Evaluation of small-scale habitat manipulation to reduce the impact of the whirling disease parasite in streams

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Abstract

The effects on trout of the whirling disease parasite *Myxobolus cerebralis* were evaluated to observe whether they could be ameliorated by intervening with physical habitat manipulations. Physical stream habitat was modified at field sites in Spring Creek and Williams Fork River, Colorado, USA to reduce or eliminate habitat for the invertebrate oligochaete host of *M. cerebralis*, *Tubifex tubifex*. Data were collected before and after habitat modifications on total oligochaete and *T. tubifex* biomass, actinospore production from oligochaete samples, surface water actinospore concentrations, and prevalence and intensity of myxospore development in brown trout, *Salmo trutta*. Oligochaete biomass estimates lacked precision due to inherently patchy distribution of the target organisms. Oligochaetes quickly re-occupied a portion of habitat at the Williams Fork River site, but oligochaete biomass was depressed for nearly a year at the Spring Creek site. All *T. tubifex* in Spring Creek belonged to a susceptible lineage, but in the Williams Fork River there was a mix of susceptible and non-susceptible *T. tubifex*. Actinospore detection in filtered surface water samples showed consistent but minor reduction in density in Williams Fork River and no difference or even higher densities in Spring Creek after habitat modification. Myxospore prevalence and intensity of infection in brown trout appeared to decrease in Williams Fork River after habitat modification, but there is evidence that a similar decrease also occurred at a control site in that stream. Spring Creek showed no effect for these metrics. The differing responses may have been influenced by *T. tubifex* lineage differences. The habitat manipulations did not show sufficient promise to encourage further efforts in Colorado.

Keywords: *Myxobolus cerebralis*, *Tubifex tubifex*, brown trout

Introduction

Myxobolus cerebralis, the causative agent of salmonid whirling disease first described by Höfer (1903), was initially reported to have negative impacts on self-sustaining feral fisheries among rainbow trout *Oncorhynchus mykiss* populations in Colorado and Montana (Nehring and Walker, 1996; Vincent, 1996; Nehring et al., 1998). More recently, research in Yellowstone National Park suggests the parasite has likely played a role in the decline of the Yellowstone cutthroat trout *O. clarki bouvieri* population in Yellowstone Lake (Koel et al., 2006).

The effects of *M. cerebralis* on the trout farming industry stimulated much of the early research on the parasite (Hedrick et al., 1998). Those efforts resulted in reliable methods to minimize or eliminate disease outbreaks in aquaculture facilities (see, e.g., O'Grodnick, 1979; Hoffman, 1974). In contrast, little

attention was given to the ecology of the parasite in natural waters; however, once impacts to wild populations of rainbow trout were documented, *M. cerebralis* ecology was vigorously investigated by a large community of researchers. Parasite effects on the oligochaete host were examined (Stevens et al., 2001; Steinbach-Elwell et al., 2006), along with the longevity of infection within the oligochaete host (Gilbert and Granath, 2001) and dose-response assessments of infection prevalence among *Tubifex tubifex* and other oligochaetes (Steinbach-Elwell et al., 2009a). The spatial/temporal nature of actinospore production was examined in a reservoir (Nehring et al., 2002), and also in rivers with regard to salmonid life histories (Sandell et al., 2001; Downing et al., 2002). Parasite effects on a number of trout species and life history stages were assessed (see, e.g., Hedrick et al. 1999a, 1999b, 2001a, 2001b; Thompson et al., 1999; Hiner and Moffitt, 2001; Wagner et al., 2002) revealing that many salmonid species or sub-species were vulnerable to infection, whereas others were less so or even refractory. Parasite effects on fish hosts were usually elevated if fish were exposed at a young age. Information was obtained on parasite distribution (Baldwin et al., 1998; Arsan et al., 2007) as well as threats associated with its potential spread (Hiner and Moffitt, 2002; Arsan et al., 2007). For a recent review of the voluminous research conducted on this topic, one may consult Steinbach-Elwell et al. (2009b).

The research summarized above filled many gaps in our knowledge of *M. cerebralis* and its effects on salmonid and oligochaete hosts. However, it has lead to few instances of active intervention attempting to halt the spread or reduce the impact of the parasite. Bartholomew et al. (2007) reported the apparent successful arrest of parasite spread after the closure of the surface-water portion of a fish rearing facility. Arndt and Wagner (2004) demonstrated the possibility of using sand filtration techniques to remove parasite life-stages from hatchery supply water. These efforts focused on fish-rearing facilities; I am aware of no instances of attempted physical field interventions in free-flowing waters.

One possible field intervention involves disruption of the parasite life cycle by reducing the population of the oligochaete host. Previous research demonstrated eutrophic impoundments could be point sources of infectivity (Thompson et al., 2002). Organically enriched environments in rivers are known to harbor dense populations of *T. tubifex* (Aston, 1973), so depositional habitats with organic content were sought out in the study streams as probable areas of *T. tubifex* proliferation. The best available oligochaete habitat was selected on the basis of low water velocity and deposition of sediments and organic materials.

The aim of this study was to determine whether parasite activity in free-flowing waters could be ameliorated with physical habitat manipulations. Physical habitat was modified in streams to reduce habitat for *T. tubifex,* then oligochaete communities, actinospore densities in surface waters, and myxospore concentrations in brown trout *Salmo trutta* were monitored and compared to pre-treatment and control site values to evaluate whether the parasite's impact was reduced.

Study Sites –

Two sites in Colorado containing substantial localized areas of *T. tubifex* habitat were selected from Williams Fork River and Spring Creek (Figure 1) for habitat manipulation. Williams Fork River is a 5th-order tributary of the Colorado River in Grand County, and Spring Creek is a 3rd-order tributary of the Taylor River in Gunnison County. Both streams may be characterized as Type 'C' channels (Rosgen, 1994) at the study locations.

