma was also exhibited in both rainbow and brown trout fry at some sites in the 1994 study on the Colorado River. Gas bubble trauma has been studied in great detail by other investigators on other streams (White et al 1991) As a result of these findings, gas saturation levels were monitored on a monthly basis at a number of locations on many streams throughout 1995 and 1996. These data are contained in Table 79 and Table 80.

Perhaps the most notable thing about the data is the fact that (with rare exception) gas supersaturation in all streams and at all sites is largely a diurnal phenomenon and is clearly linked with algal photosynthetic activity. During the daylight hours the percent oxygen supersaturation is higher than total gas supersaturation in the majority of instances. In most other instances (during daylight hours) oxygen supersaturation approximates the total gas saturation. The only significant exception is for the Gunnison River below Crystal Dam during June and July 1995 (Table 79). Crystal Dam was spilling at that time. It was at this site that the highest levels of total gas saturation observed in 1995 were documented. During this time, fishermen reported catching large rainbow and brown trout with large clear bubbles beneath epidermal tissues, inside the buccal cavity, and on the gill arches and gill lamellae. Observations of these external symptoms of gas bubble trauma (GBT) are consistent with the

Table 79. Dissolved gas measurements from several rivers in Colorado in 1995. In-column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the percent total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
Arkansas River at Stockyards Bridge (Salida)						
7/27/95	2000	586	597	+11	101.9	101.8
8/21/95	2130	592	594	+3	100.5	96.8
9/7/95	1400	588	597	+10	101.7	102.7
10/18/95	1900	582	580	-1	99.7	97.5
Cache la Poudre River above Poudre Ponds Rearing Unit						
6/15/95	1440	578	589	+11	102.0	101.6
7/26/95	1215	569	582	+13	102.3	104.7
8/10/95	2000	567	568	+1	100.3	97.6
9/21/95	1525	570	581	+11	102.0	101.2
10/6/95	1500	566	586	+21	103.6	104.6
Cache la Poudre River at Black Hollow Creek						
6/15/95	1410	583	598	+15	102.7	103.6
7/26/95	1245	573	584	+11	102.0	102.8
8/10/95	2020	571	575	+3	100.6	96.6
9/21/95	1500	575	576	+2	100.3	101.3
10/6/95	1520	571	574	+4	100.6	101.0
Cache la Poudre River at Hombres Ranch						
6/15/95	1320	589	604	+15	102.5	102.9
7/26/95	1300	581	593	+12	102.0	102.4
8/10/95	2045	579	584	+5	100.9	96.9
9/21/95	1430	583	588	+5	100.8	99.1
10/6/95	1550	579	586	+8	101.4	101.3
Cache la Poudre River at Kelly Flats Campground						
6/15/95	1200	598	613	+16	102.5	102.0
7/26/95	1315	589	599	+10	101.7	102.0
8/10/95	2125	586	587	+1	100.2	96.1
9/21/95	1230	594	596	+2	100.4	101.5
10/6/95	1630	586	586	0	100.1	99.9
Dolores River, 1.5 km below McPhee Dam						
9/19/95	1115	593	641	+49	108.4	121.2
Dolores River, 5.3 km below McPhee Dam						
9/19/95	1200	595	636	+42	107.1	126.3
Dolores River at Metaska Day Use Area						

Table 79. Dissolved gas measurements from several rivers in Colorado in 1995. In-column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the percent total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
12/18/95	1440	587	606	+20	103.3	121.3
12/18/95	1630	587	598	+12	102.0	113.7
12/18/95	2125	590	579	-9	98.2	95.6
12/19/95	0815	587	573	-13	97.6	94.5
Dolores River at Ferris Canyon Campground						
12/18/95	1400	589	603	+15	102.5	111.1
12/18/95	1655	588	588	0	100.0	103.5
12/18/95	2155	590	568	-20	96.3	92.3
12/19/95	0740	589	569	-19	96.6	91.6
Dolores River at Bradfield Bridge						
12/18/95	1330	593	606	+15	102.4	111.8
12/18/95	1720	592	589	-2	99.5	103.0
12/18/95	2225	592	577	-13	97.5	95.9
12/19/95	0700	593	582	-10	98.1	99.7
East River above Roaring Judy State Fish Rearing Unit						
6/8/95	1300	563	568	+6	101.1	102.3
6/12/95	1145	574	586	+12	102.0	101.8
7/25/95	1015	561	569	+7	101.3	101.3
8/7/95	1100	561	572	+12	102.1	108.3
9/7/95	1700	561	563	+3	100.6	95.7
9/18/95	1330	558	566	+9	101.6	96.9
10/18/95	1000	558	565	+8	101.4	96.9
East River at Almont						
6/8/95	1330	566	573	+7	101.2	100.3
6/12/95	1300	578	591	+14	102.4	100.2
7/25/95	1055	565	573	+9	101.6	100.5
8/7/95	1140	564	580	+16	102.8	108.4
9/7/95	1720	564	565	+2	100.4	98.0
Gunnison River 8 km downstream of Almont at County Road 10 Bridge						
6/8/95	1400	569	578	+9	101.5	99.9
6/12/95	1415	580	594	+14	102.5	104.1
6/12/95	1445	580	593	+13	102.3	104.6
8/7/95	1230	567	580	+14	102.4	105.4
9/7/95	1800	567	570	+3	100.7	97.7
10/18/95	0845	567	561	-4	99.1	97.1
Gunnison River below Crystal Dam						

Table 79. Dissolved gas measurements from several rivers in Colorado in 1995. In-column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the percent total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
6/7/95	1030	597	656	+59	109.9	111.9
7/24/95	1220	598	687	+89	114.8	120.0
8/4/95	1000	596	642	+47	107.9	107.8
9/13/95	1015	598	592	-5	99.0	75.4
9/15/95	1615	593	584	-8	98.6	76.9
Gunnison River at East Portal Gunnison Tunnel						
6/7/95	1055	598	660	+62	110.3	110.5
9/15/95	1620	593	587	-5	99.0	80.4
Gunnison River at USGS Gage below East Portal Gunnison Tunnel						
6/7/95	1125	598	647	+50	108.3	107.0
7/24/95	1300	598	683	+86	114.4	114.6
8/4/95	1030	597	637	+41	106.9	106.7
9/13/95	1040	598	610	+12	102.1	91.3
9/15/95	1635	594	601	+7	101.3	90.8
Gunnison River from Chukar Trail to Ute Park						
6/7/95	1420	622	657	+35	105.7	107.8
6/7/95	1700	622	651	+29	104.7	106.9
7/24/95	1600	623	661	+38	106.1	112.3
8/14/95	1115	619	639	+20	103.3	109.0
8/14/95	2200	620	631	+12	102.0	101.0
8/15/95	0600	619	630	+11	101.9	97.5
9/25/95	1700	620	634	+15	102.4	106.4
9/26/95	0630	618	618	0	100.0	94.5
9/26/95	1500	619	641	+23	103.7	112.6
9/27/95	0630	618	619	+1	100.1	96.6
9/27/95	2230	620	620	+1	100.1	96.6
9/28/95	0600	619	621	+2	100.4	94.4
3/13/95	1000	613	622	+10	101.6	107.7
Rio Grande at Marshall Park						
6/13/95	1045	562	573	+11	102.0	103.6
7/28/95	1430	552	575	+22	104.3	104.0
8/31/95	1730	552	563	+12	102.2	103.5
9/5/95	0800	552	552	0	99.8	92.3
10/16/95	0900	545	553	+8	101.6	105.1
Rio Grande at Wason Ranch near Creede						

