VARIATIONS IN THE PREVALENCE AND INTENSITY OF *MYXOBOLUS CEREBRALIS* INFECTIONS IN CUTTROAT AND RAINBOW TROUT IN THE SOUTH FORK BOISE RIVER

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Introduction

Whirling disease, caused by *Myxobolus cerebralis*, was first documented in Idaho in 1985 (Elle, 1997). Wild fish sampling and sentinel exposures have since identified its presence in 10 major river drainages. In 1997, the Idaho Department of Fish and Game conducted sentinel testing on the South Fork of the Boise River at two sites below Anderson Ranch Reservoir. Newly emerged rainbow trout (*Oncorhynchus mykiss*) fry were used at both sites, and exposures were conducted for ten days during July. The upper site (just below Anderson Dam) tested negative for the parasite, while 45% of the fish exposed 18km downstream at Danskin Bridge
tested positive, exhibiting mild to moderate infection levels (Elle, 1998). The results of the study showed that severity of infection and prevalence of *M. cerebralis* can vary within a small geographic area.

In 1998, we conducted field exposures on the South Fork of the Boise River to further investigate the differences observed in 1997. We revisited the previous sites and increased the sampling area by adding a site downstream and a site between the two 1997 sites. Our objectives were to: 1) determine whether the Anderson site remained negative, 2) test whether there was a difference in disease prevalence among the other sites, 3) determine whether there was a difference in species response between rainbow and cutthroat trout (*O. clarki*), and 4) determine whether newly emerging fry of the same species are more susceptible to the parasite during different months. The last objective may indicate whether a life history including earlier emergence is beneficial in avoiding infection by the parasite. We also wanted to collect information on temperature regimes and aquatic benthic invertebrate communities to determine whether these factors could be correlated with prevalence or intensity of infection.

Methods

We selected four locations within a 25 kilometer section of the SF Boise River downstream of Anderson Ranch Reservoir. The uppermost site, as in 1997, was located approximately 300 meters below Anderson Ranch Dam. The intermediate site chosen between the 1997 sites was located 12.5 kilometers downstream of the Anderson site, just below the Cow Creek inlet. We also repeated exposures at Danskin Bridge, which is 5.6 km below the Cow Creek site. The lowermost site was 7 km downstream of the Danskin, located between the Rock Creek and Trail Creek inlets.

The South Fork of the Boise River is regulated by releases from Anderson Ranch Dam with hypolimnetic flows that averaged approximately 3200 CFS during June and decreased to 1600 CFS in July and August. Hypolimnetic releases resulted in temperature difference between the sites, with the uppermost site remaining colder, averaging daily temperatures ranging from 7.5°C in June and warming slightly during the exposures to an average temperature of 8.3°C during August. During the study, the average daily temperature at Cow Creek was 9.7°C, Danskin averaged 10.2°C, and the Trail Creek average was 10.6°C (Table 1). Diurnal fluctuations varied among the sites, with temperatures at Anderson remaining fairly constant and the daily fluctuation increasing downstream.

Table 1. Sum of electronic temperature records at each exposure site in 1998. Intervals between readings was 15 minutes. No data were available for June due to equipment failure.

Site	Mean daily temp °C		SD of all records		Range of records	
	July	August	July	August	July	August
AN	7.57	8.28	0.60	0.24	6.9 - 10.5	8.0 - 8.9
CW	9.84	9.38	1.26	1.01	7.7 - 13.1	8.3 - 11.4
DA	10.25	9.69	1.41	1.33	7.7 - 13.4	8.1 - 12.2
TR	10.8	10.4	1.62	1.75	7.8 - 14.2	8.1 - 13.6

This region of the South Fork is low to moderate gradient with substrate size decreasing downstream. The Anderson site consists of large rubble, small boulders, and some coarse gravel with little fine particulate matter. The substrate at the Cow Creek site is predominantly rubble and cobble with fine sediments and sand settling out in eddies and along shallow shorelines. The Danskin site is characterized by small cobble and rubble with fine particulates along shallow, slower-moving shorelines. At the Trail Creek exposure site, the channel is split and exposure boxes were placed in a side channel that was partially (70%) blocked by a beaver dam. The substrate is dominated by fine sediments and is high in organic debris.

