FISHERY MANAGEMENT INTERVENTIONS TO REDUCE *MYXOBOLUS CEREBRALIS* INFECTIONS IN FISH PONDS ON THE CAP K RANCH (2000 – 2013)

A FINAL REPORT

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INTRODUCTION AND BACKGROUND HISTORY

Whirling Disease - Whirling Disease (WD), a debilitating malady than can have detrimental effects on some species of trout and salmon, is caused by the myxozoan parasite *Myxobolus cerebralis*. Although WD was first observed in cultured rainbow trout in Europe in the late-1800s (Plehn 1905), the complex two-host life cycle of this metazoan parasite remained an enigma until first described by Wolf and Markiw (1984). Clinical signs of the disease can include bulging eyes, skeletal deformities, shortened gill covers, blacktail, and “whirling behavior,” where severely infected trout can swim in tightly concentric circles. Trout infected by the parasite produce myxospores that are either shed into the natural environment while alive (Nehring et al. 2002) or are released when the carcasses of dead fish decompose. These myxospores can infect *Tubifex tubifex* (*Tt)* that are susceptible to infection by the parasite. Infected worms subsequently produce triactinomyxon (TAM) actinospores of *M. cerebralis (Mc)* that are semi-buoyant and float or tumble in the water column, and infect *Mc*-susceptible trout or salmon upon contact. The life cycle of the parasite is temperature dependent and can take up to a year or more for completion in both hosts. Cutthroat trout, brook trout, and rainbow trout, three species of salmonids that occur in Colorado, are highly susceptible to infection and can experience population-level impacts once the parasite becomes established in ponds, lakes or streams in the state (Thompson et al. 1999). Brown trout can become infected upon exposure, but have a high level of resistance as a species. Individual brown trout fingerlings that are either severely infected or have lower resistance can develop severe clinical signs of WD including blacktail and deformities of the vertebral column resulting in lordosis and/or scoliosis. However, there are no known cases among brown trout in Colorado where the cumulative impacts are detectible at the population level. The parasite was accidentally brought into Colorado in shipments of live trout from Idaho in the 1980s (Obmascik 1995).

The Fryingpan River - The 22 km reach of the Fryingpan River between Ruedi Dam and Basalt, Colorado became a destination fishery for fly fishermen from around the world seeking a high quality angling experience in 1978 after the Colorado Division of Wildlife (CDOW) implemented a catch-and-release regulation on a short reach of the stream. Wild rainbow, brown and brook trout populations flourished under the restrictive angling regulations. Density, biomass and numbers of quality size trout (≥35 cm or 14 inches) of all three species increased dramatically over the next decade. This was due in part to the augmentation of the food supply in the river immediately below the outfall of Ruedi Dam. After the dam was retrofitted with a run-of-the river hydroelectric power plant in the mid-1980s, deep water opossum shrimp *Mysis relicta* in the reservoir became entrained in the water column near the penstock intake for the turbines and were discharged into the river (Nehring 1991). During the 1980s, this fishery became an economic boon to the local economy, accounting for an estimated 3.6 - 4.8 million dollars in annual spending in the town of Basalt (Nehring 1991).

During the mid-1990s, dramatic declines in the wild rainbow trout population were initially documented in the river near confluence with Taylor Creek, a site where electrofishing studies had been regularly conducted since the 1970s. The wild rainbow trout population at this site declined more than 90% between 1994 and 1998, concurrent with the first detection of *Mc* myxospores in trout in the river at this site (Nehring and Thompson 2001).

Initial Water Filtration Studies In 1997 and 1998, aquatic researchers with the CDOW developed a water filtration technique to quantify the density of TAMs of the *Mc* parasite in water (Thompson and Nehring 1999). 2000). Water filtration studies were initiated at three sites on the Fryingpan River in 1998 to determine whether or not water being released from Ruedi Dam or some other site was a source of infection. The objective of these efforts was to document the location(s), seasonality and periodicity of TAM production in the river. Monthly filtrations between August 1998 and June 2000 demonstrated that water released from Ruedi Dam was not a point source of infection at all. No TAMs were detected emanating from Ruedi Dam or Rocky Fork Creek, tributary to the Fryingpan River below the outfall of Ruedi Dam during this time period. However, significant numbers of TAMs were consistently detected in a very small volume of water flowing into the Fryingpan River from a series of fish ponds on the Cap K Ranch (Nehring 1999, Nehring et al. 2000, Nehring 2006). Subsequent monthly water filtration efforts were initiated in November 2001 and continued through October 2002, at 31 sites throughout the Fryingpan River basin. Despite this year-long study, detection of TAMs was a rare occurrence except for the water flowing through the ponds 1 through 4 and out of pond 6 on the Cap K Ranch into the river (Nehring 2003). See Figures 1 through 10 in the Appendix for details.

Upon becoming aware of the impact upon the Fryingpan River rainbow trout fishery in late 1999, the owners of the Cap K Ranch allowed CDOW aquatic researchers virtually unlimited access to the ponds to conduct studies into the dynamic of *Mc* infection on the ranch and develop management strategies to reduce the level of infection . This collaboration continued through 2013 and led to implementation of "Best Management Practices" (BMPs) on the ranch that have dramatically reduced levels of infectivity in the ponds. Reduced infection levels should benefit the Fryingpan River fishery as well. This report documents the results of this 14-year collaboration.

METHODOLOGIES AND STUDY DESIGN ON THE CAP K RANCH

This WD research project has been a 14-year case study in fisheries forensic science. A multi-faceted adaptive management approach was used to gather the data and produce the information needed to provide insight(s) into the dynamic of *M. cerebralis* infection in trout on the ranch. Armed with that information, BMPs were developed to reduce the level of *M. cerebralis* infection. A number of fisheries techniques or tools used in the process of formulating and evaluation of the effectiveness of the BMPs included the following:

Monthly water filtration studies – Monthly water filtration was initiated on the outlets of ponds 1, 2, 3, 4 and 6 in January 2000 to assess the level of TAM production in each of the ponds. The volume of water filtered and concentrated on each sampling occasion during the first 4 years of sampling was 1,900 L (500 gallons). During 2004, the sample volume was reduced to 120 L (30 gal.) and two replicate samples were filtered per sample site and period. After that the sample volume filtered was increased to 1,900 L again to maintain consistency with the original filtration protocol for the remainder of the study. All filtrates were screened by stereo-zoom microscopy for TAMs. Numbers of TAMs observed were standardized and reported as TAMs/L, following the protocol developed by Thompson and Nehring (2000). The estimates of TAM densities (n/L) for all filtration events on the ponds at the ranch are summarized in Table 1A through 6A in the Appendix. The shape, surface area and layout of the ponds on the ranch are shown in Figure 11 in the Appendix.

Electrofishing Operations - Bank, backpack and boat electrofishing gear was used to collect trout for disease sampling and develop trout population estimates in some of the ponds in some years. Population estimates were developed at least once each year in ponds 1 and 2 between 2006 and 2013, twice in pond 3 (April 2004 and November 2012), annually in pond 4 since October 2007 once, in pond 6 (June 2005) and numerous times in pond 5 from 2004 through 2012. The electrofishing results and populations estimates are summarized in Appendix tables 7A through 12A. Annual bank and backpack electrofishing to remove as many brook trout fry as possible from the ponds during the spring months after ice-out began in 2001 and continued through 2008. These efforts consistently demonstrated that pond 2 is the only significant site of brook trout fry production. Backpack electrofishing for brook trout fry removal in the spring has not been conducted since 2008, in order to assess the capability of the sterile tiger trout (an artificial cross between a female brown trout and a male brook trout) to control brook trout recruitment through predation.

Trout Stocking Operations – Various numbers, size and species or strains of trout were stocked into many of the ponds between 2002 and 2009 to accomplish a number of objectives and goals in the study. First, we wanted to study the dynamics of *Mc* infection in rainbow trout versus the brook trout in some of the ponds. To do this we needed to stock enough trout to be sure that we were able to collect the numbers, sizes and species of fish needed for disease testing at appropriate times in the study. Second, stocking known numbers, sizes and species of trout (usually marked in some manner) allowed us to study growth rates, survival rates, infection severity and determine whether or not natural reproduction and recruitment of "wild" rainbow trout was occurring in any of the ponds during the study. Third, we wanted to evaluate the growth rates, survival rates, infection severity and reproductive capabilities of the HOFER strain rainbow trout compared to Tasmanian (TAS) rainbow trout and brook trout in the same ponds. HOFER rainbow trout, derived from brood stocks of rainbow trout that have been exposed to the *Mc* parasite in aquaculture in Germany for approximately 120 years, have a very high degree of resistance to infection (Hedrick et al. 2003; Schisler et al. 2006). Fourth, we wanted to determine whether or not Tiger trout could control excessive levels of spawning and fry recruitment among brook trout in pond 2. Tiger trout, produced by fertilizing brown trout eggs with sperm from a male brook trout, are voracious predators (Greg Brunjak, Manager of Mount Massive Lakes Trout Club, Leadville, Colorado; personal communication). Tiger trout fingerlings were stocked into ponds 1, 2, 3 and 4 in 2006, 2007, 2008 and 2012.

The overarching goals of the study were to 1) reduce the ambient levels of *Mc* TAM production in the ponds, and 2) decrease the prevalence and severity of infectivity in the fish populations in the ponds through the development and implementation of “Best Management Practices” (BMPs). These “BMPs” were as follows:

1.Conduct bi-weekly brook trout fry removal in pond #2 each year from late-March through mid-May (2001 – 2008) to control brook trout fry production and recruitment to reduce the number of YOY brook trout becoming infected with *Mc.*

2. Remove as many juvenile and adult brook trout from ponds 1 and 2 each fall prior to spawning, thereby reducing the number of YOY brook trout produced in pond #2 each spring. Transplant the captured brook trout (not utilized for disease testing) into pond 3 for sport fishing recreation opportunities.

3 Remove brown trout from ponds 1, 2, and 5 captured during fall electrofishing operations (2002 – 2013) to control predation on brook trout and prevent recycling of *Mc* myxospores back into the ponds and reduce TAM production. Transplant captured brown trout into ponds 3 and 4 for sport fishing opportunities.

4. Screen off the outlet of pond # 2 to prevent upstream migration of adult brook trout from ponds 3 and 4.

5. Construct a wetland biofilter on the outlet of pond # 6 to “scrub” *Mc* TAMs from the effluent prior to discharge to the Fryingpan River. Conduct monthly water filtrations to evaluate the efficacy and efficiency of the biofilter to remove TAMs from the water.

6. Enhance trout spawning habitat at the channel inlets to ponds 1 and 2, between ponds 3 and 4, and downstream of pond 4.

7. Stock HOFER rainbow trout that have been shown to be highly resistant to *Mc* infection (Schisler et al. 2006) into ponds 1 through 4. The purpose is to establish a “wild” rainbow trout population that is a) highly resistant to *Mc* infection, and b) can sustain itself through natural reproduction. If successful, this would further reduce ambient levels of *Mc* myxospores and TAM production in all of the ponds while providing sport fishing recreation opportunities without any need to purchase fish.

8. Stock “Tiger Trout” to control brook trout population densities in ponds 1 through 4, and in pond # 2 in particular. The Tiger trout is an aggressive predator that has proven very effective as a biological control agent on overly productive brook trout in small ponds. (Greg Brunjak, Manager, Mt. Massive Lakes Trout Club, personal communication).

The implementation, efficacies and outcomes of these management strategies and BMPs are presented below.

RESULTS AND DISCUSSION

Water Filtration Studies – Results of the monthly water filtration studies conducted on the ponds and at four sites on the Fryingpan River are summarized in Tables A1 through A6 and Figures 1 through Figure 10 in the Appendix. The time period over which filtrations were conducted varied as the objectives of the study changed over time. Filtrations were initiated at the outlets of ponds 1, 2, 3 and 4 on the ranch beginning in January 2000 (Figures 1 through 4 in the Appendix). TAMs were detected during 22 of 25 filtrations conducted on the outflow of the ponds between January and June 2000. However, TAM densities in the water flowing out of pond 2 were 10 to 250 times greater than densities detected in the discharge from any of the other ponds during that 6-month period (Appendix Figures 1 through 4). This empirical evidence suggested the infection cycle in pond 2 may be key to understanding the dynamics of infection in the ponds, and the primary point of attack for reducing infectivity in the system.

Dynamics of Myxobolus cerebralis Infections in the Cap K Ranch Ponds – A Hypothesis - The water filtration data, together with the fish population sampling data from the ponds collected during 2001 and 2002, lead to the development of the hypothesis that brook trout reproduction in pond 2 was out of control and the critical factor responsible for the high level of TAM production emanating from pond 2. Brook trout, the dominant salmonid species in ponds 1 through 4 from 2000 through 2006, are highly susceptible to infection by *M. cerebralis*

(O'Grodnick 1979; Thompson et al. 1999) and can suffer high levels of acute mortality during the first year of life when exposed to *Mc* TAMs (Thompson e t al. 1999). They develop large numbers of myxospores that can be consumed by the *Mc*-susceptible lineages of *T. tubifex*, the aquatic oligochaete host of the *Mc* parasite. Boat electrofishing and fyke netting surveys conducted during 2002 demonstrated that each lake supported a small number of large brown trout. We hypothesized that young-of-the-year (YOY) and yearling brook trout infected by the parasite were vulnerable to predation by large (≥ 2 kg) piscivorous brown trout in the ponds. Myxospores present in the YOY and yearling brook trout consumed by predaceous brown trout would be released in the feces and become readily available for ingestion by *T. tubifex* in the ponds. Myxospores have been shown to pass through the alimentary canals of both cold and warm-blooded vertebrates and remain viable (El-Matbouli et al. 1992; Taylor and Lott 1978). Myxospores can also be released into pond sediments from the carcasses of infected trout. Accordingly, implementation of BMPs 1 through 3 began in 2001 and 2002.

Reduction of ambient levels of TAM production through implementation of "BMPs" - It is evident from the water filtration data summarized in Figures 1 through 6 that as BMPs 1 through 3 began to be implemented in 2001 and 2002 on the ponds, and particularly on ponds 1 and 2, that the frequency of detection of TAMs decreased through time and that the density (TAMs/L) has generally declined in the later years (2003 – 2007) compared to 2000 and 2001 (see Tables 1A and 2A and Figures 1 through 6 in the Appendix).

Similarly, the data in Table 1 indicates that there has been an overall downward trend in cranial myxospore concentrations for brook trout in ponds 1 through 4 since 2002, concurrent with the declines in the rate of detection and overall densities of TAMs observed in the filtrates collected from ponds 1, 2 through 4 between 2000 and 2007. In the fall of 2002, the mean cranial myxospore concentrations ranged from 136,000 to 183,000 among 4 of 5 lots of brook trout collected from these ponds. In contrast, the mean cranial myxospore concentration ranged from 4,900 to 68,000 among 7 of 8 lots of brook trout collected during the fall of 2007. Compared with the earliest years of

Table1. Mean cranial myxospore concentrations among YOY and older (≥ age 1+) brook trout collected from ponds 1,2, 3 and 4 on the Cap K Ranch from 2002 through 2013.

