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Using Sediment Core Samples to Examine the Spatial Distribution of *Myxobolus cerebralis* Actinospore Production in Windy Gap Reservoir, Colorado

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Abstract.—Studies of the whirling disease epizootic in the upper Colorado River drainage have suggested that Windy Gap Reservoir is a source of the fish-infective actinospore of *Myxobolus cerebralis*. We divided the reservoir into four quadrants (12 zones) and conducted a core-sampling study to determine the spatial dynamics of actinospore production within the reservoir from late June to early November 1998. Core samples of reservoir substrate containing aquatic oligochaetes were collected, held for 24 h in filtered lake water, and then examined microscopically for actinospore production. Actinospores were produced from core samples taken in every quadrant, but the samples from one quadrant gave the overall highest estimates of actinospore production. This quadrant was in a nearly direct line between the reservoir's inlet and outlet and encompassed parts of the historic river channel. Of the four full months of the study (July–October), average actinospore production tended to be highest in July, but the differences among months were not statistically significant. We hypothesize that *M. cerebralis* myxospores are carried into the reservoir by runoff events in the Fraser and Colorado River drainages and that once they are in the reservoir they settle out and provide the basis for infection of the abundant *Tubifex tubifex* population.

Throughout most of the 20th century, the 40-km reach of the upper Colorado River in Middle Park, Colorado, supported a thriving wild trout fishery. From the 1940s to the early 1990s, wild rainbow trout *Oncorhynchus mykiss* were the dominant species. Brown trout *Salmo trutta* accounted for the remainder of the salmonid population.

Myxobolus cerebralis, the myxosporean parasite that can cause salmonid whirling disease, was detected during routine inspections of state and private fish hatcheries in Colorado in late 1987. In acute infections, whirling disease is a debilitating malady that can affect susceptible salmonids. Young rainbow trout are particularly vulnerable to the parasite (O'Grodnick 1979; Markiw 1991).

In 1988, the Colorado Division of Wildlife (CDOW) implemented a statewide testing program to determine the extent of the dissemination of *M. cerebralis* among captive and free-ranging trout species. During this investigation, the parasite was first detected in the upper Colorado River drainage in Middle Park. Stocked rainbow trout obtained from private ponds at two separate locations upstream from Windy Gap Reservoir tested positive for the parasite (Walker and Nehring 1995). One collection came from the Willow Creek drainage 1 km upstream of Willow Creek Reservoir (lo-

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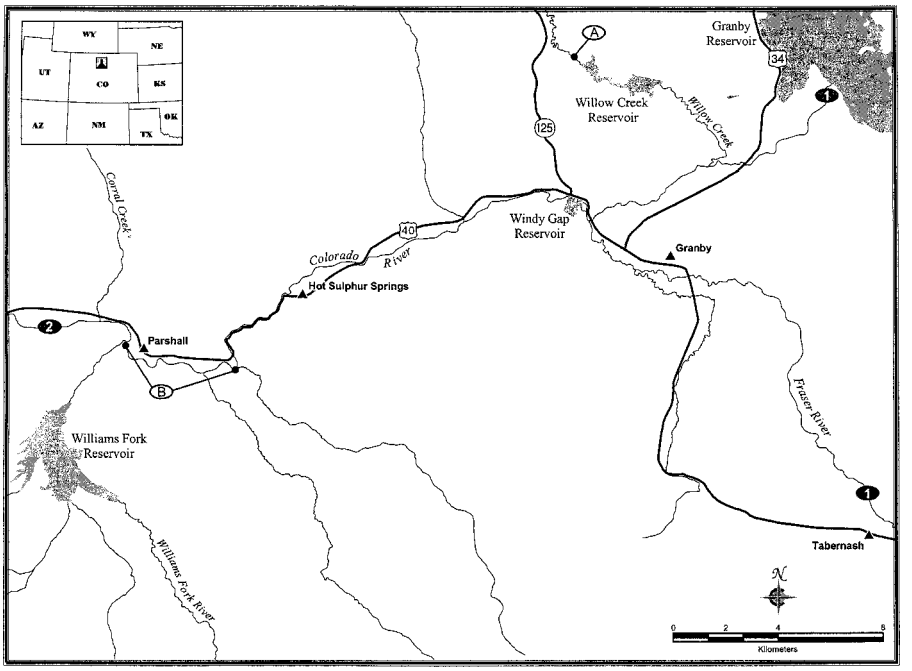

