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[Article]

Myxobolus cerebralis in Native Cutthroat Trout of the Yellowstone Lake Ecosystem

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Abstract.—The exotic parasite *Myxobolus cerebralis* was first detected in native adult Yellowstone cutthroat trout *Oncorhynchus clarkii bouvierii* from Yellowstone Lake in 1998, seriously threatening the ecological integrity of this pristine, naturally functioning ecosystem. We immediately began to assess the prevalence and spatial extent of *M. cerebralis* infection in Yellowstone cutthroat trout within Yellowstone Lake and to determine the infection risk of age-0 Yellowstone cutthroat trout, the relative abundance and actinospore production of tubificid worms, and the basic environmental characteristics of tributaries. During 1999–2001, juvenile and adult Yellowstone cutthroat trout were infected throughout Yellowstone Lake; the highest prevalence (15.3–16.4%) occurred in the northern and central regions. Exposure studies in 13 streams indicated that Pelican and Clear creeks and the Yellowstone River were positive for *M. cerebralis*; the highest prevalence (100%) and severity was found in Pelican Creek during mid-July. Sexually mature individuals of the oligochaete *Tubifex tubifex* were most abundant in early summer, were genetically homogenous, and were members of a lineage known to produce moderate to high levels of *M. cerebralis* triactinomyxons. Only 20 of the 3,037 sampled tubificids produced actinospores after 7 d in culture, and none of the actinospores were *M. cerebralis*. However, one non-actinospore-producing *T. tubifex* from Pelican Creek tested positive for *M. cerebralis* by polymerase chain reaction. Stream temperatures at Pelican Creek, a fourth-order, low-gradient stream, were over 20°C during the first exposure period, suggesting that *T. tubifex* were capable of producing triactinomyxons at elevated temperatures in the wild. Although the infection of otherwise healthy adult Yellowstone cutthroat trout within Yellowstone Lake suggests some resistance, our sentinel cage exposures indicated that this subspecies may be more susceptible to whirling disease than previous laboratory challenges have indicated, and *M. cerebralis* may be contributing to a significant recent decline in this population.

Whirling disease, caused by the exotic parasite *Myxobolus cerebralis*, is responsible for severe declines in wild trout populations in the Intermountain West (Bartholomew and Reno 2002). In Colorado (Nehring and Walker 1996) and other states where infection has been severe, whirling disease has had a significant negative economic impact on the recreational fishing industry, especially in areas where tourism is important for local and state economies. In Montana, the number of wild rainbow trout *Oncorhynchus mykiss* declined 70–90% in the Madison River after the introduction of *M. cerebralis* (Vincent 1996), and the parasite has since spread to many other drainages in the western portion of the state, resulting in population-level effects (E. Vincent, Montana Department of Fish, Wildlife, and Parks, personal communication). The parasite was first documented in Wyoming waters in 1988 and has spread to at least seven river drainages. Although fish have been observed with gross putative clinical signs of disease, no population-level losses have been documented to date (M. Stone, Wyoming Game and Fish Department, personal communication).

In Yellowstone National Park, examination of wild trout for whirling disease—particularly those trout

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inhabiting border waters—began in earnest in 1995 through the U.S. Fish and Wildlife Service's Wild Fish Health Survey. *Myxobolus cerebralis* was first detected in a 1998 collection of native Yellowstone cutthroat trout *Oncorhynchus clarkii bouvierii* from Yellowstone Lake in the park interior. The prevalence and severity of *M. cerebralis* in this system and the potential impact that the disease may have on native Yellowstone cutthroat trout populations are unknown.

The Yellowstone cutthroat trout is considered a keystone species, as it is an integral component of the greater Yellowstone ecosystem. The endangered grizzly bear *Ursus arctos horribilis* is dependent on Yellowstone cutthroat trout as a significant source of protein during the spring and midsummer of each year (Reinhart and Mattson 1990; Gunther 1995). The diet of the threatened bald eagle *Haliaeetus leucocephalus* in Yellowstone National Park consists of about 25% fish (Swenson et al. 1986). In fact, numerous avian and terrestrial species use Yellowstone cutthroat trout as an energy source in the Yellowstone Lake ecosystem (Schullery and Varley 1995). Nonnative lake trout *Salvelinus namaycush*, discovered in 1994 in Yellowstone Lake (Kaeding et al. 1996), pose an additional threat; significant declines in native Yellowstone cutthroat trout caused by cumulative impacts of exotic species could have ecosystem-level effects.

The ecology of *M. cerebralis* is complex, as it involves both a salmonid and the oligochaete *Tubifex tubifex* as hosts (Wolf et al. 1986; Gilbert and Granath 2003). The distribution and severity of whirling disease in wild trout populations have been highly variable (Nickum 1999). The risk of infection varies among salmonid species (Hedrick et al. 1999; MacConnell and Vincent 2002; Vincent 2002) and strains (Wagner et al. 2002; Hedrick et al. 2003), salmonid life history strategies (Downing et al. 2002; Ryce 2003), *T. tubifex* genetic clades (Stevens et al. 2001; Beauchamp et al. 2002), several environmental factors (Hedrick et al. 1998; Hiner and Moffitt 2001), and the interactions among all these parameters (Kerans and Zale 2002). When challenged with triactinomyxons in the laboratory, Yellowstone cutthroat trout appear to be moderately susceptible to whirling disease in comparison to other, highly susceptible species, such as rainbow trout (Hedrick et al. 1999). However, field sentinel exposures of Yellowstone cutthroat trout in Montana have shown higher infection intensities than laboratory exposures (Vincent 2002). No previous studies have examined the genetic composition of *T. tubifex* in Yellowstone National Park, but samples from the Madison River indicated the presence of a clade that is moderately susceptible to the transmission of whirling disease (Kerans et al. 2004). Environmental factors that have been most important in influencing *M. cerebralis* severity in the field have included stream temperature regime (Baldwin et al. 2000; Hiner and Moffitt 2001), flow (Vincent 2001), and sediment composition (Krueger 2002).

The waters of Yellowstone National Park provide a unique opportunity to study whirling disease in native cutthroat trout. The spawning streams vary widely in their thermal, hydrological, and geological characteristics within a relatively undisturbed region that is free from the confounding effects of land use. We hypothesized that *M. cerebralis* infection prevalence and severity in the upper Yellowstone Lake basin, above the upper falls of the Yellowstone River, would be related to Yellowstone cutthroat trout life history strategies; the presence, abundance, and infection of tubificid oligochaetes; and stream environmental gradients. The overall goal of this study was to describe patterns in infection risk of Yellowstone cutthroat trout in this complex system. Specific objectives were to (1) determine the prevalence and spatial extent of *M. cerebralis* infection in juvenile and adult Yellowstone cutthroat trout within Yellowstone Lake, (2) assess the *M. cerebralis* infection risk of age-0 Yellowstone cutthroat trout in spawning tributaries, (3) determine the relative abundance, phylogeny, and *M. cerebralis* infection of tubificid oligochaetes in spawning tributaries, and (4) relate the *M. cerebralis* infection risk to basic environmental characteristics of spawning tributaries. Improving our understanding of relationships among whirling disease infection and ecological factors will allow resource managers to focus efforts and funding on waters that have high disease potential.

Study Area

The Yellowstone Lake drainage includes 2,605 km^2 in Yellowstone National Park and adjacent U.S. Forest Service lands in Wyoming (Figure 1). Yellowstone Lake has a surface area of 34,000 ha, a shoreline length of 239 km (Kaplinsi 1991), and an average depth of 40 m (Benson 1961). At an elevation of 2,357 m, Yellowstone Lake is the largest high-elevation (above 2,000 m) lake in North America. In addition to native Yellowstone cutthroat trout and longnose dace *Rhinichthys cataractae*, the ichthyofauna of the lake basin currently includes four nonnative, introduced species, including lake trout, lake chub *Couesius plumbeus*, redside shiners *Richardsonius balteatus*, and longnose suckers *Catostomus catostomus*.