The Williams Fork River site was an irrigation diversion that supplied water to headgates on both banks of the river (Figure 2). Prior to modification the diversion consisted of a mass of rock placed across the stream perpendicular to the flow. The resulting flow pattern created a low velocity, depositional backwater habitat with high oligochaete densities. This area was estimated to be about 160 m^2 before manipulation.

The Spring Creek treatment site consisted of about 110 meters of channel that was undergoing disturbance-induced changes downstream of a road crossing (Figure 2). Situated at a bend in the stream with a small tributary entering on the outside of the bend, the site was aggrading and consisted of a shallow riffle through the bend. Material deposited at the confluence of the smaller tributary resulted in a large, back-eddying area of sediment deposition. The depositional habitat in this area comprised an estimated 132 m^2.

A control site, in Spring Creek, was located 14 kilometers downstream of the treatment site. It was also an area of sediment deposition on a bend of the stream. The lower portion of the bend contained large rocks and woody debris that slowed water velocities along the bend and induced deposition. There was a pool on this bend, in contrast to the treatment site, but it was located in the center of the stream and the near-bank habitat was similar to that seen at the treatment site. Oligochaete, actinospore, and myxospore data were collected at this site. Myxospore data only were collected at an additional control site in each stream, located upstream of the treatment sites by 1 kilometer in Williams Fork and 4.2 kilometers in Spring Creek.

Methodology

Habitat modification –

The Williams Fork irrigation diversion was rebuilt in June 2002 using a cross-vane design (Rosgen, 2001). Three closely spaced cross-vane structures were constructed to reduce the steam bed slope compared to the former diversion. The east bank headgate was moved about 12 meters closer to the stream and the previous backwater was buried (Figure 2).

Modification at the Spring Creek treatment site occurred in October 2002. The channel was realigned and shaped at the upper end to provide a hydrologically stable entrance into the bend, and the large eddying backwater was buried (Figure 2). The confluence of the small tributary was moved to the downstream end of the bend, near the end of the formerly eroding bank section. Three "J-hook" vanes (Rosgen, 2001) constructed with rocks and logs were placed to guide water through the section while protecting endangered banks. Live willow transplants were used to protect the newly reconfigured confluence from high-water failure. The near-bank region of the structures (zones of reduced water velocities) and the associated downstream pools were areas monitored to determine whether such structure/pool combinations achieve the desired goal of discouraging development of oligochaete habitat over time while providing bank protection.

Baseline data were collected from each site to describe preexisting oligochaete assemblages, surface water actinospore concentrations, and prevalence and severity of parasite infection in age 1+ brown trout. Following habitat changes, post-manipulation data were collected in the same fashion as the baseline data.

Oligochaete sampling –

Oligochaetes were sampled from what was judged to be the best oligochaete habitat at each study site on three separate occasions prior to and after habitat modifications. The control site in Spring Creek was sampled on five occasions. On each occasion, six samples were obtained by a kicknet technique. An area of 0.5 m^2 was thoroughly disturbed with the sampler's feet for 60 seconds while holding a 250-micrometer mesh kicknet just downstream in the current to capture the organisms dislodged from the substrate. Substrates were sampled for only 30 seconds for two samples from Spring Creek and four samples from Williams Fork, and the results were standardized to match the 60-second samples. Each sample was placed in a 4-liter pail and covered with water, labeled, and allowed to sit overnight. The following day, the overlying water was filtered through 20-micrometer Pecap® screen to concentrate actinospores and actinospore density was estimated using techniques previously described (Thompson and Nehring, 2000). The resulting data were examined using analysis of variance (ANOVA) on ranked values because of the severe non-normality of the data. The analyses were conducted to examine site differences, the effect of the habitat improvements, and any potential interaction. Statistical significance levels were set at α=0.05. Seventy-eight of the 102 water filtrates were also tested using the polymerase chain reaction (PCR) technique described by Schisler et al. (2001) to confirm the identity of actinospores observed as those of *M. cerebralis*.

After removing as much water as possible, each sediment sample was preserved with 10% buffered formalin and shipped to a private lab for further analysis by oligochaete taxonomists. There, each sample was washed using a 250-micrometer mesh sieve to remove the fixative. Samples containing more than one liter of material were subsampled using a modified Caton subsampler (Caton 1991). The sample was evenly distributed on a gridded screen with marked six-centimeter squares, contained in a slightly larger tray of water. The screen was lifted out of the water tray so sample contents settled onto the screen.

Grids were randomly selected for analysis. Both subsamples and entire samples were examined in small portions by placing material into a gridded petri dish and examined using a dissecting microscope at 12x magnification.

Two samples were randomly selected from each sampling occasion and all the oligochaetes were removed, counted and weighed. In the remaining four samples, a minimum of 50 oligochaetes (if available) from each of five groups (tubificids with hair and pectinate chaetae, tubificids with bifid chaetae, enchytraeids, lumbriculids, and lumbricids) were removed, counted and placed in small petri dishes of water, while the remainder were left in the sample but enumerated. *Naididae* were not removed because they are very small (Juget and Lafont, 1994; Kathman and Brinkhurst, 1999), are not confused with *T. tubifex* and contribute minimally to total oligochaete biomass. After each sample was examined and oligochaetes removed and counted, each group of oligochaetes that had been removed was weighed. The oligochaetes were blotted on a paper towel, placed into a tared aluminum weighing pan and weighed to the nearest gram. The total weight was divided by the number of oligochaetes weighed, and the weight per oligochaete recorded.