Table 79. Dissolved gas measurements from several rivers in Colorado in 1995. In-column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the percent total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
6/13/95	1230	565	579	+15	102.6	100.2
7/28/95	1340	557	574	+16	102.9	104.6
8/31/95	1645	555	569	+15	102.7	105.1
9/6/95	0830	557	557	0	100.0	94.1
10/17/95	1530	551	570	+20	103.6	103.5
Rio Grande at Coller Wildlife Area						
6/13/95	1330	570	585	+14	102.5	99.4
7/28/95	1245	563	580	+16	103.0	106.0
9/31/95	0815	560	560	0	100.0	95.4
9/6/95	0800	560	560	0	99.9	94.2
10/17/95	1415	557	583	+26	104.6	107.0
Rio Grande at State Bridge						
6/13/95	1710	575	591	+16	102.8	98.7
7/28/95	1030	569	576	+7	101.3	103.8
8/31/95	1445	567	580	+21	103.7	102.8
9/6/95	0915	566	566	0	100.0	94.9
10/17/95	1100	563	570	+7	101.4	99.4
South Fork Rio Grande (SFRG) at Beaver Creek Bridge						
6/13/95	1530	570	581	+12	102.2	98.2
7/28/95	1200	563	573	+10	101.8	103.6
8/31/95	1615	561	570	+9	101.7	100.2
9/7/95	0820	561	562	+1	100.2	96.4
10/17/95	0900	557	556	-1	99.9	99.0
Beaver Creek (SFRG Drainage) 1 km downstream of Beaver Creek Reservoir						
6/13/95	1430	565	574	+10	101.7	98.7
7/28/95	1120	557	563	+10	101.8	103.6
8/31/95	1550	555	558	+3	100.6	97.6
9/7/95	0800	554	554	0	100.0	94.1
10/17/95	1530	548	551	+3	100.5	98.8
Beaver Creek 1 km upstream of South Fork Rio Grande confluence						
6/13/95	1500	568	576	+9	101.5	98.0
7/28/95	1100	560	565	+5	101.0	105.7
8/31/95	1530	559	567	+9	101.6	101.0
9/7/95	0750	558	559	+2	100.3	98.3
10/16/95	1300	553	557	+6	100.9	99.9
South Platte River below Cheesman Dam						

Table 79. Dissolved gas measurements from several rivers in Colorado in 1995. In-column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the percent total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
6/14/95	1330	602	621	+19	103.2	101.0
7/26/95	1800	592	616	+25	104.3	101.8
8/11/95	1415	590	607	+17	102.8	106.0
8/24/95	1125	596	628	+33	105.5	110.5
9/20/95	1830	593	604	+11	101.9	99.4
10/9/95	1025	591	602	+11	102.0	101.2
South Platte River above Deckers Bridge						
6/14/95	1330	606	621	+16	102.6	103.9
7/26/95	1715	596	612	+18	103.1	101.0
8/11/95	1300	595	616	+21	103.6	111.9
8/24/95	0800	597	607	+10	101.7	106.7
9/20/95	1900	598	594	-3	99.4	94.9
10/9/95	0940	596	603	+8	101.4	104.5
11/21/95	0830	596	600	+4	100.6	83.5
South Platte River 1 km below Horse Creek confluence						
6/14/95	1500	606	621	+15	102.5	103.4
7/26/95	1700	597	613	+16	102.8	101.1
8/11/95	1240	595	614	+19	103.1	109.8
9/20/95	1915	598	596	-1	99.7	91.2
10/9/95	0915	597	599	+3	100.5	101.8
South Platte River near Scraggy View						
6/14/95	1615	608	624	+17	102.7	103.7
7/26/95	1630	599	620	+21	103.6	106.4
8/11/95	1155	598	627	+29	104.8	112.5
9/20/95	0730	606	595	-5	98.9	95.9
10/9/95	0835	600	598	-1	99.7	101.6
South Platte River below Spinney Mountain Dam						
7/27/95	1800	553	575	+22	104.1	104.6
9/20/95	1545	546	567	+21	103.9	97.9
9/20/95	1600	547	568	+22	104.1	104.9
Taylor River 0.1 km above East River confluence						
6/12/95	1245	578	590	+13	102.2	104.0
7/25/95	1040	565	571	+6	101.2	100.4
8/7/95	1120	564	573	+9	101.6	102.8
9/7/95	1730	564	568	+4	100.8	99.4
9/18/95	1520	561	564	+3	100.6	97.8
10/18/95	0900	563	561	-1	99.6	97.4

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
Arkansas River at Stockyards Bridge (Salida)						
7/17/96	1640	584	603	+19	103.3	110.6
8/14/96	2045	588	589	+1	100.1	97.9
Cache la Poudre River above Poudre Ponds Rearing Unit						
7/13/96	815	573	375	+2	100.4	101.6
7/16/96	1430	568	583	+15	102.7	108
9/19/96	1300	565	586	+22	104	99
Cache la Poudre River at Black Hollow Creek						
7/13/96	1010	579	583	+6	101	101.2
7/16/96	1530	573	580	+8	101.4	109.6
9/19/96	1200	570	580	+12	102	97
11/21/96	1600	566	562	-1.00	99.5	104.5
Cache la Poudre River at Hombre Ranch						
7/13/96	1035	586	589	+3	100.6	97.2
7/16/96	1510	580	587	+7	101.3	110.7
9/19/96	1050	577	583	+7	101.2	105.2
11/21/96	1330	576	579	+4	100.7	109.4
Cache la Poudre River at Kelly Flats						
7/13/96	1200	594	602	+8	101.5	99.04
7/16/96	1710	588	596	+9	101.5	109.1
11/21/96	1230	583	581	+7	101.1	113.7
Cache la Poudre River at Big Bend						
11/21/96	1645	561	558	-2.00	99.5	102.9
Rio Grande River at Marshall Park						
8/15/96	1215	551	569	+19	103.5	91
10/9/96	1700	547	556	+9	101.6	109.2
Rio Grande River at Beaver Creek Culvert						
5/10/96	1045	555	560	+5	100.9	106.5
5/10/96	1400	555	560	+5	100.9	101.7
7/17/96	1915	557	556	+0	100.1	101.2
8/15/96	945	562	570	+9	101.5	103.6
10/10/96	1200	558	559	+2	100.4	111.2
11/18/96	1330	553	544	-9.00	99.1	105.9
Rio Grande River at Beaver Creek Below Reservoir						