A total of 124 tributaries to Yellowstone Lake have been identified, but many of these are ephemeral (Gresswell et al. 1997). Yellowstone cutthroat trout of the upper Yellowstone Lake basin primarily exhibit an

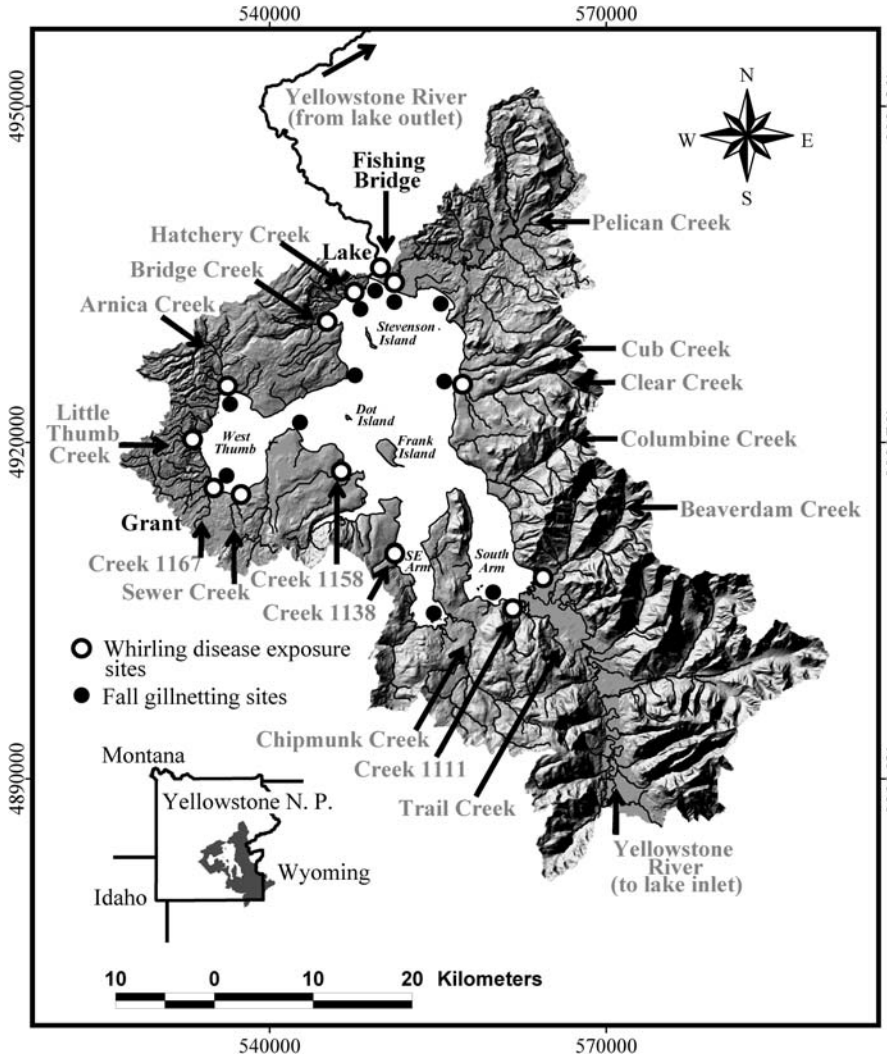

FIGURE 1.—Map of Yellowstone Lake and the upper Yellowstone River drainage within Yellowstone National Park, showing the locations of the 11 cutthroat trout lake gill-netting sites and the 12 streams and Yellowstone River near Fishing Bridge, where Yellowstone cutthroat trout sentinel fry exposure and tubificid studies were conducted in 1999–2001.

adfluvial life history strategy (Gresswell and Varley 1988), although other movement patterns exist (Kaeding and Boltz 2001). Spawning has been documented in 68 tributaries, but 16 of these are used only during years with above-average stream discharge (Jones et al. 1987; Gresswell et al. 1997).

Yellowstone Lake tributary basins are predominantly subalpine and contain lodgepole pine *Pinus contorta*, subalpine fir *Abies lasiocarpa*, Engelmann spruce *Picea engelmannii*, whitebark pine *Pinus albicaulis*, and Douglas-fir *Pseudotsuga menziesii* forest types and abundant open meadows. A wide range of stream lengths, bed gradients, slope aspects, and unique geothermal features greatly influence the timing and magnitude of snowmelt runoff and stream thermal characteristics. In general, tributary streams on the western shore of Yellowstone Lake (Little Thumb and Arnica creeks and others) experience earlier snowmelt runoff events (peak flows in late May) and greater temperatures than those on the eastern shore (Clear and Columbine creeks and others), which have later peak flows, typically occurring in mid-June. Many tributary basins have recently been influenced by natural fire disturbance (Farnes 1996), potentially influencing their suitability for *M. cerebralis* through nutrient (nitrogen and phosphorus) enrichment (Brass

et al. 1996; Robinson and Minshall 1996) or changes in retention of organic matter (McIntyre and Minshall 1996).

Methods

Infection Prevalence in Yellowstone Lake

Field collection of Yellowstone cutthroat trout.—Juvenile and adult Yellowstone cutthroat trout were collected from throughout Yellowstone Lake by gill netting in 1999–2001. In September, multi-mesh experimental gill nets were set overnight at 11 sites located throughout the lake in water that was 2–6 m deep (5 nets per site, 55 nets total each year; Koel et al. 2005). In addition, Yellowstone cutthroat trout that were incidentally killed during lake trout removal operations on Yellowstone Lake (Bigelow et al. 2003) were examined for *M. cerebralis* by removing their heads just behind the opercle. Lake trout gill nets were set predominantly in the West Thumb and near Stevenson, Dot, and Frank islands but also in many other locations, including the South and Southeast arms (Figure 1). From the date of first ice-out (late May or early June) through the end of October, nets were placed on the lake bottom in waters that were primarily 45–50 m deep. Examination of scales allowed for age estimation of all Yellowstone cutthroat trout.

Laboratory examination.—Juvenile and adult Yellowstone cutthroat trout head samples were examined for the presence of *M. cerebralis*. Screening of each head occurred initially by the pepsin–trypsin digest (PTD) method to examine for the presence of myxospores (Andree et al. 2002). One other *Myxobolus* species is also known to infect Yellowstone cutthroat trout in Yellowstone Lake. It is neurotropic and similar in size to *M. cerebralis*. Because of this, if *M. cerebralis* was suspected by PTD screening then the spore digest suspension was used for confirmation of *M. cerebralis* by the nested polymerase chain reaction (PCR) technique (Andree et al. 1998).

Infection Risk in Tributary Streams

Site selection.—Selection of tributary streams for examination of *M. cerebralis* infection risk was done after a comparison based on basin characteristics. Preexisting information on basin size, aspect, slope, precipitation yield, stream order and length at specific elevations (<2,438 and <2,396 m), geological characteristics, and forest composition were compiled for 54 known Yellowstone cutthroat trout spawning tributaries to Yellowstone Lake. Sources of these data included geographical information systems databases provided by the National Park Service and U.S. Geological Survey. We conducted principal components analysis (PCA; Krebs 1999) to determine similarities among spawning stream watersheds for the purpose of selecting sites for sentinel cage fish exposures. After this analysis, 12 tributaries and the Yellowstone River near the lake outlet were selected for examination of *M. cerebralis* infection risk in age-0 Yellowstone cutthroat trout (exposure streams). The tributaries were selected to best represent the ranges in large-scale environmental gradients (including temperature and flow) that potentially existed given the logistical challenges of conducting sentinel fish exposures in many remote areas of the Yellowstone Lake basin.

Spawning migrations and estimates of fry emergence timing.—Yellowstone cutthroat trout migrate out of Yellowstone Lake and up tributary streams to spawn during May–July of each year, and the timing of this migration varies among streams (Gresswell et al. 1997). The daily mean numbers of upstream- and downstream-migrating Yellowstone cutthroat trout spawners over the period of record for each stream were compiled for Pelican and Clear creeks from National Park Service historical records. Migrating Yellowstone cutthroat trout were counted daily at fixed weirs located near the stream mouth during 1964–1983 at Pelican Creek and during 1977–2001 at Clear Creek. Temporal patterns of migration and available temperature information for each stream were used to estimate potential fry emergence dates (based on known incubation periods for Yellowstone cutthroat trout at various temperatures) and optimal periods for sentinel fish exposures. Sentinel fish exposure periods from mid-July through mid-October were selected to encompass the times when wild-reared fry would be emerging and vulnerable to *M. cerebralis* infection in tributary streams.

Yellowstone cutthroat trout sentinel cage exposures.—Cage exposures were conducted on exposure streams during 1999–2001 (Figure 1). The cages were 1-m-high, 0.5-m-diameter cylindrical enclosures constructed of 5-mm galvanized wire mesh. Yellowstone cutthroat trout fry were obtained from the Wyoming Game and Fish Department broodstock (Le Hardy Rapids, Yellowstone River origin, 25–50 d posthatch). Fry (60–80 per cage) were exposed during 10-d periods within each study stream. A single exposure period was used on 13 streams in 1999. Three exposure periods were used on four of seven tributaries in 2000 (a single exposure period on the remaining three streams) and on all 13 streams examined in 2001. Initial exposures in each year began after peaks in flow and spawning of adfluvial Yellowstone cutthroat trout. Multiple exposure periods captured the timing of wild-reared fry emergence in each exposure stream (July–September)

and provided the best opportunity to document peak *M. cerebralis* triactinomyxon release. After completion of the 10-d exposure periods, sentinel fry were maintained in 38-L aquaria at 13°C for an infection development period of 90 d (El-Matbouli et al. 1999). Upon completion of this period, fish were lethally sampled after anesthetization with tricaine methanesulfonate (MS-222).

Laboratory examination.—Samples for detection of *M. cerebralis* in sentinel cage exposure fish were obtained by removing heads just behind the opercles. Heads were bisected along the sagittal line, and one half was immediately preserved in Davidson's fixative for histological analysis of paraffin-embedded tissues and hematoxylin- and eosin-stained sections. The other half was retained for PTD screening. If *M. cerebralis* was suspected by PTD screening, the spore digest suspension was used for confirmation of *M. cerebralis* by PCR. Histological confirmation was accomplished to describe pathology associated with the presence of *M. cerebralis* in cranial cartilage. The observation of lesions and cartilage disruption at the cellular level provided an assessment of infection severity ranked with the MacConnell–Baldwin scale (Baldwin et al. 2000). Sentinel cage mortalities, most of which were in a decomposed condition, were not assayed for the presence of *M. cerebralis*.

