

# Correlation of environmental attributes with histopathology of native Yellowstone cutthroat trout naturally infected with *Myxobolus cerebralis*

Silvia Murcia<sup>1,4,\*</sup>, Billie L. Kerans<sup>1</sup>, Elizabeth MacConnell<sup>2</sup>, Todd M. Koel<sup>3</sup>

<sup>1</sup>Department of Ecology, 310 Lewis Hall, Montana State University, Bozeman, Montana 59717, USA

<sup>2</sup>US Fish and Wildlife Service, Bozeman Fish Health Center, Bozeman, Montana 59715, USA

<sup>3</sup>Center for Resources, Fisheries and Aquatic Sciences Section, Yellowstone National Park, Wyoming 82190, USA

<sup>4</sup>Present address: Department of Sciences and Natural Resources, Instituto de la Patagonia, Universidad de Magallanes, Punta Arenas, Chilean Antarctic Province, Chile

**ABSTRACT:** Infection by the invasive parasite *Myxobolus cerebralis* (causing whirling disease in salmonids) is strongly influenced by a stream's physico-chemical characteristics, which might affect host pathology. We examined whether environmental variables of a *M. cerebralis*-positive tributary to Yellowstone Lake, Yellowstone National Park, USA, correlated with the histopathology of naturally infected native cutthroat trout *Oncorhynchus clarkii bouvieri*. Host inflammatory response and cranial cartilage lesions were the main correlates with whirling behavior. Canonical correlation analyses showed that the prevalence of trout with severe lesions in the cranial and jaw cartilages was highest in stream sites with a combination of high temperature and low specific conductivity. Our results reveal that environmental components can affect when and where a pathogen resides within the host, and manifestation of disease. Recognition of the synergism among environmental and histopathology factors most conducive to whirling disease will increase our prediction and detection abilities for *M. cerebralis* in salmonid hosts.

**KEY WORDS:** Whirling disease · Pathogen · Invasion–environment synergism · Canonical correlation · Yellowstone ecosystem

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## INTRODUCTION

The occurrence and severity of disease depend on a range of factors including characteristics of the environment, the host, the pathogen, and their interactions (Snieszko 1974, Hedrick 1998). Laboratory studies with terrestrial (e.g. Brown et al. 2003, Greer et al. 2005) and aquatic host organisms (e.g. Sousa & Gleason 1989, Jokela et al. 2005) show that environmental factors, such as warming temperatures and abnormal water chemistry, can significantly influence parasitic infection and diseases (Schaperclaus 1992, Pickering 1993, Harvell et al. 2002). A variety of stressor inputs can increase stress hormone release (corticosteroids), which reduces natural and acquired resistance to infections (Pickering 1993). Such environmental stres-

sors can affect the physiology, reproduction, and survival of parasites and their hosts (Schaperclaus 1992, Harvell et al. 2002), thus directly increasing host populations' susceptibility to parasitism and disease, or decreasing their ability to survive infection (Sousa & Gleason 1989). In addition, the synergism of multiple environmental stressors may have worse effects on host–parasite dynamics than would single stressors (e.g. Valtonen et al. 1997). Fish tolerance to pathogens, for instance, will tend to be lower when multiple stresses operate at the same time (Myers 1995, Lafferty & Kuris 1999). Natural populations are often exposed to seasonal or stochastic environmental stress, thus often exposed to multiple stressors, which in parasite-infested systems increases disease risk among host populations.

\*Email: silvia.murcia@umag.cl

Hydrothermal and high elevation streams are examples of systems where the physical and chemical environments are variable (e.g. severe temperature or pH fluctuations) and can inflict additional stresses on the native biota beyond seasonal changes. In Lake Wabamun in Alberta, Canada, thermal effluents facilitated parasite transmission between hosts throughout the year and increased prevalence of certain parasites (Sankurathri & Holmes 1976). Synergisms between infection by metacercaria and exposure to pesticides and herbicides were reported as the cause of vertebral deformities in frogs (Kiesecker 2002). These are examples of environments where natural (e.g. hydrothermal) and anthropogenic (e.g. pollutant) stressors often have a strong influence on parasite–host interactions and development of diseases.

However, synergisms between the effects of environmental stressors and development of disease from parasitic infection in wild fish are seldom examined through a histopathology approach (but see Roubal 1994). Histopathology is key to identifying target organs of parasitic infection and pathogens' mode of action at the biochemical level of organization (severity of disease), which may affect ecosystem function at the population level of organization (Schwaiger 2001). Histopathological anomalies in fish, for example, are frequently used as indicators of chemical pollution in marine and fresh water environments (Schwaiger 2001, Wester et al. 2002, Kent et al. 2004), identifying risks of population declines. But we found no examples in the scientific literature of studies on the potential relationships between environmental stress and histopathology of parasitic infection in the wild.

*Myxobolus cerebralis*, the exotic parasite causing salmonid whirling disease, has devastated wild trout populations throughout the intermountain west of the USA over the last decade (Vincent 1996, Koel et al. 2006). Given the unpredictable nature of invasions, the ability to identify local abiotic conditions influencing pathology of infection by *M. cerebralis* among native trout can facilitate development of efficient management tools. Effects of parasite invasion differ across sites, and the ability to characterize and predict its impact requires information about host and parasite responses to local conditions and to each other (Hedrick 1998). This information can lead to increased vigilance at the sites of most likely establishment, which raises detection rates and, consequently, can expedite response to invasion (Hulme 2006).

A stream's physico–chemical characteristics influence infection by *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*, the species most frequently examined. For example, infection prevalence was positively correlated to water temperature in laboratory studies (Halliday 1976, Markiw 1992) and in

field studies in Montana (Baldwin et al. 2000, Krueger et al. 2006), Idaho (Hiner & Moffitt 2001), and Utah (de la Hoz Franco & Budy 2004). In addition, decreasing severity of whirling disease was correlated to increasing stream flow (Hallett & Bartholomew 2008), and conductivity was positively correlated to increased prevalence of infection and clinical signs (whirling swimming, black tails, skeletal deformities) in sentinel rainbow trout (Sandell et al. 2001). It is possible that environmental components affect when and where the parasite resides within the fish, and thereby manifestation of disease. The actinospores infective to the fish have a chemotactic specificity for salmonids and selectively bind to host living tissue, as shown with rainbow trout (El-Matbouli et al. 1999). Physico–chemical constituents of a stream, such as conductivity (Sandell et al. 2001), dissolved solids, or water velocity (Hallett & Bartholomew 2008), could further influence *M. cerebralis'* host recognition, attachment, and burrowing rate into skin (e.g. El-Matbouli et al. 1995, 1999).

