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[Article]

Avian Piscivores as Vectors for *Myxobolus cerebralis* in the Greater Yellowstone Ecosystem

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Abstract.—*Myxobolus cerebralis*, the cause of whirling disease in salmonids, has dispersed to waters in 25 states within the USA, often by an unknown vector. Its incidence in Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* within the highly protected environment of Yellowstone Lake, Yellowstone National Park, is a prime example. Given the local abundances of piscivorous birds, we sought to clarify their potential role in the dissemination of *M. cerebralis*. Six individuals from each of three bird species (American white pelican *Pelecanus erythrorhynchos*, double-crested cormorant *Phalacrocorax auritus*, and great blue heron *Ardea herodias*) were fed known-infected or uninfected rainbow trout *O. mykiss*. Fecal material produced during 10-d periods before and after feeding was collected to determine whether *M. cerebralis* could be detected and, if so, whether it remained viable after passage through the gastrointestinal tract of these birds. For all (100%) of the nine birds fed known-infected fish, fecal samples collected during days 1–4 after feeding tested positive for *M. cerebralis* by polymerase chain reaction. In addition, tubificid worms *Tubifex tubifex* that were fed fecal material from known-infected great blue herons produced triactinomyxons in laboratory cultures, confirming the persistent viability of the parasite. No triactinomyxons were produced from *T. tubifex* fed fecal material from known-infected American white pelicans or double-crested cormorants, indicating a potential loss of parasite viability in these species. Great blue herons have the ability to concentrate and release viable myxospores into shallow-water habitats that are highly suitable for *T. tubifex*, thereby supporting a positive feedback loop in which the proliferation of *M. cerebralis* is enhanced. The presence of avian piscivores as an important component of aquatic ecosystems should continue to be supported. However, given the distances traveled by great blue herons between rookeries and foraging areas in just days, any practices that unnaturally attract them may heighten the probability of *M. cerebralis* dispersal and proliferation within the Greater Yellowstone Ecosystem.

Whirling disease, caused by the exotic parasite *Myxobolus cerebralis* (Myxozoa: Myxosporea), is responsible for wild trout population declines across the Intermountain West (Bartholomew and Reno 2002). In addition, captive hatchery trout stocks, in which *M. cerebralis* was first documented in the United States (Bergersen and Anderson 1997), have experienced losses as a result of infection. Stocking of infected hatchery trout has been a primary mode of *M. cerebralis* introduction to many streams, rivers, and reservoirs (Meyers et al. 1970), but the dispersal vector to waters where trout have not been stocked, including to waters within the protected interior of large natural areas such as Yellowstone National Park (Koel et al. 2006), is most often unknown (Baldwin et al. 1998). Piscivorous birds are common and extremely mobile; can disperse propagules of aquatic plants, algae, and invertebrates (Proctor 1959; Charalambidou and Santamaria 2005); and have long been suspected of disseminating *M. cerebralis* as they feed and move freely between infected and uninfected trout populations (Halliday 1976). However, documenting this

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phenomenon has been extremely challenging because of ecological and life history complexities of the parasite, hosts, and birds. A better understanding of potential dispersal vectors will have implications for the management of whirling disease in wild and captive trout populations throughout the Greater Yellowstone Ecosystem and elsewhere.

In the United States in the early 1900s, piscivorous birds (e.g., American white pelican *Pelecanus erythrorhynchos* and double-crested cormorant *Phalacrocorax auritus*) were blamed for declines in important fisheries (Munro 1927) and, in some cases, were removed as a result (McEneaney 2002). Subsequent research documenting the effects of piscivorous birds on fish populations has been primarily associated with hatcheries and aquaculture facilities (Lagler 1939; Pitt and Conover 1996; Glahn et al. 1999), where economic consequences of declines can be extreme (King 2005; Dorr 2006). These and other studies on wild fish populations (Derby and Lovvorn 1997; Hodgens et al. 2004) have produced estimates of trout consumption by birds and have advanced our understanding of the movement and feeding strategies exhibited by many of these bird species. Most recently, this line of research has revealed the potential of piscivorous birds to act as possible vectors for fish pathogens (Waterstrat et al. 1999), including whirling disease (Dorr and Taylor 2003).