Mature tubificids with hair and pectinate chaetae, as well as representative immature ones, were mounted in CPMC mounting medium for identification. In cases where there were no mature oligochaetes, several immature ones were mounted to ensure that they were not *Rhyacodrilus* spp (possible to differentiate from *T. tubifex* or *Ilyodrilus templetoni* in the immature stage). To identify tubificids with bifid chaetae, 5 to 10 mature specimens were mounted. Oligochaetes with hair and pectinate chaetae were divided into three groups: mature *T. tubifex*, mature *I. templetoni*, and immature (unidentified). Oligochaetes were considered mature when penis sheaths were present. If no penis sheaths were present but other characters could be used to indicate that they were most likely one of the two species, they were labeled as immature of that species; otherwise immature oligochaetes were apportioned to groups based on proportions of adults identified.

Actinospore sampling –

Surface water samples filtered through 20-micrometer Pecap® screen were collected each month from each site, with few exceptions. Through June 2004, the volume of water filtered on each occasion was 1900 liters; thereafter it was reduced to 114 liters because the lower volume was found to be more efficient at detecting actinospores (Lukins et al., 2007; Thompson unpublished data). The concentrates were examined for actinospores and density was estimated with protocols from Thompson and Nehring (2000). These samples were also tested by PCR (Schisler et al., 2001) to confirm the identity of the actinospores observed. Samples from 12 months preceding and following habitat modifications were compared with a one-tailed paired t-test (Ho: No difference, Ha: densities lower after treatment, $\alpha = 0.05$).

Myxospore monitoring –

Brown trout were chosen as the salmonid sentinel species because they develop myxospores but rarely die from *M. cerebralis* infection. Moreover, insufficient numbers of rainbow trout survived in the study streams to allow lethal collections. Samples of age 1+ brown trout were collected at each site during late summer or fall. Myxospore data were collected at both treatment sites, the Spring Creek control site, and additional control sites in each stream. Trout heads were individually tested for presence and abundance of *M. cerebralis* myxospores by the pepsin-trypsin digest method (Markiw and Wolf, 1974). The resulting data were subjected to analysis of variance (ANOVA) for each stream on ranked data because of severe non-normality. Analyses were conducted both with and without individual fish in which no myxospores were detected, and included least-squares means comparisons for the status*site effect to evaluate whether site infectivity changed after habitat modification. Statistical significance levels were set at $\alpha=0.05$.

Results

Post-construction, the estimated oligochaete habitat at the Williams Fork site was reduced to about 32 m^2 at irrigation diversion headgates from the estimated pre-construction area of 160 m^2. The estimated oligochaete habitat at the Spring Creek site was reduced to about 0 m^2 from the estimated pre-construction area of 132 m^2.

Oligochaete response –

Oligochaete assemblages at all sites were characterized by irregular, patchy distribution, so precision of the biomass estimates was generally poor (Table 1). Nearly all identified Tubificidae with hair and pectinate chaetae were *T. tubifex*; 100% in the Williams Fork (n=386) and 99.7% in Spring Creek (n=343), where one *I. tempeltoni* specimen was identified from the control site. The oligochaete assemblage was thoroughly dominated by *T. tubifex* and *Limnodrilus hoffmeisteri* at every site, with an estimated 97.8% of oligochaetes belonging to these two taxa.

Williams Fork oligochaete samples produced actinospores in 56% of those obtained prior to modification and 22% after modification. Spring Creek oligochaete samples from the treatment site produced actinospores in 89% of samples prior to and 61% of samples after modification. Samples from the control site in Spring Creek yielded actinospores in 83% of samples. All actinospores resembled those of *M. cerebralis* morphologically, and 67% of samples in which actinospores were observed tested positive for *M. cerebralis* DNA by the PCR test, confirming that *T. tubifex* at each location were producing *M. cerebralis* actinospores.

Modeling actinospore production from oligochaete samples revealed site effects (F = 18.40, df = 2, $p < 0.0001$) and a status*site interaction (F = 10.70, df = 2, $p < 0.0001$). Status alone was not a significant effect (F = 1.15, df = 1, $p = 0.2860$). Least-squares means comparisons indicated actinospore production from oligochaete samples collected at treatment sites (Table 1) was significantly reduced at Spring Creek ($p = 0.0009$) but not at Williams Fork ($p = 0.0708$). Conversely, the control site oligochaete samples produced more actinospores in the post-construction evaluation period than in the pre-construction period ($p = 0.0039$). Oligochaete collections from the control site produced fewer actinospores than those from the Spring Creek treatment site before construction ($p = 0.0030$) but more afterward ($p = 0.0013$). Actinospore production from control site oligochaete samples was not different than from the Williams Fork treatment site prior to construction ($p = 0.2895$), but was significantly higher after construction ($p = 0.0001$).

The Williams Fork oligochaete assemblage was dominated by *T. tubifex* both before and after the modifications. Oligochaete density was initially greatly depressed following construction in the Williams Fork, but the remaining habitat was fully re-occupied by the time the second sample was obtained 3.5 months post-construction. In October 2002, assemblages of oligochaetes were readily observed in front of the west irrigation headgate. Fewer, smaller assemblages were detected in front of the east irrigation headgate, the area targeted for substantial habitat reduction. Oligochaete density was much less in November, likely a result of the October sampling and associated disturbance.

In Spring Creek, larger proportions of the samples consisted of oligochaetes other than *T. tubifex* compared to the Williams Fork (Table 1). The habitat modifications implemented in Spring Creek also resulted in reduced oligochaete biomass. However, at this site the reduction appeared to be more lasting, indicating that the modifications were more effective than in the Williams Fork. The last monitoring samples, collected nearly a year after completion of the habitat manipulations, still showed oligochaete biomass to be considerably lower than was observed before construction.