Tubificid and Actinosporean Examination

Field collection of tubificids.—Each exposure stream was sampled for live tubificids three times during 2001. To the extent possible, collections occurred during the 10-d period when Yellowstone cutthroat trout fry were being held in sentinel cages. Oligochaetes were collected within 30 m upstream of each sentinel cage location. Collections were made by sieving sediments through 1-cm-mesh hardware cloth; the supernatant was poured onto a 250-μm Nalgene sieve to collect oligochaetes from the fine sediments. Undamaged oligochaetes were placed in plastic containers by means of a transfer pipet and were stored in cool stream water until processing. If a single oligochaete was not located in a 30-m reach within 1 h, a 30-m reach downstream of the sentinel cage was selected. If a single oligochaete was detected, the collection effort would persist for one additional hour or until 300 oligochaetes were collected, whichever occurred first.

Laboratory examination.—Oligochaetes were examined under a dissecting microscope; those with external morphology similar to *T. tubifex* (with hair chaetae; Kathman and Brinkhurst 1998) were placed into wells of tissue culture plates with 2 mL of dechlorinated water. The plates were maintained at 15–18°C for 7 d with three to four water changes per week. Each well was examined microscopically for actinospore production every 2 d. Tubificids that produced actinospores were sacrificed; the anterior 15 segments were prepared for microscopy, and the remainder was prepared for DNA extraction. Actinospores produced by each tubificid were frozen in 1.5-mL microfuge tubes for DNA extraction and PCR analysis. In addition, after 7 d, a maximum of 24 non-actinospore-producing tubificids was randomly chosen from each collection site and was sacrificed for DNA extraction and *M. cerebralis* PCR analysis.

Myxobolus cerebralis–specific PCR analysis.—Genomic DNA was isolated from tubificids with hair chaetae and from actinospores by use of NucleoSpin tissue kits (Clontech Laboratories, Inc., Mountain View, California). The species-specific nested *M. cerebralis* PCR (Andree et al. 1998) was performed on actinospore and tubificid DNA and positive plasmid controls.

Genetic analysis of Tubifex tubifex.—Seventeen mature *T. tubifex* individuals were morphologically identified from the more than 3,000 tubificids with hair chaetae. These mature individuals were found in three geographically distant streams (Pelican and Beaverdam creeks and Creek 1167; Figure 1). Seven randomly amplified polymorphic DNA (RAPD) primers were used to assess the genetic heterogeneity of these *T. tubifex*, as described in Kerans et al. (2004). The 16S mitochondrial DNA (mtDNA) region was amplified from the genomic DNA of the 17 mature *T. tubifex*, and each individual was assigned an mtDNA lineage, as described in Beauchamp et al. (2002). The internal transcribed spacer (ITS1) region was amplified, as described in Kerans et al. (2004), from two mature *T. tubifex*: one individual from Pelican Creek (GenBank number AY550886–95) and one from Beaverdam Creek (GenBank number AY550885). The variability of the ITS1 sequences within *T. tubifex* individuals in the Yellowstone National Park population was also tested by performing two sets of PCRs. One set of PCRs was performed as described, whereas the other set contained 10% dimethyl sulfoxide (DMSO) to facilitate denaturation throughout the PCR (Varadaraj and Skinner 1994). Five clones from the 10% DMSO reactions and five clones from non-DMSO-containing reactions were sequenced for the Pelican Creek individual. The PCR products were cloned by use of the Zero Blunt TOPO PCR Cloning Kit (Invitrogen Corp., Carlsbad, California) and were sequenced by Davis Sequencing, LLC (Davis, California).

Phylogenetic analysis of Tubifex tubifex.—Analysis of the ITS1 sequences obtained from the mature Pelican Creek *T. tubifex* showed 13 mutations within two specific ITS1 types (AY550886–95). The number

of nucleotide differences was within the expected error rate for the *Taq* polymerase used in this experiment (Smith et al. 1997); therefore, we used a consensus sequence for each of the ITS1 types in the phylogenetic analysis. The ITS1 consensus sequences obtained from a mature *T. tubifex* individual from Pelican Creek and the partial ITS1 sequence obtained from a Beaverdam Creek *T. tubifex* (AY550885) were aligned with sequences of *Limnodrilus hoffmeisteri* and *Ilyodrilus templetoni* and the consensus sequences of the other geographic isolates of *T. tubifex* (Kerans et al. 2004) by use of ClustalW in MacVector version 6.5.3 (Accelrys, Ltd., San Diego, California). Default settings were used except for gap-opening penalties, where different pairwise penalty/multiple penalty combinations were used: 5/10, 10/10, 15/15, 15/30, 15/40, 15/50, 15/60, 30/15, 30/30, 30/40, 30/50, and 30/60. These alignments were analyzed by maximum parsimony in PAUP version 4.0 (Sinauer Associates, Inc., Sunderland, Massachusetts) (program settings = heuristic search, random addition sequence with 100 replicates, tree bisection reconnection [TBR] branch swapping, and MulTrees on).

Environmental Characteristics of Tributaries

Water temperatures were recorded hourly by digital temperature data loggers (Onset Computer Corp., Bourne, Massachusetts) to determine daily and seasonal thermal regimes of each stream near sentinel cages in 2001. Recent and historical hydrological characteristics of the Yellowstone Lake basin were assessed by use of the U.S. Geological Survey stream discharge gauge (site 6186500; discharge recorded hourly) on the Yellowstone River downstream of the lake outlet near Fishing Bridge. Stream physical features were quantified near sentinel fish cages in areas where oligochaetes were sampled. Habitat assessments were conducted once per exposure stream in the summer of 2001 by assessing the relative quantity and quality of natural structures that could provide ecological niches, such as vegetative cover, streambank stability, sediment deposition, channel characteristics, and riparian zone width (Barbour et al. 1999).

Substrates of 5 of the 13 exposure streams were quantified by means of Wolman pebble counts (Wolman 1954) during July–September 2001. A 36-m reach upstream from each sentinel cage was sectioned into 13 transects located 3 m apart; pebbles were collected and measured from each transect. Counts were not conducted on the other eight streams because they were impossible to ford, their substrate was composed of only fine sediments, or they were too narrow to achieve a minimum pebble count sample size with reasonable effort. Bottom sediment total organic analysis was conducted once per exposure stream during July–September 2001. Three pooled samples of bottom sediment were obtained by scraping the top 5-cm sediment layer with a trowel. The samples were kept at −20°C until the total volatile solids content was determined based on the standardized American Public Health Association (APHA) method 2540 B (APHA et al. 1989). This method was performed in triplicate on 10 g of dry sediments.

Water samples were collected from the lower reaches of all exposure streams during July and September 2001. Analyses to determine nutrient and other chemical characteristics of waters were conducted by the Great Lakes Water Center, University of Wisconsin–Milwaukee. A Yellow Springs Instruments, Inc. (Yellow Springs, Ohio; YSI model 85), multiparameter meter was used to collect field measurements of dissolved oxygen concentration, percent oxygen saturation, and specific conductance at each sentinel cage site during each of the three exposure periods in 2001. Measurement of pH was done with an Oakton Instruments (Vernon Hills, Illinois) pH Tester.

Results

Infection Prevalence in Yellowstone Lake

Juvenile and adult Yellowstone cutthroat trout infected by *M. cerebralis* were collected throughout Yellowstone Lake. Eighty-nine of the 453 fish tested in 1999–2001 were infected. The mean proportion of infected fish was higher in the northern region (15.3% near the mouth of Pelican, Clear, and Hatchery creeks and the Yellowstone River outlet) and central region of the lake (16.4% near Stevenson, Dot, and Frank islands) than in the West Thumb (6.5%) or south arms (7.3%; Figures 1, 2). A high degree of variation occurred among years, as 87 of the 89 total infected fish were collected in 1999 and 2000; only two infected fish were found in 2001 in the northern region of the lake. Fish of all age-classes were infected in 1999 but not in 2000 or 2001 (Figure 2). Juvenile and adult Yellowstone cutthroat trout that tested positive for *M. cerebralis* showed no significant external signs of the disease and otherwise appeared healthy.