Myxobolus cerebralis occurs from New York to Alaska and has been reported in 25 states; this has generated great concern among fisheries managers and the angling public (WDI 2008). In Colorado (Nehring and Walker 1996), Montana (Vincent 1996), and other states where infection has been severe, whirling disease has had a negative economic impact on the recreational fishing industry, especially in areas where tourism is important for local and state economies (Nickum 1999). The parasite was first documented in Wyoming waters in 1988 and has been detected in at least seven river drainages. In 2007, *M. cerebralis* was discovered at the Ten Sleep Fish Hatchery (Wyoming Game and Fish Department), resulting in the destruction of 0.5 million trout in an attempt to rid the hatchery of the parasite. Similar efforts have been required at hatcheries elsewhere in the United States. Complexities associated with attempts to manage *M. cerebralis* include its obligate two-host life cycle, which involves (1) infection of a salmonid fish by the myxosporean stage; (2) infection of the oligochaete *Tubifex tubifex* by the actinosporean stage; and (3) two intermediary spore stages, the myxospore and triactinomyxon (TAM; Hedrick et al. 1998).

Within Yellowstone National Park, *M. cerebralis* was first detected in 1998 in native Yellowstone cutthroat trout *Oncorhynchus clarkii bouvieri* collected from Yellowstone Lake (Koel et al. 2006). A subsequent Yellowstone cutthroat trout decline, exacerbated by introduced lake trout *Salvelinus namaycush* and drought (Koel et al. 2005), has coincided with intensive research on the effects of *M. cerebralis* in this system. Experimental exposures of Yellowstone cutthroat trout fry within sentinel cages placed in Yellowstone Lake tributaries have resulted in much higher infection rates and severity than previously noted for cutthroat trout *O. clarkii* in laboratory exposures (Hedrick et al. 1999), and histological examinations provide strong evidence that Yellowstone cutthroat trout are extremely susceptible to *M. cerebralis* (Murcia et al. 2006). The Yellowstone cutthroat trout spawning population of Pelican Creek, Yellowstone Lake's second largest tributary, declined primarily as a result of *M. cerebralis* infection (Koel et al. 2006). The vector of *M. cerebralis* dispersal deep into the protected interior of Yellowstone National Park remains unknown, as does the pathogen's introduction to many other waters of the region (Baldwin et al. 1998). Suspected vectors have included humans (anglers and their gear; Gates et al. 2008) and wildlife that are capable of moving sediment, water, fish, or fish parts among streams.

Despite past nonindigenous species introductions, Yellowstone National Park continues to preserve what is arguably the largest naturally functioning ecosystem remaining in the lower contiguous 48 states. As such, there are many avian and terrestrial wildlife species in the Yellowstone Lake area during periods of open water (May–November). The Yellowstone cutthroat trout are often found near the surface of Yellowstone Lake and spawn each spring in tributary streams (Gresswell and Varley 1988), making them accessible to many wildlife species, including piscivorous birds (Schullery and Varley 1995; McEneaney 2002). For example, the Molly Islands of Yellowstone Lake host a large and historically significant American white pelican colony each year (approximately 570 breeding birds; Diem and Pugesek 1994). This avian piscivore uses Yellowstone Lake, the Yellowstone River, Pelican Creek, and many other tributary streams regularly for feeding and resting (Kaeding 2002; M. B. Davenport, Yellowstone National Park, unpublished completion report). Other piscivorous birds in the ecosystem include the great blue heron *Ardea herodias* and double-crested cormorant (McEneaney 2007), the latter of which also have a resident nesting colony on the Molly Islands, with approximately 100–200 nesting attempts made each year. In radiotelemetry studies conducted on Yellowstone cutthroat trout from the Yellowstone River, American white pelicans were a

cause of Yellowstone cutthroat trout mortality, and several radio tags were retrieved from American white pelican roosting areas along the Yellowstone River and the Molly Islands (Kaeding and Boltz 2001; Koel et al. 2004). Estimates of Yellowstone cutthroat trout consumed by all piscivorous avifauna on Yellowstone Lake were 81,000–114,000 kg/year (Davenport, unpublished completion report).