Oligochaete biomass estimates at the Spring Creek control site were less variable than at treatment sites with the exception of an initial high estimate and one low estimate. The low biomass values observed in July 2003 were likely a result of a natural loss of suitable oligochaete habitat at the control site following the spring runoff-induced loss of woody debris that had previously encouraged sedimentation.

Actinospore response –

Actinospores were detected in surface water samples on 27 of 52 (51.9%) of sampling occasions prior to habitat modification in the Williams Fork versus seven of 49 (14%) afterwards (Figure 3). Mean density was lower in the 4.5 years following construction compared to the 4.5 years prior to construction, but the difference was insignificant (before: 0.06 l^{-1}, 95% CI 0.027, 0.093; after: 0.035 l^{-1}, 95% CI 0.000, 0.075). There were an equal number of actinospore density estimates ≥ 0.2 l^{-1} before and after construction, possibly attributable in part to the increased efficiency of the post-June 2004 water sampling volume.

Surface water filtration indicated that habitat manipulation in Spring Creek did not result in reduced frequency of actinospore detection or densities following construction. Post-construction monitoring resulted in actinospore detection on 29 of 55 (52.7%) sampling occasions compared to six of 13 (46.2%) occasions during pre-construction sampling, and several occasions showed higher actinospore densities than those seen during pre-construction sampling (Figure 3). The one-tailed paired t-test of the 12 months preceding construction versus the 12 months following construction showed that the mean differences were not significant (p = 0.90, 95% lower bound for mean difference = -0.75 actinospores l^{-1}).

The control site in Spring Creek exhibited a similar pattern of increased frequency of detection and higher density estimates. The one-tailed paired t-test of the 12 months preceding construction at the treatment site versus the 12 succeeding months indicated that the mean differences were not significant (p = 0.56, 95% lower bound for mean difference = -0.228 actinospores l^{-1}).

Myxospore response –

Myxospore data were considered pre-treatment through 2003 because age 1+ brown trout collected in fall 2003 would have hatched and emerged in 2002 prior to habitat modifications in either stream. Pre-manipulation data on myxospore prevalence and concentration in age 1+ brown trout revealed that a substantial proportion of the juvenile brown trout population was infected with the parasite in each stream prior to habitat modifications, but more so in Spring Creek (Figure 4). Prevalence varied considerably in the Williams Fork samples over the course of the study but trended downward during the post-manipulation period, whereas it remained high in Spring Creek samples.

The Williams Fork ANOVA indicated significant site, status, and interaction effects when using all the data, but when using only fish in which myxospores were observed just the status effect was significant (Table 2). Specific a priori least-squares means comparisons showed that the upper control Williams Fork site exhibited no effect (p = 0.8321) but the treatment site exhibited decreased myxospore concentrations after treatment (p = 0.0009) when using all the data. Using only fish with myxospores (Figure 4), the upper site tended toward a decrease in myxospore burden but was not significant (p = 0.0925), and the treatment site showed the same effect as with all the data (p = 0.0002). In Spring Creek, no significant effects were detected with either the full or reduced data sets, nor did a priori least squares means comparisons indicate that any Spring Creek site experienced a change in myxospore prevalence or infection intensity (Figure 4).

Discussion

Habitat modifications in these two streams generated mixed results. The oligochaete assemblage at the Williams Fork study site continued to exhibit dense assemblages dominated by *T. tubifex* after habitat modification, despite reductions in the amount of habitat remaining after modification. In Spring Creek, oligochaete densities at the treatment site were consistently lower after habitat modification. The differences in oligochaete densities between the two streams may have been due to habitat differences; the irrigation headgates in the Williams Fork still provided oligochaete habitat after removal of the backwater, whereas there was little oligochaete habitat in the treatment site in Spring Creek after habitat modifications.

Although actinospore production from oligochaete samples was not diminished to a degree that achieved statistical significance at the Williams Fork treatment site, both treatment sites exhibited downward-trending actinospore production as opposed to the increased actinospore production seen at the Spring Creek control site. However, it is possible that the reduction was not a lasting result since a likely explanation for the reductions could have been the physical removal of many mature (and some proportion infected) oligochaetes during habitat modification. These oligochaetes would have been replaced primarily by reproduction from remaining oligochaetes, and recent evidence suggests that immature and juvenile *T. tubifex* produce fewer actinospores than adults (Shirakashi and El-Matbouli, 2009). Consequently the relatively short term over which oligochaete response was measured may have been insufficient to fully judge the efficacy of habitat modifications by this metric.

Actinospore response was also inconsistent between the streams, as frequency of detection decreased in Williams Fork but increased in Spring Creek. A number of other streams were being

sampled by the same technique over this same time frame (Thompson, unpublished data), and nearly all were exhibiting decreased frequency of detection from 2004 onward. Spring Creek was the exception with increased actinospore density and frequency of detection. Possible explanations for the actinospore reduction occurring in so many waters in Colorado may include a significant change in trout stocking policy in 2003. The amended policy prohibited stocking trout exposed to *M. cerebralis* in cold water streams or reservoirs occupied by self-sustaining trout populations. That change was followed by waning infectivity over succeeding years, although that result has not been proven to be cause and effect. The Williams Fork never received fish from a facility that was positive for the *M. cerebralis* parasite, but Williams Fork Reservoir upstream of the study site received catchable rainbow trout from positive facilities in 1994, 2000, and 2001. Spring Creek received fish from a known positive facility in 1995 and Spring Creek Reservoir was stocked with catchable rainbow trout from positive facilities in 1992-1996 and again in 1999.