Aquatic benthic invertebrates were collected after exposures were completed in August to assess the community structure at each site. A D-frame kicknet was used to collect samples from the substrate surrounding the exposure boxes. Each sample consisted of an area of $1m^2$ and 10 cm deep. Three samples were taken from the Anderson, Cow Creek, and Danskin sites and two replicates were taken from Trail Creek. All samples were washed through a #60 sieve and invertebrates and materials were preserved in 10% neutral buffered formalin for storage. Samples were sorted, identified, and recorded by taxonomic order in the laboratory. In larger samples, representative subsamples were removed and abundance extrapolated using a correction factor.

Henry's Lake cutthroat trout or Kamloops rainbow trout (Troutlodge, Sumner, WA) were used in field exposures. Feeding fry (10-15 days post button-up) were transported from the University of Idaho wet laboratory to each site. Two replicates of a species were exposed at each site and control groups were reared at the University of Idaho for comparison. We placed each sentinel box in suitable fry habitat with comparable flow rates and river depth. Fish were exposed for ten days, then collected and transported back to the University of Idaho for rearing.

We reared all exposed fish for 84 days (or approximately 1000 temperature units), and fed them daily rations of BioOregon Grower twice daily based on estimated fish weights. Daily observations were made to document onset and prevalence of black tails and whirling behavior. We removed portions of each replicate at 28d and 56d post-exposure and measured weights, lengths, and examined each fish for clinical signs (black tails, exophthalmia, and head/jaw deformities). At the end of rearing, we examined all fish and randomly selected ten fish per replicate for histological analysis. The heads of these fish were fixed in 10% neutral buffered formalin, prepared for paraffin histology, sectioned sagitally (5µm thickness), and stained with hematoxylin and eosin. Fish were then examined by light microscopy for spores and pathology typical of *M. cerebralis* infections and scored for severity of infection using Baldwin's scale (Kiryu and Moffitt, 1998).

We used SAS (version 6.11) for all statistical analyses. Species of fish (RB or CT) and month (June, July, or August) were combined to form four treatment groups: JuneCT, JulyCT, JulyRB, and AugustRB. Due to the loss of rainbow trout in July, we could only examine a difference in species response during the July exposure. We also examined monthly variation for each species between two concurrent months:

cutthroat in June and July and rainbow in July and August. We used Analysis of Variance (ANOVA) and chi-square tests to perform statistical analyses; PROC INSIGHT was also used to observe trends and patterns in response variables between main effects.

Results

Disease prevalence was variable among the sites, with prevalence increasing downstream. As in 1997, all fish exposed at Anderson tested negative for the parasite (Table 1). All four treatment groups (JuneCT, JulyCT, JulyRB, and AugustRB) exhibited 40% disease prevalence at Cow Creek. Disease prevalence at Danskin ranged from 40% to 55% for the four treatments. The Trail Creek location produced fish exhibiting the highest disease prevalence, with 90% infection of cutthroat exposed in June and 100% disease prevalence for all fish exposed in July and August.

Table 1. Summary of disease prevalence and histological ranking for rainbow and cutthroat trout exposed in the South Fork of the Boise River during June, July, and August of 1998.

Month	Site	Species	Number of Fish Examined	Histological Ranking 0	1	2	3	4	Prevalence
June	AN	CT	20	20	0	0	0	0	0
	CW	CT	20	12	2	2	1	3	40
	DA	CT	20	9	2	6	2	1	55
	TR	CT	20	2	0	2	1	15	90
July	AN	CT	10	10	0	0	0	0	0
	CW	CT	10	8	1	0	0	1	40
	DA	CT	10	5	0	2	2	1	40
	TR	CT	10	0	0	0	0	10	100
	AN	RB	10	10	0	0	0	0	0
	CW	RB	10	6	1	1	1	1	40
	DA	RB	10	6	0	1	1	2	50
	TR	RB	10	0	0	0	0	10	100
August									
	AN	RB	10	10	0	0	0	0	0
	CW	RB	10	6	0	0	2	2	40
	DA	RB	10	6	1	1	1	1	40
	TR	RB	10	0	0	0	0	10	100

When comparing the intensity of pathology and histological scoring, there was no significant difference in average gross pathology among the four treatment groups. There were also no significant differences between the rainbow and cutthroat trout exposed during July. When analyzing each species separately, there were no differences within each species between monthly exposures. However, when analyzing the site difference (model: average histology = treatment location), there was a significant difference ($P=0.0001$) among the four locations, with the Trail Creek site having significantly higher ($P\leq0.05$) severity of infection than the other locations.