Once inside the fish, the pathogen's affinity for cartilaginous tissue causes variable lesion severity depending on characteristics of the host. In rainbow trout, severe and lethal pathology occurs in cranial cartilage, but other salmonids survive *Myxobolus cerebralis* infection when lesions occur in cartilage of the caudal peduncle area (e.g. *Prosopium williamsoni*; MacConnell & Vincent 2002) or the fin rays and gill arches (e.g. *Salmo trutta*; Hedrick et al. 1999a,b, Baldwin et al. 2000). Besides characteristics of the salmonid host, the oligochaete host, and the parasite, environmental stressors (Hedrick 1998) may also influence location and severity of *M. cerebralis* infection and dispersal of whirling disease within a system.

Little is known, however, about factors influencing the pathology of native Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri*, or its potential to survive *Myxobolus cerebralis* infection, especially in high-country streams of the intermountain west. Here, we examine the relationships between a suite of environmental factors and histopathology of *M. cerebralis* infection in Yellowstone cutthroat trout exposed in the wild. *M. cerebralis* was detected in Yellowstone Lake in 1998 and over the last decade has posed a growing threat on the declining cutthroat trout population (Koel et al. 2005, 2006, Murcia et al. 2006). The Yellowstone Lake ecosystem is the last stronghold for one of the most ecologically and economically valued inland cutthroat trout populations in North America.

We examined Yellowstone cutthroat trout exposed at 4 different times in 3 sites of a *Myxobolus cerebralis*-positive tributary to Yellowstone Lake: Pelican Creek (Koel et al. 2006, Murcia et al. 2006). Unlike our prior work (Murcia et al. 2006), we correlate infection with a set of environmental predictor variables, placing

histopathology of this parasitic infection in the wild, natural context. Here, we focus on the 4 most important infection response variables for survival (cranium, lower and upper jaw cartilages, and inflammation; Murcia et al. 2006) of cutthroat trout, across multiple sites and exposure times. Our concurrent studies at these particular sites and exposure times in Pelican Creek identified only lineage III *Tubifex tubifex* (susceptible to *M. cerebralis*; Baxa et al. 2008) and abundance of infected *T. tubifex* to be relatively uniform (J. Alexander, Montana State University, pers. comm.). We can therefore assume, in this particular context, that environmental factors are a chief influence on the fish infection responses observed because densities of, and infection in, *T. tubifex* were similar throughout the study sites. Our objectives were to determine whether histopathology of infection in Yellowstone cutthroat trout was associated with (1) prevalence of whirling behavior (fish spin until exhaustion) and (2) environmental variables, and (3) whether such associations differed among exposure sites and exposure times.

## MATERIALS AND METHODS

**Study area and field exposures.** Pelican Creek, at the north end of Yellowstone Lake (Fig. 1), is the lake's second largest tributary (2400 m above sea level). It flows southwest for 53 km from its headwaters to the lake. The stream is low gradient, meandering through a valley of sub-alpine meadows and grass-covered banks. Many of Pelican Creek's over 100 tributaries are hydrothermal springs. The lack of canopy cover throughout most of the stream and warm, hydrothermal influences, lead to high temperature and primary production.

We examined *Myxobolus cerebralis* infection in Yellowstone cutthroat trout using histopathology of cranium, lower and upper jaw, inflammation, and whirling behavior. Variables were measured in fry after their exposure to the parasite in sentinel cages (1 m height  $\times$  0.5 m diameter) in their natural environment. Parasite-free Yellowstone cutthroat fry (4 to 6 wk old) were obtained from the Wyoming Game and Fish Department's broodstock, originating from the Yellowstone River and Clear Creek, a large tributary on the lake's eastern shore.

Field exposures were conducted as in Murcia et al. (2006) but at 3 sites (upper, middle, lower; Fig. 1) in the lower 13 km of Pelican Creek. Locations for sentinel cages were selected to represent a wide range of environmental characteristics (e.g. water temperature, velocity) and typical native fry habitat, while considering logistic constraints and minimizing the probability of cages being discovered, or tampered with, by park visitors or wildlife. Two cages, 50 to 60 m apart, were

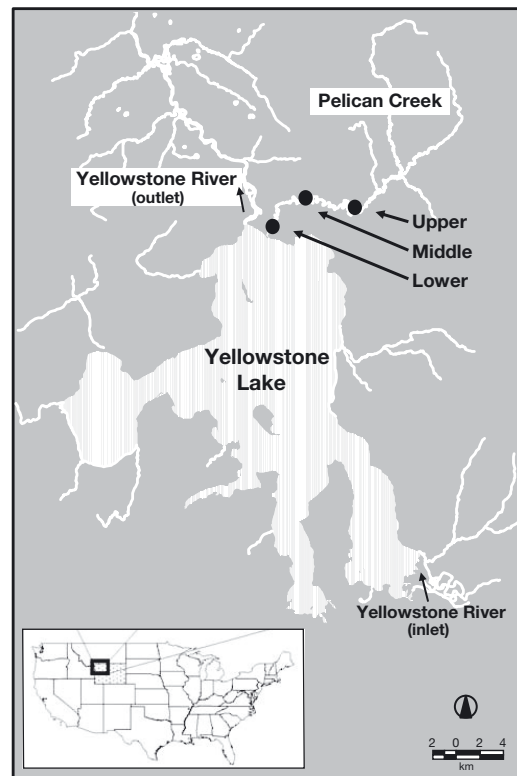


Fig. 1. Yellowstone Lake, Yellowstone National Park, Wyoming, USA. Study sites (●) in Pelican Creek (*Myxobolus cerebralis*-positive tributary) where four 10 d exposures of cutthroat trout *Oncorhynchus clarkii bouvieri* were performed using duplicate sentinel cages in July, August, and September 2002, and July 2003

used at each site and 60 fry were deployed in each. Cages and fry remained in the stream for 10 d in July (10 to 20), August (7 to 17), and September (August 28 to September 7) 2002. In 2003, fry were exposed in July (8 to 19) because high temperatures and several wild fires in August and September led to high fry mortality or precluded access to the sites entirely. For each exposure, a control group of 60 Yellowstone cutthroats were reared in well-water for 10 d at the park's Aquatic Resources Laboratory. At the end of each exposure, we transported the fry to the Wild Trout Research Laboratory at Montana State University, Bozeman, MT, and reared them in separate tanks at 12 to 13°C for 90 d. Holding fish for 90 d allows for sufficient parasite development for examinations to determine prevalence and severity of infection (Murcia et al. 2006).