FIGURE 1.—Map of the upper Colorado River basin study area. The area covered by the map is approximately 820 km^2. The upstream boundaries of the study area, denoted by 1s, are the Colorado River at the base of Granby Dam and the Fraser River near the town of Tabernash, Colorado. The downstream boundary of the study area, denoted by 2, is approximately 2 km west of the confluence of Corral Creek and the Colorado River. Privately cultured stocked rainbow trout collected at location A in 1988 were found to be infected with the *Myxobolus cerebralis* parasite. This is the most probable point from which *M. cerebralis* became enzootic in Windy Gap Reservoir and subsequently throughout much of the Colorado and Fraser River basins. Location B is the reach of the Colorado River from which wild adult rainbow trout infected with *M. cerebralis* were first collected in April 1992.

cation A in Figure 1); the other collection was from the Colorado River drainage upstream of Granby Reservoir. It is highly unlikely that the actinospores of the *M. cerebralis* parasite that were produced upstream of Granby Reservoir would survive entrapment in the lake and escape into the Colorado River. The reservoir is deep, oligotrophic, and more than 2,900 ha in area. Therefore, location A is the most likely point from which the parasite spread into the Colorado and Fraser River drainages. Adult wild rainbow trout in the river downstream of Windy Gap Dam have been tested annually since 1983 during annual spawning operations. The parasite was first detected in such fish in April 1992 at a point 15 km downstream of Windy Gap Dam (location B in Figure 1).

Intensive research in the upper Colorado River drainage in 1994 implicated whirling disease as a major factor (and possibly the decisive factor) in the disappearance of three year-classes of wild rainbow trout beginning in 1991 (Walker and Nehring 1995). The downstream boundary of the study area was 2 km west of the confluence of Corral Creek and the Colorado River. The upstream boundaries were Granby Dam on the Colorado River and the Fraser River near Tabernash, Colorado (Figure 1).

Systematic sampling of rainbow and brown trout fry populations in the river during 1994–1996 showed an increasing incidence and severity of whirling disease in samples taken closer to Windy Gap Dam (Schisler 1999). This strongly suggested a source of high infectivity near Windy Gap Reservoir. Water filtration studies throughout 1997 and 1998 revealed high levels of actinospores of *M. cerebralis* in the outflow of the reservoir (Thompson and Nehring 2000). Sentinel fish exposures also confirmed higher infectivity at exposure sites closer to Windy Gap Reservoir than at sites upstream and further downstream (Thompson et al. 2002). Zendt and Bergersen (2000) estimated the mean oligochaete density in Windy

Gap Reservoir to be 47,539 worms/m^2, 73% of which were *Tubifex tubifex*, the oligochaete host of *M. cerebralis* (Wolf and Markiw 1984). About 1.2% of the *T. tubifex* they sampled were infected with *M. cerebralis*.

These lines of evidence led us to conclude that Windy Gap Reservoir was a focus of *M. cerebralis* infectivity. We hypothesized that this shallow reservoir provided excellent habitat for *T. tubifex* and, in particular, that the large shallow flats on the southern end of the reservoir were the areas of most intense infection.

Study Site

Windy Gap Reservoir is a 42-ha impoundment on the upper Colorado River 0.5 km downstream from its confluence with the Fraser River near the town of Granby, Colorado. Completed in 1985, the reservoir has a maximum volume of 5.49×10^5 m^3. Average depth at full pool is about 1.3 m, with a maximum depth of about 6 m. Because of the reservoir's small volume and shallow depth, its total volume is exchanged one to three times per day, depending on the rate of inflow.