Date	Pond # 1		Pond # 2		Pond # 3		Pond #4	
(mm/dd/yy)	YOY	≥ age 1+	YOY	≥ age 1+	YOY	≥ age 1+	YOY	≥ age 1+
10/26/2002	Ns	166,000*****	183,000	79,700	Ns	135,900	Ns	149,800
10/28/2004	118,500	142,700	69,400	23,111	Ns	Ns	Ns	Ns
10/31/2005	40,600	44,000	35,300	Ns	3,500	15,100	Ns	81,100******
10/30/2006	Ns	89,400	Ns	57,900	Ns	Ns	25,300	55,600
10/30/2007	20,100	255,300	63,000	19,600	4,900	20,500	64,700	68,000
10/30/2008	9,700	97,300	20,700	47,300	Ns	Ns	263,200	28,900
11/06/2009	7,100	67,200	Ns	Ns	Ns	Ns	Ns	Ns
11/05/2010	Ns	Ns	24,400	11,429	Ns	Ns	Ns	Ns
4/20/2012	Ns	Ns	Ns	14,187	Ns	Ns	Ns	Ns
11/05/2012	Ns	Ns	15,600	34,000	Ns	36,200	Ns	Ns
4/03/2013	Ns	Ns	Ns	Ns	Ns	17,600	Ns	Ns
11/06/2013	Ns	Ns	14,944	0	Ns	Ns	0	Ns

***:** This sample is an average of 2 lots of trout from pond 1 collected in the fall of 2002
******: This sample was collected June 2005
Ns: No sample collected

testing, mean myxospore concentrations had declined by 94% among YOY brook trout in pond 1 in 2009, while the decline among brook trout ≥ age 1+age was 60% for the same time period. Similarly, cranial myxospore concentrations among YOY and age 1+ and older brook trout in pond 2 declined by 91% and 57%, respectively, between 2002 and 2012. These data, together with the results of the pond water filtration studies support the hypothesis that ambient levels of *M. cerebralis* infectivity in the ponds 1 and 2 have declined substantially over the 2002-2013 time period since implementation of BMPs were first initiated. These results strongly support the conclusion that the multi-faceted adaptive management approach has substantially ameliorated the ambient level of *Mc* infectivity in the entire system of ponds on the ranch. Appendix Tables 13A through 17A contain a detailed chronology of the trends and differences in myxospore levels in cranial tissues of all strains and species of trout in the ponds on the ranch.

Implementation of BMP 1(spring removal of brook trout fry) began in the spring of 2001 and was conducted every year through 2008. Removal by electrofishing was conducted beginning in late March or early April depending upon the time of surface ice melting on the ponds. Fry were captured and removed around the entire perimeter of pond 2 at two to 3-week intervals. This continued until such time that the remaining brook trout fry were moving into deeper water and were no longer susceptible to capture by backpack electrofishing. Backpack electrofishing during 2001 revealed large numbers (> 3,000) of brook trout fry were produced in pond 2 (0.26 ha or 0.64 acres). Very little brook trout fry production was occurring in any other pond. A few were produced annually in the channels between the ponds (< 100 at most sites). However, the excessive fry production in pond 2 was more than adequate to "seed" all ponds in the system, since all of the ponds were connected in series. More than 2,000 brook trout fry were annually removed from pond 2 from 2002 through 2006. In the spring of 2007 a total of 1,109 brook trout fry were removed during 5 electrofishing operations between March 14 and June 15. This suggests that the removal of juvenile (YOY) and adult brook trout from ponds 1 and 2 during fall electrofishing may finally be reducing brook trout spawning success. (See Tables 7A and8 A in the Appendix for details on the numbers of juvenile and adult brook trout removed from ponds 1 and 2 each fall from 2002 through 2013).

Between April 9 and July 21, 2008, 1,608 brook trout fry were captured and removed by electrofishing on five separate occasions. A total of 1,550 (96.3%) of the YOY brook trout were captured around the perimeter of from pond 2 and the channel connecting pond 1 and 2. Only 12 YOY brook trout were captured in the inlet channel to pond 1. No brook trout fry were collected from the perimeter of ponds 1 or 3. These data are *prima facie* evidence that virtually all of the brook trout fry production within the Cap K Ranch ponds originates from pond 2. There was no effort to remove brook trout fry by backpack electrofishing after 2008 in order to assess the effectiveness of tiger trout to function as "biological control agents" on the brook trout fry in ponds 1 and 2.

Implementation of BMP 2, (removal of juvenile and adult brook trout prior to spawning in ponds 1 and 2) began in the fall of 2002 and has been conducted at least once annually every year through 2013 (see Tables 7A and 8A in the Appendix). Four hundred and five and 455 brook trout were removed from ponds 1 and 2 (respectively) in the fall of 2002. Fewer numbers were removed during 2003 and 2004 suggesting that the removal of adult spawners in 2002 had met with some initial success. However, number of brook trout captured in ponds 1 and 2 in the fall increased substantially in 2005, 2006 and 2007. There are at least two plausible explanations for this phenomenon. First, brook trout fry removal efforts in the spring of these 3 years may have been less successful than in 2002, 2003 and 2004. This does not seem to be the most plausible explanation given that only 1,109 brook trout fry were captured in pond 2 during the spring of 2007 and 1,550 during the spring and summer of 2008. The more plausible explanation for this increase in abundance of brook trout in these ponds between 2005 and 2007 is that there was a compensatory increase in the survivorship of YOY brook trout with implementation of BMPs 1 through 3, and particularly BMP 3.

Implementation of BMP 3 (removal of all juvenile and adult brown trout from ponds 1, 2, 5 and 6) was completely successful in ponds 1 and 2 by the fall of 2004 (see Tables 7A and 8A in the Appendix) as none were captured in either pond during fall electrofishing operations in 2005, 2006 or 2007. During 2004, 7 brown trout ranging from 520 to 660 mm TL were removed from pond 2. It is very likely that the survival of brook trout fingerlings that managed to escape capture by electrofishing during the spring months in 2005, 2006 and 2007 probably increased in the absence of piscivorous predators. Indeed, numbers of YOY fingerling/juvenile brook trout captured during the fall electrofishing operations increased dramatically in ponds 1 and 2 during this period, but not in ponds 3, 4, 5 or 6. (See Tables 9A through 12A for comparison and details, but especially Table 10A). It is noteworthy that large brown trout (≥ 500 mmTL) have always been captured during periodic boat electrofishing operations in ponds 3, 4, 5 and 6 from 2005 through 2012.

Implementation of BMP 4 (screening of the culvert between ponds 2 and 3) was accomplished in the spring of 2005 when it was discovered that some adult brook trout were able to migrate from pond 3 into pond 2 by swimming up the steep gradient culvert separating the two ponds. A steel plate screen perforated with 12 mm diameter holes with 13 mm spacing was placed over the outlet of pond 2 to prevent upstream migration of fish. Since then, it has been impossible for brook trout from ponds 3 and 4 to migrate back into ponds 1 and 2 by swimming through the culvert.

Construction of the "wetland biofilter" during the spring of 2003 marked the implementation of BMP 5. The data shown in Appendix Figure 7 indicate that the wetland biofilter was functioning quite well for almost 2 years after initial construction. However, by the spring of 2005, TAMs were being detected in filtrates collected from the outlet tubes of the wetland. It is unclear why this was happening but two explanations are plausible. First, as water percolated and filtered through the sand bed, some of the sand could have been transported into the 3/8 inch pea gravel beds above and below the sand beds, creating subterranean channels for unfiltered or inadequately filtered water to pass. Second, pressurizing of the bed with an air compressor to agitate the bed and disrupt compacted organic material may have created air pockets that then became filled with water, started to wash sand out, and created alternative pathways for unfiltered or inadequately filtered water to pass. In addition, mechanical aeration of the bed was expensive, time consuming and did not work for more than about 30-60 days. In the final analysis, we decided "version 1.0" wetland biofilter required too much "hands-on" maintenance, was undersized for the volume of water that required filtering during the summer irrigation season, and the surface colonization of the wetland filter by soft-stemmed and hard-stemmed bulrushes was very slow due to the alternate flooding and drying cycles that the surface was experiencing. This was a learning experience.

Based upon this experience, another wetland biofilter (version 2.0) was constructed at the Bel-Aire State Wildlife Area (SWA) in the spring of 2007 on the White River, 20 miles east of Meeker. Soft-stemmed and hard-stemmed bulrushes in the new wetland were far more densely populated just 4 months post construction than occurred in the wetland biofilter on pond 6 after 5 years. The key to rapid colonization of the surface by these bulrushes on the wetland biofilter at the Bel-Aire SWA seems due to the continual hydration of the surface of the wetland at a relatively shallow depth (3-6 inches) over most of the first summer of growth. Water has continued to flow through the filter on pond 6 periodically during the summer months when there has been adequate inflow. However, maintenance has been discontinued. By 2012- 2013, the surface of the filter was heavily colonized by vegetation, much of it by soft-stemmed and hard-stemmed bulrushes.

Enhancement of spawning habitat for rainbow trout in the channels upstream of pond 1, between ponds 1 and 2, and ponds 3 and 4 in September 2004 marked the implementation of BMP 6. The data in Appendix Tables 7A and 8A suggest rainbow spawning success increased in 2006 and 2007 compared to the period from 2002 through 2005. Numbers of YOY wild rainbows captured in pond 1 increased slightly in 2006 and 2007. Numbers of YOY wild rainbows captured in pond 2 increased exponentially in 2006 and 2007 compared to 2002-2005. Since there has been no stocking of unmarked fry or fingerling rainbow trout at any time in the ponds, these rainbow trout are definitely the result of natural reproduction that occurred in the spring of 2006 and 2007. Wild, unmarked (YOY) rainbow trout have been captured in ponds 1 and 2 each year during fall electrofishing operations since at least 2006. Since 2008 however, survival and recruitment of "wild" YOY rainbow trout into the fall has been uneven (at best) in ponds 1 and 2. That may be due to predation by tiger trout. The average size of the tiger trout in pond 2 had reached 380 mmTL (total length) with a maximum length of 440 mmTL in 2010, and an average length of 413 mmTL and a maximum length of 454 TL in 2011.

Implementation of BMP 7 was initiated in June 2005 with the stocking of approximately 1,000 HOFER rainbow trout into ponds 1 through 4. Additional stocking occurred in June 2006, May 2008, June 2009 and July 2012 (See Appendix Table 18A for details). The objective is to establish a "wild" rainbow trout population that is highly resistant to *Mc* infection, and can sustain itself through natural reproduction. If successful, this would further reduce ambient levels of *Mc* myxospores and TAM production in all of the ponds while providing sport fishing recreation opportunities without any need to purchase fish. After 5-6 years of intensive research, testing and evaluation that began in 2001-2002, it was conclusively demonstrated that the HOFER strain rainbow trout is highly resistant to *Mc* infection (Schisler et al. 2006). Acquired from a commercial trout farm in Bavaria, Germany, empirical evidence suggests that the HOFER rainbow has probably been exposed to *M. cerebralis* and heavy selection pressures for approximately 120 years. The significant unknown regarding this strain of rainbow trout was whether or not it has the ability to grow, survive and successfully reproduce in the natural environment. Fall electrofishing results for 2007, 2008 and 2009 indicated the HOFER trout survive and grow extremely well in all of the ponds (See Tables 7A through 10A in the appendix for details**).** Moreover, male and female Hofer rainbow trout from the 2005 and 2006 introductions sacrificed for WD disease testing from ponds 1 and 2 in October 2008 were sexually mature. Given that the number of age 3and 4 (2005 and 2006 year classes) HOFER trout far outnumber the "wild" rainbow trout of the same age in ponds 1 and 2, it was hoped that HOFER trout reproduction would occur in the spring of 2009.

On July 15, 2009, 21 YOY rainbow trout fry were captured from the inlet to pond 1 and the spawning channel between ponds 1 and 2 and transplanted into pond # 5. This was done to make sure that all of the naturally spawned rainbow fry would not be lost to predation by tiger trout. Prior to transplanting these fry into pond 5, boat electrofishing operations were conducted to remove any large brook and brown trout from the pond to minimize losses to predation. These fry ranged from 30 – 40 mmTL (1.2 – 1.6 inches) at the time of transplanting. Fourteen of these fish were recaptured in pond 5 during the November 2009 electrofishing operations. Amazingly, 12of the 14 fish ranged in length from 212 – 250 mmTL (8.3-9.8 inches)! Their weight ranged from 135 to 227 g! All of these fish were very silvery in color with hardly any parr marks (dark vertical bands on the sides of the body) or black spots. This coloration pattern is very characteristic of fast growing, juvenile HOFER rainbow trout. The other two rainbows had bright parr marks, a bright red band along the lateral line, and substantial numbers of black spots. This coloration is characteristic of the unmarked "wild" rainbow trout fingerlings that we have seen produced in ponds 1 and 2 since 2002. These two rainbow trout were substantially smaller (155 and 175 mmTL) and weighed 43 and 61 g, respectively, and were very similar in length and coloration to "wild" unmarked rainbow fingerlings captured in ponds 1 and 2 in November 2009, two days prior to the electrofishing of pond #5. The silvery coloration of the 12 larger unmarked rainbows recaptured from pond 5 strongly suggests that successful spawning and hatching of HOFER rainbow trout took place in the inlet channels of both ponds 1 and 2 in the spring of 2009. Fin clips were collected from all of these fingerlings and preserved in 70% ethanol for subsequent DNA screening for determination of parentage.

The same sampling process was carried out during the summer and fall seasons of 2010, 2011 and 2012. Results of the DNA screening using microsatellites technology for the samples collected between 2009 and 2011 are summarized in Table 2. Those results indicate that reproduction by pure HOFER strain rainbow trout occurred in all three years. Indeed, 69.8% of the unmarked "wild" rainbow trout were shown to have the genetic markers indicating they were the progeny of male and female HOFER parents.

Table 2. Genetic assessment to determine the probable parentage of unmarked "wild" rainbow trout fry produced in ponds 1 and 2 on the Cap K Ranch between 2009 and 2011.