**Histopathology and whirling behavior.** On Day 90 post-exposure, 10 fry were randomly selected from each aquarium and observed for 30 to 60 s for whirling behavior prior to being sacrificed in tricaine methane-sulfonate. We did not record prevalence of skeletal deformities and black tails because these clinical signs were seldom present (Murcia et al. 2006). Fry heads

were removed and half heads prepared for testing by nested polymerase chain reaction (PCR) for *Myxobolus cerebralis* DNA (Andree et al. 1998) in pools of 5 as in Murcia et al. (2006). Nested PCR was used to detect infection presence/absence and thereby identify which fry heads required histological analysis to determine location and severity of pathology. If a sample tested positive for *M. cerebralis* DNA by PCR, all 5 heads in the sample were microscopically analyzed using standard histological techniques (Humason 1979) as in Murcia et al. (2006). We also analyzed a random sub-sample of 30 PCR-negative half heads to verify lack of infection. We examined lesions in cartilage of the cranium, lower and upper jaw, and inflammation to measure pathology because, in our prior studies, lesions were rarely observed in cartilage of the nares, vertebrae, and gill arches (Murcia et al. 2006). Microscopic lesions and inflammation were scored on a 6 category scale of no infection/inflammation, minimal, mild, moderate, moderately severe, and severe (Andree et al. 2002). Fish in the moderate and above categories had several to all cartilage regions with mild to severe degeneration and necrosis, and granulomatous inflammation impacting surrounding tissues, including bone displacement in severe cases (for detailed category description see Andree et al. 2002). Tissue sections were evaluated blindly—with no knowledge of sentinel cage site or PCR results.

The proportions of fry with moderate to severe inflammation and lesions in cranial and jaw cartilage were used for statistical analysis. Infection severities of moderate to severe were considered to be biologically meaningful because below that there is little to no impact on surrounding tissue (Murcia et al. 2006), or population-level impacts (Vincent 2002).

**Environmental characteristics.** We measured environmental attributes of the study streams as predictor variables to identify potential factors influencing pathology of *Myxobolus cerebralis* in the wild. Daytime (08:00 to 20:00 h) water temperature ( $^{\circ}\text{C}$ ), specific conductivity ( $\mu\text{S}$ ), and dissolved oxygen concentration ( $\text{mg l}^{-1}$ ) were measured at each cage location during each exposure using a YSI (Yellow Springs Instruments) model 85 multiparameter meter. Water velocity ( $\text{m s}^{-1}$ ) was measured by setting random transects across the stream channel, perpendicular to the flow direction and adjacent to each cage, and channel depth (m) and current velocity were measured with a Marsh-McBirney Flo-Mate 2000 flow meter at 10 to 15 equidistant intervals. Measurements were averaged into one mean depth and velocity measurement per cage location. Due to a malfunctioning flow meter, we could not measure water velocity during the September exposure at the Pelican upper site. Instead, the average of Cage 1 and the average of Cage 2 from the

other 3 exposures at this site were used as a surrogate of a velocity measure at Cages 1 and 2, respectively, for September.

**Statistical analyses.** Correlations (Pearson correlation coefficients) were used to determine which of the histopathology measures was best associated with prevalence of whirling behavior. Residuals were examined for homogeneity of variance and no transformation was needed. Canonical correlations were used to examine potential relationships between the set of histopathology (response) variables and the set of environmental (predictor) variables simultaneously (James & McMulloch 1990, Manly 2005). In canonical correlation analysis, linear combinations of the attributes (canonical variables) are created for each data set such that the correlations between canonical variables of the 2 data sets are maximized. These combinations are analogous to the eigenvectors of principal component analysis (Manly 2005). Eight new canonical variables were created, 4 from the environmental attributes ( $\text{ENV}_1, \text{ENV}_2, \text{ENV}_3, \text{ENV}_4$ ) and 4 from the histopathology response variables ( $\text{PAT}_1, \text{PAT}_2, \text{PAT}_3, \text{PAT}_4$ ). The canonical correlations (between  $\text{ENV}_1$  and  $\text{PAT}_1$ , between  $\text{ENV}_2$  and  $\text{PAT}_2$ , etc.) give an overall feel for the degree of association between the environmental and histopathology variables (James & McMulloch 1990, Manly 2005). Only canonical correlations with  $p$ -values  $< 0.05$  were considered to be significant. All statistical analyses were carried out with the statistical software program SAS 9.1 (SAS Institute 2007).

## RESULTS

### Histopathology and whirling behavior

Across all 4 exposures, whirling behavior occurred at an average rate of 13% of the fish at the upper site, 30% at the middle, and 54% at the lower (all ratios reported as number of fry whirling/total fry examined). The highest prevalence of whirling behavior was observed at the lower site during the July and September 2002 exposures (Table 1). Moderate to severe inflammation and cranial lesions were most frequent at the lower site during all exposures (Table 1). However, cartilage of the jaws was also highly infected during the July 2002 and 2003 exposures at all sites, except the middle site in July 2002 (Table 1). The prevalence of whirling behavior was positively correlated with inflammation ( $r = 0.56$ ,  $p = 0.005$ ,  $n = 24$ ; Fig. 2a) and with lesions in the cranium ( $r = 0.50$ ,  $p = 0.01$ ; Fig. 2b). The correlations between prevalence of whirling and lesions in lower jaw ( $r = 0.44$ ,  $p = 0.03$ ) and lesions in upper jaw ( $r = 0.39$ ,  $p = 0.06$ ,  $n = 24$ ) exhibited similar patterns, although the latter was not significant.

Table 1. *Myxobolus cerebralis* infecting *Oncorhynchus clarkii bouvieri*. Proportion of Yellowstone cutthroat fry with inflammation and infection severity greater than moderate in each cartilage, and proportion of fry exhibiting whirling behavior, after four 10 d natural exposures to *M. cerebralis* in Pelican Creek, Yellowstone National Park, in 2002 and 2003. Mean proportions for duplicate cages at each site ( $\pm 1$  SE)