The widespread decrease in actinospore densities may have occurred because of shifts in the *T. tubifex* assemblage from susceptible lineages (Sturmbauer et al., 1999; Beauchamp et al., 2001, 2002) to non-susceptible ones. Samples collected for this study revealed that the oligochaete assemblages differed dramatically between the two streams. The Williams Fork contained a mix of three lineages; two are known to be resistant to the *M. cerebralis* parasite in laboratory exposures (R. B. Nehring, Colorado Division of Wildlife, Montrose, CO, USA, pers. comm.). The susceptible lineage III component of the Williams Fork samples usually constituted less than 50% of the oligochaete DNA detected. In contrast, only DNA of the susceptible lineage III *T. tubifex* was detected in Spring Creek. Stevens et al. (2001) asserted that variability in whirling disease severity could be influenced by the composition of the *Tubifex* community, although they were addressing differences among populations from different geographic areas but of the same lineage. Beauchamp et al. (2005) found sites dominated by less susceptible or non susceptible lineages of *T. tubifex* corresponded to lesser impacts on the trout populations. The phenomenon of a long-term shift in lineage composition toward non-susceptible *T. tubifex* has been observed in the wild in Windy Gap Reservoir, Colorado (R. B. Nehring, Colorado Division of Wildlife, Montrose, CO, USA, pers. comm.), and it resulted in decreased frequency of detection and densities of actinospores below the impoundment. That no such lineage shift has occurred in Spring Creek may explain why surface water actinospore detection frequency and densities were not diminished.

Exploratory oligochaete sampling in both streams, ancillary to the current study, revealed other suitable habitat areas harboring infected *T. tubifex*. Such areas, though small in surface area compared to those removed by habitat modifications, contained oligochaetes that produced actinospores (data not shown). Six of seven sites in the Williams Fork were shown to contain oligochaetes producing actinospores. Twelve sample sites in Spring Creek all contained oligochaetes producing actinospores. Exploratory sampling sites were distributed upstream and downstream of the treatment sites in both streams.

Differences observed in oligochaete assemblages may also have affected cranial myxospore concentrations. The Williams Fork results suggested that the habitat modifications had a desirable effect on fish infectivity at the treatment site since there was a significant decrease in myxospore concentration among positive fish following habitat modifications. However, the decrease in mean myxospore concentration at the additional upper control site among positive fish was marginally significant (p =0.093). This suggests the fish inhabiting the entire drainage may have experienced a decrease in infection pressure/exposure over the course of this study, and the process was accelerated at the treatment site as a result of habitat modification. The results in the Williams Fork contrast with those in Spring Creek. There was no evidence whatever that habitat modification had a desirable effect on the prevalence and intensity of infection among brown trout in Spring Creek. The observed differences in oligochaete density as well as lineage composition in the Williams Fork (Winkelman et al., 2005) may be a plausible explanation for the more favorable response there.

Myxospore prevalence and mean concentrations were consistently higher in Spring Creek samples than in Williams Fork samples throughout the study. This is likely related to the different oligochaete communities in the two streams, with the lineage III-dominated Spring Creek supporting the observations of Beauchamp et al. (2005) that increased effects on resident trout populations are associated with *Tubifex*

communities dominated by susceptible lineages. Beauchamp et al. (2006) also demonstrated that pure cultures of susceptible *T. tubifex* produced more actinospores than cultures that contained a mix of susceptible and non-susceptible *T. tubifex*, so the higher rate and intensity of infection among trout in Spring Creek is not surprising.

Brown trout are known to be resistant to disease caused by *M. cerebralis* (Hedrick et al., 1999b), whereas the cutthroat trout native to Colorado and the rainbow trout so ubiquitously introduced are far more susceptible (Thompson et al., 1999, 2002; Hedrick et al., 1999b). Had there been sufficient numbers of more susceptible species to collect for this study, there may have been less evidence of effect on myxospore numbers.

Conclusions

Despite the hints of success obtained in this study, small-scale habitat alterations seem unlikely to achieve widespread success. Habitat modification is expensive and many affected areas are not easily accessible with the equipment needed. Moreover, it would be impossible and undesirable to remove all oligochaete habitat from a given reach. As experienced in this study, numerous small habitat areas conducive to oligochaete presence are nearly certain to be present in most reaches of trout streams. Such areas are likely sufficient to facilitate continued infection even if large areas of habitat are removed. The habitat manipulations did not show sufficient promise to encourage further efforts in Colorado. However, if a habitat removal strategy is used, it should be preceded by *T. tubifex* lineage analyses to determine whether resistant or non-susceptible lineages are present.