Year	**Known HOFER Rainbow Trout**			**Unmarked "wild" Rainbow Trout**					
	N	**HOFER**	**Unknown**	**N**	**HOFER**	**Unknown**	**"CRR"**	**F1's**	**BC[a]**
2009	5	5	0	24	24	0	0	0	0
2010	3	3	0	60	48	6	1	0	5
2011	2	2	0	55	25	8	2	12	8
Totals	**10**	**10**	**0**	**139**	**97**	**14**	**3**	**12**	**13**

Note ([a]): BC = back cross in some combination of HOFER, CRR, F1 or B2H

During 2012, much of western Colorado was subject to a severe drought. Flows out of the springs on the ranch feeding into ponds 1 and 2 were severely reduced. To insure that the ponds did not overheat during the hot summer months, the ranch manager installed two large siphons on the irrigation canal delivering cold Fryingpan River water for irrigation of hay meadows and pastures. The siphons ran water onto the pastures surrounding the ponds. Despite the augmented inflow, no rainbow trout fry were captured in the spring-fed inlet channel to pond 1 or in the channel between ponds 1 and 2 in July 2012. However, more than 100 rainbow trout fry were captured by backpack electrofishing in the spring inlet arm at the SE corner of pond #3 (see Figure 11 in the Appendix). Twenty fry were saved for whole-body microsatellite DNA analysis to determine the parentage of the fry and 50 were transplanted into pond 5 for growth and then re-collection in November 2012. Fin clips were collected from eight unmarked wild rainbow trout and three HOFER strain rainbows (marked with an adipose clip) for DNA analysis. These samples have not been analyzed as of January 2014. No wild rainbow trout fry were found during backpack electrofishing operations in July 2013. The reasons for the lack of natural reproduction in 2013 are unknown.

The stocking of 75 tiger trout fry into pond 2 in the March 2006, marked the implementation of BMP 8. Additional tiger trout fingerlings were stocked into ponds 1 through 4 in 2007, 2008 and 2012. See Table 18A in the Appendix for the stocking rate for each pond. Tiger trout were introduced into the ponds to function as a biological control agent to reduce numbers of brook trout in ponds 1 through 4 and control the production of brook trout fry in pond 2. Tiger trout are sterile hybrid trout produced when brown trout eggs are fertilized with sperm from male brook trout. These fish are aggressive, very colorful, easily caught on a fly rod, and highly predatory on YOY brook trout. They have been very effective as a biological control agent on overly productive brook trout in small ponds at the Mt. Massive Lakes Trout Club near Leadville, CO (Greg Brunjak, club manager, personal communication). Since they are sterile, there will be no problem with overpopulation because of natural reproduction. All of these traits are compatible with the management objectives.

Despite the most intensive fall electrofishing efforts ever in ponds 1 and 2, the numbers of YOY brook trout captured in October 2008 were more than 90% lower than October 2007. This is strong empirical evidence that tiger trout predation upon brook trout fry was intense during 2008. However, "wild" YOY rainbow trout numbers in pond 2 in October 2008 were at an all-time low as well, most probably due to tiger trout predation.

In November 2009, substantial numbers of YOY brook trout fingerlings were captured in both ponds 1 and 2 compared to October 2008. This was not totally unexpected since there were no backpack electrofishing operations in the spring of 2009 conducted on pond # 2. We removed 169 YOY brook trout from pond #2 in November 2009. However, this was far less than the 391 brook trout fingerling removed from the pond in October 2006, at a time when we had been removing brook trout fry in the spring by backpack electrofishing. This suggested that the tiger trout were functioning as "biological" control agents that would become more efficient predators as they increase in size. Indeed, that has been the case. Electrofishing results for November 2013 reveal that the numbers of YOY brook trout captured in ponds 1 and 2 were near an all-time low since the population estimation efforts were initiated in 2006. The results of all trout population estimation efforts for all ponds and all years in which electrofishing efforts were conducted are summarized in Tables 7A through 12A in the Appendix.

***Myxobolus cerebralis* cranial myxospore concentrations and assessment of resistance –** Results of pepsin-trypsin digest (PTD) tests summarizing cranial myxospore concentrations for TAS, HOFER and "wild" rainbow trout, together with brook and "tiger" trout from ponds 1 through 6 between July 2000 and November 2013 are shown in Appendix Tables 13A through 17A. Spore concentrations have declined dramatically over the past 11 years among brook trout in ponds 1 through 4 since systematic sampling began in October 2002. The decrease in mean myxospore levels in cranial tissues of YOY brook trout in pond 2 has been the most dramatic, declining by 92% in November 2013, compared with October 2002. Prevalence of infection has declined from 100% to 40-50% over the same time period. Pond 2 has always been the most severely infected (see Figure 2 and Table 2A in the Appendix). These decreases in the prevalence and severity of infection among brook trout are undoubtedly the result of the implementation of several BMPs, including the removal of predatory brown trout from ponds 1 and 2, reductions in the number of YOY and juvenile brook trout populating ponds 1 and 2, and the stocking of Tiger trout to reduce the survival of YOY brook trout through the first 6 months of life in pond 2 through predation, thereby reducing the total production and input of *Mc* myxospores into parasite life cycle.

Cranial myxospore levels for 8 age 1+ "wild" (unmarked) rainbow trout captured in pond #2 in the fall of 2007 averaged 8,611, ranging from 1,111 – 64,444 among fish with detectible levels of spores. This was lower than observed in 15 other groups of rainbow trout (except for known HOFER strain rainbows) in any pond between 2002 and 2007. The average myxospore level observed in this group of unmarked rainbow trout was also lower than detected in 27 of 28 groups of brook trout ≥ age1+ collected from any of the ponds between 2002 and 2007. These data suggest that there may have been some spawning activity by male HOFER strain rainbow trout in pond # 2 as early as the spring of 2006. Only 3 of 8 rainbows in this sample had detectible myxospores (Appendix Table 14A).

The very low level of prevalence of infection and cranial myxospore levels among the HOFER rainbow trout in pond 2(compared to all of the other trout) is truly remarkable (Appendix Table 14A) and indicative of the high resistance to infection by the *Mc* parasite . Cranial myxospores were detected in only 13.6% of HOFER trout (14 of 103) sacrificed for PTD testing between the fall of 2005 and November 2013.

In contrast, the prevalence and severity of infection among the TAS strain rainbow trout was clearly demonstrated early in this study (see Tables 13A through 17A in the Appendix), a reconfirmation of similar results from other studies (Walker and Nehring 1995; Thompson and Nehring, unpublished data). Catchable-sized TAS rainbow trout marked with VIE (visual implant elastomer) tags were stocked into the Fryingpan River near the Taylor Creek confluence in March 2002. After 18 months of continuous exposure to ambient levels of infection in the river, these same trout (when tested by PTD) were 100% infected, with a mean cranial myxospore burden of 1.4

million, and ranging from 25,900 to 11.3 million in the most severely infected fish. For these reasons, use of the TAS rainbow trout broodstock has been discontinued in Colorado's state hatchery system.

The prevalence and severity of infection among the hybrid Tiger trout is also extremely high, an unexpected outcome given that brown trout have substantial resistance to infection by the *Mc* parasite. This would seem to be an unexpected manifestation of hybrid vigor, as no clinical signs of whirling disease have ever been observed in the Tiger trout in the ponds on the Cap K Ranch.

On April 5, 2010, we began an experiment to determine whether or not the "wild" Cap K rainbow trout had any resistance to infection by the *Mc* parasite. Eggs and sperm were collected from wild" (unmarked) rainbow trout and Hofer (adipose fin-clip marked) rainbow trout from ponds 1 and 2 on the ranch. Gametes from males and females of each strain were used to create individual single-pair crosses of all possible combinations. The pairings to be tested were as follows:

1. Male Hofer X female Hofer (HXH)
2. Male Hofer X female Cap K "wild" rainbow (F1)
3. Female Hofer X male Cap K "wild" rainbow (F1)
4. Female Cap K rainbow X male Cap K rainbow (Wild)

After water hardening, the eggs were transported to the CDOW Parvin Lake Research Laboratory for experimental exposure and testing. The eggs of a few HXH pairings were infertile, and for those reasons additional spawns of HOFER rainbow trout were taken at the Poudre Hatchery on April 26, 2010. Pairings of pure TAS rainbow trout were also included in the study as a "known laboratory" control against which all of the other crosses could be compared. After incubation and hatching, surviving fry were subjected to controlled laboratory exposures of the *Mc* parasite for comparisons of resistance among all possible single-pair crosses of each strain of rainbow trout. Exposure took place on July 14, 2010 at approximately 5 weeks post-hatch and 2.5 weeks post-swim-up. The exposure dose rate was 2,000 TAMs/fish. Percent mortality, myxospore counts and histological evaluations were the metrics for assessing the differences in response to exposure to *M. cerebralis.* The experiment was terminated on November 2, 2010. The results of the experiment are summarized in Table 3. Mean percent mortality was lowest among the HOFER treatment group, highest among the TAS rainbow trout, with the wild (Cap K) rainbow trout treatment group suffering an intermediate level of mortality. The same trends held trout for mean myxopsore counts and the histology scores. These data support the hypothesis that the Cap K rainbow trout may have developed a substantial amount of resistance to *Mc* infection after approximately 25 years of continuous exposure to the parasite. The owners of the Cap K Ranch introduced rainbow trout into the ponds only once in the mid-1980s. Our electrofishing studies that began in 2002, clearly demonstrated that natural reproduction was on-going in ponds 1 and 2 throughout the study.

Table 3. Summary of the results of exposure of four separate stocks or strains of rainbow trout to a single, fixed dose of 2,000 triactinomyxon actinospores *Myxobolus cerebralis.* Test exposure groups included pure Tasmanian (TAS), pure Hofer, wild Cap K and F1 crosses (Cap K X Hofer) rainbow trout.

Strain	Mortality			Myxospore Counts			Histology Scores		
	N	Mean (%)	St. Dev.	N	Mean (%)	St. Dev.	N	Mean (%)	St. Dev.
Hofer	3 (60)	61.7	15.3	3 (19)	9,627	14,394	3 (10)	0.9	0.9
F1	3 (60)	90.0	13.2	2 (6)	0.0	0.0	1 (3)	1.3	---
Wild	6 (120)	79.2	9.7	6 (23)	22,139	28,907	6 (12)	1.1	1.6
TAS	3 (60)	91.7	5.8	2 (3)	95,551	14,812	2 (2)	3.5	0.7

Means and standard deviations shown above are using the tank as the sampling unit.
Values in parenthesis are the total number of individuals.

Biological Data Age and Growth, Longevity and Survivorship - Age and growth analyses for fish scales collected from HOFER, "wild" unmarked rainbow, brown and "tiger" trout from ponds 1, 2 and 4 are shown in Appendix Tables 20A through 22A for 2008 and 2009. These data indicate all of these species and strains of trout were growing very well, strong empirical evidence that the ponds have not been overstocked or over populated and not straining the food base or carrying capacity of any of the lakes.

CONCLUSIONS AND RECOMMENDATIONS

Reductions in the Ambient Level of TAM Production in Ponds on the Cap K Ranch – Ambient levels of TAM production in the ponds on the ranch declined rapidly beginning in 2001- 2002, concurrent with the reductions in brook trout population numbers in ponds 1 and 2 and have remained low ever since. Beginning in April 2012 and continuing through March 2013, monthly water filtrations were conducted at the outlet of pond #2 to determine whether or not ambient levels had declined further compared with monthly estimates of TAMs from April 2006 through March 2007 (Table 2A). Although the average TAM density for the 2012-2013 sample period was substantially lower, a paired t-test statistical analysis revealed the decline was not significant. Moreover, the average TAM density for the 2012-2013 period was higher than that observed from April 2004 through March 2005. These results indicate that TAM densities have remained low and relatively stable since 2004, supporting the conclusion that the implementation of the BMPs and adaptive management strategies has worked well.

Control of Brook Trout Reproduction - The near absence of YOY fingerling brook trout in ponds 1 and 2 in the fall of 2013 (compared to 2006 through 2010) suggests that predation by tiger trout has been very effective in controlling brook trout fry production. No brook trout fry have been removed by electrofishing since the spring of 2008 to see if tiger trout predation alone could be effective in controlling excessive recruitment. The results suggest that tiger trout predation has been effective. As long as Tiger trout are present in the ponds, it should be possible to completely control the brook trout population in ponds 1 and 2 by removing juvenile and adult brook trout by periodic fly fishing during the open water months. All brook trout captured by fly fishing should be killed or put into pond 3. Without the fly fishing effort, it is quite probable that the brook trout population might get out of hand and overpopulate pond 2 again in just 2-3 years.

Natural Reproduction of Hofer Rainbow trout – Hofer rainbow trout were first stocked into the ponds on the Cap K Ranch in June 2005, with subsequent introductions occurring in 2006, 2008, 2009 and 2012. Genetic assessment to determine the probable parentage of unmarked “wild” rainbow trout fry produced in ponds 1 and 2 on the Cap K Ranch between 2009 and 2011 was conducted at the University of California-Davis. These tests confirmed that 70% (97 of 139) of the “wild” unmarked fingerling rainbow trout captured by electrofishing over that 3-year period were the result of natural reproduction among male and female Hofer rainbow trout. These results confirm that the pure Hofer rainbow trout are (when protected from angler harvest) capable of surviving in the natural environment until they reach sexual maturity and spawning successfully.

Enhancement of Spawning Channels - The full length and gradient slope of the stream channel between ponds 1 and 2 was not fully utilized to provide maximum spawning habitat in 2004. According to the GPS-generated map of the Cap K Ranch ponds size and layout in Appendix Figure 11, the total channel length between ponds 1 and 2 is approximately 133 feet. However, the upstream portion of the channel is very wide with a high gradient, shallow and filled with large angular rock. The high width:depth ratio, high gradient and the lack of spawning gravel makes this section unsuitable both for spawning habitat and fry habitat. These shortcomings could easily be eliminated by adding the correct size spawning gravel after significantly narrowing, deepening and meandering the narrowed channel in the current channel bed. This would approximately double the amount of optimum spawning habitat in this reach with very little cost, other than “sweat equity”. The spawning channel should be fenced after improvement to prevent degradation by cattle and/or horses during periods of grazing during in the spring to fall months. The outlet of pond 1 could have a small drop structure installed to prevent upstream migration of brook trout fry and adult trout of any kind.

Narrowing and deepening of the downstream end of the stream channel connecting ponds 3 and 4 could provide more and better spawning habitat for trout in both ponds 3 and 4. The same procedure could be completed on the outlet channel downstream of pond 4 as well, again, with very little cost except for “sweat equity”. Any spawning habitat enhancement should be protected by fencing to minimize collapse of the banks into the channel resulting from grazing by cattle and horses. Gravels ranging in size from 0.5 inches to 2.0 inches are the preferred size. These can be purchased locally from sand and gravel companies, as was done in 2004.

Annual Flushing of Spawning Gravels – Sediments and clogging of spawning gravels with organic matter should be dislodged once each spring, immediately after ice out by shooting compressed air into the gravels through a hand-held nozzle connected to a high pressure canister or K bottle (commercial high pressure gas cylinder) .This would flush out sediment, clean out and break up any armoring or aggregation of the spawning gravels prior to the onset of rainbow trout spawning activity. This should significantly increase the embryonic development and hatching success of rainbow trout eggs deposited in the gravels in the spawning channel. This only needs to be done once each spring, and not in the fall. Brown and brook trout would utilize these areas for spawning in the fall, unless there was an effort to keep them screened out. However, we have seen very little evidence of successful spawning by either of these species in the channels constructed in the fall of 2004. It appears that most of these eggs suffocate in the interstitial spaces in the gravel in mid-winter as the surface area becomes covered with diatoms and algal masses, which reduces, restricts, and ultimately shuts off intra-gravel water flow and oxygen to the developing embryos.