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
8/15/96	910	556	560	+5	100.8	104.7
Rio Grande River at Wason Ranch						
5/10/96	1000	553	559	+6	101	110
South Fork of the Rio Grande River at Beaver Creek Bridge						
5/10/96	1110	558	567	+9	101.7	109.3
7/17/96	1935	559	560	+2	100.3	98.6
8/15/96	945	562	570	+9	101.5	104.3
10/11/96	1000	559	565	+7	101.3	111.5
11/18/96	1350	555	544	-9.00	98.2	111.2
Rio Grande River at State Bridge						
5/10/96	1300	564	582	+18	103.2	109.3
7/17/96	1830	564	573	+10	101.7	96.2
8/14/96	2310	568	569	+5	100.3	81.7
8/15/96	1005	567	572	+6	101.1	86
11/18/96	1230	561	568	+8	101.5	111.5
Rio Grande River at Coller Wildlife Area						
7/18/96	750	559	560	+1	100.1	92.9
8/15/96	1100	561	587	+26	104.6	103.5
Rio Grande River at Upper Shocking Station near Campground						
7/18/96	840	550	556	+7	101.3	96.1
10/10/96	1015	554	559	+5	101	112.2
Rio Grande River at Ute Bluff Bridge						
10/9/96	1015	554	559	+5	101	112.2
Rio Grande River at 1/4 mile Above Beaver Creek						
10/10/96	1200	558	559	+2	100.4	111.2
South Fork of the Rio Grande at Columbine Picnic Area						
10/10/96	1540	553	566	+14	102.5	115.4
South Fork of the Rio Grande above Falls						
10/10/96	1640	542	538	-2.00	99.5	110.8
Colorado River near Sentinel Tanks						
5/13/96	1300	573	595	+22	104	113.2
Colorado River at Drowsy Water HQ						
5/16/96	1140	568	598	+30	105.4	119.5
Colorado River at Hitchin Post Bridge						

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
5/16/96	1240	560	599	+32	105.6	111.9
8/12/96	1615	571	591	+20	103.6	106.9
10/14/96	1600	565	588	+24	104.1	116.9
11/13/96	1635	566	577	+13	102.2	113.4
Colorado River at Kemp/Breeze						
8/12/96	1430	578	599	+21	103.7	115
10/17/96	0945	573	580	+7	101.3	112.7
10/17/96	1100	571	594	+24	104.1	115.0
10/17/96	1300	572	595	+24	104.2	110.3
10/17/96	1500	572	596	+25	104.3	109.5
10/17/96	1630	572	589	+19	103.1	103.4
11/13/96	0920	568	572	+5	100.9	118.2
11/13/96	1315	565	592	+28	100.0	110.1
10/17/96	1630	572	589	+19	103.1	103.4
11/22/96	1205	564	588	+25	104.5	117.5
Colorado River at Sheriff Ranch						
8/12/96	1535	573	600	+27	104.7	97.2
8/12/96	2125	573	568	-4.00	99.2	86.7
10/14/96	1420	567	583	+19	103.3	108.6
11/13/96	1500	567	588	+22	103.7	132.8
11/22/96	1020	562	569	+11	101.9	114.3
Colorado River at Windy Gap Bridge						
9/12/96	0900	568	571	+4	100.8	94.7
9/12/96	1040	568	592	+27	104.9	104.8
9/12/96	1215	568	600	+34	105.8	112.5
Colorado River at Chimney Rock						
8/12/96	1555	572	592	+20	103.6	107.7
10/14/96	1510	567	591	+25	104.5	109.2
11/13/96	1535	567	585	+20	103.5	119.1
Colorado River at Lone Buck						
8/12/96	1510	577	607	+30	105.3	111
10/14/96	1220	573	597	+25	104.3	112.2
Colorado River above Windy Gap						
8/12/96	1635	571	584	+14	102.5	101.8

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
11/12/96	955	557	569	+13	102.4	116.3
Colorado River near Carmichaels						
8/12/96	1700	568	583	+16	102.9	105.5
Colorado River near Parshall						
10/14/96	1715	573	582	+10	101.7	114.2
Colorado River at Hot Sulphur						
11/22/96	1100	562	566	+5	100.9	109.4
Colorado River at Horn Ranch						
9/12/96	1530	564	574	+11	101.9	96.8
East River at Roaring Judy						
5/9/96	1500	556	559	+3	100.5	100.4
7/18/96	1200	563	574	+12	102.1	103.2
8/15/96	1615	562	571	+9	101.6	93.5
8/15/96	0910	563	562	0.00	99.9	91.2
8/16/96	0725	564	562	+0	99.9	96.4
10/2/96	1030	562	569	+8	101.5	92.4
11/20/96	0930	555	552	-1.00	99.8	106.7
Taylor River at Almont						
5/9/96	1530	559	563	+4	100.8	104
7/18/96	1220	566	574	+9	101.7	106.9
8/15/96	1625	565	570	+5	100.9	101.9
8/15/96	925	566	563	-2.00	99.9	100.7
8/16/96	735	567	564	-1.00	99.7	103.5
10/2/96	1615	563	568	+5	100.9	101.7
East River at Almont						
7/18/96	1235	566	581	+15	102.7	107.4
8/15/96	1635	565	576	+11	102	104.3
8/15/96	935	566	560	-5.00	98.9	93.2
8/16/96	745	567	564	-2.00	99.5	99.9
10/2/96	1635	564	577	+14	102.5	105.9
Gunnison River at Almont Campground						
8/15/96	1650	566	579	+14	102.9	105.7
8/15/96	940	567	561	-5.00	99.1	96.6
8/16/96	755	567	563	-3.00	99.3	100.2