Infection Risk in Tributary Streams

Classification of tributaries by PCA indicated that factors such as basin size, basin slope, precipitation yield, stream length, stream length at elevations less than 2,438 m, and andesite geology were most important in classifying streams (Table 1; Figure 3). Pelican, Clear, and Beaverdam creeks had watersheds that were relatively large in size (>8,000 ha) and were third or fourth order (Table A.1 in the appendix). Pelican Creek was the largest watershed, covering

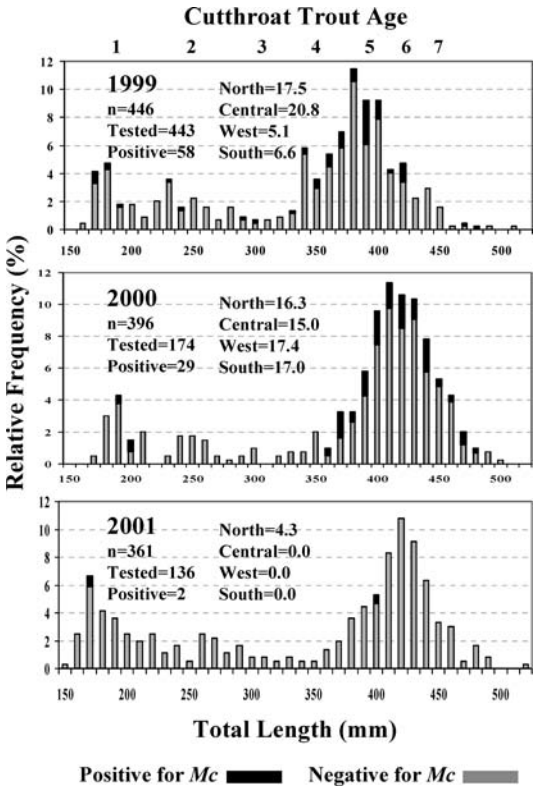

FIGURE 2.—Length-frequency distributions of Yellowstone cutthroat trout collected in September by gill netting in Yellowstone Lake, showing the total number of trout (*n*), the number of trout tested, and the number that tested positive for the presence of *Myxobolus cerebralis* (*Mc*). The proportions (%) of trout that were positive within each lake zone (north, central, west, and south) are provided.

17,656 ha. The watershed of Beaverdam Creek had the highest maximum elevation at 3,414 m. In contrast, Creeks 1158 and 1167 had relatively small watersheds (<150 ha) and were first-order streams. Arnica, Little Thumb, and Bridge creeks were intermediate in size and in terms of other significant factors that were important in explaining PCA axis 1 (Table 1; Figure 3).

Historical records indicated that most Yellowstone cutthroat trout migrated up Pelican Creek from mid-May through the third week in June, peak migration occurring the first week of June (Figure 4). The spawning migration at Clear Creek has generally occurred a few weeks later in the year, and it demonstrates a bimodal spawning peak that typically occurs during the third week in June. Downstream movements of adult fish returning to Yellowstone Lake from Pelican Creek occurred from mid-June to mid-July, and returns from Clear Creek occurred from late-June to late-July (Figure 4).

Sentinel Yellowstone cutthroat trout fry showing

TABLE 1.—Loadings of Yellowstone Lake tributary basin characteristics on principle components analysis (PCA) axes 1 and 2. Loadings with absolute values greater than 0.3, used to explain relationships among streams, are shown in bold italics. The cumulative variance explained is given in parentheses for each axis.

Basin characteristic	PCA axis 1 (39.3%)	PCA axis 2 (56.1%)
Basin size (ha)	***0.336***	0.186
Stream order	0.292	0.178
Basin aspect (°)	0.203	−0.259
Basin elevation (m)	0.256	−0.298
Basin slope	0.038	***−0.399***
Precipitation yield (m^3)	***0.337***	0.175
Stream length (km)	***0.319***	0.235
Stream length < 2,438 m	0.292	***0.315***
Stream length < 2,396 m	0.294	0.266
Geology–sedimentary (%)	−0.037	−0.07
Geology–surficial (%)	−0.065	0.216
Geology–andesite (%)	0.232	***−0.349***
Geology–rhyolite (%)	−0.060	0.131
Forest–lodgepole pine (%)	−0.274	0.176
Forest–whitebark pine (%)	0.295	−0.258
Forest–Englemann spruce and subalpine fir (%)	0.241	−0.227
Forest–Douglas-fir (%)	0.026	−0.142
Forest–none (%)	0.151	0.058

histological evidence of and testing positive for the presence of *M. cerebralis* by PCR were first obtained from the Yellowstone River during August 1999 (Table 2). None of the other 12 exposure streams examined that year showed evidence of the parasite. In 2000, histological examination and PCR confirmed the presence of *M. cerebralis* in Pelican Creek (strong infection) and Clear Creek (weak infection); fish held in the Yellowstone River and the other four exposure streams were not infected during that year, even though

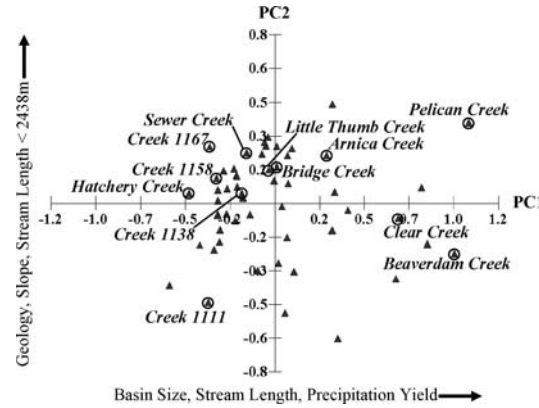

FIGURE 3.—Two-dimensional scatterplot of 54 Yellowstone Lake spawning tributaries on principal component axes 1 (PC1) and 2 (PC2), describing basin factors most important in defining tributaries (also see Table 1). Whirling disease exposure studies were conducted on 12 tributaries (circled data markers).

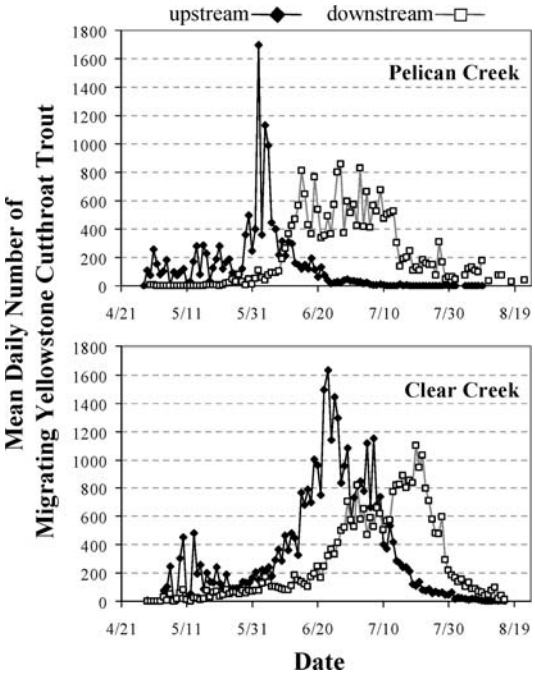

FIGURE 4.—The mean daily number of Yellowstone Lake adfluvial Yellowstone cutthroat trout that were enumerated at migration traps while moving upstream or downstream in Pelican Creek (1964–1983) and Clear Creek (1977–2001).

multiple exposure periods were used in an attempt to span potential peak infection periods. Studies conducted in 2001 with multiple exposure periods provided further evidence that the infection of *M. cerebralis* in Pelican Creek was severe, as Yellowstone cutthroat trout fry exposed during all periods were infected and showed clinical signs of the disease; all of the fish exposed during period 1 (July 12–23) were infected, and the mean histological ranking of severity was 4.00 (maximum of 5.00) on the MacConnell–Baldwin scale (Table 2). Fish exposed at the Yellowstone River also became infected (as in 1999), but histological examination indicated that the infection was weak (<0.40). Clear Creek and all other exposure streams examined in 2001 showed no signs of the parasite.

Tubificid and Actinosporean Examination

Throughout the 2001 sampling season, a high number of tubificids were consistently found (>100 tubificids/h) in Beaverdam, Sewer, and Little Thumb creeks and Creek 1167; the most difficulty in locating tubificids occurred in Clear and Hatchery creeks and Creek 1111, where collections were 13.3 tubificids/h or less (Table 3). Collections in late August and early September yielded the greatest number of tubificids. A total of 632 tubificids was found in the first exposure period in mid-July and 994 were collected during mid-August, whereas 1,411 were collected in the late-August to early-September sampling period. Few of the tubificids collected were sexually mature, making morphological identification of most of the individuals difficult. The sexually mature oligochaetes with hair chaetae were identified as *T. tubifex*, *I. templetoni*, and individuals of the genus *Rhyacodrilus*. Of the 17 sexually mature *T. tubifex* collected, 14 were found in

TABLE 2.—Results of sentinal-fry exposure studies from streams in which Yellowstone cutthroat trout fry tested positive for *Myxobolus cerebralis* during 1999–2001. Prevalence (prev) is the proportion of individuals examined that were infected. Severity is the average histological score from laboratory examination and is based on a scale of 0–5 (5 = the most severe infection). Pelican Creek was not tested in 1999. A single exposure period occurred on all tested streams in 1999 and on Pelican Creek in 2000.