Although there were no significant differences between the treatment groups when analyzing gross pathology, there were differences in the development of clinical signs. Cutthroat trout exposed in June developed significantly less clinical signs (model: average number of clinical signs/fish = treatment location) than any other exposure group ($P\leq0.05$). Location of exposure was also determined significant in the development of clinical signs, with fish exposed at Trail Creek exhibiting significantly higher responses ($P\leq0.05$). Cutthroat trout were more likely to develop exophthalmia than were rainbow trout with similar infections (Figure 1). In the July exposure, cutthroat trout exposed at Cow Creek and Danskin had a significantly higher occurrence of exophthalmia than rainbow trout (Fisher's Exact $P \leq 0.001$).

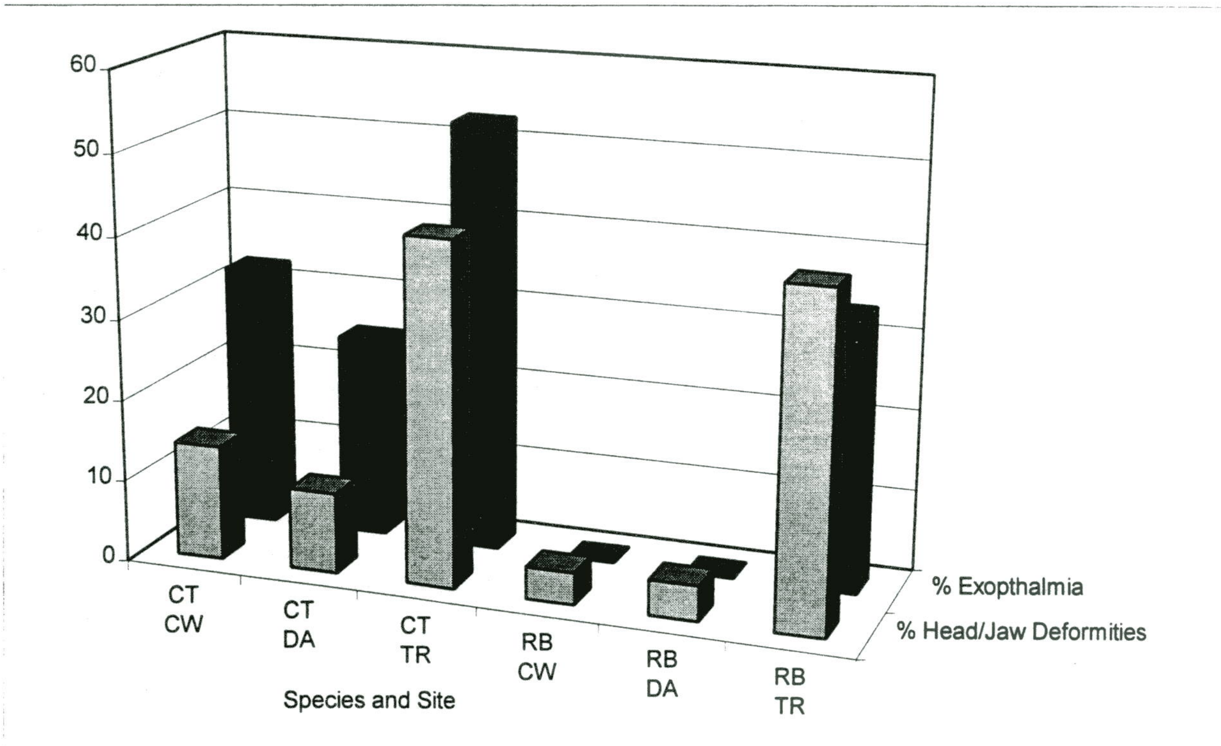

Figure 1. Comparison of exophthalmia and head/jaw deformities between rainbow and cutthroat trout exposed at Cow Creek, Danskin, and Trail Creek in July.