Wetland Biofilter Outlet Modification – Although the wetland biolfilter installed at the outlet of pond 6 did not prove to be a long-term solution to reduce or eliminate TAM production and release into the Fryingpan River, it has become thoroughly colonized by hard and soft stem bulrushes and other vegetation that is serving to filter some of the water that runs through ponds 5 and 6 during the spring to fall period. As currently constructed, brown trout from the Fryingpan River can swim up the overflow drainage channel and into pond 6. This will result in an increase in TAM production when the trout die under the ice of pond 6 when it almost dries up during the winter months due to lack of inflow. To prevent this scenario from happening, an L-shaped vertical drain pipe constructed out of 24 inch diameter steel culvert piping should be reinstalled at the outlet of pond 6 to block upstream migration of brown trout from the Fryingpan River into the pond. Additionally, the overflow drainage channel should be cutoff completely, and all of the water flowing out of pond 6 should then drain through the L-shaped culvert and/or percolate through the substrate of the wetland biofilter.

Brook Trout Spawning Operations – The Cap K Ranch ponds sustain a substantial brook trout population that has been tested numerous times since 2006 for the presence of prohibited pathogens, including bacterial kidney disease. Every time, the tests for all pathogens except the *Mc* parasite have come back negative. Pond 3 has a brook trout population that exceeds 1,100 according to the 2012 population estimate. This population could be an easy egg-take operation for Colorado Parks and Wildlife, if the owners of the Cap K Ranch were agreeable to allow that to happen. The owners might well be amenable to allowing access for such a spawning operation in return for periodic stocking of Tiger trout to maintain predation pressure on the brook trout populations in ponds 1 and 2 together with advice and assistance in maintaining the Hofer rainbow trout population through natural reproduction.

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Figure 1. TAMs/L versus temperature in Cap K Ranch pond 1, January 2000 through June 2007.

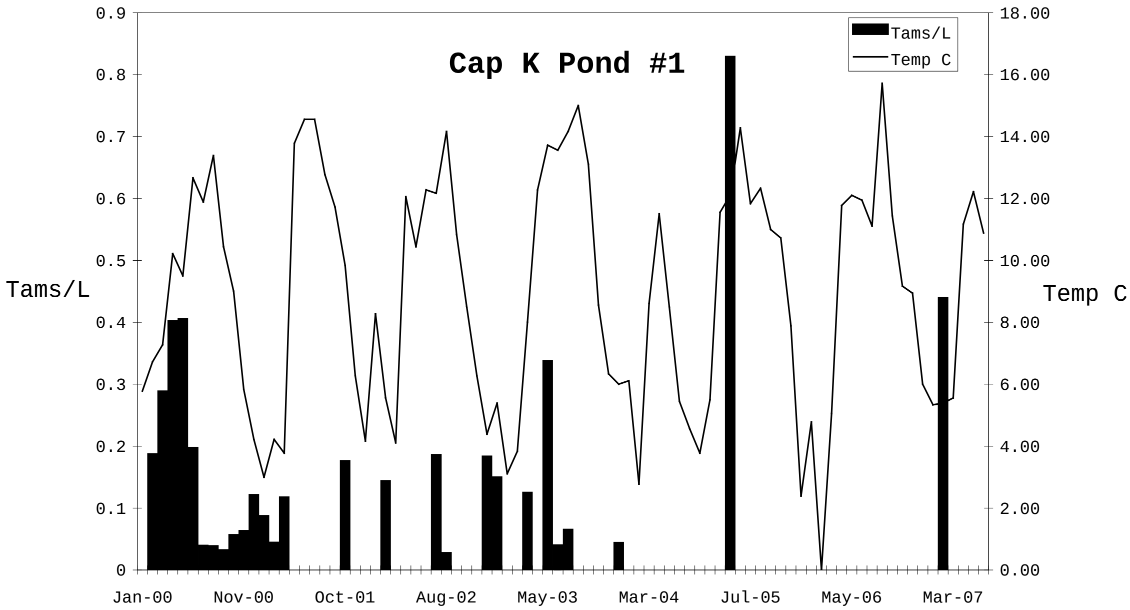

Figure 2. TAMs/L versus temperature in Cap K Ranch pond 2, January 2000 through June 2007.

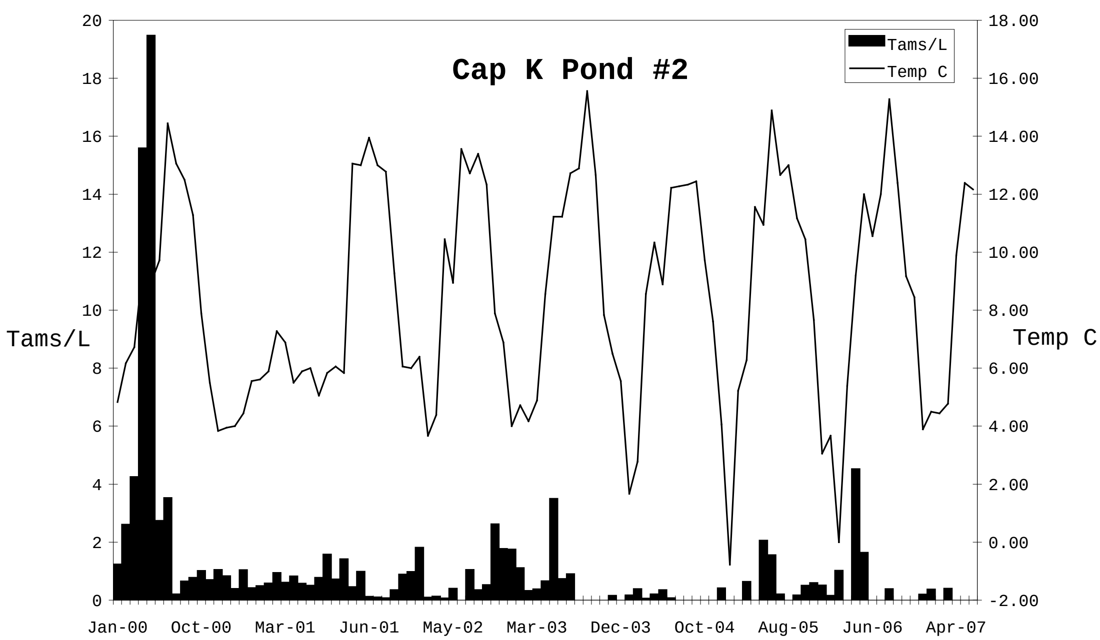

Figure 3. TAMs/L versus temperature in Cap K Ranch pond 3, January 2000 through June 2003.

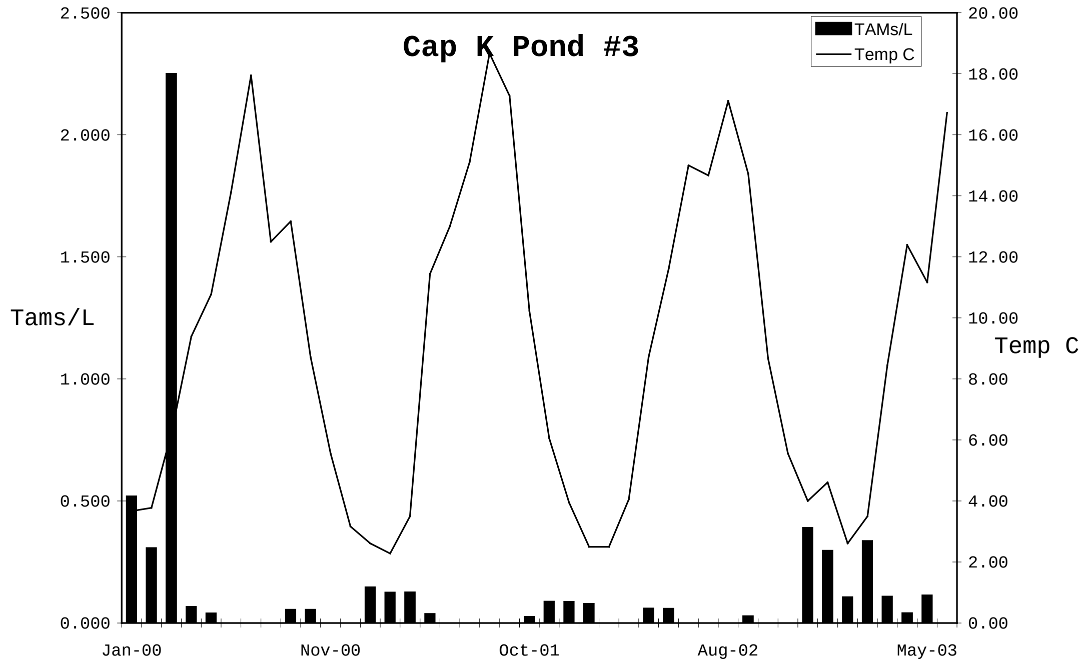

Figure 4. TAMs/L versus temperature in Cap K Ranch pond 4, January 2000 through December 2006.

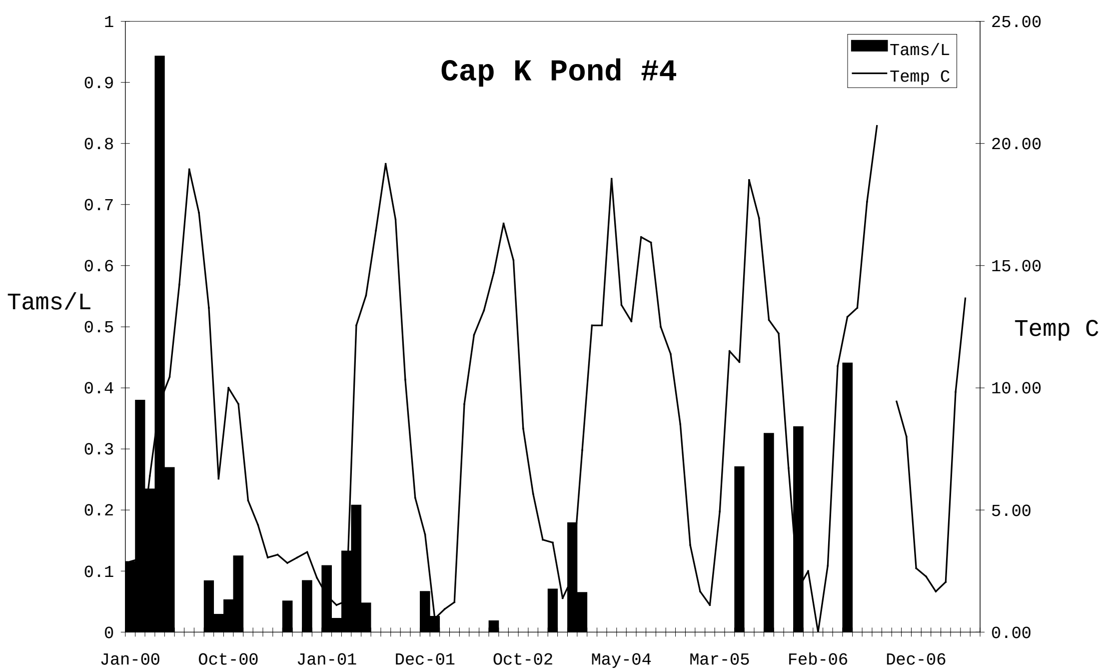

Figure 5. TAMs/L versus temperature in Cap K Ranch pond 5, May 2002 through April 2007.

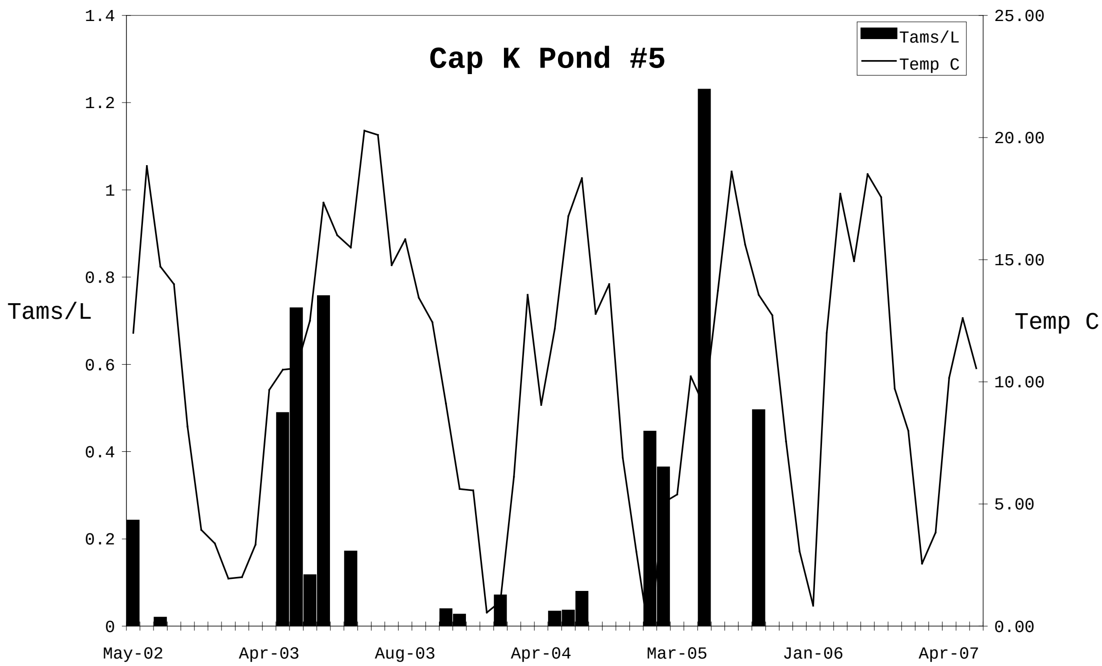

Figure 6. TAMs/L versus temperature in Cap K Ranch pond 6 outlet, August 1998 through June 2002 before the wetland biofilter was constructed.