Windy Gap Reservoir has never been stocked with trout and is closed to fishing. Brown trout is the predominant salmonid species in both the Colorado and Fraser rivers above the reservoir. Brook trout *Salvelinus fontinalis* and rainbow trout have occasionally been encountered during sampling surveys in these streams. The CDOW has not stocked any trout that tested positive for *Myxobolus cerebralis* in the upper Colorado River drainage, either upstream or downstream of Windy Gap Reservoir.

In 1998, we initiated an investigation with the objective of documenting the spatial pattern of the production of actinospores of *M. cerebralis* in Windy Gap Reservoir.

Methods

We used core samples to study the spatial dynamics of *M. cerebralis* actinospore production in Windy Gap Reservoir. We did not attempt to describe or quantify the aquatic oligochaetes contained in these core samples. Average oligochaete abundance data in Zendt and Bergersen (2000) suggested that each core sample would contain several hundred *T. tubifex*. We hypothesized that *T. tubifex* infected with *M. cerebralis* would release actinospores when placed in a container with an overlying layer of water. In previous laboratory experiments, small populations of *T. tubifex* infected with *M. cerebralis* have been shown to release actinospores continuously for more than a year (Nehring et al. 2002). We began sampling on June 18, 1998, and continued through November 5, when ice cover prevented further sampling.

We constructed several shallow-water core samplers similar in design to that described by Gale (1971). The inside diameter of the iron core sampling tube was 8.26 cm, and its cross-sectional area was 53.52 cm^2. Handle lengths ranging from 1.0 to 6.4 m allowed sampling of virtually all locations in the reservoir.

We divided the lake into 12 zones of approximately equal surface area. Core sampling followed a stratified systematic protocol, with three samples drawn from three different zones in the lake each sampling day. Sampling locations in each zone were determined visually and the proximate location noted with the aid of a handheld global positioning system (GPS) device. We made no corrections for satellite selective availability. At each sampling location, four cores were drawn from the substrate, resulting in a total cross-sectional area for each composite core sample of 214 cm^2. The typical substrate depth collected for the core samples ranged from 5 to 20 cm, depending on the degree of compaction. *Tubifex tubifex* respire by waving the posterior portion of the body in the water column; therefore, they were largely found in the upper 3–5 cm of sediment nearest the mud–water interface.

Each composite core sample was placed into a 19-L bucket and covered with 10–13 L of filtered lake water. Atmospheric air was bubbled into the water through an air stone to provide aeration and circulation. After 24 h, the entire volume of water covering the composite core sample was filtered and the concentrate examined for the presence of actinospores. Filtrate volumes averaged 61 mL over the course of the study. The minimum filtration volume was 28 mL. The maximum filtration volume was 252 mL, but most filtrates were between 30 and 90 mL.

We subsampled each filtrate by drawing ten 2-mL aliquots, which were each stained with 60 μL of crystal violet biological stain. From each aliquot of the stained filtrate we pipetted 84.8 μL of water (80.0 μL in actual sample volume) onto a gridded petri dish, placed a 24-mm × 40-mm cover slip over the water, and examined the entire volume for actinospores. Stained actinospores were identified and enumerated using a variable magnification (40−100×) stereozoom microscope. The total number of actinospores found in each of the 10 subsamples was recorded, and the mean number