Exposure site	Exposure period	Whirling behavior	Histopathology			
			Cranium	Upper jaw	Lower jaw	Inflammation
<b>Upper</b>	Jul 2002	0.32 ( $\pm 0.18$ )	0.45 ( $\pm 0.45$ )	0.25 ( $\pm 0.25$ )	0.25 ( $\pm 0.25$ )	0.45 ( $\pm 0.45$ )
	Aug 2002	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )
	Sep 2002	0.20 ( $\pm 0.2$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )
<b>Middle</b>	Jul 2003	0.0 ( $\pm 0.0$ )	1.00 ( $\pm 0.0$ )	0.59 ( $\pm 0.09$ )	0.50 ( $\pm 0.20$ )	1.00 ( $\pm 0.0$ )
	Jul 2002	0.50 ( $\pm 0.1$ )	0.40 ( $\pm 0.0$ )	0.10 ( $\pm 0.10$ )	0.10 ( $\pm 0.10$ )	0.85 ( $\pm 0.05$ )
	Aug 2002	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )
	Sep 2002	0.30 ( $\pm 0.3$ )	0.30 ( $\pm 0.10$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.30 ( $\pm 0.10$ )
<b>Lower</b>	Jul 2003	0.40 ( $\pm 0.0$ )	1.00 ( $\pm 0.0$ )	0.89 ( $\pm 0.11$ )	0.95 ( $\pm 0.05$ )	0.95 ( $\pm 0.05$ )
	Jul 2002	0.80 ( $\pm 0.0$ )	1.00 ( $\pm 0.0$ )	0.45 ( $\pm 0.05$ )	0.90 ( $\pm 0.10$ )	0.40 ( $\pm 0.0$ )
	Aug 2002	0.10 ( $\pm 0.1$ )	0.30 ( $\pm 0.30$ )	0.0 ( $\pm 0.0$ )	0.0 ( $\pm 0.0$ )	0.30 ( $\pm 0.30$ )
	Sep 2002	0.80 ( $\pm 0.0$ )	0.70 ( $\pm 0.30$ )	0.40 ( $\pm 0.20$ )	0.20 ( $\pm 0.0$ )	0.90 ( $\pm 0.10$ )
	Jul 2003	0.45 ( $\pm 0.05$ )	0.85 ( $\pm 0.05$ )	0.79 ( $\pm 0.09$ )	0.90 ( $\pm 0.10$ )	0.85 ( $\pm 0.05$ )

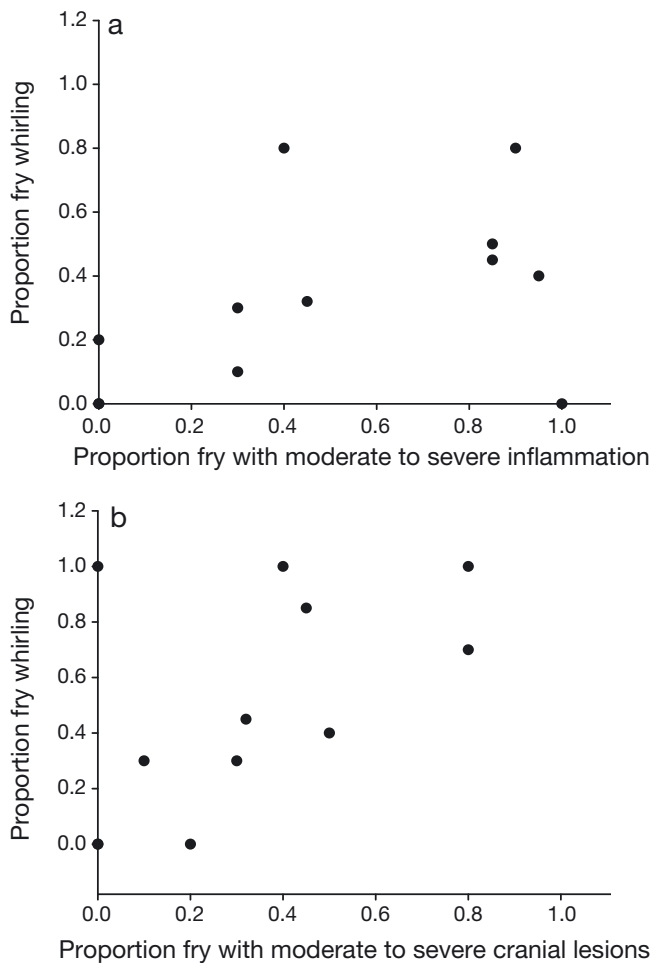


Fig. 2. *Myxobolus cerebralis* infecting *Oncorhynchus clarkii bouvieri*. Correlation between the proportion of fry whirling and proportion of Yellowstone cutthroat trout with moderate to severe (a) inflammation and (b) lesions in cranial cartilage. Shown are means of 2 cages per site per exposure in Pelican Creek (*M. cerebralis*-positive tributary to Yellowstone Lake)

### Histopathology and environment

Daytime temperature was generally high and specific conductivity low during the July exposures in 2002 and 2003 at all sites, except the upper site in July 2003 and the middle site in July 2002 (Table 2). Velocity was lowest at the lower site during the July exposures in 2002 and 2003, and at the middle site in August, when depth was also the lowest (Table 2).

We found a high correlation between the first pair of canonical variables ( $r = 0.819$ , eigenvalue = 2.03,  $F_{20,50.7} = 1.87$ ,  $p = 0.038$ ), and low correlations between the second, third, and fourth pairs of canonical variables (all eigenvalues  $< 0.7$ , and all  $p > 0.376$ ); thus, only the first pair of canonical variables ( $ENV_1$  and  $PAT_1$ ) was interpreted because it explained the strongest relationship between pathology and environmental characteristics. The environmental variables that loaded strongly on  $ENV_1$  were water temperature and specific conductivity (Table 3). Locations and times with high water temperature tended to have low specific conductivities. All histopathology variables loaded positively on  $PAT_1$ , with the lower jaw having the strongest correlation (Table 3).

Although interpretation of canonical correlations must be with caution given the explorative or descriptive nature of the analysis, the correlation between the first pair of canonical variables suggested that the frequency of moderate to severe lesions in cranium and jaw cartilage (especially, lower jaw) was high in areas with high temperatures and low specific conductivity. These patterns were observed in both replicate cages at the lower site in both July exposures, both replicate cages at the middle site in July 2003, and only 1 replicate cage of the upper site in July 2002. These sites had the highest values of the first canonical variable for infection,  $PAT_1$ , and the environment,  $ENV_1$  (Fig. 3). A

Table 2. *Myxobolus cerebralis* infecting *Oncorhynchus clarkii bouvieri*. Environmental attributes measured at 3 study sites in Pelican Creek, Yellowstone National Park, during four 10 d exposures of Yellowstone cutthroat fry to *M. cerebralis* in 2002 and 2003. Means for duplicate cage locations at each site ( $\pm 1$  SE)