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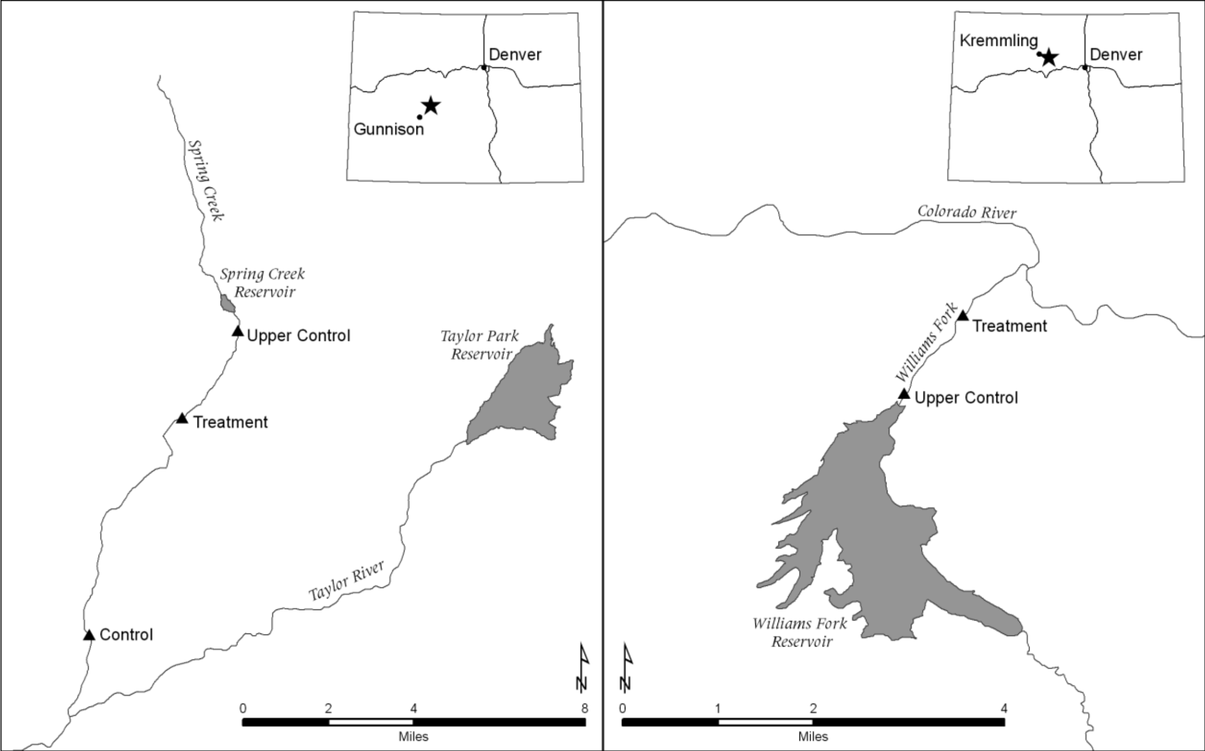


Figure 1. Location of the study sites in Colorado, with detail to show treatment and control sites from both rivers ("upper control" sites were only for myxospore evaluation).

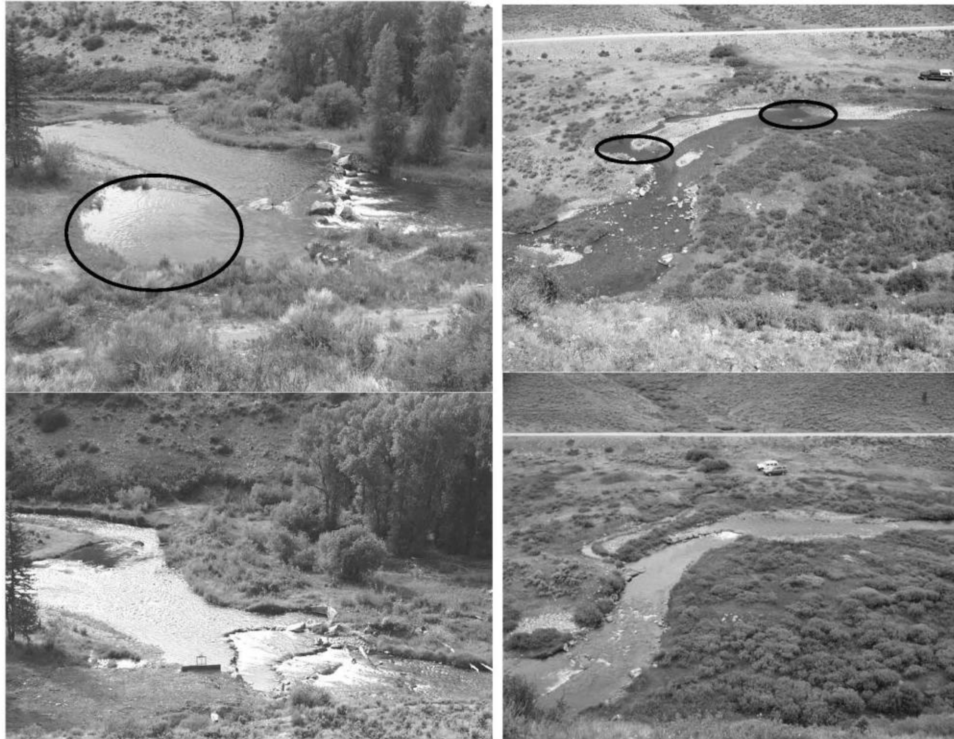


Figure 2. The treatment sites before and after habitat modifications. Williams Fork River streamflow is from upper left to lower right (left panels), and Spring Creek streamflow is from right to left (right panels). Areas of depositional habitat removed in each stream are circled in the upper photographs.