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
10/2/96	1645	564	577	+3	100.6	100
Gunnison River at Costello's Bridge						
5/9/96	1530	559	563	+4	100.8	104.8
11/20/96	1000	557	552	+5	98.9	112.2
Gunnison River at Way Camp						
7/18/96	1255	569	581	+13	102.2	104
Gunnison River Below Crystal Dam(1km below dam)						
7/18/96	1515	597	609	+13	102.2	102.5
8/16/96	0945	599	604	+6	101.0	91.5
Gunnison River Below Crystal Dam (1km below dam) Cont.						
10/4/96	0920	596	627	+32	105.4	91.4
10/4/96	1045	595	632	+37	106.3	102.0
Gunnison River Below Crystal Dam (gauging station)						
7/18/96	1550	598	617	+20	103.3	111.1
8/16/96	1000	599	607	+9	101.5	96.3
10/4/96	1145	597	635	+40	106.8	111.4
Gunnison Gorge at Ute Park (Island Pool)						
9/23/96	1245	624	651	+28	104.4	114.1
9/23/96	1330	624	653	+29	104.7	114.8
9/23/96	1630	621	644	+24	103.9	109.2
9/23/96	2100	620	622	+2	100.3	99.3
9/24/96	0600	620	619	+0	99.9	100.0
Dolores River at Metaska						
5/29/96	1400	588	619	+13	105.3	120.5
7/29/96	1400	596	639	+44	107.5	139.9
7/29/96	1240			+48	109.3	142.9
8/28/96	1750	600	621	+23	106.3	118.5
8/28/96	2115	594	589	-3.00	99.4	101.6
8/29/96	0710	594	585	-8.00	98.5	87.9
9/30/96	1630	592	634	+42	107.2	138.0
10/1/96	0930	591	601	+11	101.9	109.5
11/19/96	1300	591	620	+32	105.3	122.3
Dolores River at Lone Dome Weir						
5/29/96	1530	590	637	+47	108.0	134.5

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
11/19/96	1220	592	602	+12	102.1	119.9
Dolores River at Bradfield Bridge						
5/30/96	1610	592	625	+33	105.5	117.7
7/29/96	945	600	620	+21	103.5	79.5
7/29/96	1005	600	620	+21	103.6	104.7
8/28/96	1612	600	620	+20	103.4	111.5
8/28/96	1000	598	598	+1	100.1	97.5
8/29/96	630	600	591	-6.00	98.8	94.6
11/19/96	1155	592	611	+19	103.2	117.2
Dolores River at Ferris Canyon						
7/29/96	1140	598	637	+39	106.5	113.7
9/30/96	1810	592	607	+16	102.7	109.2
10/1/96	1300	594	625	+32	105.5	120.2
Dolores River near Irrigation Weir (Thermograph Location)						
8/28/96	1510	595	614	+19	104.0	116.2
8/28/96	2135	596	595	+1	100.1	90.1
8/29/96	0650	596	588	-7.00	98.5	100.0
Dolores River at Mile 8.8 Trap						
9/30/96	1730	600	617	+31	105.2	117.7
Dolores River at Cabin Canyon Campground						
10/1/96	1500	595	629	+38	106.4	120.9
South Platte River at Sugar Creek Road						
7/17/96	1130	599	625	+27	104.5	129.2
8/14/96	0815	604	599	-4.00	99.2	94.1
South Platte River Below Horse Creek						
7/17/96	1200	597	630	+34	105.7	131.9
South Platte River at Upper Deckers Shocking Station						
7/17/96	1230	597	635	+39	106.6	129.5
8/13/96	2145	602	598	+3	99.4	103.9
8/14/96	840	602	607	+7	101.1	106.1
11/20/96	1415	590	603	+15	102.4	125.6
South Platte River Below Cheeseman Dam						
7/17/96	1305	592	612	+21	103.6	109.7
8/14/96	1425	597	613	+17	102.9	104.1

Table 80. Dissolved gas measurements from several rivers in Colorado in 1996. In the column headings, P_T refers to total gas pressure in the water, ΔP is the difference between P_T and barometric pressure, and TDG% is the total dissolved gas saturation.

Date (mo/day/yr)	Time (military)	Barometer mm Hg	P_T mm Hg	ΔP TDG	TDG % Saturation	O_2 % Saturation
South Platte River Above Wigwam						
8/14/96	1045	600	626	+28	104.6	108.5
South Platte River Below Spinney Mtn.						
8/14/96	1730	555	573	+19	103.4	100.9
South Platte River Near Scraggy View Campground						
11/20/96	1505	592	604	+14	102.4	124.2

gas saturation levels documented in the river, according to the findings of White et al (1991). That investigation was a seven year study of the effects of acute gas supersaturation and the associated GBT induced in rainbow and brown trout in the Bighorn River downstream from Yellowtail Dam, Montana.

Gas supersaturation measurements in 1996 (Table 80) largely reaffirmed the findings from 1995. Gas supersaturation on most Colorado streams is largely a daytime phenomenon resulting from algal photosynthetic activity. The highest total gas supersaturation observed during 1996 was on the Dolores River, reaching 109.3% at 1240 hours on July 29, 1996. Oxygen saturation was 142.9% at that time. It is interesting that we have never observed exophthalmia in YOY rainbow or brown trout collected from the Dolores River during these episodes of elevated gas saturation. Similar levels of gas supersaturation were observed in the Dolores River in 1995 (Nehring and Thompson 1996). Interestingly, among the 20 trout fry from the river examined for evidence of tissue damage caused by *M. cerebralis* using histological techniques, no tissue abnormalities of any kind were detected. Tissue abnormalities due to gas embolism are easily recognizable in histological cross sections of affected fish, as shown in studies by Machado et al, 1987. These findings are strong empirical evidence that healthy young rainbow and brown trout can handle the low level chronic gas supersaturation caused by photosynthetic activity during daylight hours.

The lowest total dissolved gas supersaturation levels observed during the summer of 1995 and 1996 were on the East River at Almont, Beaver Creek, the South Fork of the Rio Grande, and Cache la Poudre rivers. Many of the YOY feral rainbow trout collected from all of these rivers were suffering from moderate to severe exophthalmia when collected and preserved for histological sectioning. Histological sectioning confirmed that most of these fish had sustained tissue damage consistent with parasitism by *M. cerebralis* (see Table 18 in Nehring and Thompson 1996 for details).

The suggestion that GBT or gas bubble disease is a significant operative mechanism in the failure of rainbow trout recruitment in the Cache la Poudre, Colorado, Dolores, Gunnison, Rio Grande, and South Platte rivers is not supported by our findings. Rather, we believe GBT (as documented in the upper Colorado River) may be an exacerbating stressor that could accelerate the degenerative processes and ultimate death set in motion by the debilitating effects of parasitism by *Myxobolus cerebralis*, the myxosporean parasite that causes whirling disease. Except for the short period of time when Crystal Dam was spilling on the Gunnison River and causing mechanically-induced hyperbaric saturation of the water with both nitrogen and oxygen, the levels of total gas supersaturation seen in this study in 1995 and 1996 are not high enough to be causing chronic or acute GBT.