Exposure site	Exposure period	Temperature (°C)	Specific conductivity ( $\mu$ S)	Dissolved oxygen ( $\text{mg l}^{-1}$ )	Velocity ( $\text{m s}^{-1}$ )	Depth (m)
<b>Upper</b>	Jul 2002	18.5 ( $\pm 0$ )	278.1 ( $\pm 0.05$ )	9.2 ( $\pm 0.01$ )	0.20 ( $\pm 0.06$ )	0.34 ( $\pm 0.03$ )
	Aug 2002	11.7 ( $\pm 0.05$ )	329.9 ( $\pm 2.00$ )	10.8 ( $\pm 0.26$ )	0.12 ( $\pm 0.04$ )	0.27 ( $\pm 0.05$ )
	Sep 2002	11.3 ( $\pm 0.05$ )	280.8 ( $\pm 0.05$ )	6.82 ( $\pm 0.14$ )	0.16 ( $\pm 0.03$ )	0.27 ( $\pm 0.05$ )
	Jul 2003	14.7 ( $\pm 0.15$ )	286.3 ( $\pm 1.00$ )	8.47 ( $\pm 0.06$ )	0.16 ( $\pm 0.02$ )	0.31 ( $\pm 0.02$ )
<b>Middle</b>	Jul 2002	16.1 ( $\pm 0.10$ )	236.3 ( $\pm 1.30$ )	10.8 ( $\pm 0.05$ )	0.18 ( $\pm 0$ )	0.32 ( $\pm 0.05$ )
	Aug 2002	18.5 ( $\pm 0.15$ )	361.8 ( $\pm 1.60$ )	12.0 ( $\pm 0.20$ )	0.04 ( $\pm 0.02$ )	0.18 ( $\pm 0.06$ )
	Sep 2002	11.0 ( $\pm 0.05$ )	313.1 ( $\pm 0.50$ )	7.39 ( $\pm 0.07$ )	0.13 ( $\pm 0.01$ )	0.28 ( $\pm 0.02$ )
	Jul 2003	21.0 ( $\pm 0$ )	303.7 ( $\pm 2.40$ )	12.9 ( $\pm 0.40$ )	0.19 ( $\pm 0.02$ )	0.27 ( $\pm 0.01$ )
<b>Lower</b>	Jul 2002	22.4 ( $\pm 0.05$ )	300.9 ( $\pm 0.20$ )	7.2 ( $\pm 0$ )	0.16 ( $\pm 0.01$ )	0.27 ( $\pm 0.04$ )
	Aug 2002	13.7 ( $\pm 0.10$ )	308.4 ( $\pm 0.75$ )	8.94 ( $\pm 0.01$ )	0.23 ( $\pm 0.02$ )	0.23 ( $\pm 0.04$ )
	Sep 2002	12.4 ( $\pm 0.05$ )	318.7 ( $\pm 0.10$ )	8.15 ( $\pm 0.05$ )	0.24 ( $\pm 0.11$ )	0.25 ( $\pm 0.02$ )
	Jul 2003	20.0 ( $\pm 0$ )	285.1 ( $\pm 1.05$ )	7.4 ( $\pm 0.01$ )	0.09 ( $\pm 0.05$ )	0.47 ( $\pm 0.28$ )

Table 3. Correlations between environmental attributes measured at Pelican Creek, Yellowstone National Park, and their canonical variables ( $\text{ENV}_1$ ) and the correlations between pathology response measures for *Myxobolus cerebralis*-infected Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* and their canonical variables ( $\text{PAT}_1$ ). Env. var.: environmental variables; Path. var.: pathology variables. High correlation given in **bold**

Env. var.	Correlations $\text{ENV}_1$	Path. var.	Correlations $\text{PAT}_1$
Temperature	<b>0.915</b>	Cranium	<b>0.840</b>
Specific conductivity	<b>-0.811</b>	Upper jaw	<b>0.817</b>
Dissolved oxygen	-0.035	Lower jaw	<b>0.982</b>
Velocity	0.138	Inflammation	0.786
Depth	0.263		

time effect is probable because July tends to show high scores for the first pair of canonical variables (except for July 2002 at the middle site, and 1 replicate at the upper site). A slight site effect for infection is possible as the upper site, for example, consistently shows the lowest  $\text{PAT}_1$  scores, with only 1 cage exception (Fig. 3).

## DISCUSSION

Parasitic infection may render hosts more susceptible to adverse environmental conditions than otherwise healthy individuals (Snieszko 1974, Schaperclaus 1992, Jokela et al. 2005). In turn, very small environmental changes may dramatically modify host responses to parasitism (Lenihan et al. 1999, Thomas & Blanford 2003, Jokela et al. 2005). The use of histopathology is important to understand internal response to parasitic infection, and thus potential impacts at the

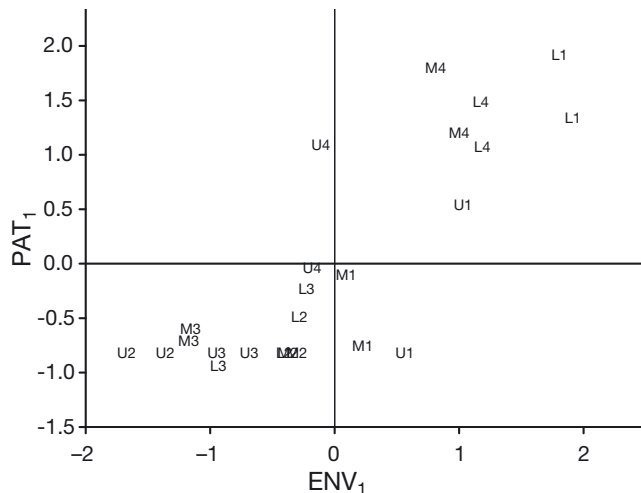


Fig. 3. Canonical correlations between canonical variables  $\text{ENV}_1$  and  $\text{PAT}_1$  representing linear combinations of 5 environmental predictor variables and 4 pathology response variables measured at 3 sites during four 10 d sentinel Yellowstone cutthroat fry exposures in Pelican Creek, Yellowstone National Park. Sites: L = Lower, M = Middle, U = Upper. Exposure: 1 = July 2002, 2 = August 2002, 3 = September 2002, 4 = July 2003. (One cage at the Lower and both at the Middle site overlap for August:  $L_2$  and  $M_2$ , respectively)

population level (Schwaiger 2001, Vincent 2002). Our study tested whether exposure of Yellowstone cutthroat to *Myxobolus cerebralis* in a naturally stressful environment influenced infection pathology (location and severity of lesions) and whirling behavior over multiple sites and exposure times.