Stream	Year	Period	Dates	Prev (%)	Severity
Pelican Creek	2000	1	12–23 Sep	94	2.76
	2001	1	12–23 Jul	100	4.00
		2	7–17 Aug	75	1.00
		3	29 Aug–7 Sep	94	2.72
Clear Creek	1999	1	12–23 Aug	0	0.00
	2000	1	12–23 Sep	2	0.02
		2	25 Sep–5 Oct	0	0.00
		3	9–19 Oct	0	0.00
	2001	1	12–23 Jul	0	0.00
		2	7–17 Aug	0	0.00
		3	29 Aug–7 Sep	0	0.00
Yellowstone River	1999	1	12–23 Aug	14	0.20
	2000	1	12–23 Sep	0	0.00
		2	25 Sep–5 Oct	0	0.00
		3	9–19 Oct	0	0.00
	2001	1	14–23 Jul	20	0.40
		2	7–17 Aug	7	0.07
		3	29 Aug–7 Sep	0	0.00

TABLE 3.—Numbers of tubificids with hair chaetae selected from bulk live oligochaete samples taken near Yellowstone cutthroat trout sentinel cage sites over three time periods and observed for actinospore production for 7 d. Areas not sampled are indicated "ns.'' Triactinomyxon-type actinospores were produced by all infected tubificids except that isolated from Beaverdam Creek, which produced synactinomyxon-type actinospores. The diagnostic *Myxobolus cerebralis* (*Mc*) nested polymerase chain reaction test was used to assay for *Mc* infection.

			Number (%) of tubificids		
Stream	Orientation to cage	Tubificids per hour	Observed	Producing actinospores	*Mc* positive
Pelican Creek	Upstream	94.5	189	0	1
	Downstream	28.0	28	0	0
Clear Creek	Upstream	0.0	0	0	0
	Downstream	0.3	1	0	0
Beaverdam Creek	Upstream	259.0	777	1 (0.13)	0
	Downstream	ns	ns	ns	ns
Creek 1111	Upstream	13.3	40	0	0
	Downstream	ns	ns	ns	ns
Creek 1138	Upstream	84.3	253	0	0
	Downstream	ns	ns	ns	ns
Creek 1158	Upstream	47.0	141	0	0
	Downstream	ns	ns	ns	ns
Sewer Creek	Upstream	0.0	0	0	0
	Downstream	200.0	200	1 (0.50)	0
Creek 1167	Upstream	139.0	278	1 (0.36)	0
	Downstream	132.0	264	0	0
Little Thumb Creek	Upstream	40.0	80	1 (1.25)	0
	Downstream	196.0	392	1 (0.26)	0
Arnica Creek	Upstream	17.7	53	5 (9.43)	0
	Downstream	40.0	40	3 (7.50)	0
Bridge Creek	Upstream	61.3	184	6 (3.26)	0
	Downstream	51.0	51	0	0
Hatchery Creek	Upstream	0.0	0	0	0
	Downstream	10.0	10	0	0
Yellowstone River	Upstream	17.0	34	1 (2.94)	0
	Downstream	22.0	22	0	0
Total			3,037	20	1
Mean		64.6	38	1	0

early July and the remaining three were collected in early September.

Twenty of the 3,037 collected tubificids produced actinospores during 7-d observation periods. Both the synactinomyxon and triactinomyxon types of actinospores (Lom et al. 1997) were observed. Actinosporean production by tubificids was variable within and among the streams sampled. Arnica Creek exhibited the highest prevalence of infection at 7.50–9.43% of the 93 total tubificids observed (Table 3). Surprisingly, the *M. cerebralis*–specific nested PCR failed to detect this parasite in either tubificid or actinosporean genomic DNA preparations, indicating that none of the triactinomyxons found were *M. cerebralis*. The nested PCR assay also failed to detect *M. cerebralis* in 66 and 40 randomly sampled immature tubificids with hair chaetae from Pelican Creek and the Yellowstone River, respectively. We repeated the *M. cerebralis* nested PCR several times and repeatedly failed to detect *M. cerebralis* in any of the infected or immature worms or any actinosporean preparations, although we detected *M. cerebralis* in actinosporean-producing tubificids collected from other *M. cerebralis* endemic areas and in our positive plasmid controls. However, one sexually mature *T. tubifex* collected from Pelican Creek in early July was not shedding triactinomyxons but tested positive for *M. cerebralis* infection, indicating the presence of infected worms in this stream.

The identical patterns of seven RAPD DNA loci indicated that the 17 *T. tubifex* from Pelican and Beaverdam creeks and Creek 1167 were genetically homogeneous (Figure 5a).

Analysis of mtDNA lineages of the sexually mature *T. tubifex* (Strumbauer et al. 1999; Beauchamp et al. 2002) showed that all these individuals belonged to mtDNA lineage III (Figure 5b). The presence of lineage III *T. tubifex* in whirling disease endemic regions has been associated with high levels of whirling disease (Beauchamp et al. 2002). Phylogenetic analysis of the 11 ITS1 sequences from a mature *T. tubifex* from Pelican Creek and another mature individual from Beaverdam Creek indicated that the *T. tubifex* populations in Yellowstone National Park were genetically similar to lineages of *T. tubifex* that produce moderate to high levels of *M. cerebralis* triactinomyx-

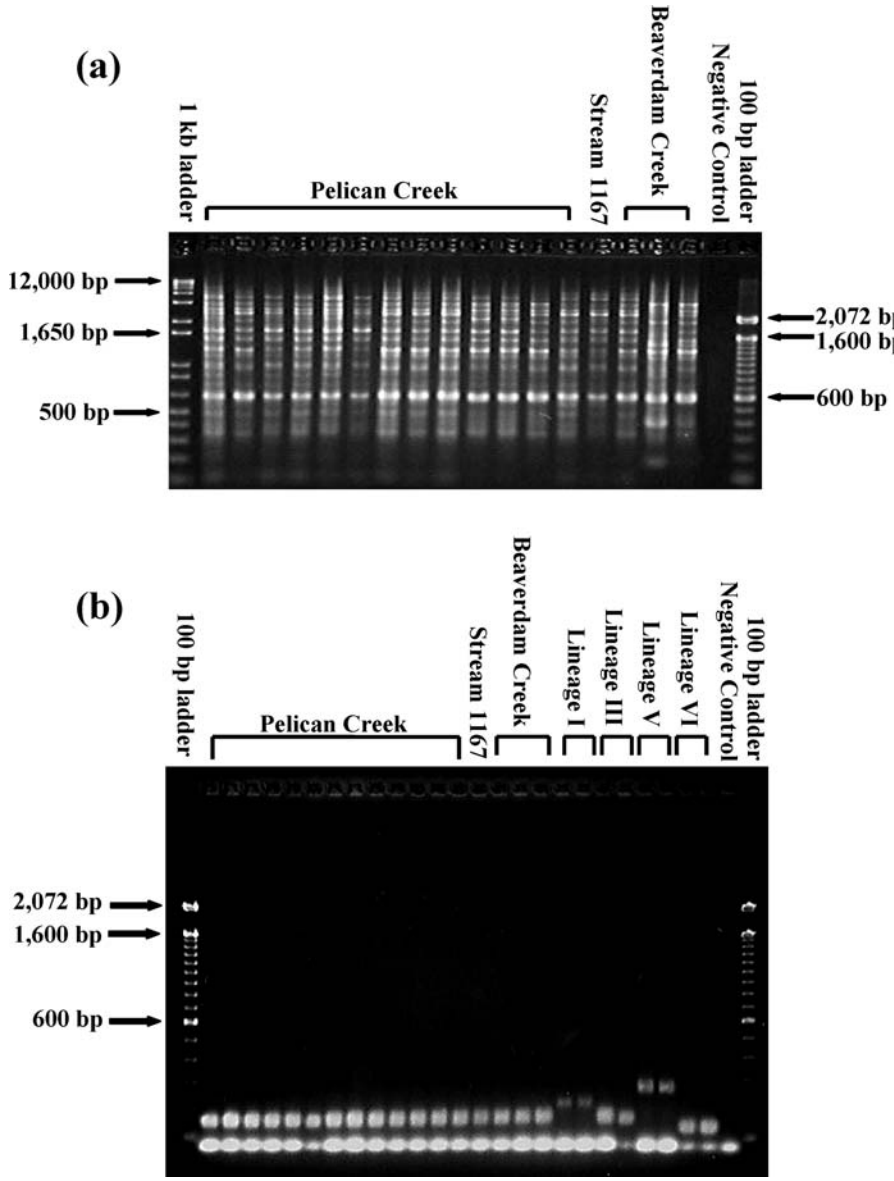

FIGURE 5.—Genetic analysis of *Tubifex tubifex* from Yellowstone National Park, including **(a)** randomly amplified polymorphic DNA banding patterns of sexually mature *T. tubifex* (the polymerase chain reaction [PCR] amplification products generated by primer 5′-CCTGGCGGTA-3′) collected from Pelican Creek, Creek 1167, and Beaverdam Creek and **(b)** PCR amplification products of the mitochondrial DNA 16S ribosomal DNA lineage-specific primers of *T. tubifex* from Pelican Creek, Creek 1167, and Beaverdam Creek. Examples of PCR amplification products from lineage I primers (196 base pairs [bp]; Gallatin River), lineage III primers (147 bp; Mount Whitney, California), lineage V primer (320 bp; Great Lakes, Ontario), and lineage VI primers (125 bp; Logan River, Utah), are shown. The negative controls were water.

ons in laboratory experiments (Figure 6; Kerans et al. 2004).