This is especially true when the gas responsible for hyperbaric saturation is oxygen (produced by photosynthesis during daylight hours) and not nitrogen. Rucker (1975) demonstrated that as the percentage composition of oxygen increased in supersaturated water, the lethal effects of nitrogen supersaturation declined in rainbow trout. Garey and Rahn (1970) demonstrated that oxygen gas tension in the tissues of rainbow trout cycled rhythmically in concert with the rising and falling of oxygen tensions in the surrounding water in synchrony with photosynthetic activity. Gas tensions in rainbow trout tissues increased during the day when environmental oxygen levels were high, and declined during the night as oxygen levels fell.

Triactinomyxon (TAM) spore filtration

Over the past two years we have developed and field tested a technique to filter and quantify the number of waterborne TAM spores in streams. We have been able to detect these spores that infect the young trout in almost all streams that we have sampled, including Beaver Creek (tributary to the South Fork of the Rio Grande), the Big Thompson, Cache la Poudre, upper Colorado, East, Gunnison, and Middle Fork of the South Platte rivers, as well as Spring Creek (tributary to the Taylor River), South Cottonwood Creek (tributary to the Arkansas River), and Wigwam Creek (tributary to the South Platte River). We have documented TAM spore densities ≥ 26 TAMS/liter (100 TAMS/gallon) in raw water from some locations on the upper Colorado River. Release of the TAM spores from the *Tubifex tubifex* aquatic worm appears to be highly correlated with water temperature. Water temperatures between 10 °C and 15 °C are the range where TAM concentrations are the highest. However, we have detected low densities of TAMS in the Gunnison and Williams Fork rivers during the winter months when water temperatures are as low as 3 °C to 5 °C. Moreover, in streams downstream of small lakes or impoundments with a long history (5 - 10 years) of stocking of *MC*-infected catchable rainbow trout we have found TAM densities as high as 3.12/L (11.8/gallon) even during the mid-winter period when water temperatures are at 0°C (32° F).

We have been using the DNA-based PCR technique (Andree et al 1997) in concert with water filtration/microscopic identification techniques to quantify the number of TAM spores that were found in 1893 L (500 gallons) of water. This technique has proven to be a highly accurate and precise quality control check to determine whether or not actinospores identified as the

Table 81. Comparison of the number of triactinomyxon (TAM) actinospores counted by stereozoom microscope scanning of 20 - 80 μL aliquots (total volume 1.6 mL) drawn from concentrated filtrates of field water samples of up to 1893 liters of volume with the relative index of PCR electrophoretic banding intensity on parallel samples of 1.6 mL aliquots drawn from the same concentrated filtrate.

TAM Spore Count Range	Microscope TAM Count Occurrence	Relative Intensity of PCR Banding Pattern					
		-	w+	+	++	+++	inh*a*
Net Washes-negative sample	0	10	0	0	0	0	0
Net Washes-positive sample	1 - 29*b*	12	0	0	0	0	0
Unknown negative controls*c*	6	6	0	0	0	0	0
0	104	74	21	7	1	0	1
1	30	19	8	3	0	0	0
2	13	2	3	5	2	0	1
3 - 4	13	3	4	2	1	0	3
5 - 14	34	0	3	17	13	0	1
15 - 49	40	0	1	14	25	0	0
50 - 99	24	0	0	6	14	0	4*d*
100 - 500	9	0	0	2	5	2	0
501 - 5000	11	0	0	0	4*e*	7	0

a inh = negative test results are believed to be the result of an inhibitory effect on the PCR assay.

b These samples had 1 - 29 TAMS counted in our standardized protocol. Water samples drawn from the net wash water all tested negative by PCR analysis. This is strong empirical evidence that the chance of cross contamination of samples by an unwashed net is small.

c The samples were known to be negative for TAM DNA by the field collection team but were of unknown status to laboratory personnel.

d These samples were collected from riverine habitats containing fecal material from cattle (in one instance) and immediately downstream of beaver ponds (in two cases) containing fecal material of beaver. However, we have noted in the majority of instances, collections of samples from these exact sample locations at other times have not resulted in such strong inhibitory reaction(s).

e Laboratory personnel believe these samples would score +++ in the absence of any inhibitory effect on the PCR reaction.

triactinomyxon spores of *M. cerebralis* were indeed that species. The results of these quality control checks on well over 300 samples are summarized in Table 81. These data indicate that (in most instances) the PCR test gives a reliable indication of the relative number of TAM spores counted with our standard microscope identification/enumeration protocol. There is definitely a very clear trend of increasing strength in the PCR banding pattern with increasing numbers of TAM spores enumerated in a parallel sample from the same filtrate. The data also show that in a very few instances (4 of 306 samples), the PCR test can give a false negative result, apparently due to some inhibitory factor that can prevent replication of the target DNA in the thermocycling process.

We find this filtration technique to be an extremely useful tool for determining the seasonality and periodicity of TAM spore production and for locating microhabitat "hot zones" of infectivity. We are currently using this technique together with core sampling in lakes and rivers and GPS (Global Positioning System) technology to map the exact location of these "hot zones" of infectivity. In the future, this technique may allow fishery managers to determine when and where fingerling trout can best be stocked into waters testing positive for the *M. cerebralis* parasite to avoid exposure to excessive densities of the organism.

RECOMMENDATIONS AND CONCLUSIONS

The data we have gathered from numerous streams and study sites across Colorado clearly indicate that whirling disease, caused by the myxosporean parasite *Myxobolus cerebralis*, is having a devastating effect on recruitment and survival of YOY rainbow trout in the Cache la Poudre, Dolores, Colorado, Gunnison, Rio Grande, and South Platte rivers. Impacts on wild rainbow trout in the Roaring Fork River have been significant as well. Among the ten rivers that have been a part of this study, only the wild rainbow trout population of the Big Thompson River appears totally unaffected by this parasite at the end of 1997. It is unclear whether or not population-level impacts are occurring with wild rainbow trout on the Fryingpan River. Over the past four decades, the Taylor River never has had a bonafide wild rainbow trout population. We estimate that approximately 400 km (250 miles) of stream are currently affected. It is possible that more streams are affected but have not been studied as well, if at all.

In addition to the aforementioned rivers, *M. cerebralis* and the attendant tissue damage caused by the parasite has been detected in the Arkansas, Blue, and Conejos rivers. While there is little evidence of population level effects as of the end of 1997, these streams do not have self-sustaining wild rainbow trout populations. Without a viable rainbow trout population, it is difficult to determine what the actual level of infectivity might be.