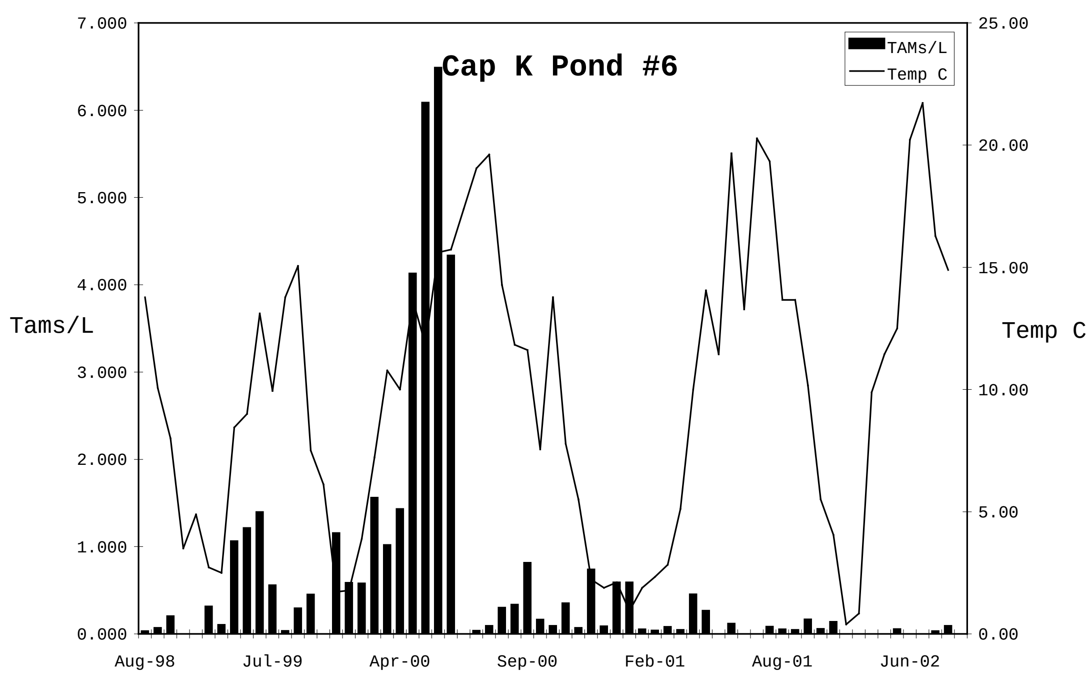

Figure 7. TAMs/L versus temperature in Cap K Ranch wetland biofilter outlet (below pond 6), May 2003 through October 2005.

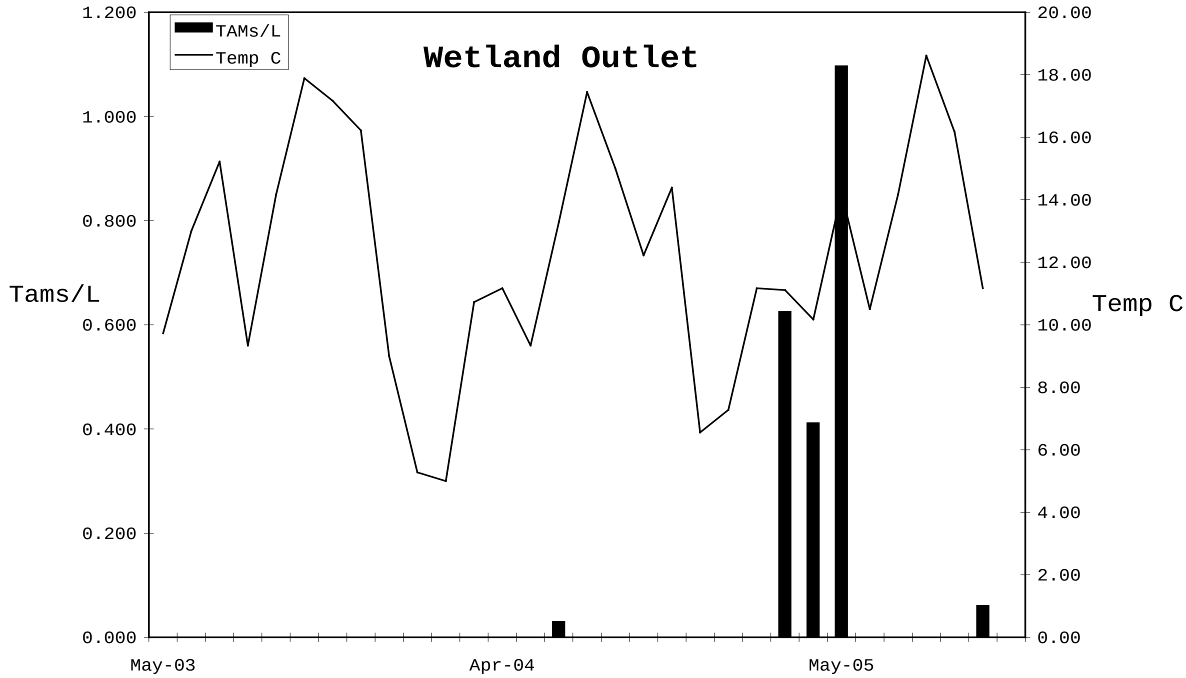

Figure 8. TAMs/L versus temperature, Fryingpan River at Ruedi Dam outlet, October 1998 through August 2005.

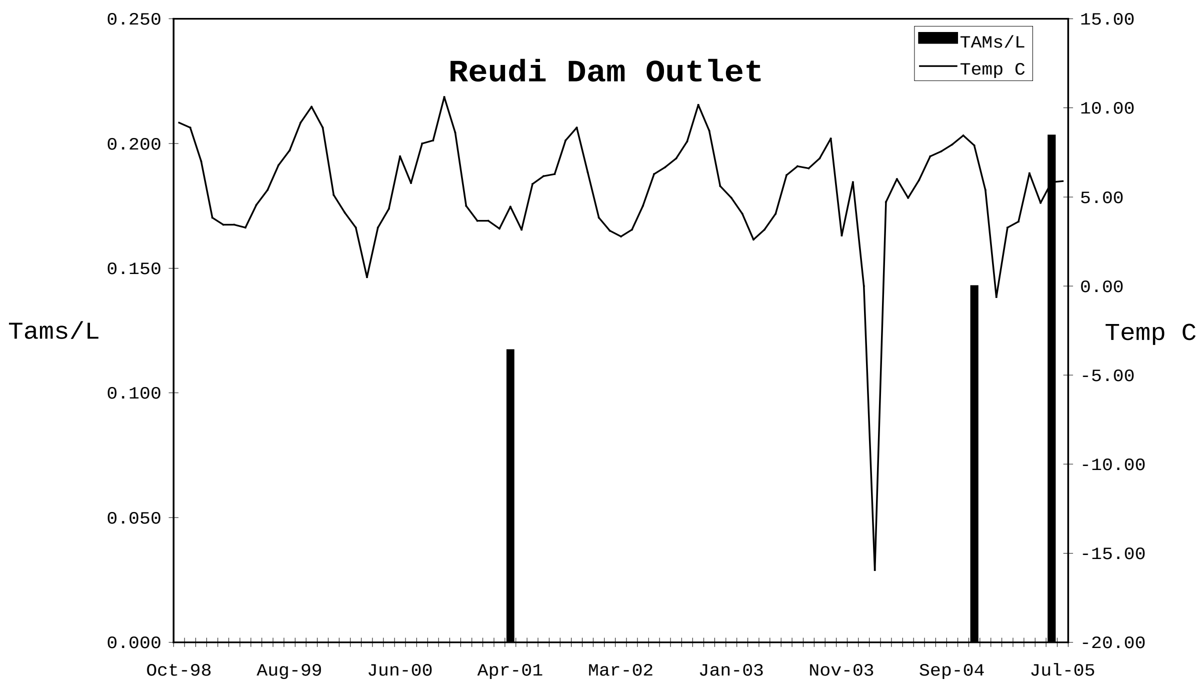

Figure 9. TAMs/L versus temperature, Fryingpan River 1.2 miles upstream of Cap K Ranch pond 6 outlet, November 1999 through December 2006.

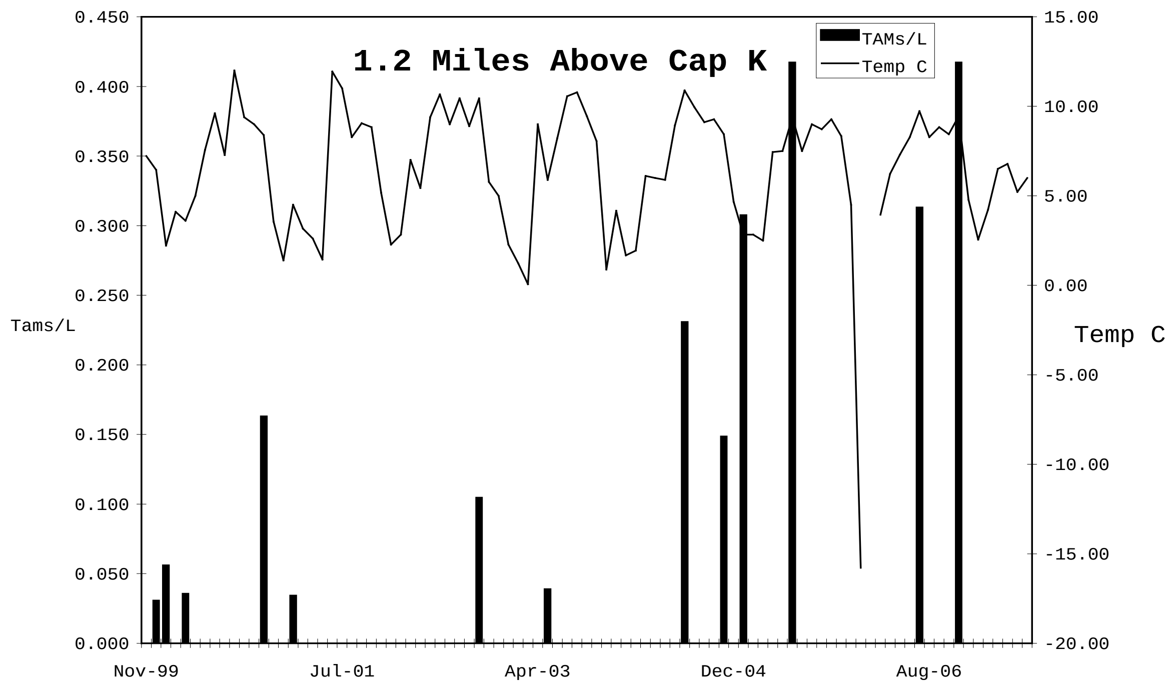

Figure 10. TAMs/L versus temperature, Fryingpan River at Taylor Creek Island Pool, August 1998 through June 2007.

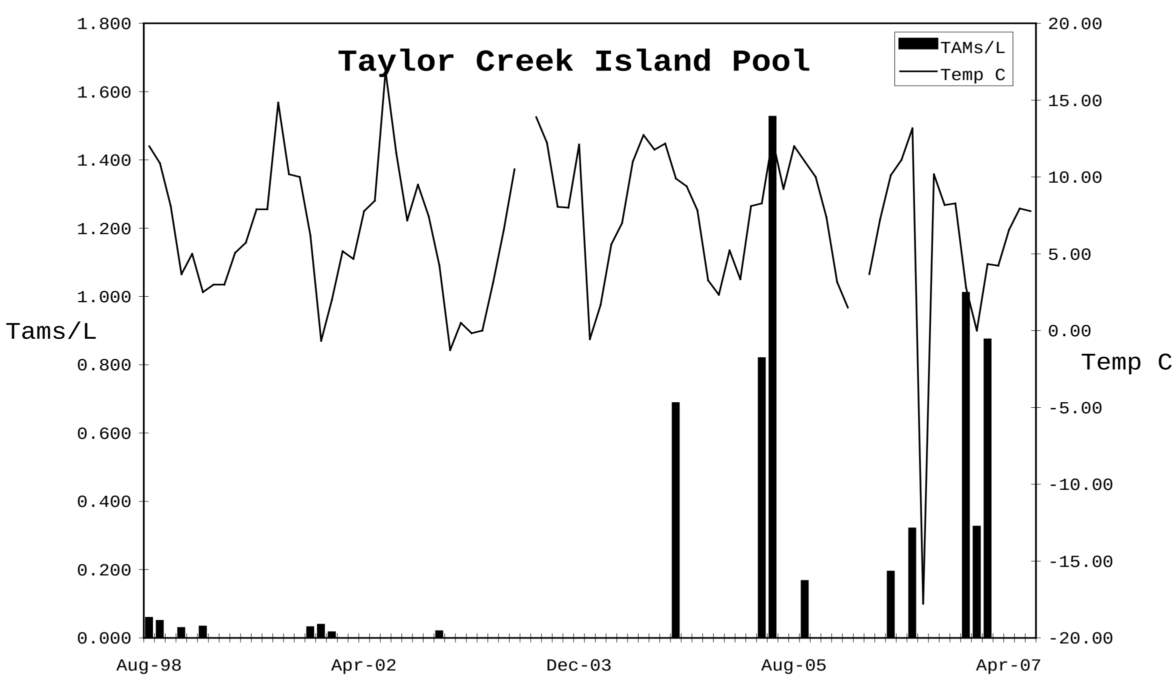

Figure 11. GPS-generated surface map(s) on Cap K Ranch ponds 1 through 6.

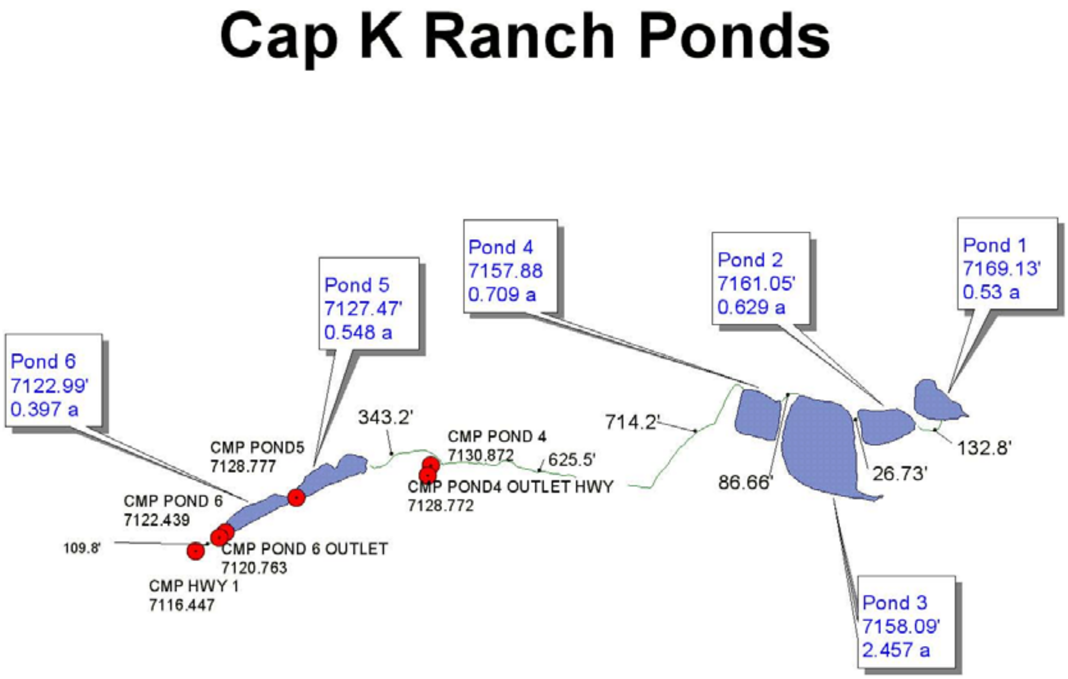

Table 1A. Month and year comparisons of estimated densities (N/L) of triactinomyxon (TAM) actinospores in pond #1 on the Cap K Ranch, Fryingpan River basin, January 2000 through May 2007.