Environmental Characteristics of Tributaries

Water temperature profiles obtained during 2001 indicated strong temperature variation among streams (Figure 7). For example, the daily mean temperature during July and August was 11.3, 13.5, and 18.0°C at Hatchery, Clear, and Pelican creeks, respectively. The conditions in 2001 were those of an extreme drought for Yellowstone National Park and the surrounding region. Many tributary streams decreased to zero or

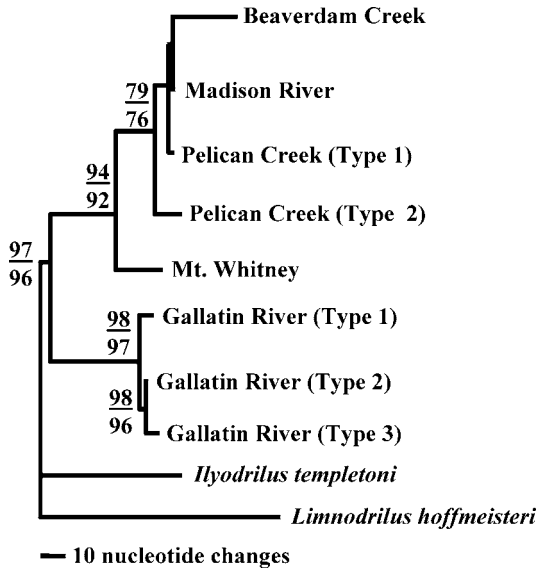

FIGURE 6.—Phylogenetic relationship of *Tubifex tubifex* from Pelican and Beaverdam creeks with other known *T. tubifex* isolates based on the ITS1 sequence. The most parsimonious tree generated using pairwise and multiple-gap penalties of 15/15 is shown. The results (%) of 100 bootstrap (numbers above bars) and jackknife (numbers below bars) replicates are shown near the nodes.

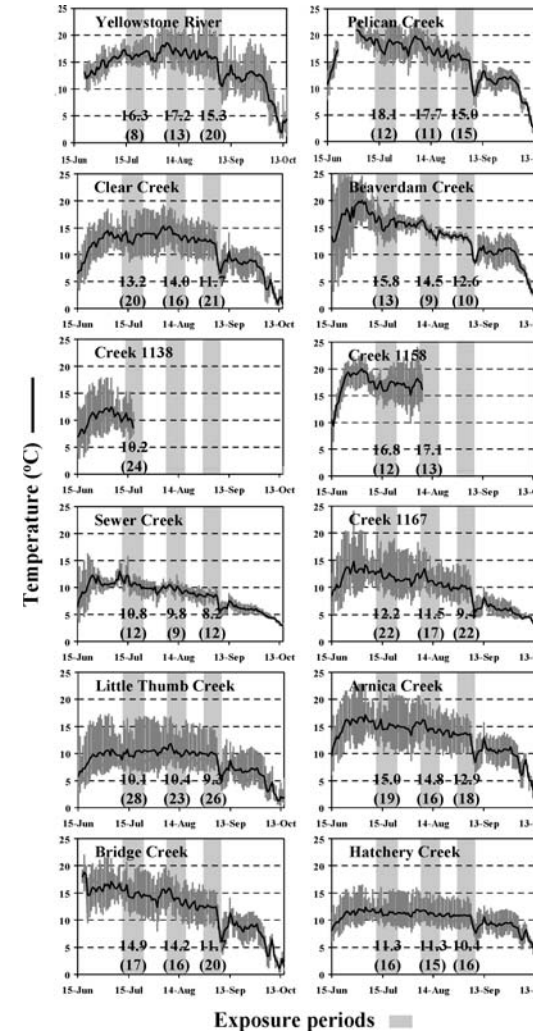

FIGURE 7.—Daily mean and range of temperatures (hourly; °C) in Yellowstone Lake tributaries and the Yellowstone River near Fishing Bridge, where Yellowstone cutthroat trout sentinel fry exposure and tubificid studies were conducted in 2001. Shaded areas represent exposure periods, and overall mean water temperatures are provided for each period. The coefficient of variation is given in parentheses.

near-zero surface flow and became disconnected from the lake. This situation occurred at Beaverdam and Sewer creeks and at creeks 1158 and 1167. The highest mean hourly temperatures during the three exposure periods were at Pelican Creek: 18.1, 17.7, and 15.0°C for periods 1–3, respectively. Relatively high temperatures were also recorded at the Yellowstone River, Beaverdam Creek, and Creek 1158 (Figure 7). Pelican Creek and Beaverdam Creek temperatures increased dramatically in mid-June to near 20°C or above. Sewer, Little Thumb, and Hatchery creeks were relatively cool streams (<12°C) that had only moderate among-day variation in mean daily temperature through the summer. The coefficient of variation (CV) of hourly temperatures within the exposure periods was highest (>22%) in Little Thumb Creek and Creek 1167, both located in the West Thumb.

Stream discharge measured by the U.S. Geological Survey gauge on the Yellowstone River near Fishing Bridge provided an indication of hydrological conditions for the lake basin in 1999–2002. Peak discharge was 190 m^3/s in 1999, 120 m^3/s in 2000, and only 71 m^3/s in 2001. The 2001 water year was unique, and interpretation of thermal and/or chemical characteristics of streams should be done with this in mind. The peak discharge in 2001 was 46% below the long-term average peak discharge for this stream at this location. The abnormally low flows probably had an effect on the overall habitat quality of streams. The exposure streams were assessed a score based upon habitat conditions that were likely to influence the presence and abundance of tubificids. In general, the streams with the lowest scores, such as Beaverdam Creek and Creek 1158, had poor-quality riparian zones and tended to have more abundant fine sediments (Table A.2).

Mineral substrates were classified as boulder,

cobble, pebble, or gravel (Wolman 1954). Clear and Sewer creeks and creeks 1111 and 1138 were predominantly composed of pebble, while Creek 1158 had smaller mineral substrates composed predominantly of gravel and small pebbles. The organic analysis of stream sediments indicated that there were significant differences between and within streams, and the organic content ranged from 0.62% to 9.27% (Table A.2). The sediment collected at locations downstream of sentinel cages on Pelican Creek and the Yellowstone River had a higher organic content than samples collected at upstream locations. High organic content was not a factor related to high abundance of tubificids in these streams.

Chemical analysis of surface waters indicated the highest degree of variation (CV $>$ 200%) among tributaries for chloride and sulfate (Table A.2). Pelican Creek had ammonium concentrations far above most other streams during all three exposure periods in 2001. Pelican Creek and the Yellowstone River had chloride concentrations far above those of other streams, and sulfate was extremely high (1,034 μM) in Pelican Creek. The total phosphorus was high ($>$2 μM) in Pelican Creek during the first exposure period but was also high in Beaverdam Creek and creeks 1111, 1158, and 1167 during later exposure periods. Overall, these results suggest that Pelican Creek had the highest potential for biological productivity among the lake tributaries studied.

Discussion

Biological Aspects of Myxobolus cerebralis Infection Risk

Age-0 Yellowstone cutthroat trout reared in sentinel exposure cages in Pelican and Clear creeks and the Yellowstone River were infected by *M. cerebralis* during at least one exposure period. Whereas the infections at the Yellowstone River site (1999 and 2001) and Clear Creek (2000) were relatively light, the fish exposed at Pelican Creek were severely infected and showed clinical signs of whirling disease in laboratory aquaria. These streams were located along the north and east-central shores of Yellowstone Lake; all other exposure streams tested negative for *M. cerebralis*. Interestingly, the proportion of juvenile and adult Yellowstone cutthroat trout from within Yellowstone Lake that tested positive for *M. cerebralis* in 1999 varied spatially and was highest in the north (17.5%) and central (20.8%) sections of the lake. Benson and Bulkley (1963) demonstrated that two-thirds of fish tagged in Pelican Creek were recaptured by anglers in the northern section of the lake. Most fish (75%) that were tagged in tributary streams of the South Arm and West Thumb were later recaptured in the subbasins fed by these streams. Previous studies have also shown the fidelity of these adfluvial fish to particular spawning tributaries; straying of spawning Yellowstone cutthroat trout has typically been less than 3% among tributaries (Gresswell et al. 1997). The higher infection prevalence in the northern and central sections of the lake in 1999 may have been due to Pelican Creek and, to a lesser extent, the Yellowstone River and Clear Creek as sources of *M. cerebralis*.

There was also significant temporal variation in *M. cerebralis* infection of juvenile and adult Yellowstone cutthroat trout within Yellowstone Lake, as many fish from the lake were infected in 1999 and 2000 but only two fish tested positive in 2001; both of these infected fish were collected from the north section of the lake. The reason for this significant temporal variation is not known. The infected fish from Yellowstone Lake were in good condition and otherwise appeared healthy. Our results suggest at least some resilience of this subspecies to whirling disease, as a significant number of fish have evidently been surviving and recruiting to the spawning population even though infected (perhaps at an older age) by *M. cerebralis*. Population-level declines have only recently been noticed, and it is likely that *M. cerebralis* has only recently invaded this system. Stocking in Yellowstone National Park for sport fishery purposes ended in 1955 (Varley 1981), prior to the 1956 detection of *M. cerebralis* in the United States (Bartholomew and Reno 2002). Now, a serious concern is the potential for this parasite to increase in its prevalence, further diminishing the ability of Yellowstone cutthroat trout to survive to spawning age.