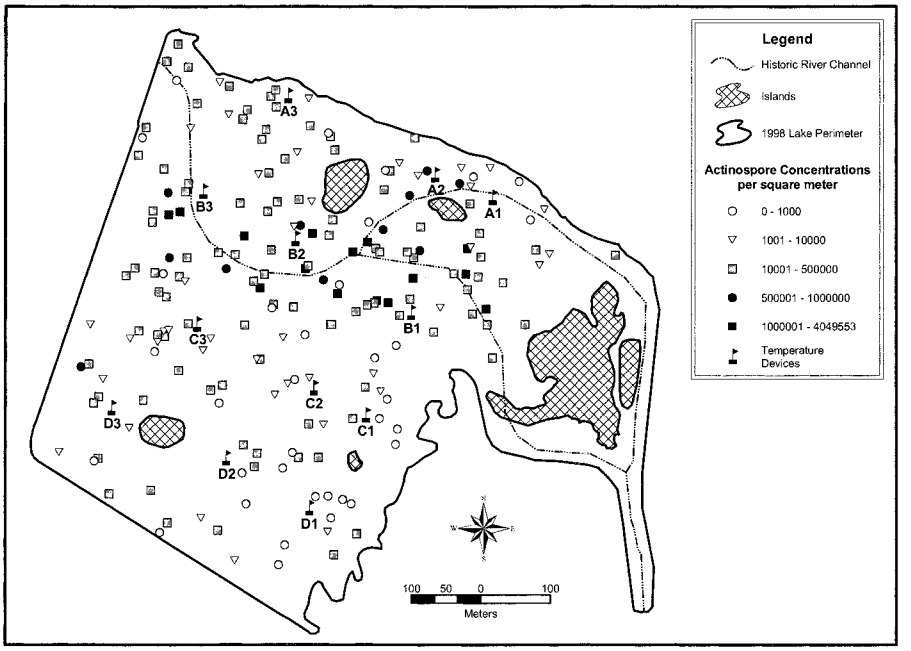

FIGURE 2.—Map of Windy Gap Reservoir showing the locations of islands, the historic river channel, and the spillway. Each of the four quadrants comprised three zones designated by common letters (A–D). Circles, triangles, and squares mark sample locations; also shown are temperature monitor locations, which are labeled A1 through D3.

per slide was used to estimate the total number of actinospores contained in the concentrated filtrate by the formula

$$(\text{actinospores}/80\ \mu\text{L}) \times (1{,}000\ \mu\text{L/mL}) \times (\text{mL filtrate}) = \text{actinospores in filtrate.}$$

The result was the estimate of the number of actinospores produced from each composite core sample in the 24-h period. Each estimate was standardized according to the formula

$$\text{actinospores}/214\ \text{cm}^2 \times (10^4\ \text{cm}^2/\text{m}^2) = \text{actinospores}/\text{m}^2.$$

We report the results as actinospores/m^2.

Actinospores of *M. cerebralis* were easily differentiated from the other actinospores observed during the study based on their overall conformation and general size and shape as shown in El-Matbouli and Hoffmann (1998). For identification of the non–*M. cerebralis* actinospores, we used a dichotomous key provided by Michael Kent (Oregon State University) that was in draft form at the time of the study. The key is now available in published form (Kent et al. 2002).

On 42 occasions, we pipetted 0.8-mL samples from the concentrated filtrates for testing by a single-round modification of the polymerase chain reaction (PCR) test developed by Andree et al. (1998). The intensity of the PCR band was subjectively rated on a five-point scale ranging from 0 (PCR negative) to 4 (a very intense signal). Ratings were assigned independently by two evaluators, and the rare discrepancies were resolved by mutual consultation. The primary purpose of the PCR testing was to provide quality assurance that the actinospores identified by stereozoom microscopy were actually *M. cerebralis*.

Temperature monitors were deployed in each of the 12 sampling zones to record water temperature at 2-h intervals from June to early November 1998. The devices were in contact with the substrate, thereby monitoring the water temperatures experienced by the aquatic oligochaetes.

For analysis, the 12 zones were converted to 4 quadrants parallel to the direction of water flow through the reservoir (Figure 2). Quadrant A was adjacent to the north shore of the reservoir. Quadrant B was roughly superimposed on the old river channel and adjacent to quadrant A. Quadrant C was south of and adjacent to quadrant B, while quadrant D was the southernmost quadrant adja-

TABLE 1.—Numbers of samples obtained each month in each quadrant of Windy Gap Reservoir, Colorado, from June 18 to November 5, 1998. The numbers of samples yielding nonzero estimates are in parentheses.