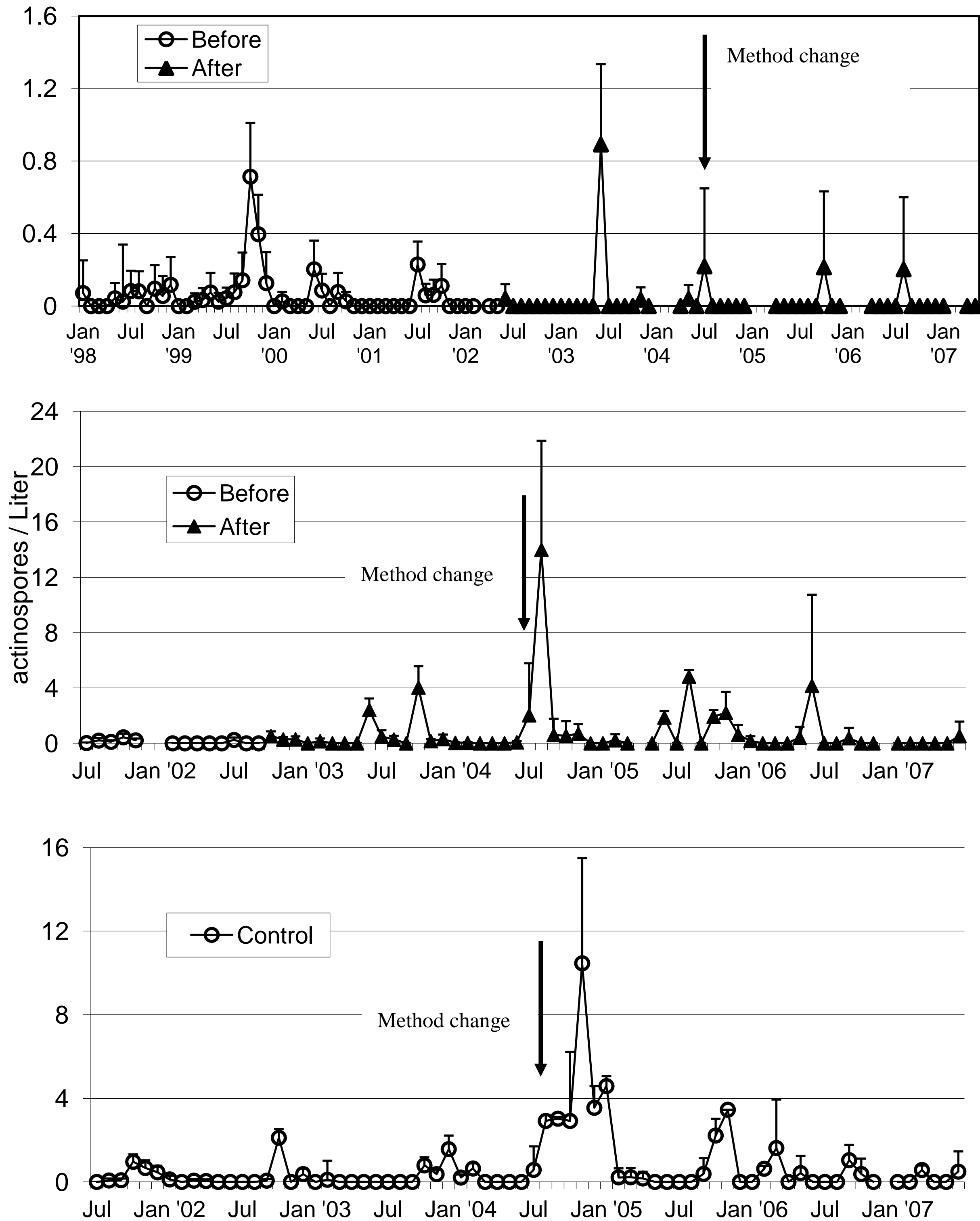
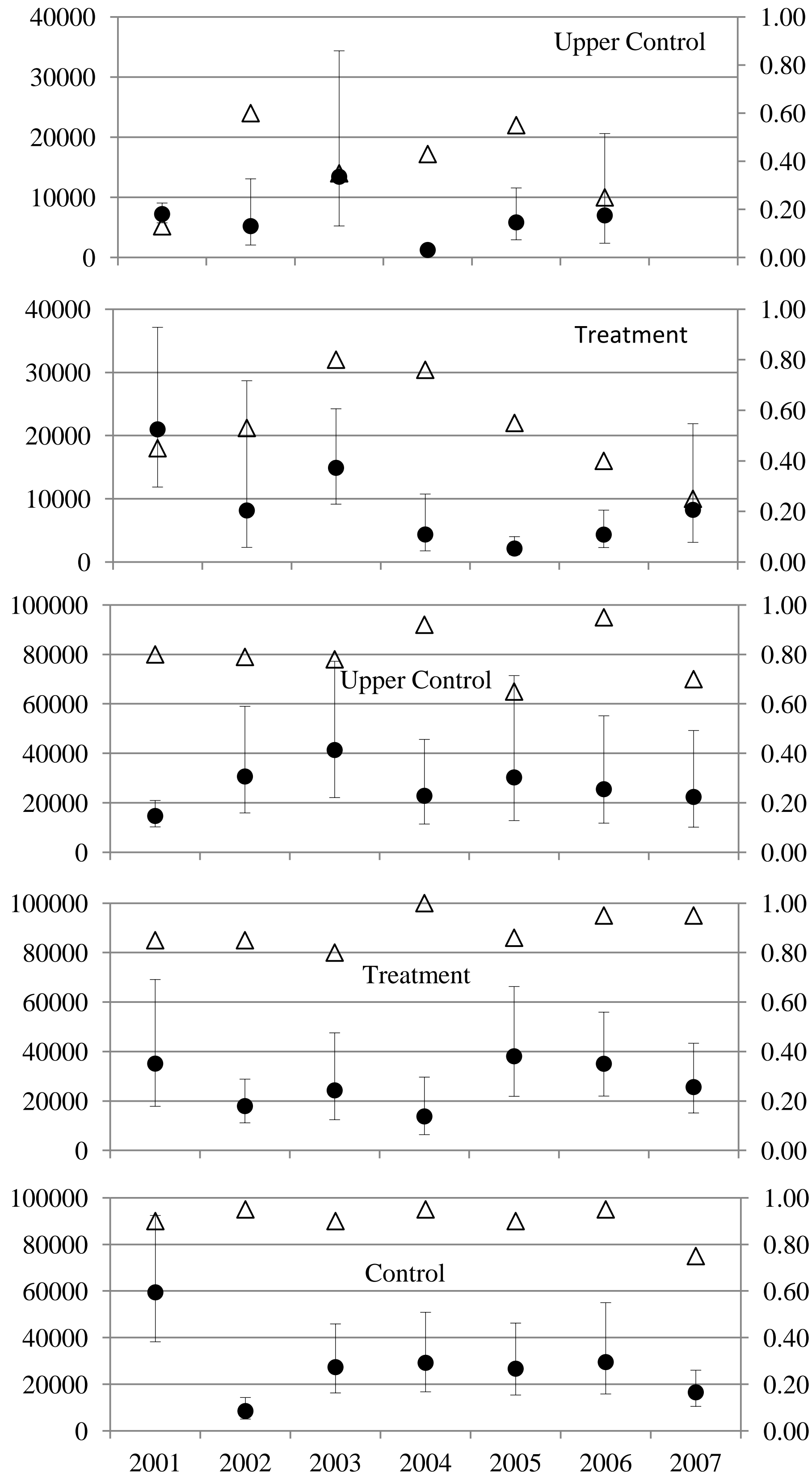