Recent studies in Idaho and Colorado suggested that a relationship may exist between the abundance of *T. tubifex* and infection risk in salmonids (Hiner and Moffitt 2001; Nehring and Thompson 2003). Recent studies in Montana showed a significant relationship between the density of infected worms and *M. cerebralis* infection risk in salmonids (Krueger 2002). The relationship in Yellowstone Lake tributaries is not so clear. Certainly, *T. tubifex* is required for the presence of *M. cerebralis*, and the results from Pelican Creek may indicate that low numbers of tubificids can evidently support severe infection of native Yellowstone cutthroat trout. Alternatively, the distribution of *M. cerebralis*–infected tubificids may be clumped or the infection source may exist some distance upstream from our exposure site. Moreover, the finding of a *M. cerebralis*–positive *T. tubifex* individual that (1) is genetically similar to laboratory strains that produce moderate to high numbers of triactinomyxons (Kerans et al. 2004) and (2) belongs to an mtDNA lineage that

has been associated with high salmonid infection levels in whirling disease endemic regions (Beauchamp et al. 2002) is consistent with severe infection rates in Pelican Creek.

Actinosporean production was low among streams except Arnica Creek, where prevalence was relatively high at 7.5–9.4% of the 93 tubificids observed. Infection rates elsewhere have also been low (Brinkhurst 1996) at 2.6% in Montana (Rognlie and Knapp 1998) and 0.4–1.5% in Colorado (Beauchamp et al. 2002). All of the actinosporeans examined by this study were negative for the presence of *M. cerebralis* genes. Preliminary 18S ribosomal RNA sequence analysis of several of the actinospores from Sewer and Bridge creeks suggest that they were most similar to *M. algonquinensis* and other myxosporeans known to infect cyprinids of the genus *Notropis*, while an actinospore from Arnica Creek was most similar to *M. truttae*, a myxosporean known to infect brown trout *Salmo trutta* (C.R., unpublished data). It is unknown whether these myxozoans are native to the upper Yellowstone Lake basin or whether they have recently been introduced. Also unknown are the life history strategies allowing them to persist in this system. Brown trout were once stocked in Duck Lake (a small, historically fishless lake near the West Thumb) in 1938, but the lake was poisoned in 1967 to remove the threat of this species entering Yellowstone Lake (Varley 1981). Brook trout *Salvelinus fontinalis* were introduced to Arnica Creek but were chemically removed from the stream in 1985 (Gresswell 1991). It is possible that the stocking of nonnative fishes early in the history of Yellowstone National Park (Varley 1981), prior to *M. cerebralis* being introduced to the United States, contributed to the introduction of relatively unknown myxozoans. Future research should address these other myxozoans to improve our understanding of host–parasite interactions and the potential interaction between these species and *M. cerebralis* in this system.

Environmental Aspects of Myxobolus cerebralis Infection Risk

Pelican Creek, where infection was most severe and occurred during all three exposure periods in 2001, was a fourth-order stream and the largest tributary to Yellowstone Lake in terms of stream length (53.5 km), total drainage size (17,656 ha), and precipitation yield. Pelican Creek had far more length at lower elevations (<2,396 m) than any of the other exposure streams. Open-lake sediments comprised a large area of the lower Pelican Creek valley and the area between Pelican Creek and the Yellowstone River; lake levels have historically been much higher and once flooded lower Pelican Creek and the Yellowstone River near the present-day lake outlet. Surficial deposits in the area of Fishing Bridge on the Yellowstone River, where exposed fishes tested positive with a light infection of *M. cerebralis* in 1999 and 2001, were largely lake-derived sediments of gravelly sand (Richmond 1977).

In contrast, other exposure streams were typically much smaller than Pelican Creek in total basin size, stream order, length, and precipitation yield. Clear and Beaverdam creeks, which were moderate in size, were different than Pelican Creek geologically, as andesite and rhyolite were more predominant. The dominant forest type at Beaverdam Creek was whitebark pine, whereas the drainages of Pelican and Clear creeks were mostly forested by lodgepole pine. These basin characteristics can potentially influence stream chemical composition; a high degree of variation in water chemistry was noted among exposure streams. Levels of ammonium, chloride, sulfate, and phosphorus were generally much higher in Pelican Creek than in the other streams. Specific conductivity was much higher (319–322 μS/cm) in Pelican and Beaverdam creeks and may indicate the higher overall productive potential of these streams; this parameter has been significantly correlated with *M. cerebralis* infection prevalence in Oregon, where specific conductivities were in the same range as those of Yellowstone Lake tributaries (Sandell et al. 2001).

During the first exposure period in 2000, a single Yellowstone cutthroat trout fry at Clear Creek was lightly infected by *M. cerebralis*, but the parasite did not reappear in this stream in 2001. The variation in thermal patterns among tributaries is known to affect the timing of spawning migrations of adfluvial Yellowstone cutthroat trout from Yellowstone Lake (Gresswell et al. 1997). Peak spawning migrations at Pelican Creek took place during the first week of June, whereas the migrations at Clear Creek occurred several weeks later. The emergence of fry much later in the season and the environmental setting at Clear Creek may be somewhat incompatible with successful *M. cerebralis* life cycle establishment.

Seasonal variation in *M. cerebralis* infection prevalence and the severity of sentinel fry occurred among exposure periods on the Yellowstone River and Pelican Creek. This variation suggests seasonal patterns of infection risk, although many questions remain. In Montana (Baldwin et al. 2000) and Oregon (Sandell et al. 2001), the prevalence of infection varied seasonally and was significantly higher later in the calendar year. In Yellowstone Lake tributaries, sentinel fry infection was most prevalent and (especially) severe early in the season (mid-July). The fry infection did not seem to

correlate with tubificid abundance at exposure sites, as most tubificids were collected in late August and early September, and only one tubificid, collected in early July, tested positive for *M. cerebralis* infection. Although there was some variation in the age of fry exposed, our research conducted from 2002 to the present in Pelican Creek suggests that the pattern in seasonal infection risk remains, even when fry that are very similar in age and size are exposed among periods (S.M., unpublished data).

Other studies in the Intermountain West have demonstrated the relationship between *M. cerebralis* infection and stream temperature in the field (Baldwin et al. 2000; Hiner and Moffitt 2001; Krueger 2002). However, mean water temperatures during the exposure periods of these studies were 6.2–10.8°C. Stream temperatures of Pelican Creek and the Yellowstone River during sentinel fish exposure periods in 2001 were among the highest recorded among all exposure streams. In particular, Pelican Creek, with a drainage aspect largely to the south, had waters that warmed quickly (to over 20°C in June 2001), and elevated temperatures (>15°C) remained through early September. The mean temperatures of exposure periods 1–3 at Pelican Creek were 18.1, 17.2, and 15.3°C. The highest infection severity occurred during period 1, which was characterized by the highest mean temperature. This is significant because a temperature of 15°C has been considered most optimal for triactinomyxon development, but a transfer to 20°C has stopped the production of the parasite in *T. tubifex* in laboratory incubations (El-Matbouli et al. 1999). No triactinomyxons were found when *T. tubifex* were incubated at 20°C; however, the warming of waters from 9°C to 17°C increased infection rates and induced earlier triactinomyxon release (Blazer et al. 2003). Pelican Creek was well above 20°C during parts of most days of exposure periods 1 and 2. Evidence suggests that tubificids in this system are capable of triactinomyxon production at high water temperatures, possibly above those previously documented. Tubificids could also be releasing triactinomyxons during the night, when water temperatures declined somewhat in Pelican Creek.

Conclusions

Pelican Creek, which once supported nearly 30,000 upstream-migrating Yellowstone cutthroat trout (Jones et al. 1982), now appears to be the center of *M. cerebralis* infection in the upper Yellowstone Lake basin. There has been significant among-year and within-season variation in infection prevalence and severity of exposed fry at Pelican Creek and other infected streams, and the host–parasite and ecological interactions in this system have been unclear. The *T. tubifex* strain found during this study is genetically similar to laboratory strains known to produce moderate to high levels of triactinomyxons, suggesting that the establishment of whirling disease in the Yellowstone *T. tubifex* populations poses a substantial threat to the Yellowstone cutthroat trout. Moreover, other myxozoans exist in the lake basin and are also infecting tubificids and (unknown) fish hosts.

Myxobolus cerebralis apparently tolerates higher mean water temperatures than have been documented for most other systems. The unique geothermal influences of Pelican Creek have perhaps concentrated tubificids and *M. cerebralis* infection (many areas upstream of the exposure reach are thermally heated and remain without ice cover throughout the winter). If additional information regarding locations of infected tubificids in Pelican Creek could be determined, management action could potentially be taken (especially if the distribution is highly clumped) to reduce *M. cerebralis* infection risk in this stream. The temperature effects on *M. cerebralis* in both hosts are of interest, and studies aimed at relating infection prevalence in *T. tubifex* and Yellowstone cutthroat trout to temperature and other environmental characteristics would be useful for predicting the risk of whirling disease establishment in other park watersheds in the future.