Month	Quadrant A	B	C	D	Total
Jun	5 (3)	9 (9)	2 (0)	5 (5)	21 (17)
Jul	13 (11)	12 (12)	14 (10)	11 (4)	50 (37)
Aug	14 (12)	16 (15)	11 (9)	10 (5)	51 (41)
Sep	5 (5)	14 (13)	8 (8)	1 (1)	28 (27)
Oct	12 (12)	15 (15)	9 (9)	11 (8)	47 (44)
Nov	2 (2)	5 (4)	2 (2)	0 (0)	9 (8)
Total	51 (45)	71 (68)	46 (38)	38 (23)	206 (174)

cent to the dike on the southern edge of the lake. We used SAS PROC GLM (SAS Institute 1989) to examine the influence of quadrant and month on the production of actinospores by two-way analysis of variance (ANOVA). The numbers of actinospores per square meter were $\log_e$ transformed to stabilize variances. To further ensure the normality of the data, only those samples that yielded actinospores were included in the ANOVA. The proportion of positive samples in each quadrant was tested by chi-square contingency analysis. An α level less than or equal to 0.05 was considered statistically significant. As preliminary analysis of mean temperature data indicated that temperatures were not significantly different among quadrants, temperature was not included as a factor in the ANOVA.

Results

The actual numbers of samples drawn each month varied among quadrants (Table 1). A total of 206 samples were collected and analyzed; 174 produced detectable numbers of actinospores of *M. cerebralis*. Statistical analysis was limited to 149 positive samples collected from July 1 to October 31 because of incomplete quadrant sampling coverage for June and November. Discrepancies between visual and GPS placement of samples in quadrants resulted for six samples; all of these were collected near boundaries between quadrants. We maintained visual zone placement for the purposes of data analysis but used GPS coordinates to place the points on the map (Figure 2).

For the entire sampling period, 88% of the samples from quadrant A were positive, as were 96% from quadrant B, 83% from quadrant C, and 61% from quadrant D (Table 1). Chi-square analysis indicated that the proportions of positive samples were different among quadrants ($\chi^2 = 24.191$, df $= 3$, $P < 0.001$). Quadrant D accounted for most

TABLE 2.—Mean monthly temperature (°C) in each quadrant from June 18 to November 5, 1998. Data are from three temperature monitors deployed within each quadrant.

Quadrant	Jun	Jul	Aug	Sep	Oct	Nov
A	10.5	14.4	15.3	13.7	7.0	4.9
B	10.0	13.2	13.5	11.9	6.4	5.0
C	9.3	11.4	12.9	12.6	6.9	5.1
D	10.1	12.7	14.1	13.5	7.3	5.2

of this effect, as it clearly had a lower proportion of positive samples. An analysis without that quadrant showed no significant differences ($\chi^2 = 5.538$, df $= 2$, $P = 0.063$). Water temperature did not vary greatly among quadrants (Table 2), with a maximum spread of 3.0°C in July. Some of the temperature monitors became imbedded in the lake substrate within a month of deployment and showed less daily variation than those that did not become imbedded. Most of the monitors that became imbedded were deployed in quadrants C and D. Mean monthly temperature from June through September was 10–15°C, a range that is conducive to parasite development (El-Matbouli et al. 1999).

Individual estimates of the number of actinospores per square meter for every core sample collected were plotted on a surface map of the reservoir (Figure 2). The overall ANOVA model indicated that actinospore production was a dynamic phenomenon ($F = 3.24$, df $= 15$, $P = 0.0001$). The quadrant of collection was the only variable of importance in the model ($F = 11.56$, df $= 3$, $P = 0.0001$). Quadrant B was the source of the highest average actinospore production (Table 3). Least-squares means comparisons indicated that the differences between quadrant B and quadrants A and C were highly significant ($P = 0.006$) and that that between quadrant B and quadrant D were almost significant ($P = 0.0636$). In contrast, month of collection was not a significant factor in the model ($F = 0.94$, df $= 3$, $P = 0.422$), and actinospore production did not differ among months

TABLE 3.—Estimated average number of actinospores per square meter by quadrant from core samples yielding actinospores from July 1 to October 31, 1998.