On the upper Colorado River during the 1996 field season, we observed far greater numbers of juvenile brown trout that were suffering significantly from the debilitating effects of clinical whirling disease than we had observed in 1994 and 1995. This may indicate that the ambient level of infectivity in some portions of this stream may still be increasing. Interestingly, we saw fewer significantly affected brown trout in 1997 than we did in 1996. Clearly, there are differences among and between years and rivers that affect the dynamics of transmission of this disease from *T. tubifex* worms to the fish that remain enigmatic. Much work remains to be done.

The sentinel fish studies that have been conducted over the past four years have yielded significant insights into many facets of the etiology and epidemiology of this disease as it applies to salmonids in the intermountain west. We have shown that Colorado's native trouts (the greenback, Rio Grande, and Colorado River cutthroat trout) are highly susceptible to this parasite. When exposed at a very young age, i.e., 1-2 weeks post emergence from the gravel, brook trout are also highly vulnerable to the parasite. Wild rainbow trout from the Colorado River (CRR) are also severely affected by the parasite. Infected CRR trout produce the highest levels of *MC* myxospores of all salmonids tested over the past four years. However, CRR trout have survived longer and at somewhat higher levels than similarly exposed brook trout, greenback, Rio Grande, and Colorado River cutthroat trout. Except for brown trout, Snake River cutthroat trout appear to have greater resistance to the debilitating effects of the parasite than any of the other salmonids tested in Colorado. Moreover, our data shows that (statistically) they do not produce any more *MC* myxospores than brown trout.

In 1998 and 1999, additional sentinel fish testing is being done on Snake River cutthroat trout. If these experiments reconfirm the findings from our 1996/1997 tests with this species, it may be that this fish is a better choice (than rainbow trout) for stocking in *MC* positive habitats. Compared to rainbow trout they should survive better and produce significantly fewer myxospores which would reduce the level of *MC* myxospore loading of the environment. In many lacustrine habitats, Snake River cutthroat trout grow well, are long-lived, and they provide a good catch rate for anglers. According to many hatchery superintendents and technicians, Snake River cutthroat trout are also easy to rear in an aquaculture environment.

We recommend that more field biologists become trained in the techniques of TAM spore filtration, quantification, and identification using the techniques we have developed. We believe that a greater field effort utilizing this technique over the next few years will greatly aid in determining 1) what are the risk factors that can pre-dispose a stream or stream reach to population level effects of whirling disease, and 2) determine the specific microhabitat characteristics that foster high levels of TAM spore production and release. Armed with such information, we become better informed for making wise decisions regarding the management of coldwater fishery resources in the future.

Study Title: Stream Fisheries Investigations

Job Title: Native Salmonid Stream Fishery Investigations Job No. 3

Job Objective: Determine the microhabitat preferences of Colorado's native cutthroat trout for water depth, water velocity, substrate, and cover in allopatry and in sympatry with other salmonids, and attempt to establish high quality (≥ 35 cm) cutthroat trout populations in one or more large streams.

Period Covered: July 1, 1993 through June 30, 1998.

INTRODUCTION

In Colorado, cutthroat trout restoration projects have been a part of stream fisheries management for almost two decades. Three sub-species of cutthroat trout, the Greenback - GBN (*Oncorhynchus clarki stomias*), the Colorado River - CRN (*O. c. pleuriticus*), and the Rio Grande - RGN (*O. c. virginalis*) have been reintroduced into many areas across the state. While many headwater streams (near timberline) support viable populations of these fish there are no lotic environments that produce large numbers of quality size (≥ 14 inches or 35 cm) cutthroat trout.

Maintenance and protection of cutthroat trout populations in lower elevation streams has been difficult due to interactions with other salmonids, particularly brook and brown trout. These two fall-spawning species appear to put the spring spawning cutthroat trout at a disadvantage. However, the mechanisms causing the cutthroat trout (in sympatry with brook or brown trout) to decrease in density and biomass are not well understood. Is the mechanism solely predation by brown trout or brook trout at the fry/fingerling life stage or are there behavioral interactions at the juvenile and adult life stages as well, as other studies suggest (Wang and White 1994). Does variability in habitat play a major role?

Finally, almost nothing is known about the habitat preferences of native cutthroats for water depth, velocity, substrate, and cover. Are their habitat preferences for depth, velocity, substrate, and cover constant among the three sub-species of cutthroat trout? Does cutthroat trout habitat use change in sympatry with brook or brown trout? Past and current cutthroat trout recovery efforts have met with some success. However, finding answers to these questions should enhance our ability to recover these species. Ultimately, finding answers to these questions may determine whether or not the CDOW (as a management agency) is successful at recovering these fish to the degree that allows removal from the "species of special concern" list(s).

Finding solutions to these perplexing problems is the primary reason for this study.

Segment objectives originally outlined for the 1993/1994 study period were:

1. Conduct baseline population estimates on one to three larger rivers where the management objective is to establish a high quality cutthroat trout population.

2. Stock Colorado River Native (CRN) cutthroat trout fingerlings in one or more study streams if fingerlings are available.

3. Based on input from regional management biologists, select one or two small streams (second or third

order) for each of the three sub-species of CRN, GBN, and RGN cutthroat trout where underwater observation is possible from July through September.

Work on any of these objectives was officially postponed from the 1995/1996 segment up until the present time. Some work under segment objectives 1 and 2 above was accomplished through the efforts of regional fishery management personnel. Some work was also accomplished as a result of work completed under Job 1 (*Electrofishing Injury Investigations)* and Job 2 (*Special Regulations Evaluations).* As we became increasingly aware of the threat that whirling disease posed for wild salmonid populations in Colorado no effort was ever directed towards accomplishment of segment objective 3.

METHODS

Study Sites

The Dolores and Fryingpan rivers were selected as study streams for the introduction of CRN cutthroat trout fingerlings. In the past, both streams have demonstrated the biotic potential to produce quality size (≥ 35 cm) cutthroat trout. Snake River native (SRN) cutthroat trout stocked in the Dolores River as fingerlings from 1984 through 1991 grew to sizes ≥ 50 cm in length and up to 2 kg in weight. Similarly, CRN cutthroat trout ≥ 40 cm in length periodically have been captured in the upper reaches of the Fryingpan River just downstream from the confluence with Rocky Fork Creek, a tributary to the Fryingpan River downstream from Ruedi Dam. This creek supports an A+ population of wild CRN cutthroat trout.