Vincent (2001) predicted population-level losses of wild rainbow trout in systems with histological infection grades exceeding 2.5. This study has shown that infection rates during the emergence period of Yellowstone cutthroat trout fry in Pelican Creek have the potential to significantly affect this fishery. Angler survey data from throughout the stream and recent efforts to capture upstream-migrating adult Yellowstone cutthroat trout are indicating a substantial decline in this spawning population; the Yellowstone Lake population overall is currently at extremely low levels (Koel et al. 2004, 2005). It is possible that a reduction in the Pelican Creek spawning population may be partly responsible for the loss of infected juvenile and adult fish in lake gillnetting samples in 2001.

Investigations during 1999–2001 have indicated that *M. cerebralis* has established itself in waters supporting ecologically important Yellowstone cutthroat trout. This establishment and the potential proliferation of the parasite add a significant additional threat to a population that is already imperiled due to predation and competition by introduced, nonnative lake trout. Although laboratory challenges and previous field studies have suggested that Yellowstone cutthroat trout are only moderately susceptible to *M. cerebralis* infection, results of our research indicate that this subspecies may be very susceptible. Variation in

infection prevalence has been shown among single-dose and sustained *M. cerebralis* challenges for Chinook salmon *O. tshawytscha* and rainbow trout (Sollid et al. 2003), and a similar phenomenon may be occurring here. Additional work should be done to compare the *M. cerebralis* resistance among potentially unique cutthroat trout from isolated populations. Perhaps inherent resistance to this parasite exists and could be used to support ongoing broodstock development programs for conservation efforts in Yellowstone National Park and the neighboring states.

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Appendix follows

Appendix: Basin and Water Characteristics

TABLE A.1.—Tributary basin physical characteristics for streams used in Yellowstone cutthroat trout sentinel fry exposure, tubificid, and myxozoan studies in the upper Yellowstone Lake basin. Geology included sedimentary (sedi), surficial (surf), andesite (ande), and rhyolite (rhyo). Dominant forest types included lodgepole pine (LPP), whitebark pine (WBP), Engelmann spruce–subalpine fir (SF), Douglas-fir (DF), and nonforested (NF).

Stream	Basin size (ha)	Stream order	Aspect (°)	Elevation (m)	Basin slope	Precipitation yield (m^3)
Pelican Creek	17,656	4	177	2,540	0.0059	152,134,570
Clear Creek	5,768	3	219	2,638	0.0351	55,555,833
Beaverdam Creek	8,397	4	191	2,735	0.0705	92,589,350
Creek 1111	22	1	146	2,539	0.3617	148,273
Creek 1138	421	1	139	2,458	0.0285	3,408,363
Creek 1158	143	1	123	2,384	0.0125	904,875
Sewer Creek	422	2	123	2,434	0.0271	3,539,173
Creek 1167	134	1	43	2,404	0.0238	1,145,858
Little Thumb Creek	968	3	120	2,516	0.0255	9,072,245
Amica Creek	4,289	3	173	2,523	0.0184	36,749,673
Bridge Creek	1,671	2	136	2,489	0.0367	12,416,473
Hatchery Creek	49	1	82	2,386	0.0382	309,563
Mean	3,328	2	139	2,504	0.0570	30,664,520
Coefficient of variation (%)[a]	158	55	34	4	171	156

[a] 100 × SD/mean.

TABLE A.2.—Mean habitat score, organic content of sediments (SedOrg), chemical characteristics of water samples, and field measurements taken at sentinel cage exposure reaches (July–September 2001) on tributaries to Yellowstone Lake and the Yellowstone River near the lake outlet. Chemical constituents included ammonium (NH_4^+), silica dioxide (SiO_2), soluble reactive phosphorus (SRP), chloride (Cl^-), sulfate (SO_4^{2-}), total phosphorus (TP), filterable (dissolved) phosphorus (FP), and particulate phosphorus (PP). Mean field measurements are dissolved oxygen (O_2; mg/L), percent oxygen saturation (%O_2), specific conductance (Cond; μS/cm), pH, stream width (m), and velocity (vel; in August and September only; cm/s); ns = not sampled.

			Chemical constituent (μM)								
Stream	Habitat score	SedOrg (%)	NH_4^+	SiO_2	SRP	Cl^-	SO_4^{2-}	TP	FP	TP − FP	PP
Pelican Creek	118	1.78	2.9	1,039.8	0.13	714	1,034.2	0.76	0.25	0.52	0.48
Clear Creek	149	1.35	0.2	316.1	0.10	9	35.7	0.22	0.15	0.07	0.06
Beaverdam Creek	87	9.27	0.2	914.0	0.19	12	204.0	2.38	0.33	2.06	1.55
Creek 1111	172	6.47	0.7	614.7	1.99	11	15.4	2.68	2.11	0.57	0.32
Creek 1138	157	2.16	0.1	473.9	0.13	10	8.2	0.36	0.24	0.11	0.10
Creek 1158	89	0.62	1.9	432.4	0.73	40	4.3	2.17	0.97	1.20	0.97
Sewer Creek	136	4.11	0.5	325.4	0.24	10	29.2	1.23	0.39	0.84	0.57
Creek 1167	103	1.67	0.4	908.2	0.71	19	29.8	1.73	0.84	0.89	0.49
Little Thumb Creek	148	1.77	0.1	472.7	0.06	11	14.3	0.34	0.13	0.21	0.15
Arnica Creek	144	2.49	0.1	945.4	0.23	33	19.3	0.52	0.38	0.15	0.12
Bridge Creek	131	1.45	0.0	1,626.3	0.30	27	20.1	0.69	0.41	0.27	0.16
Hatchery Creek	122	1.38	0.4	687.5	0.09	18	20.1	0.33	0.21	0.12	0.13
Yellowstone River	103	4.02	0.3	188.2	0.21	130	88.8	0.62	0.36	0.26	0.16
Overall mean	128	2.96	0.6	708.0	0.39	80	117.1	1.08	0.52	0.56	0.41
Coefficient of variation (%)[a]	21	83	168	54	136	237	235	83	103	106	107

[a] 100 × SD/mean.

TABLE A.1.—Extended.

Stream	Length (category)					Geology (%)				Dominant forest type (%)				
	Length (L; km)	L < 2,438 m	% < 2,438 m	L < 2,396 m	% < 2,396 m	Sedi	Surf	Ande	Rhyo	LPP	WBP	SF	DF	NF
Pelican Creek	53.5	41.60	78	28.75	54	0	60	24	16	58	12	8	0	21
Clear Creek	15.8	5.50	35	3.60	23	0	40	35	25	63	26	4	0	7
Beaverdam Creek	15.0	7.10	47	4.80	32	0	29	71	0	16	73	1	0	11
Creek 1111	0.9	0.50	56	0.42	47	0	36	64	0	100	0	0	0	0
Creek 1138	3.5	2.40	70	0.95	28	0	19	0	81	99	0	0	0	1
Creek 1158	1.9	1.90	100	1.90	100	0	100	0	0	100	0	0	0	0
Sewer Creek	2.9	2.90	100	1.50	52	0	98	0	2	100	0	0	0	0
Creek 1167	2.6	2.60	100	1.70	65	0	100	0	0	100	0	0	0	0
Little Thumb Creek	7.1	1.30	18	1.03	15	0	44	0	56	100	0	0	0	0
Amica Creek	7.5	5.90	79	3.50	47	0	24	0	76	96	0	1	0	3
Bridge Creek	5.3	2.60	49	1.89	36	0	27	0	73	99	0	0	0	1
Hatchery Creek	1.1	1.10	100	1.00	91	0	78	0	22	99	0	0	0	1
Mean	10	6	69	4	49	0	55	16	29	86	9	1	0	4
CV (%)[a]	150	180	41	184	53	0	58	165	112	31	232	223	0	169

TABLE A.2.—Extended.

Stream	Field measurements					
	O_2	$\%O_2$	Cond	pH	Width	Vel
Pelican Creek	8.21	84.7	322	8.1	18	11.9
Clear Creek	9.27	86.3	58	8.6	7	14.3
Beaverdam Creek	13.20	135.7	319	9.7	10	0.0
Creek 1111	7.80	77.3	84	8.2	2	25.0
Creek 1138	9.06	81.2	33	8.0	1	4.9
Creek 1158	8.98	101.2	70	7.4	4	0.0
Sewer Creek	7.35	66.0	48	8.0	2	0.0
Creek 1167	7.01	69.8	55	7.9	2	0.0
Little Thumb Creek	8.04	74.5	33	8.0	1	0.6
Arnica Creek	7.79	78.1	86	8.3	2	32.9
Bridge Creek	7.16	70.6	102	8.1	1	1.2
Hatchery Creek	7.97	75.5	42	8.3	1	3.1
Yellowstone River	7.94	81.5	88	8.7	102	ns
Overall mean	8.44	83.3	103	8.3	12	7.8
CV (%)[a]	19	22	96	7	234	139