Results of correlation analyses suggested a strong relationship between the host's moderate to severe inflammatory response and cranial lesions, and whirling behavior. These results were expected because when a pathogen invades cranial cartilage, the host's inflam-

matory response may restrict nerves along the lower brain stem and spinal cord causing the whirling motion observed amongst infected trout and salmon (Hedrick et al. 1999b, Rose et al. 2000). Though this may be a reasonable hypothesis, lesions in cartilage of the lower jaw also correlated with prevalence of whirling behavior (and may correlate with lesions in cranium, thus not necessarily precluding this mechanism). Exceptions occurred in some sites and exposures, however, where fry showed whirling signs when neither inflammation, cranial nor jaw lesions were severe. This suggests that factors other than *Myxobolus cerebralis* infection, such as other parasites, environmental stressors, or physiological deficiencies, may also have caused whirling and abnormal swimming behavior (Schaperclaus 1992, Margolis et al. 1996) among those fry. Alternatively, fry could have severely damaged cartilage and inflammation of surrounding tissue throughout the head and exhibit no overt clinical signs of disease (e.g. Thompson et al. 1999, Sipher & Bergersen 2005). Hence, although clinical signs are an important gauge of overall fish health, they may not be a main indicator of *M. cerebralis* pathology or population survival potential, as they may not always reflect internal damage to vital cartilage regions (Vincent 2002) such as cranium and jaws.

Based on the canonical correlation, the proportion of Yellowstone cutthroat fry with moderate to severe lesions in cartilage of the lower jaw strongly correlated with a combination of high water temperature and low specific conductivity. This was most notable at the lower site during both July exposures and the middle site in July 2003. That suggests strong relationships between exposure to environmental stress and increased infection pathology, with decreased immunocompetency associated with environmental stress (e.g. Kiesecker 2002). The influence of temperature stress on *Myxobolus cerebralis* infection in rainbow and cutthroat trout is well recognized (Markiw 1992, Vincent 2002, de la Hoz Franco & Budy 2004, Krueger et al. 2006). But, few studies have examined its relationship with conductivity (but see Sandell et al. 2001), or their potentially synergistic effects—or additional stressors—and salmonid vulnerability to whirling disease. In Sandell et al. (2001), only conductivity correlated with infection prevalence among sentinel rainbow trout, but not temperature, pH, or total dissolved solids—perhaps because these were not analyzed concurrently but individually against presence/absence of infection.

Although prior research shows an optimal temperature range of 15 to 17°C for *Myxobolus cerebralis* spore production, infection and development in the fish host (Halliday 1976, MacConnell & Vincent 2002), we did not find a high proportion of moderately to highly

infected fish in that temperature range (e.g. middle site in July 2002, 16.1°C). The fact that we were not correlating infection severity with temperature alone but in concert with several other factors might explain these results. Although our measurements could not capture diel temperature variation, the high daytime temperatures we recorded at sites where fish were severely infected are well above the metabolically optimal range requirements of trout (Dickerson & Vinyard 1999, Leprieur et al. 2006). This probably worsened the physiological condition of sentinel trout and increased their vulnerability to parasitic infection. At the sites where temperature was highest, specific conductivity remained under 305 µS. Thus, we cannot argue for the importance of one factor over the other, but perhaps the overlap of the 2, compounded with the presence of the parasite in the system, as the likely source for the onset of disease (e.g. Hedrick 1998). Synergisms may arise in the wild when 2 or more environmental factors interact in such a way that the outcome is not additive but multiplicative (Myers 1995, Kiesecker 2002).

Fish were most severely infected at the middle and especially lower site of Pelican Creek, which probably resulted from the cumulative effects of high numbers of myxospores, warm water (e.g. from extended sun exposure), and similar biotic (e.g. oligochaetes) or physico-chemical stressors accumulated and carried throughout the drainage from upstream. This is a common pattern of *Myxobolus cerebralis* infection in the wild whereby infection prevalence and severity increase in a downstream progression (Hiner & Moffitt 2001, Sandell et al. 2001, Hubert et al. 2002). For example, we observed severe jaw and cranial infection in fry from the lower site in September even under low temperature (12.4°C) and high specific conductivity (319 µS). At this time in the season, increased water conductivity probably results from increased summer productivity (Holmes 1990, Edwards & Helvey 1991) or diminishing melt effects, and subsequent increased influences of hydrothermal discharge, or a combination of all. We suggest that pathology may correlate differently with single environmental attributes than with several factors in unison. We further propose that conclusions on fish host pathology should not be drawn based on site or exposure time alone because pathology in the cutthroat trout host differed between sites and with exposure times. This may imply important spatio-temporal factors in host infection pathology that could otherwise be missed if evaluated on a seasonal or spatial basis alone.

Unlike findings in Oregon (Sandell et al. 2001) and Montana (Krueger et al. 2006), *Myxobolus cerebralis* infection in our study system did not relate to high specific conductivity, except at the lower site in September (Table 2). But a low conductivity range in Pelican

Creek (285 to 310  $\mu\text{S}$ ) is probably comparable to the high ends of the range in other systems (e.g. Sandell et al. 2001, Krueger et al. 2006). An example is the severe infection pathology observed in July 2003 at the middle and lower site where specific conductivity (304 and 285  $\mu\text{S}$ , respectively) would be considered low for Pelican Creek, but high in other systems. This could indicate a conductivity threshold range of 180 or 200  $\mu\text{S}$  to ~300  $\mu\text{S}$  below and above which prevalence and severity of *M. cerebralis* declines considerably in some systems (e.g. Sandell et al. 2001, Krueger et al. 2006). Those prior studies, however, were conducted with rainbow trout, and did not examine the collective effects of conductivity and other environmental factors against the collective host pathology responses.

We propose that *Myxobolus cerebralis* infection cannot be examined in the same manner in all salmonids, but that it should be species- (e.g. Hedrick et al. 1999a,b) and context-dependent (e.g. local potential drivers of disease). That is an examination should include the histology of various cartilage regions in tandem and study potentially synergistic effects of environmental drivers of disease. For whirling disease diagnostic and sampling purposes, examination of cranial cartilage should be an effective means of assessing pathology in most salmonids because this organ is most consistently and intensely damaged. Biologically, however, lesions in the jaw are extremely important for survival and feeding purposes, and should also be assessed.