Population Estimation Procedures

Population estimation procedures include walk electrofishing of 4-6 sites on the Fryingpan River and boat electrofishing on the Dolores River. Electrofishing methodologies for the Stream Fisheries Investigations project, previously described in the Methods section of the Special Regulations Evaluations study final report (Nehring 1987) will not be reiterated here.

Population estimates were conducted on a 17.7 - 19.7 km reach of the Dolores River between McPhee Dam and the Bradfield Bridge in July 1993, June 1994, July 1995, June 1997, and June 1998. Discharge levels were too low during 1996 to facilitate boat electrofishing. Population estimates were completed at several study sites on the Fryingpan River each fall between 1993 and 1997, usually during mid-November. Trout population estimates, density (n/ha), and biomass (kg/ha) were determined using procedures previously reported (Nehring 1980). Since 1985 we have used a computer software program called GOLDMEDL to generate these statistics. We used a Bausch and Lomb Microprojector together with the computer software package DISBCAL (Missouri Department of Conservation) to read fish scales and back calculate the length of the fish at each age. Age and growth information was used to subdivide the trout population estimates into age specific cohorts and construct life tables for each population on the Dolores River (see Appendix Table I).

Fingerling Stocking

Previous studies have shown that survival of stocked fingerling salmonids can be increased by boat distribution (Nehring 1993). During this study we have made every effort to employ boat distribution of fry/fingerling salmonids where ever and when ever possible. All fry/fingerlings stocking on the Dolores River has been done using the boat distribution method.

On October 4, 1993, 30,000 CRN cutthroat trout fry, averaging 3.1 cm in length, were boat distributed throughout the 11 km reach of the Dolores River immediately downstream from McPhee Reservoir. In addition, approximately 5,200 Colorado River (CRR) and 9,900 Tasmanian (TAS) rainbow trout fingerlings were stocked in the 8.4 km reach of river from the irrigation weir diversion to Bradfield Bridge as part of standard regional management practices. The CRR fingerlings averaged 7.4 cm in length at stocking while the TAS fingerlings averaged 7.2 cm in length.

In early October 1994, 30,000 - 3.5 cm (total length) CRN trout fry were boat distributed in the upper 11 km reach of the Dolores River immediately downstream of McPhee Dam. No CRN fingerlings were stocked in 1995. We felt that the small size at which they had been stocked was contributing to poor survival. Therefore, the fry scheduled for stocking in October 1995 were held over the winter as stocked in early March 1996. We stocked 38,940 CRN fingerlings that averaged 6.15 cm (2.42 inches total length) in the upper 11 km reach of the Dolores River. No fingerlings were available for stocking in 1997.

On July 20, 1993, approximately 7000 fingerling CRN cutthroat trout averaging 11.3 cm in length, were stocked by hand (from a stocking truck) at more than 20 sites throughout a 23.4 km reach of the Fryingpan River between Ruedi Dam and Basalt, Colorado. In addition, 10,000 CRR trout fingerlings averaging 8.1 cm in length were also stocked as part of standard regional fisheries management practices. No CRR or CRN fingerlings were stocked in the Fryingpan River (as a part of this study) after 1993 because of shortages in the availability of these fish within the hatchery system.

RESULTS AND DISCUSSION

Dolores River

Survival of the 1993 CRN, CRR, and TAS Fingerling Plants

During the June 1993 and July 1994 population estimation procedures, rainbow trout ranging in size from 10 to 19 cm were captured in the Dolores River, indicating that rainbow trout fingerlings stocked in 1992 and 1993 had survived in significant numbers. In 1993, rainbow trout in the 10-19 cm size range were captured throughout the 19.7 km reach of stream from the Metaska Picnic Area to Bradfield Bridge. In July 1994, 76% of the rainbow trout in the 10-19 cm size range were captured in the 8.4 km reach of river downstream from the irrigation weir to Bradfield Bridge. No fingerling rainbow trout were stocked upstream of the irrigation weir in 1993. These data indicate that there was measurable survival of the TAS and CRR fingerling plant from 1993. Moreover, this plant of fish did not disperse upstream from the stocking site in significant numbers.

During the June 1994 population estimation procedures on the 11 km reach of river from the Metaska area to the irrigation weir, no CRN fingerlings were captured or observed during the electrofishing process. Either the CRN fry (3.1 cm at the time of stocking) had not grown to a large enough size to be vulnerable to capture by June 1994, or overwinter survival was minimal to non-existent. Backpack electrofishing surveys in the upper reach of river in late-October or November 1993 revealed the CRN fry were present in high abundance in the Metaska area of the river at that time.

By July 1995, the CRN trout fry stocked in October 1993 were large enough to be vulnerable to capture by boat electrofishing. Our CRN cutthroat trout population estimate for the 19.7 km reach of river was 71 fish, indicating extremely poor survival of these fry. Estimated survivorship from October 1993 through July

20, 1995 is 0.237%. In contrast, the estimated size of the 1993 cohort of "wild" rainbow trout population (survivors of the stocking of 15,100 CRR and TAS rainbow trout fingerlings) was 310, with lower and upper 95% confidence limits of 131 and 489, respectively. The best estimate of survivorship from that stocking effort is 2.1%, and could be as low as 0.9% or as high as 3.2%. Thus, survivorship of the larger rainbow trout fingerlings is probably one order of magnitude (10 times) better than that of the CRN cutthroat trout fry that were only 3.1 cm in length at the time of stocking.

These findings strongly suggest that it is imperative that larger CRN fingerlings must be stocked to enhance survivorship. Fingerling CRN cutthroat trout should be overwintered in a whirling disease (WD)-free hatchery and stocked as 8-9 cm fish in the spring of the year on the ascending limb of the discharge hydrograph.

An encouraging aspect of the 1995 electrofishing operation conducted below McPhee Dam during July 1995 was the capture of 49 age 1+ wild rainbow trout. These fish ranged in size from 9 cm to 18 cm, and could not be from any other source except natural reproduction, as no fingerling rainbow trout of any kind were stocked in the Dolores River in 1994. Although the U.S. National Fish Hatchery at Hotchkiss stocked McPhee Lake with fingerling rainbow trout during August and September 1994, they were larger at the time of stocking than these fish were when collected in July 1995. The population estimate for this group of fish was 577 for the reach of river from Metaska Day Use Area to Ryman Draw. Colorado River rainbow (CRR) trout fingerlings were first stocked in the Dolores River in 1989; therefore, it is a certainty that sexually mature CRR trout capable of natural reproduction have been present in the river since 1992 or 1993.

Bank electroshocking surveys in October 1996 and boat electrofishing surveys in June 1997 corroborated the findings from 1995. Age 1+ wild rainbow trout again turned up in the samples. No rainbow trout fingerlings were stocked in the Dolores below McPhee Dam after 1993. Fry electrofishing surveys during September and October (1993 - 1997) confirm the presence of rainbow fry at sampling stations throughout the river.