Month	2000	2001	2002	2003	2004	2005	2006	2007	2012	2013
Jan	0	0.086	0	0.149	0	0	0	0		
Feb	0.19	0.043	0.143	0	0	0	0	0.439		
Mar	0.29	0.116	0	0	0	0	0	0		
April	0.40	0	0	0.062 [a]	0	0.828	0	0		
May	0.40	0	0	0.337	Ns	0	0	0		
June	0.20	0	0	0.039	Ns	0	0	Ns		
July	0.038	0	0.185	0.064	Ns	0	0	Ns		
Aug	0.038	0	0.026	0	Ns	0	0	Ns		
Sept	0.031	Ns	0	0	Ns	0	0	Ns		
Oct	0.055	0.175	0	0	Ns	0	0	Ns		
Nov	0.062	0	0	0	0	0	0	Ns		
Dec	0.120	0	0.182	0.043	0	0	0	Ns		
Mean	0.152	0.038	0.045	0.056	0	0.069	0			

Ns – no sample collected
[a]**:** Average of 2 samples on two different days

Table 2A. Month and year comparisons of estimated densities (N/L) of triactinomyxon (TAM) actinospores in pond #2 on the Cap K Ranch, Fryingpan River basin, January 2000 through May 2007 and April 2012 through March 2013.

Month	2000	2001	2002	2003	2004	2005	2006	2007	2012	2013
Jan	1.21	0.803	1.785	1.082	0.139	0	0.126	0.340	Ns	0.052
Feb	2.58	0.361	0.060	0.291	0.356	0	0.987	0	Ns	0
Mar	4.22	0.776[b]	0.096	0.351	0.028	0.603	0	0.367	Ns	0
April	17.6[a]	0.423	0.038	2.046 [a]	0.176	0	4.49	0	1.294	
May	2.71	0.951	0.370	0.703	0.317	2.037	1.612	0	0.653	
June	3.49	0.0911	0	0.874	0.045	1.524	0	Ns	0.0859	
July	0.172	0.067	1.017	0	0	0.175	0	Ns	0.164	
Aug	0.617	0.043	0.318	0	0	0.137	0.356	Ns	0	
Sept	0.745	Ns	0.495	0	0	0	0	Ns	0.0657	
Oct	0.981	0.317	2.588	0	0	0.471	0	Ns	0.151	
Nov	0.670	0.855	1.743	0.125	0	0.565	0	Ns	0	
Dec	1.74	0.950	1.718	0	0.385	0.482	0.170	Ns	0.360	
Mean	3.06	0.512	0.852	0.456	0.121	0.500	0.645		0.235 [c]	

Ns – no sample collected
[a]**:** Average of 2 samples on two different days
[b]**:** Average of 13-24 hour composite samples of 500 gallons of water filtered over a 24-hour period between March 1 and March 18, 2001. Daily estimates of TAM densities ranged from 0.39 to 1.55 TAMs/L across the 18-day period.
[c]**:** 2012 mean TAM density was calculated using the January to March 2013 data for missing 2012 data.

Table 3A. Month and year comparisons of estimated densities (N/L) of triactinomyxon (TAM) actinospores in pond #3 on the Cap K Ranch, Fryingpan River basin, January 2000 through June 2003.

Month	2000	2001	2002	2003	2004	2005	2006	2007	2012	2013
Jan	1.15	0.144	0.075	0.293						
Feb	0.30	0.122	0	0.103						
Mar	0.56	0.123	0	0.333						
April	0.63	0.0343	0.056	0.0715						
May	0.036	0	0.055	0.110						
June	0	0	0	0						
July	0	0	0							
Aug	0	0	0							
Sept	0.052	Ns	0.025							
Oct	0.052	0.022	0							
Nov	0	0.0841	0							
Dec	0	Ns	0.386							
Mean	0.232	0.048	0.050							

Ns – no sample collected

Table 4A. Month and year comparisons of estimated densities (N/L) of triactinomyxon (TAM) actinospores in pond #4 on the Cap K Ranch, Fryingpan River basin, January 2000 through June 2003.

Month	2000	2001	2002	2003	2004	2005	2006	2007	2012	2013
Jan	0.11	0.107	0.024	0.069						
Feb	0.38	0.0205	0	0						
Mar	0.23	0.131	0	0.177						
April	0.40	0.206	0	0.032 [a]						
May	0.27	0	0	0						
June	0	0	0	0						
July	0	0	0.017							
Aug	0	0	0							
Sept	0.082	Ns	0							
Oct	0.027	0	0							
Nov	0.007[b]	0	0							
Dec	0	0.065	0							
Mean	0.126	0.048	0.0034							

Ns – no sample collected

[a]**:** Average of 2 samples on two different days

[b]**:** The average of 7 -500 gallon samples collected over an 11-day period from November 11-17, 2000.

Table 5A. Month and year comparisons of estimated densities (N/L) of triactinomyxon (TAM) actinospores in pond #5 on the Cap K Ranch, Fryingpan River basin, July 20020 through June 2003 and April through August 2012.

Month	2000	2001	2002	2003	2004	2005	2006	2007	2012	2013
Jan				0						
Feb				0						
Mar				0						
April				0.243					0	
May				0.423					0	
June				0.375					0.101	
July			0						0	
Aug			0.018						0	
Sept			0						Ns[d]	
Oct			0						Ns[d]	
Nov			0							
Dec			0							
Mean										

Ns – no sample collected

Table 6A. Month and year comparisons of estimated densities (N/L) of triactinomyxon (TAM) actinospores in pond #6 on the Cap K Ranch, Fryingpan River basin, August 1998 through October 2002 and June through August 2012.

Month	1998	1999	2000	2001	2002	2003	2006	2007	2012	2013
Jan	Ns	0.305	1.28	0.401 [a]	0					
Feb	Ns	0.094	0.93	0.037 [a]	0					
Mar	Ns	Ns	0.63	0.055 [a]	0					
April	Ns	1.057	1.695	0.350 [a]	0					
May	Ns	1.215	5.100	0	0.046	0				
June	Ns	1.389	5.405	0.055 [a]	0	0			0	
July	Ns	0.550	0.014 [a]	0	0				0	
Aug	0.024	0.026	0.188 [a]	0.036	0.0231				0	
Sept	0.061	0.290	0.569 [a]	Ns	0.0845				Ns[d]	
Oct	0.195	0.440	0.121 [a]	0.159	0				Ns[d]	
Nov	0	0	0.202 [a]	0.050	Ns					
Dec	0	1.15	0.794 [a]	0.131	Ns					
Mean	0.056	0.592	1.41	0.116	0.0154					

Ns – no sample collected

[a]**:** Average of 2 samples on two different days

[d]**:** no water flowing in or out of pond 6 and the pond leve

Table 7A. Total trout captured (highlighted by asterisk) for each species/strain and trout population estimates (in parentheses) for 2002 through 2013 for Pond #1 on the Cap K Ranch.

Date (mm/yyyy)	**Total Brook Trout**	**YOY Brook Trout**	**TAS Rainbow Trout**	**Cap K “Wild” Rainbow Trout[a]**	**Adipose HOFER Rainbow Trout**	**Tiger Trout**	**Wild Brown Trout**	**Splake**	**Total All Trout**
11/2002	405*	51*	43*	50*	---	---	3*	---	501*
6/2003	58*	34*	17*	44*	---	---	0	---	119*
4/2004	95*	20*	21*	39* **(2)**[a]	---	---	2*	---	159*
10/2004	38*	20*	4*	5* **(1)**[a]	---	---	1*	3	51*
10/2005	85*	14*	3*	35* **(1)**[a]	47*	---	0	6	176*
10/2006	83*(107)	26*(69)	0	31* (61) **(3)**[a]	66*(147)	0	1(1)	1(1)	194* (277)
10/2007	192*(332)	63*(74)	0	61* (85) **(7)**[a]	80*(105)	48*(85)	0	1(1)	382* (588)
10/2008	72*(306)	6*(6)	0	538*(59) **(0)**[a]	93*(99)	64*(68)	0	0	279* (306)
11/2009	82* (136)	66*(93)	**“Wild”**	28* (58) **(3)**[a]	98* (177)	40*(53)	2*(2)	1(1)	251* (430)
11/2010	106* (138)	55* (92)	**HOFER**	29*(32) **(3)**[a]	54*(56)	36* (41)	1* (1)	0	226* (259)
11/2011	59* (Ne)	11*(11)	12*(13)	13* **(2)**[a]	32*(33)	27*(28)	1*(1)	0	135* (170)
11/2012	119* (133)	47* (Ne)	0	26* **(15)**[a]	82*(83)	35*(35)	1*(1)	1*(1)	264*(274)
11/2013	82* (94)	2* (2)	0	18*(18) **(4)**[a]	38*(52)	31*(32)	1*(1)	0	189*

Note(*): All brook trout and brown trout captured in pond # 1 highlighted with an asterisk in a given year were killed for disease sampling, given away for food, or transferred to pond # 3 for recreational fishing.

Note (**[a])**: Numbers in parentheses in **BOLD** print are the actual number of “wild” (unmarked) YOY Cap K rainbow trout fingerlings captured during the fall electrofishing operations in pond #1. From 2010 and all subsequent years genetic microsatellite DNA testing (conducted at U. of CA-Davis) indicated the vast majority of these fish are progeny of HOFER strain rainbow trout parents, proving that pure HOFER rainbow trout are capable of surviving to maturity and spawning successfully under natural conditions when protected from angler harvest.

Note: Ne= no estimate due to inadequate depletions through 16 - 20 minute electrofishing passes.

Table 8A. Total trout captured (highlighted by asterisk) for each species/strain and trout population estimates (in parentheses) for 2002 through 2013 for Pond #2 on the Cap K Ranch.

Date mm/yyyy	**Total Brook Trout**	**YOY Brook Trout**	**TAS Rbow Trout**	**Cap K “Wild” Rainbow Trout[a]**	**Adipose HOFER Rainbow Trout**	**Tiger Trout**	**Wild Brown Trout**	**Splake**	**Total All Trout**
11/2002	455*	66*	42*	20* (0)	---	---	5*	---	522*
6/2003	95*	10*	8*	23* (0)	---	---	3*	---	129*
4/2004	219*	15*	47*	49* (2)	---	---	6*	---	321*
10/2004	78*	55*	2*	12* (4)	---	---	1*	3*	96*
10/2005	328*(Ne)	43*(54)	4*(4)	43* (Ne)	8*(163)	---	0	14*(21)	469*(Ne)
10/2006	625*(641)	390(483)*	0	56* (87)	61*(66)	11*(Ne)	0	7*(Ne)	642*(819)
10/2007	303*(541)	122*(153)	0	99* (113)**(59)**[a]	35*(45)	37*(Ne)	0	1*(1)	470*(684)
10/2008	123*(327)	12*(21)	**YOY**	74*(356 **(1)**[a]	63*(96)	58*(84)	0	5*(Ne)	323*(660)
11/2009	301* (360)	196* (297)	**Cap K**	49*(57)	110*(123)	40*(42)	0	2*(2)	502*(579)
11/2010	360*(483)	210* (360)	**RBT**	41* (99) **(12)**[a]	52*(68)	25*(26)	0	1*(1)	479*(638)
11/2011	128* (190)	35*(Ne)	(43)	34* (49) **(6)**[a]	47*(60)	18*(20)	0	0	227*(317)
11/2012	411* (555)	92* (Ne)	0	25*(30) **(1)**[a]	100*(141)	40*(41)	0	0	576*(743)
11/2013	283* (410)	20* (Ne)	0	20* (0)	58*(59)	29*(29)	0	0	389*(465)

Note(*): All brook trout and brown trout captured in pond # 2 highlighted with an asterisk in a given year were killed for disease sampling, given away for food, or transferred to pond # 3 for recreational fishing.

Note (**[a]**): Numbers in parentheses in **BOLD** print are the actual number of “wild” (unmarked) YOY Cap K rainbow trout fingerlings captured during the fall electrofishing operations in pond # 2. From 2010 and all subsequent years genetic microsatellite DNA testing (conducted at U. of CA-Davis) indicated the vast majority of these fish are progeny of HOFER strain rainbow trout parents, proving that pure HOFER rainbow trout are capable of surviving to maturity and spawning successfully under natural conditions when protected from angler harvest.

Note: Ne= no estimate due to inadequate depletions through 16 - 20 minute electrofishing passes.

Table 9A. Total trout captured by species/strain (2004) and trout population estimates (2012) by species/strain for Pond #3 on the Cap K Ranch.

Date (mm/yyyy)	**Total Brook Trout**	**YOY Brook Trout**	**TAS Rainbow Trout**	**Cap K “Wild” Rainbow Trout**	**Adipose HOFER Rainbow Trout**	**Tiger Trout**	**Wild Brown Trout**	**Splake**	**Total All Trout**
4/2004	748	75	49	55	0	0	2	0	854
11/2012	1,152	63	0	65	431	92	41	2	1,800

The population estimates were conducted using the Peterson Mark and Recapture
Population estimation procedure by marking known numbers of fish capture by fyke netting and marking for a number of days, and then conducting boat electrofishing operations for the “recapture” runs `

Table 10A. Total trout captured (highlighted by asterisk) and population estimates (in parentheses) for 2006 through 2013 by species/strain for Pond #4 on the Cap K Ranch.

Date (mm/yyyy)	Total Brook Trout	YOY Brook Trout	TAS Rainbow Trout	Cap K "Wild" Rainbow Trout	Adipose HOFER Rainbow Trout	Tiger Trout	Wild Brown Trout	Splake	Total All Trout
10/2006	46*	7*	1*	3*	22*	1*	5*	0	77*
10/2007	66*(81)	7*(7)	0	11*(53)	66*(79)	8*(8)	14*(14)	1*(1)	165*(195)
10/2008	36*(40)	5*(5)	0	5*(6)	48*(49)	14*(16)	15*(15)	1*(1)	119*(125)
11/2009	25*(33)	11*(16)	0	4*(6)	69*(77)	19*(23)	13*(13)	1*(1)	131*(149)
11/2010	37*(53)	13*(17)	0	3*(3)	38*(44)	17*(18)	9*(9)	0	104*(120)
11/2011	14*(17)	2*(3)	0	5*(5)	22*(Ne)	8* (Ne)	9*(9)	1*(1)	60*(103)
11/2012	74*(101)	21*(Ne)	0	0*	46*(54)	32*(38)	14*(15)	0	177*(214)
11/2013	57*(70)	5*(Ne	0	3*(Ne)	43*(56)	31*(34)	12*(12)	0	146*(173)

Note(*): Numbers highlighted with an asterisk for pond 4 are numbers captured, NOT a population estimate.