Though current knowledge of the immune response to *Myxobolus cerebralis* in the salmonid host is still limited, initial response is reportedly stimulated early in the infection with macrophage defense just below the skin surface and during the active feeding phase of the parasite: during cartilage destruction (El-Matbouli et al. 1999, MacConnell & Vincent 2002). In Yellowstone cutthroat trout, moderate to severe lesions were consistently most prevalent in lower jaw cartilage. It is possible that the parasite enters its host through the gills (operculum) and preferentially resides and consumes the nearby cartilage, at the lower jaw, instead of taking the longer migration to reach rib, vertebral, or some further cartilaginous tissue. Alternatively, in traveling to those areas, *M. cerebralis* in cutthroat trout might encounter greater resistance from the host immune response, such as dense eosinophilic granular leukocytic response in nerve ganglia (El-Matbouli et al. 1995, Hedrick et al. 1999a, MacConnell & Vincent 2002), and thus greater difficulty reaching the upper jaw, cranial, and further cartilage. The diverse location of parasite lesions observed among salmonid species and subspecies (Hedrick et al. 1999a) may consequently reflect the different host immune responses of fish under variable ambient conditions and diverse geographic areas.

Often, invasive species are opportunists taking advantage of environmental degradation (Hulme 2006), and as global climate continues to change, so will the environmental conditions within Yellowstone Ecosystems (e.g. Cook et al. 2004). Continued research and monitoring are necessary to identify new biotic and abiotic stressors (in tandem) that might facilitate pathogenic invasions, to improve our alertness and predictions, and define areas of high risk, inside and outside park boundaries.

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#### LITERATURE CITED

- Andree KB, MacConnell E, Hedrick RP (1998) A nested polymerase chain reaction for the detection of genomic DNA of *Myxobolus cerebralis* in rainbow trout *Oncorhynchus mykiss*. *Dis Aquat Org* 34:145–154
- Andree KB, Hedrick RP, MacConnell E (2002) A review of the approaches to detect *Myxobolus cerebralis*, the cause of salmonid whirling disease. In: Bartholomew JL, Wilson JC (eds) Whirling disease: reviews and current topics. *Am Fish Soc Symp* 29, Bethesda, MD, p 197–211
- Baldwin TJ, Vincent RE, Silflow RM, Stanek D (2000) *Myxobolus cerebralis* infection in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) exposed under natural stream conditions. *J Vet Diagn Invest* 12:312–321
- Baxa DV, Kelley GO, Mukkatira KS, Beauchamp KA, Rasmussen C, Hedrick RP (2008) Arrested development of the myxozoan parasite, *Myxobolus cerebralis*, in certain populations of mitochondrial 16S lineage III *Tubifex tubifex*. *Parasitol Res* 102:219–228
- Brown MJF, Schmid-Hempel R, Schmid-Hempel P (2003) Strong context-dependent virulence in a host-parasite system: reconciling genetic evidence with theory. *J Anim Ecol* 72:994–1002
- Cook ER, Woodhouse C, Eakin CM, Meko DM, Stahle DW (2004) Long-term aridity changes in the western United States. *Science* 306:1015–1018
- de la Hoz Franco E, Budy P (2004) Linking environmental heterogeneity to the distribution and prevalence of *Myxobolus cerebralis*: a comparison across sites in a northern Utah watershed. *Trans Am Fish Soc* 133:1176–1189
- Dickerson BR, Vinyard GL (1999) Effects of high chronic temperatures and diel temperature cycles on the survival and growth of lahontan cutthroat trout. *Trans Am Fish Soc* 128:516–521