Quadrant	Positive samples	Average actinospores/m^2	Confidence limits Lower	Upper
A	40	188,400	104,200	340,500
B	55	549,700	342,200	883,100
C	36	64,900	36,200	116,500
D	18	113,400	37,800	340,000

TABLE 4.—Estimated average number of actinospores per square meter by month from core samples yielding actinospores from July 1 to October 31, 1998.

Month	Positive samples	Average actinospores/m^2	Confidence limits	
			Lower	Upper
Jul	37	440,000	233,500	829,000
Aug	41	238,900	130,200	432,700
Sep	27	246,900	87,600	695,700
Oct	44	213,800	124,100	368,200

(Table 4; all $P > 0.096$). The interaction between quadrant and month was not significant ($F = 1.07$, df = 9, $P = 0.389$).

Actinospore Counts

Actinospores of *M. cerebralis* accounted for more than 96% of the actinospores observed in the microscope counts; over the course of the study we counted 15,988 actinospores, of which 15,354 were identified as *M. cerebralis*. Thirty-two filtrates from the 206 sediment samples contained no actinospores. Actinospores of *M. cerebralis* were detected in filtrates from 174 samples.

Actinospores that did not fall within the size range or conformation of *M. cerebralis* were detected in 29 of 206 filtrates. Five different non–*M. cerebralis* actinospores of various configurations were encountered in the filtrates. Two were of the triactinomyxon type. One of these was approximately 50% smaller than the *M. cerebralis* actinospore and more robust in conformation, with a bulbous swelling at the terminal end of the style. The tip-to-tip distance across the caudal processes was 200–240 μm, compared with a distance of approximately 400 μm for the actinospore of *M. cerebralis* (El-Matbouli and Hoffmann 1998). The other unidentified triactinomyxon was approximately 50% larger than *M. cerebralis*, measuring approximately 600 μm across the caudal processes. Moreover, the caudal processes for this actinospore were straighter, thinner, and more rigid than those of *M. cerebralis*.

Three other actinospores were rarely encountered and very distinct in appearance compared with the triactinomyxon of *M. cerebralis*. These actinospores were of the echinactinomyxon, raabeia, and hexactinomyxon types (Kent et al. 2002). Over the course of the study, individual examples of all five actinospores were micropipetted out of the samples and tested by PCR. None of the tests were positive for the DNA of the *M. cerebralis* parasite (Andree et al. 1998).

Forty-two 0.8-mL samples containing actinospores of *M. cerebralis* were tested by PCR for the DNA of the parasite over the course of study. All samples tested positive. Nine filtrates that had originally contained actinospores were refiltered and tested by PCR to determine whether or not actinospores had passed through the 20-μm-mesh filtration screen on the first filtration. Four of the nine samples tested positive, indicating that actinospores can pass through the filtration screen in some instances. No attempts were made to correlate microscope actinospore counts and relative PCR signal strength since we only tested 23% of the samples collected. Moreover, samples with low actinospore counts and those where none were detected were not tested by PCR because of budgetary restrictions. However, in a much larger study involving more than 1,300 samples there was a strong positive relationship between the relative PCR gel-banding pattern score and the number of actinospores of the *M. cerebralis* parasite enumerated by microscope count (see Table 37 in Nehring and Thompson 2001 for details).

The variance in the number of actinospores counted among the ten 2-mL aliquots decreased as the number of actinospores counted per sample filtrate increased. For filtrates in which the total number of actinospores counted in 10 aliquots ranged from 2 to 10, the mean coefficient of variation (CV, defined as 100 · SD/mean) was 50.2%. Among samples in which the total number of actinospores ranged from 11 to 99, the mean CV was 17.5%. And among samples in which the total number of actinospores was at least 100, the mean CV was 8.0%.