From preliminary results of samples of aquatic invertebrates a trend in the number of oligochaetes per square meter was observed, with little variation in densities among the three upper sites (between 77-357/m^2), and a higher abundance at the Trail Creek site, which averaged 2395 oligochaetes/ m^2 (Figure 2). The average number of Chironomids collected per sample at the Anderson site was low (2 chironomids/square meter) and average chironomid densities of 79/m^2 and 61/m^2 were recorded at Cow Cr. and Danskin (respectively). Samples from Trail Creek had the highest average number of midges with 371/m^2.

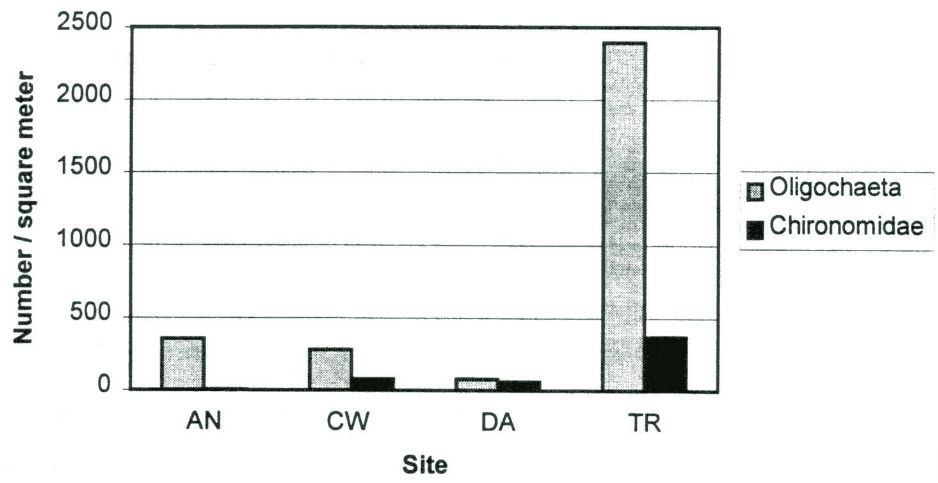

Figure 2. A comparison among the study locations of the average number of individuals per meter from the Family Chironomidae and Order Oligochaeta.

Discussion

Cutthroat trout exposed in June had a significantly lower number of clinical signs when compared to the July group of cutthroat (ANOVA $P \leq 0.05$), and no black tails or whirling behavior were observed in the June cutthroat. The average prevalence and severity of infection were lower in June, but did not show significant differences between the months (90% and 3.35 compared with 100% and 4 for June and July, respectively).

During July, no histological differences were observed between prevalence and severity of infection in cutthroat and rainbow trout, but cutthroat trout had a significantly higher number of clinical signs. Exophthalmia was more prevalent in cutthroat with lower grade infections.

Temperature differences among the four sites may be affecting disease prevalence. Average daily temperatures at Anderson were below 9°C during each exposure, and results from Vincent (1998) showed that severe infections begin at 9°C and peak at 14°C. Constantly cooler temperatures at this site, facilitated through hypolimnetic releases from Anderson Dam, could be inhibiting the release of TAMs if the parasite is present. Although average daily temperatures at Trail Creek were only slightly higher (between 10-11°C), maximum daily temperatures peaked at approximately 14°C. This peak in daily temperatures, coupled with high *Tubifex tubifex* densities, could be driving the intensity and prevalence of the disease at the lower site.

The trend among the sites in densities of chironomids may be a good indicator of organics and provide a surrogate tool for assessing probability of infection in areas where information on the *T. tubifex* community is not available. The family Chironomidae is easier to recognize than *T. tubifex*, which may aid in future field collections and studies. Information on the distribution of chironomids may also be more readily available from previously conducted studies.

Sentinel box studies should target probable parasite and worm habitat within the area of interest. Areas probable for infection of the parasite include lower gradient reaches with high organic and sediment loading. Wild population sampling may not be indicative of the disease prevalence and status within a geographic area if age one and older fish are sampled from areas of high probability of infection. If all fish reared in the sampled area suffered severe mortality due to the parasite, older fish from other rearing areas may move into the available habitat. Although we did not observe significant mortality due to *M. cerebralis* infections in the laboratory, observations of their erratic swimming behavior make it likely that fish exposed at the Trail Creek site in July and August would not have survived in the wild.

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