Note ([a]): Numbers in parentheses in **BOLD** print are the actual number of "wild" (unmarked) YOY Cap K rainbow trout fingerlings captured during the fall electrofishing operations in pond #1. From 2010 and all subsequent years genetic microsatellite DNA testing (conducted at U. of CA-Davis) indicated the vast majority of these fish are progeny of HOFER strain rainbow trout parents, proving that pure HOFER rainbow trout are capable of surviving to maturity and spawning successfully under natural conditions when protected from angler harvest.

Note: Ne= no estimate due to inadequate depletions through 16 - 20 minute electrofishing passes.

Table 11A. Total trout captured (highlighted by asterisk) by species/strain (2002 through 2005) and trout population estimates (in parentheses) for 2006 through 2012 for Pond #5 on the Cap K Ranch.

Date mm/yyyy	Total Brook Trout	YOY Brook Trout	TAS Rainbow Trout	CAP K "Wild" Rainbow Trout	Adipose HOFER Rainbow Trout	Tiger Trout	Wild Brown Trout	HOFER "wild" Rainbow Trout	Total All Trout
11/2002	1	0	19	0	0	0	31[a]	0	51
10/2004	108*(Ne)	77*(110)	4*(4)	0	0	0	2*(2)	0	113*(197)
06/2005	37*(59)	0	1	1*(1)	0	0	3*(3)	0	41*(58)
11/2006	2*(2)	0	0	0	0	0	2*(2)	0	4*(4)
10/2007	2*(2)	0	0	0	0	0	12*(12)	0	14*(14)
10/2008	1*(1)	0	0	0	0	0	7*(8)	0	9*(10)
11/2009	1*(1)	1*(1)	0	2*(2)	14*(19)	0	8*(8)	14*(Ne)	24*(25)
11/2010	3*(4)	1*(1)	0	3*(3)	4*(4)	0	5*(5)	0	15*(15)
11/2011	2*(2)	0	0	0	2*(2)	0	6*(6)	2*(2)	12*(12)
11/2012	1*(1)	1*(1)	0	0	140*168)	0	5*(5)	12*(17)	158*(187)
11/2013	ns	Ns	ns	ns	ns	ns	ns	Ns	Ns

Note(*): All brook trout and brown trout captured in pond # 5 have been killed for disease sampling, given away for food, or transferred to pond # 3 for recreational fishing.

ns: no sampling in November 2013

[a]: An actual population estimate. The average size of these brown trout was 559 mm TL, ranging in size from 410 to 630 mm TL. It was hypothesized that these large fish had been functioning as efficient predators feeding on immigrant YOY and yearling brook trout that came into the pond via the drainage ditch from pond 4. It is noteworthy only 1 brook trout (330 mm TL) was captured in November 2002 and that once these fish had been moved up to pond 3, 108 YOY and yearling brook trout were captured in October 2004, when only 8 brown trout were present in pond 5 and none of them were over 400 mmTL.

[b]: Includes 3 splake that average 230 mm TL; immigrants from pond 4.

Table 12A. Total trout captured by species/strain on June 8, 2005 in Pond # 6 on the Cap K Ranch.

Date (mm/yyyy)	Total Brook Trout	YOY Brook Trout	TAS Rainbow Trout	Cap K "Wild" Rainbow Trout	Adipose HOFER Rainbow Trout	Tiger Trout	Wild Brown Trout	Splake	Total All Trout
6/2005	25	0	0	1	0	0	71[a]	0	98

Note ([a]):The brown trout were most likely upstream migrants from the Fryingpan River that were able to swim into pond 6 once the 4 foot high vertical culvert had been removed with the completion of the wetland "biofilter" constructed in 2003 and an overflow channel for the wetland was installed to handle excess inflow.

Table13 A. Cranial concentrations of *Myxobolus cerebralis* myxospores in brook trout and Tiger trout from pond # 1 on the Cap K Ranch collected between July 2000 and November 2009.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
07/15/2000	Brook	Adult	10	6	50,450	84,083	5,056 – 312,467
10/26/2002	Brook	1+	24	24	169,542	169,542	2,656 – 980,583
10/26/2002	Brook	2+	13	13	159,526	159,526	10,450 – 749,667
10/26/2002	Brook	1+	7	4	26,684	46,697	10,622 – 84,000
04/02/2004	Brook	≥ 1+	40	33	118,472	143,603	2,778 – 1,233,333
10/28/2004	Brook	1+	9	8	142,716	160,556	1,111 – 380,000
10/31/2005	Brook	YOY	10	10	40,565	40,565	2,156 - 143,733
10/31/2005	Brook	1+	20	8	44,012	110,029	900 – 594,806
06/13/2006	Brook	1+	10	9	50,056	55,617	2,500 – 243,3333
10/30/2006	Brook	1+	10	10	89,385	89,385	3,367 – 240,717
10/30/2007	Brook	YOY	10	5	20,056	40,112	5,556 – 76,667
10/30/2007	Brook	1+	10	7	255,333	364,761	3,333 – 583,333
10/30/2008	Brook	YOY	9	3	9,683	29,048	11,244 – 56,222
10/30/2008	Brook	1+	12	9	97,311	129,748	2,811 – 595,956
11/05/2009	Brook	YOY	10	3	7,130	23,767	3,478 – 47,089
11/05/2009	Brook	1+	10	7	67,204	96,006	12,456 – 248,267
12/04/2007	PL Tiger[a]	1	38	21	7,648	13,839	2,794 – 117,367
10/30/2007	Tiger	1+	10	6	4,556	7,593	1,667 – 14,444
10/30/2008	Tiger	1+	10	10	243,442	243,442	42,167 – 666,233
11/05/2009	Tiger	2+	5	4	335,898	419,872	12,589 – 1,197,411

“Tiger” trout – a sterile hybrid produced by fertilizing the eggs of a female brown trout with the sperm (milt) of a male brook trout.

[a] PL Tiger: Tiger trout exposed to a one-time dose of 2,000 *Myxobolus cerebralis* TAMs and then held specific-pathogen free (SPF) water for 6 months. These results are shown above for comparative purposes with Tiger trout in Cap K Ranch ponds # 1 for approximately 5 months.

Table 13A (continued). Cranial concentrations of *Myxobolus cerebralis* myxospores in three strains of rainbow trout from pond # 1 on the Cap K Ranch collected between October 2002 and November 2012.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
10/26/2002	TAS o	18	20	19	61,520	64,758	6,456 – 203,600
10/26/2002	TAS r	18	20	19	99,047	122,681	15,917 – 311,500
10/30/2007	Wild Rbt	1+	8	2	141,250	556, 500	3,333 – 1,126,667
10/30/2008	Wild Rbt	2+	5	3	7,522	12,537	2,811 – 26,367
10/31/2005	HOFER	1+	10	0	0	0	------
06/13/2006	HOFER	2	10	1	667	6,667	6,667
10/30/2006	HOFER	1+	14	4	721	2,525	1,683 – 3,367
10/30/2006	HOFER	2+	10	2	7,527	37,634	3,367 – 71,900
10/30/2007	HOFER	2+	3	0	0	0	0
10/30/2008	HOFER	1+	13	1	678	8,817	8,817
10/30/2008	HOFER	4+	8	4	54,501	109,003	22,467 – 363,922
11/05/2009	HOFER	1+	10	2	344	1,722	1,572 – 1,872
11/05/2009	HOFER	3+	2	0	0	0	0
11/01/2012	HOFER	≥3+	2	0	0	0	0

Notes: YOY – "young-of the-year" trout, i.e., approximately 7-8 months of age.

Notes: "Eye marks" are elastomer visual implant fluorescent pigment sub-cutaneous eye marks behind the right or left eye. r – red eye mark; o – orange eye mark ; g – green eye mark; y – yellow eye mark.

TAS denotes Tasmanian strain rainbow trout stocked as catchable size trout in March 2002.

Wild Rbt denotes naturally spawned, unmarked rainbow trout produced in the inlet spring to pond # 1.

HOFER strain rainbow trout originating from aquaculture facilities in Germany that are known to be highly resistant to *Myxobolus cerebralis* infection(s).

Table 14A. Cranial concentrations of *Myxobolus cerebralis* myxospores in brook trout and Tiger trout from pond # 2 on the Cap K Ranch collected between October 2002 and November 2013.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
Cap K Ranch Pond # 2							
10/26/2002	Brook	YOY	25	25	183,033	183,033	19,722 – 858,700
10/26/2002	Brook	2+	20	13	79,696	122,609	17,644 – 456,467
04/07/2004	Brook	1+	39	30	44,162	57,410	556 – 338,889
10/28/2004	Brook	YOY	20	16	69,361	86,701	1,111 – 468,889
10/28/2004	Brook	1+	10	9	23,111	25,679	2,222 – 55,556
11/03/2005	Brook	YOY	10	9	35,292	44,115	2,333 – 80,500
06/13/2006	Brook	1+	7	6	180,556	210,648	556 – 536,667
10/31/2006	Brook	1+	10	10	57,907	57,907	5,050 – 175,067
10/31/2007	Brook	YOY	10	4	63,028	157,570	4,444 – 528,889
10/31/2007	Brook	1+	10	8	19,611	24,514	2,222 – 71,111
10/29/2008	Brook	YOY	11	7	20,700	32,529	2,811 – 84,333
10/29/2008	Brook	1+	9	6	47,262	70,893	16,867 – 140,556
10/29/2008	Brook	2+	7	4	77,304	135,282	26,189 – 293,028
11/05/2010	Brook	YOY	12	6	24,444	48,072	4,967 – 125,889
11/05/2010	Brook	≥1+	11	7	11,429	17,960	4,200 – 37,339
4/20/2012	Brook	≥2+	15	9	14,187	23,646	4,917 – 76,067
11/01/2012	Brook	YOY	10	4	15,588	38,971	8,711 – 62,150
11/01/2012	Brook	1+	10	8	34,043	42,554	4,183 – 97,367
4/03/2013	Brook	≥2+	10	6	42,416	70,693	4,211 – 154,233
11/06/2013	Brook	YOY	10	5	14,944	29,888	3,728-99,600
11/06/2013	Brook	≥1+	10	0	0	0	------
12/04/2007	PL Tiger[a]	1	38	21	7,648	13,839	2,794 – 117,367
10/31/2007	"Tiger"	1+	10	7	19,056	27,223	1,111 – 106,667
10/29/2008	"Tiger"	1+	10	10	435,441	435,441	87,144 – 1,678,233
11/06/2013	"Tiger"	1+	10	10	309,923	309.923	11,744 – 728,000

Notes: "Tiger" trout – a sterile hybrid produced by fertilizing the eggs of a female brown trout with the sperm (milt) of a male brook trout.

[a] PL Tiger: Tiger trout exposed to a one-time dose of 2,000 *Myxobolus cerebralis* TAMs and then held specific-pathogen free (SPF) water for 6 months. These results are shown above for comparative purposes with Tiger trout in Cap K Ranch ponds # 1 for approximately 5 months and collected on 10/31/2007 and the same cohort collected on 10/29/2008 after approximately 17 months of exposure in pond #1.

Table 14A (continued). Cranial concentrations of *Myxobolus cerebralis* myxospores in three strains of rainbow trout from pond # 2 on the Cap K Ranch collected between October 2002 and November 2013.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
Cap K Ranch Pond # 2							
10/26/2002	TAS g	1+	17	17	368,441	368,441	43,700 – 1,403,733
10/26/2002	TAS o	1+	20	20	432,369	432,369	32,167 – 1,234,833
11/03/2005	TAS g	4+	1	1	882,222	882,222	-----
10/31/2007	TAS Rbt	6+	3	1	1,481	4,444	4,444
11/03/2005	Wild Rbt	≥ 1+	8	4	65,079	130,159	9,989 – 440,611
06/13/2006	Wild Rbt	1+	1	1	22,222	22,222	------
10/31/2006	Wild Rbt	> 2+	1	1	20,950	20,950	20,950
10/31/2007	Wild Rbt	1+	8	3	8,611	22,963	1,111 – 64,444
10/29/2008	Wild Rbt	1+	10	7	75,010	107,158	2,811 – 390,744
4/3/2013	Wild Rbt	> 2+	4	1	112,396	449,583	449,583
11/03/2005	HOFER	1	10	0	0	0	-----
06/13/2006	HOFER	1+	1 0	1	333	3,333	------
10/31/2006	HOFER	1+	11	2	612	3,367	3,367
10/31/2006	HOFER	2+	10	4	2,204	5,511	1,683 – 11,783
10/31/2007	HOFER	2+	6	0	0	0	0
10/29/2008	HOFER	1+	9	5	2,499	4,498	2,811 – 11,244
10/29/2008	HOFER	2+	2	1	1,406	2,811	2,811
11/05/2010	HOFER	≥2+	10	0	0	0	0
4/20/2012	HOFER	≥3+	10	1	1,896	18,961	18,961
4/03/2013	HOFER	≥2+	14	0	0	0	0
11/06/2013	HOFER	1+	10	0	0	0	0
11/06/2013	HOFER	≥4+	1	0	0	0	0

Notes: TAS denotes Tasmanian strain rainbow trout stocked as catchable size trout in March 2002. HOFER strain rainbow trout originating from aquaculture facilities in Germany that are known to be highly resistant to *Myxobolus cerebralis* infection(s).

"Eye marks" are elastomer visual implant fluorescent pigment sub-cutaneous eye marks behind the right or left eye. r – red eye mark; o – orange eye mark ; g – green eye mark; y – yellow eye mark.

Wild Rbt denotes naturally spawned, unmarked rainbow trout produced in the stream channel connecting ponds #1 and 2.

Table 15A. Cranial concentrations of *Myxobolus cerebralis* myxospores in various species and strains of trout from pond # 3 on the Cap K Ranch collected between October 2002 and April 2013.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
Cap K Ranch Pond # 3							
10/26/2002	Brook	2+	20	20	135,894	135,894	14,500 – 289,922
04/07/2004	Brook	≥ 1+	40	31	54,811	70,726	1,111 – 450,000
11/04/2005	Brook	YOY	10	5	3,531	7,061	2,650 – 12,933
11/04/2005	Brook	1+	20	10	15,058	30,225	2,822 – 134,911
11/14/2005	Brook	YOY	31	19	6,290	10,263	1,111 – 60,556
06/13/2006	Brook	1+	10	8	95,333	119,167	2,222 – 385,000
11/02/2007	Brook	YOY	10	5	4,944	9,888	556 – 35,556
11/02/2007	Brook	1+	10	5	20,528	41,056	13,333 – 71,111
11/05/2012	Brook	≥1+	60	22	36,239	98,834	4,739 – 463,500
4/03/2013	Brook	≥2+	10	5	17,554	35,108	7,728 – 109,356
11/02/2007	“Tiger”	1+	10	9	8,806	9,784	1,111 – 36,994
04/17/2013	“Tiger”	≥2+	2	2	84,133	84,133	43,250 – 125,017
10/26/2002	TAS y	18	24	22	64,667	70,546	5,828 – 279,933
10/26/2002	TAS r	18	20	20	110,391	110,391	4,272 – 529,306
11/04/2005	HOFER	1	10	0	0	0	------
06/13/2006	HOFER	1+	10	5	2,667	5,333	833 – 10,000
11/07/06	HOFER	1+	10	0	0	0	0
11/07/06	HOFER	2+	10	1	2,525	25,250	25,250
11/02/07	HOFER	2+	2	0	0	0	0

Notes: TAS denotes Tasmanian strain rainbow trout stocked as catchable size trout in March 2002.