Discussion

This study demonstrates that many actinospores are produced in Windy Gap Reservoir, a fact that is corroborated by water filtration studies in the Colorado River above and below the reservoir (Thompson and Nehring 2000). The temperatures observed in the reservoir from June through September were 10–15°C, a range considered ideal for the development and release of the actinospore of *M. cerebralis* (El-Matbouli et al. 1999). Without a substantial trout population in the reservoir, and no history of trout stocking, the question arises, ''Where do the myxospores required for infecting the oligochaete host come from?''

Data collected by CDOW biologists provide little support for the hypothesis that salmonid fishes living and dying in Windy Gap Reservoir are the primary source of myxospores. Using daily discharge records and estimated densities of *M. cer-*

ebralis actinospores in the effluent of Windy Gap Reservoir derived from water filtration studies, Nehring et al. (2002) estimated that 9.6×10^{11} and 1.8×10^{12} actinospores were released from the lake during 12-month periods in 1997–1998 and 1998–1999, respectively. At the same time, given a population proportion of 73% for *T. tubifex* in the lake (Zendt and Bergersen 2000), an estimate of 27,000 myxospores per brown trout in the lake, and 457 as the maximum estimated number of actinospores produced by each myxospore (Nehring et al. 2002), 107,000–200,000 trout would be required each year in this 42-ha reservoir to maintain the level of actinospore production estimated in the effluent for 1997–1998 and 1998–1999. A trout density of 2,548–4,761/ha is far above the density that this small reservoir could sustain biotically. A 1992 gill-net survey of the fish population in the lake indicated that the numerical abundance of trout was low and that suckers *Catostomus* spp. dominated the fish population. That catch included 305 catostomids, 18 rainbow trout, and 24 brown trout. Another gill-net survey completed in September 2001 yielded 3 rainbow trout, 14 brown trout, 3 kokanee *O. nerka*, 5 longnose suckers *C. catostomus*, and 150 white suckers *C. commersoni*.

Assessment of the prevalence and severity of infection by *M. cerebralis* among brown and rainbow trout in Windy Gap Reservoir and the rivers upstream from the lake was not part of this study. However, salmonids were collected from the lake and the streams above it between 1998 and 2001 during other investigations on the impact of whirling disease in the upper Colorado River basin (R. B. Nehring, unpublished data). These fish were tested for the prevalence and concentration of cranial myxospores as determined by the pepsin–trypsin digest method (Markiw and Wolf 1974). These data indicate that the prevalence and severity of infection is greater in salmonids collected from the Colorado and Fraser rivers immediately upstream of the lake than in those collected in the reservoir. Six of 12 brown trout collected during the September 2001 gill-net survey in the lake tested positive for myxospores of *M. cerebralis*. The estimated mean cranial myxospore burden was 10,896. The maximum myxospore burden among the brown trout tested was 40,467. Similarly, two rainbow trout from the collection tested positive, and their mean cranial myxospore burden was 224,300.

In contrast, the prevalence of infection among age-1 brown trout collected from the Colorado River 0.5 km upstream of Windy Gap Reservoir consistently ranged between 80% and 100%. The mean cranial myxospore burden among brown trout sampled at this location was 21,600 in the fall of 1998 and 58,700 in the fall of 2000 (Nehring, unpublished data). The highest estimate of cranial myxospores was 208,700. In the Fraser River 1 km upstream of the reservoir, the prevalence of infection was 100% among all collections of age-1 rainbow and brown trout in 1998 and 1999. The mean cranial myxospore burden for brown trout was 75,900 in 1998 and 50,700 in 2000 (Nehring, unpublished data). The maximum spore levels among the brown trout tested were 341,000 in 1998 and 113,300 in 1999. Mean cranial myxospore levels were 202,400 among age-1 rainbow trout in the Fraser River upstream of Windy Gap Resevoir. The maximum cranial myxospore burden among the age-1 rainbow trout tested was 708,900.