- Edwards PJ, Helvey JD (1991) Long-term ionic increases from a central Appalachian forested watershed. *J Environ Qual* 20:250–255
- El-Matbouli M, Hoffmann RW, Mandok C (1995) Light and electron microscopic observations on the route of the triactinomyxon-sporoplasm of *Myxobolus cerebralis* from epidermis into rainbow trout cartilage. *J Fish Biol* 46: 919–935
- El-Matbouli M, Hoffmann RW, Schoel H, McDowell TS, Hedrick RP (1999) Whirling disease: host-specificity and interaction between the actinosporean stage of *Myxobolus cerebralis* and rainbow trout *Oncorhynchus mykiss*. *Dis Aquat Org* 35:1–12
- Greer AW, Stankiewicz M, Jay NP, McAnulty RW, Sykes AR (2005) The effect of concurrent corticosteroid induced immuno-suppression and infection with the intestinal parasite *Trichostrongylus colubriformis* on food intake and utilization in both immunologically naive and competent sheep. *Anim Sci* 80:89–99
- Hallett SL, Bartholomew JL (2008) Effects of water flow on the infection dynamics of *Myxobolus cerebralis*. *Parasitology* 135:371–384
- Halliday MM (1976) The biology of *Myxosoma cerebralis*: the causative organism of whirling disease. *J Fish Biol* 9: 339–357
- Harvell CD, Mitchell CE, Ward JR, Altizer S, Dobson P, Ostfeld RS, Samuel MD (2002) Climate warming and disease risks for terrestrial and marine biota. *Science* 296: 2158–2162
- Hedrick RP (1998) Relationships of the host, pathogen, and environment: implications for diseases of cultured and wild fish populations. *J Aquat Anim Health* 10:107–111
- Hedrick RP, McDowell TS, Gay M, Marty GD, Georgiadis MP, MacConnell E (1999a) Comparative susceptibility of rainbow trout *Oncorhynchus mykiss* and brown trout *Salmo trutta* to *Myxobolus cerebralis*, the cause of salmonid whirling disease. *Dis Aquat Org* 37:173–183
- Hedrick RP, McDowell TS, Mukkatira K, Georgiadis MP, MacConnell E (1999b) Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. *J Aquat Anim Health* 11:330–339
- Hiner M, Moffitt CM (2001) Variation in infection of *Myxobolus cerebralis* in field-exposed cutthroat trout in Idaho. *J Aquat Anim Health* 13:124–132
- Holmes JA (1990) Sea lamprey as an early responder to climate change in the Great Lakes. *Trans Am Fish Soc* 119:292–300
- Hubert WA, Joyce MP, Gipson R, Zafft D, Money D, Hawk D, Taro B (2002) Whirling disease among Snake River cutthroat trout in two spring streams in Wyoming. In: Bartholomew JL, Wilson JC (eds) Whirling disease: reviews and current topics. *Am Fish Soc Symp* 29, Bethesda, MD, p 181–193
- Hulme PE (2006) Beyond control: wider implications for the management of biological invasions. *J Appl Ecol* 43: 835–847
- Humason GL (1979) Animal tissue techniques. WH Freeman, San Francisco, CA
- James FC, McMulloch CE (1990) Multivariate analysis in ecology and systematics: panacea or Pandora's box? *Annu Rev Ecol Syst* 21:129–166
- Jokela J, Taskinen J, Multikainen P, Kopp K (2005) Virulence of parasites in hosts under environmental stress: experiments with anoxia and starvation. *Oikos* 108:156–164
- Kent ML, Watral VG, Whipps CM, Cunningham ME and others (2004) A digenean Metacercaria (*Apophallus* sp.) and a Myxozoan (*Myxobolus* sp.) associated with vertebral deformities in cyprinid fishes from the Willamette River, Oregon. *J Aquat Anim Health* 16:116–129
- Kiesecker JM (2002) Synergism between trematode infection and pesticide exposure: A link to amphibian limb deformities in nature? *Proc Natl Acad Sci* 99:9900–9904
- Koel TM, Bigelow PE, Doepke PD, Ertel BD, Mahony DL (2005) Nonnative lake trout result in Yellowstone cutthroat trout decline and impacts to bears and anglers. *Fisheries* (Bethesda) 30:10–19
- Koel TM, Mahony DL, Kinnan KL, Rasmussen C, Hudson CJ, Murcia S, Kerans BL (2006) *Myxobolus cerebralis* in native cutthroat trout of the Yellowstone ecosystem. *J Aquat Anim Health* 18:157–175
- Krueger RC, Kerans BL, Rasmussen C, Vincent ER (2006) Correlations among environmental features, *Myxobolus cerebralis* infection prevalence in oligochaetes, and salmonid infection risk in the Madison River, Montana. *Ecol Appl* 16:770–783
- Lafferty KD, Kuris AM (1999) How environmental stress affects the impacts of parasites. *Limnol Oceanogr* 44:925–931
- Lenihan HS, Micheli F, Shelton SW, Peterson CH (1999) The influence of multiple environmental stressors on susceptibility to parasites: an experimental determination with oysters. *Limnol Oceanogr* 44:910–924
- Leprieur F, Hickey MA, Arbuckle CJ, Closs GP, Brosse S, Townsend CR (2006) Hydrological disturbance benefits a native fish at the expense of an exotic fish. *J Appl Ecol* 43:930–939
- MacConnell E, Vincent RE (2002) The effects of *Myxobolus cerebralis* on the salmonid host. In: Bartholomew JL, Wilson JC (eds) Whirling disease: reviews and current topics. *Am Fish Soc Symp* 29, Bethesda, MD, p 95–107
- Manly BFJ (ed) (2005) Canonical correlation analysis. In: Multivariate statistical methods: a primer, 3rd edn. Chapman & Hall/CRC Press, Boca Raton, FL, p 143–161
- Margolis ML, Kent ML, Bustos P (1996) Diseases of salmonids resembling myxosporean whirling disease, and the absence of *Myxosoma cerebralis*, in South America. *Dis Aquat Org* 25:33–37
- Markiw ME (1992) Experimentally induced whirling disease I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis*. *J Aquat Anim Health* 4:40–43
- Murcia S, Kerans BL, MacConnell E, Koel TM (2006) *Myxobolus cerebralis* infection patterns in Yellowstone cutthroat trout after natural exposure. *Dis Aquat Org* 71: 191–199
- Myers N (1995) Environmental unknowns. *Science* 269: 358–360
- Pickering AD (1993) Endocrine induced pathology in stressed salmonid fish. *Fish Res* 17:35–50
- Rose JD, Marrs GS, Lewis C, Schisler G (2000) Whirling disease behavior and its relation to pathology of brain stem and spinal cord in rainbow trout. *J Aquat Anim Health* 12:107–118
- Roubal FR (1994) Histopathological and ecological aspects of *Henneguya* and *Myxobolus* (Myxosporea) infections in *Acanthopagrus australis* (Günther) (Pisces: Sparidae) from Moreton Bay, Australia. *J Fish Dis* 17:495–512
- Sandell TA, Lorz HV, Stevens DG, Bartholomew JL (2001) Dynamics of *Myxobolus cerebralis* in the Lostine River, Oregon: implications for resident and anadromous salmonids. *J Aquat Anim Health* 13:142–150
- Sankurathri CS, Holmes JC (1976) Effects of thermal effluents on parasites and commensals of *Physa gyrina say* (Mollusca: Gastropoda) and their interactions at Lake Wabamun, Alberta. *Can J Zool* 54:1742–1753

- SAS Institute (2007) SAS/STAT for Windows version 9.1. SAS Institute, Cary, NC
- Schaperclaus W (1992) Causes, development and prevention of fish diseases. In: Schaperclaus W, Kulow H, Schreckenback K (eds) Fish diseases, 5th edn. AA Balkema Publisher, Rotterdam, p 3–42
- Schwaiger J (2001) Histopathological alterations and parasite infection in fish: indicators of multiple stress factors. *J Aquat Ecosyst Stress Recovery* 8:231–240
- Sipher CR, Bergersen EP (2005) The effects of whirling disease on growth and survival of Snake River cutthroat and Colorado River rainbow trout fingerlings. *J Aquat Anim Health* 17:353–364
- Snieszko SF (1974) The effects of environmental stress on outbreaks of infectious diseases of fishes. *J Fish Biol* 6:197–208
- Sousa WP, Gleason M (1989) Does parasitic infection compromise host survival under extreme environmental conditions? The case for *Cerithidea californica* (Gastropoda: Prosobranchia). *Oecologia* 80:456–464
- Thomas MB, Blanford S (2003) Thermal biology in insect–parasite interactions. *Trends Ecol Evol* 18:344–350
- Thompson KG, Nehring RB, Bowden DC, Wygant T (1999) Field exposure of seven species or subspecies of salmonids to *Myxobolus cerebralis* in the Colorado River, Middle Park, Colorado. *J Aquat Anim Health* 11: 312–329
- Valtonen ET, Holmes JC, Koskivaara M (1997) Eutrophication, pollution, and fragmentation: effects of parasite communities in roach (*Rutilus rutilus*) and perch (*Perca fluviatilis*) in four lakes in central Finland. *Can J Fish Aquat Sci* 54:572–585
- Vincent ER (1996) Whirling disease and wild trout. *Fisheries* (Bethesda) 21:32–33
- Vincent ER (2002) Relative susceptibility of various salmonids to whirling disease with an emphasis on rainbow and cutthroat trout. In: Bartholomew JL, Wilson JC (eds) Whirling disease: reviews and current topics. *Am Fish Soc Symp* 29, Bethesda, MD, p 109–115
- Wester PW, van der Ven LTM, Vethaak AD, Grinwis GCM, Vos JG (2002) Aquatic toxicology: opportunities for enhancement through histopathology. *Environ Toxicol Pharmacol* 11:289–295

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