Boat electrofishing surveys in June 1998 confirmed that (for all practical purposes) none of the wild rainbow trout fry from the 1997 year class survived to recruit to the population as yearlings in 1998. No age 1+ juveniles were captured during the 1998 surveys. Data on the abundance of rainbow and brown trout fry observed in the Dolores River for 1997 are summarized in Tables 1 and 2. For details on trout fry abundance in 1995 and 1996 see appropriate tables in previous segment reports (Nehring and Thompson 1996, 1997).

Trout Population Estimates

Trout density and biomass statistics for the Dolores River from 1991 through 1998 are given in Table 20. The data indicate that the self-sustaining brown trout population reached an eight year peak in July 1995. This certainly seems to indicate that "pool-management" of the water allocated in McPhee Reservoir (for fishery and esthetic purposes) has had a positive impact on the trout population. Hopefully, this will continue.

Reduced recruitment can result from unusually high spring flow levels that resulted from the above average snow pack in the 1992, 1993, 1995, and 1997 water years. Brown trout fry emerge from the gravel in the months of May and June on the Dolores River. Trout fry less than 50 mm in length are weak swimmers and vulnerable to high water velocities (Nehring and Anderson 1993) that occur during extended periods of high stream discharge, as happened in 1992, 1993, 1995, and 1997. However, careful ramping of flows on the ascending and descending limbs of the annual discharge hydrograph during above normal water years can have an ameliorating effect on this problem. This may have ameliorated fluctuations in the brown trout population in the river.

However, there has been a steady decline in brown trout density and biomass since 1995. This is probably the result of above average water years and high discharge levels from McPhee Dam that have occurred in 1995, 1997, and 1998. We were encouraged by the small numbers of CRN cutthroat trout ranging up to almost 40 cm in size captured during the electrofishing survey in June 1998. This is *prima facie* evidence that at least some CRN trout are surviving, recruiting, and competing successfully in sympatry in a stream harboring an abundance of large brown trout (Wang and White 1994).

Fryingpan River

The fishery biostatistics for brown, rainbow, and brook trout for the Fryingpan River are summarized in Tables 21, 22, and 23 in the Job No. 2 (Special Regulations Evaluations) portion of this report. Fish disease outbreaks, loss of brood stocks, and severe shortages of trout to stock due to the whirling disease problem in the CDOW hatchery system, resulted in the termination of cutthroat trout stocking in this river. Since there has been no cutthroat trout stocking in the Fryingpan River since 1993, there is no additional data that needs to be presented here.

In 1997, a few native cutthroat trout were collected during electrofishing operations. As was the case for the Dolores River, a few specimens ranged from 35 cm (14 inches) and 40 cm (16 inches) in length. These fish were very colorful and in robust condition. Clearly, they were competing successfully in sympatry in the face of intense competition with brown trout. When the CDOW hatchery system begins to produce significantly more numbers of cutthroat trout, hopefully early in the 21st century, supplemental stocking of native cutthroat trout fingerlings might be a worthy management consideration.

RECOMMENDATIONS AND CONCLUSIONS

Dolores River

We recommend that plants of CRN cutthroat trout fingerlings in the Dolores River be continued on an experimental basis. Stocked fish ranging in size from 7 cm to 12 cm in length are likely to survive and compete with the dominant brown trout better than the smaller sizes stocked during this study. These fish can be stocked either in the late-summer/early-fall (September/October) period or in the spring on the ascending or descending limb of the spring discharge hydrograph. The fingerlings should be distributed by boat.

Fryingpan River

If fingerlings are available, we recommend continued stocking (on an experimental basis) of CRN cutthroat trout fingerling in the Fryingpan River, stocking fish ranging in size from 7 cm to 12 cm. Boat distribution of these fish would be desirable; however, this is probably not realistic in light of the low levels of stream flow normally occurring in the Fryingpan River. Additional problems with fences, cascading rapids sections filled with logjams and large boulders in the river may well make boat distribution of the CRN fingerlings impossible.

The monitoring of these stocking efforts will require very little extra effort since both streams are included in another 5 year research study to document the continuing effects of whirling disease on the wild trout populations in these two streams.

Study Title: Stream Fisheries Investigations

Job Title: Stream Trout Habitat Modeling Investigations — Job No. 4

Job Objective: Using the IFIM/PHABSIM computer models, existing stream trout population estimates, and IFIM stream profile data sets, determine the relationship between trout habitat availability and carrying capacity by life stage on selected sections of 14 trout streams.

Period Covered: July 1, 1993 through June 30, 1998.

The unexpectedly heavy workload encountered on the Colorado River (Special Regulations Evaluations - Job 2) as well as that resulting from the number of streams added to the project for whirling disease research during the last three segments of this five year project precluded the possibility that any work could be accomplished under this study.

Study Title: Stream Fisheries Investigations

Job Title: Technical Assistance — Job No. 5

Job Objective: To provide technical assistance in the selection, design, and evaluation of instream flow studies and issues when requested by fish biologists, managers, and researchers.

Period Covered: July 1, 1993 through June 30, 1998.

No requests for technical assistance were made by fish research or regional fisheries personnel during the 1996/1997 or 1997/1998 job segments. However, during the first three job segments, a considerable amount of time. field assistance, and technical expertise was provided to Mr. Rod Van Velson that aided in the successful completion of an extensive IFIM/PHABSIM modeling effort on a major stream habitat improvement project on the upper South Platte River above Spinney Mountain Reservoir in Park County, Colorado. That project, was Federal Aid Project F-161, entitled *Stream Habitat Investigations and Assistance.*

In September 1993, assistance in field electrofishing operations was provided on this study. In the spring of 1994, assistance was provided in the field for acquisition of water surface elevation and discharge measurements. During the fall of 1994, additional assistance was provided during electrofishing operations. In the fall of 1994 and spring of 1995, approximately 20 man-days of technical assistance were provided in the field for the acquisition of additional information on water surface elevations, channel depth, width, cell velocities, and discharge measurements required for the IFIM/PHABSIM modeling effort. During the 1995/1996 segment an additional 10 man-days of assistance were provided in the field, as well as two months of effort spent compiling, calibrating, and running the habitat simulation data sets for each of three stream study reaches.

Habitat Simulation data sets were developed, calibrated, and production runs completed using the RHABSIM (River Habitat Simulation) computer software program developed by Thomas R. Payne and Associates of Arcata, California. We found this software program to be very utilitarian in modeling the interactions between riverine hydrology and wild trout populations.

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