Figure 3. Density of actinospores observed in concentrated surface water samples collected at the Williams Fork treatment site (upper chart) and in Spring Creek at both treatment and control sites (lower chart). "Before" designates samples acquired prior to habitat modification; "After" designates those obtained following habitat modification. Error bars represent upper 95% confidence limit. "Method change" refers to the reduction in the amount of water filtered from 1900 liters per sample to 114 liters per sample. Note differing y-axis scales.



Upper Control
Treatment
Upper Control
Treatment
Control
40000
30000
20000
10000
0
100000
80000
60000
40000
20000
1.00
0.80
0.60
0.40
0.20
0.00
2001
2002
2003
2004
2005
2006
2007

Figure 4. Prevalence (open symbols, no error bars) of myxospores and mean myxospores per fish (solid symbols with 95% confidence interval, positive fish only) in samples of age 1+ brown trout taken from the study sites, the control site, and an additional upstream site ('Upper Control') on each stream. Means and confidence limits were back-transformed from the natural logarithm. Williams Fork data are depicted in the top two panels and Spring Creek data in the lower three panels. Data from 2004 and later are "post-modification". The upper control site on Williams Fork River was not sampled in 2007.

Table 1. Oligochaete biomass values (g) observed at treatment and control sites. Six oligochaete samples were obtained on each occasion. The 'Spores' column indicates the number of oligochaete samples from which actinospores were observed by microscopy after holding the oligochaetes overnight in the lab. The following value in parentheses is the mean estimate of actinospores present in the six samples. The column labeled 'PCR' refers to the number of filtered water samples that tested positive for parasite DNA.

Date	Mean Total Biomass	SE Mean	% likely *T. tubifex*	*T. tubifex* mean biomass	SE Mean	Spores	PCR
			Williams Fork (pre-construction)				
06/25/01	2.03	1.2457	83.1	1.95	1.2160	1 (92)	not done
11/05/01	3.66	1.3799	93.9	2.86	0.9224	4 (165)	not done
11/26/01	5.23	3.0515	93.1	4.96	3.0249	5 (466)	5
			Williams Fork (post-construction)				
07/17/02	0.33	0.1323	88.0	0.30	0.2244	0 (0)	1
10/21/02	13.10	6.1561	97.5	13.06	6.1360	3 (69)	3
11/19/02	2.84	0.9280	92.6	2.74	0.8958	1 (27)	2
			Spring Creek (pre-construction)				
07/23/01	1.67	0.6685	51.7	0.99	0.4811	5 (36142)	not done
10/17/01	1.06	0.4485	33.9	0.43	0.2388	5 (2276)	not done
11/15/01	2.07	0.6150	26.3	0.68	0.2616	6 (3599)	5
			Spring Creek (post-construction)				
11/07/02	0.15	0.0589	26.3	0.02	0.0068	2 (428)	2
07/14/03	0.43	0.3029	65.2	0.29	0.2056	5 (2496)	1 [a]
09/24/03	0.35	0.1243	67.0	0.18	0.0702	4 (355)	0 [a]
			Spring Creek (control site)				
04/22/02	1.82	0.9291	57.4	0.49	0.2970	4 (608)	3
07/15/02	0.62	0.2610	48.4	0.26	0.1062	5 (446)	5
11/07/02	0.99	0.7534	50.8	0.84	0.7422	5 (4426)	5
07/14/03	0.09	0.0456	59.2	0.03	0.0120	5 (2740)	3 [a]
09/24/03	0.63	0.1308	53.0	0.32	0.0814	6 (6923)	2 [a]

a: The detection of *M. cerebralis* DNA by PCR and the relative strength of signal for these samples were lower than anticipated. The use of ordinary tap water to rinse material from the filter screen likely resulted in the degradation of much of the DNA by chlorine exposure prior to processing at the lab.

Table 2. Summary ANOVA results for myxospore concentrations among age 1+ brown trout in each stream using all fish sampled and only fish containing detectable myxospore levels. Status refers to before versus after modification.

Stream	Response variable	Source of variation	*F*	df	*p*
Williams Fork	All fish	Status	5.71	1	0.0176
		Site	10.94	1	0.0011
		Status*Site interaction	4.30	1	0.0393
	Positive fish	Status	13.05	1	0.0005
		Site	2.08	1	0.1521
		Status*Site interaction	0.74	1	0.3931
Spring Creek	All fish	Status	1.10	2	0.3332
		Site	0.75	1	0.3883
		Status*Site interaction	0.86	2	0.4237
	Positive fish	Status	0.11	2	0.8936
		Site	0.00	1	0.9852
		Status*Site interaction	0.27	2	0.7632