We hypothesize that *M. cerebralis* myxospores produced in salmonid populations in the Colorado and Fraser River drainages upstream of Windy Gap Reservoir are the primary source of infectivity emanating from the reservoir. Moreover, we suggest that those myxospores are transported to the reservoir during runoff events and deposited in those areas of the reservoir receiving direct inflows from the Colorado and Fraser rivers. Quadrant B in our study encompasses most of the area receiving direct inflows. Snowmelt in April, May, and June accounts for the major runoff events in most years.

The locations of the samples yielding the highest actinospore production in the lake (Figure 2) and the seasonality and periodicity of peak actinospore release from the reservoir lend credence to this hypothesis. Originally, we expected the shallowest portion of the reservoir (quadrants C and D on the southern end) to be the primary zone of actinospore production based on its presumed superiority as *T. tubifex* habitat. However, quadrant B displayed the highest production. This quadrant encompasses parts of the historic river channel (Figure 2) and at high flows receives much direct flow around the south end of the large island that sits in the reservoir inlet. Moreover, quadrant B lies directly between the inflow and the spillway, where most water is discharged during the snowmelt runoff. This quadrant makes much sense as a primary area of deposition if myxospores are transported into the reservoir from points upstream during runoff events. Zendt and Bergersen (2000) present evidence indicating that the infection rate (as determined by PCR testing) among tubificids collected within the old river channel was 1.9 ±

0.5% (mean ± SD), compared with 0.7 ± 0.3% in other areas of the reservoir. However, they found no significant differences in oligochaete density among sampling sites. Their results revealed that infection rates among the *T. tubifex* in the reservoir were highest in May (2.7 ± 1.0%) and that they declined appreciably by August (to 0.8 ± 0.3%). The results of Zendt and Bergersen (2000) are consistent with our findings that the area of highest actinospore production was quadrant B.

Intensive sampling of water at the outlet of Windy Gap Reservoir from April 1997 to December 1998 revealed that the peak period of actinospore release was from July to December (Thompson and Nehring 2000). This pattern has remained consistent from 1997 to 2001. *Tubifex tubifex* held at 12.5°C and exposed to myxospores of *M. cerebralis* in a laboratory first released actinospores 104–113 d postexposure (Markiw 1986). In a similar experiment, positive control *T. tubifex* held at 15°C began releasing actinospores 78 d postexposure (Nehring et al. 2002). Mean water temperatures in Windy Gap Reservoir during late spring and summer 1998 were 10–15°C (Table 2). At those temperatures, *T. tubifex* that ingested myxospores deposited in the reservoir in April and May would begin releasing actinospores in July and August (Table 4), in synchrony with the onset of the peak discharge of actinospores observed in the effluent of the reservoir (Thompson and Nehring 2000).

An alternative explanation is that the myxospores were carried into the reservoir by fish-eating birds (Taylor and Lott 1978). Piscivorous birds that frequent the reservoir include the white pelican *Pelecanus erythrorhynchos*, double-crested cormorant *Phalacrocorax auritus*, common merganser *Mergus merganser*, great blue heron *Ardea herodias*, California gull *Larus californicus*, bald eagle *Haliaeetus leucocephalus*, and belted kingfisher *Ceryle alcyon*. It is possible that they contribute myxospores to the reservoir. However, the potential contributions of bird predators fail to account for the concentration of core samples producing the greatest number of actinospores in quadrant B between the reservoir inlet and the spillway. The fish-eating avian predators swim and presumably defecate all over the lake, not just in that area of the reservoir receiving inflowing water.

This study demonstrates that the numerous actinospores of *M. cerebralis* occurring in the Colorado River immediately below Windy Gap Reservoir originate in the reservoir. The great majority of those actinospores are produced during the period from the end of the spring runoff through late fall (Thompson and Nehring 2000). The fishery in the upper Colorado River downstream from Windy Gap Dam continues to suffer the ill effects of the whirling disease epizootic, with the rainbow trout population in particular exhibiting much lower levels of abundance and biomass than a decade ago. This fishery might benefit greatly if a means could be devised to sequester actinospores produced in Windy Gap Reservoir within the lake.

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