“Eye marks” are elastomer visual implant fluorescent pigment sub-cutaneous eye marks behind the right or left eye. r – red eye mark; o – orange eye mark ; g – green eye mark; y – yellow eye mark.

YOY – “young-of the-year” trout, i.e., less than 1 year of age.

“Tiger” trout – a sterile hybrid produced by fertilizing the eggs of a female brown trout with the sperm (milt) of a male brook trout.

HOFER trout are HOFER strain rainbow trout originating from aquaculture facilities in Germany that are known to be highly resistant to *Myxobolus cerebralis* infection(s).

Table 16A. Cranial *Myxobolus cerebralis* myxospore burden in rainbow trout and brook trout from pond #4on the Cap K Ranch collected between October 2002 and November 2013.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
Cap K Ranch Pond # 4							
10/26/2002	Brook	2+	13	12	149,811	162,295	7,456 – 636,194
06/08/2005	Brook	1+	8	5	81,108	129,773	24,950 – 237,722
06/07/2006	Brook	1+	6	4	6,389	9,584	5,000 – 15,556
11/07/2006	Brook	YOY	7	3	25,250	58,917	1,683 – 149,817
11/07/2006	Brook	1+	9	8	55,550	62,494	1,683 – 175,067
11/01/2007	Brook	YOY	10	8	63,694	79,618	556 – 211,111
11/01/2007	Brook	1+	10	9	68,028	75,587	7,778 – 184,444
10/28/2008	Brook	YOY	4	2	263,219	526,439	21,822 – 1,031,056
10/28/2008	Brook	1+	10	4	28,932	72,331	18,200 – 102,378
11/05/2013	Brook	YOY	1	0	0	0	------
10/26/2002	TAS o	1+	20	19	59,744	62,888	5,556 – 250,600
10/26/2002	TAS g	1+	20	17	41,010	48,247	5,494 – 230,289
10/28/2008	Wild Rbt	2+	1	0	0	0	------
11/05/2013	HOFER	1+	1	0	0	0	------
11/05/2013	“Tiger”	1+	2	0	0	0	------

Notes: YOY – “young-of the-year” trout, i.e., less than 1 year of age.

TAS denotes Tasmanian strain rainbow trout stocked as catchable size trout in March 2002.

“Eye marks” are elastomer visual implant fluorescent pigment sub-cutaneous eye marks behind the right or left eye. r – red eye mark; o – orange eye mark ; g – green eye mark; y – yellow eye mark.

Wild Rbt denotes naturally spawned, unmarked rainbow trout produced in the channel connecting ponds #3 and 4 or in the outlet of pond #4.

HOFER strain rainbow trout originating from aquaculture facilities in Germany that are known to be highly resistant to *Myxobolus cerebralis* infection(s).

“Tiger” trout – a sterile hybrid produced by fertilizing the eggs of a female brown trout with the sperm (milt) of a male brook trout.

Table 17A. Cranial concentrations of *Myxobolus cerebralis* myxospores in various species and strains of trout from ponds 5 and 6 on the Cap K Ranch collected between November 2002 and June 2005.

Collection Date Mo/Da/Yr	Species	Age (Yrs)	Sample Size		Overall Mean Myxospore Burden	Myxospores in Positive Fish	
			No.	No.+		Mean	Range
Cap K Ranch Pond # 5							
11/07/2002	TAS r	1+	21	21	336,860	336,860	6,933 – 843,922
11/07/2002	TAS y	1+	19	19	473,430	473,430	80,750 – 1,045,161
06/08/2005	Brook	1+	24	19	74,593	94,223	3,044 – 312,511
Cap K Ranch Pond # 6 (Above Biofilter)							
06/08/2005	Brook	1+	26	19	28,865	39,499	2,767 – 162,500

Notes: TAS denotes Tasmanian strain rainbow trout stocked as catchable size trout in March 2002.

“Eye marks” are elostomer visual implant fluorescent pigment sub-cutaneous eye marks behind the right or left eye. r – red eye mark; o – orange eye mark ; g – green eye mark; y – yellow eye mark.

Table 18A. Trout stocking history for the Cap K Ranch ponds, 2002 - 2012

Species or Strain	**Date mmddyy**	**Marks VIE/**	**Pond Number**					
			1	**2**	**3**	**4**	**5**	**6**
Tasmanian Rbw	03/29/2002	vie/fins	40/40	66/66	202/202	103/103	41/41	0
Splake	05/04/2004	none	100	100	0	0	0	0
Hofer Rbw 2005	06/15/2005	adipose	150	150	550	150	0	0
Hofer Rbw 2006	05/17/2006	adipose	75	75	275	75	0	0
Tiger Trout 2006	03/27/2006	none	0	75	0	0	0	0
Tiger Trout 2007	06/15/2007	none	150	250	450	150	0	0
Tiger Trout 2008	05/07/2008	none	150	250	465	150	0	0
Hofer Rbw 2008	05/07/2008	adipose	200	300	315	200	0	0
Hofer Rbw 2009	06/02/2009	adipose	150	150	575	150	0	0
Hofer Rbw 2012	07/06/2012	adipose	150	150	450	150	150	0
Tiger Trout 2012	07/06/2012	none	150	150	350	150	0	0

Table 19A. Water quality and suspended solids data for various ponds on the Cap K Ranch, tributary to the Fryingpan River approximately 8 km downstream from Ruedi Dam.

Date Mmddyy	Time (HHMM	Temp (° F)	pH	Alkalinity (mg/L)	Hardness (mg/L)	D.O. (ppm)	Suspended Solids (mL/L)
Outlet of Pond # 6							
11/10/00	1300	37.9	7.7	112	266	13.0	----
11/11/00	1400	40.9	7.6	188	291	12.0	0.1 ml/L
12/14/00	1350	36.6	7.5	120	274	9.0	< 0.1 ml/L
01/08/01	1350	35.6	7.4	137	222	9.0	< 0.1 ml/L
02/14/01	1330	36.2	7.3	137	222	8.0	0.1 ml/L
03/13/01	1130	42.0	7.6	137	239	10.0	----
03/16/01	1245	41.8	7.6	137	239	10.0	----
04/24/01	1645	59.5	7.7	137	239	10.0	----
05/09/01	1600	56.8	7.7	120	222	11.0	< 0.05 ml/L
Outlet Pond #4 or # 5							
11/11/01	1130	39.9	7.6	120	239	13.0	< 0.1 ml/L
03/09/01	1054	38.6	7.7	120	222	12.0	< 0.05 ml/L
03/15/01	1240	38.5	7.6	103	205	13.0	-----
05/09/01	1600	56.8	7.7	120	239	11.0	< 0.05 ml/L
Outlet Pond #2							
03/09/01	1238	45.1	7.6	120	239	12.0	-----
03/15/01	1200	41.1	7.6	120	239	12.0	-----

Table 20A. Length at capture and back-calculated lengths (cm) of trout from the Cap K Ranch ponds on the Fryingpan River, near Basalt, CO, October 2008.For the HOFER rainbow trout, the back-calculated length @ age 1 (L_1), is the approximate length at the time of stocking into the pond(s), which was usually in May or June of the “year class”year plus 1.

Year Class	Age	n	L_c	s.e.	L_1	s.e.	L_2	s.e.	L_3	s.e.	L_4	s.e.	L_5	s.e.	L_6	s.e.	L_7
HOFER Rainbow Trout – Pond # 1																	
2007	1+	22	24.9	0.29	16.0	0.21											
2005	3+	24	38.4	0.47	17.3	0.57	29.5	0.44	35.6	0.49							
2004	4+	23	41.9	0.37	17.3	0.42	28.7	0.42	36.1	0.62	39.5	0.44					
HOFER Rainbow Trout – Pond # 2																	
2007	1+	24	24.9	0.33	14.1	0.35											
2005	3+	10	36.8	0.49	13.9	0.38	29.2	0.92	34.5	0.43							
2004	4+	15	43.0	1.11	19.0	0.97	29.7	0.94	36.3	0.69	40.4	0.22					
HOFER Rainbow Trout – Pond # 4																	
2007	1+	11	26.6	0.30	17.5	0.83											
2005	3+	10	35.4	0.26	19.1	1.05	27.7	0.82	33.2	0.40							
2004	4+	17	37.8	0.35	17.2	0.39	26.5	0.48	31.2	0.56	35.6	0.41					
“Tiger” Trout (Brook Trout♂ X Brown Trout♀- sterile hybrid) – Pond# 1																	
2008	0+	23	19.0	0.43													
2007	1+	25	28.2	0.45	18.1	0.36											
2006	2+	2	33.3	0.70	18.3	3.05	26.2	0.50									
“Tiger” Trout (Brook Trout♂ X Brown Trout♀- sterile hybrid) – Pond# 2																	
2008	0+	21	18.8	0.45													
2007	1+	21	29.2	0.43	19.8	0.42											
2006	2+	1	16.1	----	23.3	----	32.5	----									
“Tiger” Trout (Brook Trout♂ X Brown Trout♀- sterile hybrid) – Pond# 4																	
2008	0+	1	23.3	----													
2007	1+	13	28.4	0.57	21.2	0.40											

Table 21A. Length at capture and back-calculated lengths (cm) of trout from the Cap K Ranch ponds on the Fryingpan River, near Basalt, CO, November 2009.For the HOFER rainbow trout, the back calculated length @ age 1 (L_1), is the approximate length at the time of stocking into the pond(s), which was usually in May or June of the "year class" year plus 1.

Year Class	Age	n	L_c	s.e.	L_1	s.e.	L_2	s.e.	L_3	s.e.	L_4	s.e.	L_5	s.e.	L_6	s.e.	L_7
HOFER Rainbow Trout – Pond # 1																	
2008	1+	18	25.8	0.44	12.6	0.46											
2007	2+	16	33.3	0.55	11.9	0.36	25.8	0.49									
2005	4+	15	40.2	0.64	11.6	0.46	26.6	0.41	35.1	0.60	38.0	0.61					
2004	5+	10	44.1	0.62	12.1	0.37	26.8	1.01	35.5	1.19	39.8	0.76	42.2	0.67			
HOFER Rainbow Trout – Pond # 2																	
2008	1+	13	26.2	0.25	12.3	0.37											
2007	2+	12	32.4	0.44	11.1	0.42	25.6	0.76									
2005	4+	19	39.5	0.84	11.7	0.33	25.6	0.76	32.4	0.96	36.8	0.90					
2004	5+	4	44.3	0.78	10.0	1.14	23.5	1.08	30.6	1.49	37.4	0.85	42.4	0.47			
HOFER Rainbow Trout – Pond # 4																	
2008	1+	23	25.4	0.21	12.0	0.31											
2007	2+	9	32.7	0.60	12.1	0.54	27.8	0.58									
2005	4+	16	37.9	0.48	10.9	0.40	27.1	0.45	34.1	0.82	36.2	0.63					
2004	5+	2	42.7	0.90	11.0	3.41	29.2	1.12	37.2	2.06	40.1	1.38	41.4	1.33			
Wild (Unmarked) Rainbow Trout – Pond # 1																	
2009	0+	3	13.7	0.70													
2008	1+	4	30.0	1.25	14.7	0.29											
2007	2+	18	35.3	0.39	13.4	0.43	30.0	0.57									
2006	3+	3	36.2	0.73	15.1	1.31	29.7	1.95	33.4	0.28							
Wild (Unmarked) Rainbow Trout – Pond # 2																	
2009	0+	7	13.7	0.68													
2008	1+	3	23.7	1.55	11.8	0.94											
2007	2+	26	32.3	0.45	13.1	0.32	27.6	0.38									
2006	3+	3	36.2	0.44	13.3	0.61	25.9	1.37	32.3	0.55							
Wild (Unmarked) Rainbow Trout – Pond # 4																	
2009	0+	1	11.2	-----													
2008	1+	1	30.1	-----	14.8	-----											
2007	2+	2	35.2	0.28	13.9	0.71	29.9	1.97									

Table 22A. Length at capture and back-calculated lengths (cm) of Tiger trout and Brown trout from the Cap K Ranch ponds on the Fryingpan River, near Basalt, CO, November 2009.

Year Class	Age	n	L_c	s.e.	L_1	s.e.	L_2	s.e.	L_3	s.e.	L_4	s.e.	L_5	s.e.	L_6	s.e.	L_7
				“Tiger” Trout (Brook Trout♂ X Brown Trout♀- sterile hybrid) – Pond# 1													
2008	1+	15	29.1	0.31	20.9	0.55											
2007	2+	23	34.1	0.48	13.8	0.53	28.5	0.71									
2006	3+	1	36.2	-----	14.1	-----	27.8	-----	33.7	------							
				Tiger” Trout (Brook Trout♂ X Brown Trout♀- sterile hybrid) – Pond# 2													
2008	1+	17	30.9	0.49	21.6	0.55											
2007	2+	11	36.5	0.51	17.8	1.24	31.3	0.83									
				“Tiger” Trout (Brook Trout♂ X Brown Trout♀- sterile hybrid) – Pond# 4													
2008	1+	2	29.1	1.45	18.1	2.42											
2007	2+	13	36.2	0.49	14.9	0.71	30.3	0.79									
						Brown Trout – Pond #4											
2007	2+	1	25.0	-----	11.1	-----	16.6	-----									
2006	3+	5	34.8	1.86	7.53	0.78	15.5	0.65	28.4	0.16							
2005	4+	6	40.9	1.89	9.81	0.80	21.7	1.22	32.0	2.88	37.4	1.97					
2004	5+	1	54.0	-----	16.2	----	26.0	-----	33.4	-----	40.4	-----	48.0	-----			
						Brown Trout – Pond #6											
2006	3+	3	31.2	0.82	5.38	0.70	13.4	0.70	26.4	1.47							
2005	4+	3	35.1	0.52	6.71	0.55	14.5	1.00	22.5	1.35	32.1	0.52					
2004	5+	1	38.0	-----	9.59	-----	19.5	-----	24.9	-----	32.3	-----	35.2	-----			