Several common piscivorous birds have widespread distributions in North America, and they are often encountered in large numbers on lakes, reservoirs, rivers, and coastal regions (Sauer et al. 2008). Many waters frequented by these birds are also known to support *M. cerebralis*. In an early study before the two-host life cycle of *M. cerebralis* was completely understood (Markiw and Wolf 1983), Taylor and Lott (1978) were able to infect trout in ponds with *M. cerebralis* after exposing them to waterbirds. Unfortunately, the specific mechanism resulting in this infection was unclear. Later, El-Matbouli and Hoffman (1991) demonstrated that myxospores pass through the gastrointestinal (GI) tracts of northern pike *Esox lucius* and mallards *Anas platyrhynchos* without loss of infectivity. It remained unknown whether myxospores were viable after passing through wildlife species that specifically preyed on trout, especially wildlife species with abilities to range widely among drainages, such as piscivorous birds. Research was first initiated in this regard by Barrows et al. (1999), who conducted feeding experiments to determine evacuation rates of rainbow trout in American white pelicans and bald eagles *Haliaeetus leucocephalus*. Although the work documented the time required for trout to pass through the GI tract (and therefore the potential distance *M. cerebralis* could be moved), the degree to which myxospores remained intact and viable after passage was not examined.

The overall goal of this study was to use feeding trials in a controlled environment to determine the potential role of American white pelicans, great blue herons, and double-crested cormorants as dispersal vectors for *M. cerebralis* in the wild. Our specific objectives were to experimentally determine whether *M. cerebralis* myxospores (1) can be detected and (2) can remain viable after consumption and passage through the GI tracts of these avian piscivore species. Results will provide a more complete understanding of potential *M. cerebralis* dispersal vectors and will allow for better assessment of *M. cerebralis* introduction risk to the remaining uninfected trout waters of the Greater Yellowstone Ecosystem and elsewhere in the Intermountain West.

Methods

Avian piscivore feeding trials.—We chose rainbow trout *O. mykiss* as a vehicle for carrying *M. cerebralis* myxospores during feeding trials because of their high susceptibility to *M. cerebralis* and because parasite-free Yellowstone cutthroat trout fry were not available. Age-0 rainbow trout (6 weeks posthatch) were obtained from the Ennis National Fish Hatchery (U.S. Fish and Wildlife Service), Ennis, Montana, and were infected with *M. cerebralis* by exposing each fish to 750 TAMs at the Wild Trout Laboratory (Montana State University, Bozeman). Infected rainbow trout were held in aquaria at 13°C for 11–12 months to allow for somatic growth of fish and for proliferation of the parasite. Rainbow trout that were uninfected by *M. cerebralis* (fish were free of specific pathogens and parasites) were obtained from Colorado Division of Wildlife hatcheries in Bellevue and Pueblo. At the time they were fed to birds, the infected rainbow trout from Montana were smaller (50–75 mm total length [TL]) than uninfected fish from Colorado (100–150 mm TL). Laboratory examination for the prevalence of *M. cerebralis* in subsamples of infected and uninfected rainbow trout was by polymerase chain reaction (PCR; Epp et al. 2002).

American white pelicans, double-crested cormorants, and great blue herons (up to eight individuals of each species) were captured for the experiments; for identification purposes, each bird was fitted with an aluminum leg band labeled with a unique number. The American white pelicans and double-crested cormorants were captured from night roosts and loafing sites in Mississippi, Alabama, and Arkansas. Great blue herons were captured at aquaculture facilities in Colorado. Double-crested cormorants were captured from their night roosts using a night-lighting technique (King et al. 1994). Birds of the other two species were captured on their roosting, loafing, or feeding sites by use of modified soft-catch leg-hold traps (King et al. 1998).

All birds were transported to the indoor aviary at the National Wildlife Research Center (U.S. Department of Agriculture [USDA], Animal and Plant Health Inspection Service [APHIS], Wildlife Services), Fort Collins, Colorado, and were housed in individual pens ($2.13 \times 2.44 \times 2.13$ m) containing a water tank. Environmental conditions in the aviary were ambient and uncontrolled, with room temperatures at 15–24°C. Birds were acclimated for 10 d and were fed disease-free rainbow trout. Birds were fed ad libitum during pretreatment acclimation and during the experiments. Maintenance diets were at least 600 g daily for each double-crested cormorant, 1,500 g daily for each American white pelican, and 400 g daily for each great blue heron. Fecal samples were analyzed (by PCR as described in the next section) to ensure that the birds were free of *M. cerebralis* before experimental challenges. Pens were

TABLE 1.—Average mass (g) of pre- and post-fed birds (each $n = 3$), *Myxobolus cerebralis*-infected and uninfected rainbow trout (each $n = 10$), and bird fecal material collected during days 1–4, 5–7, and 8–10 of 10-d trials with American white pelicans (AWPE), double-crested cormorants (DCCO), and great blue herons (GBHE). All (100%) fecal samples denoted by asterisks tested positive for the presence of *M. cerebralis* by polymerase chain reaction.

Species code	Bird			Rainbow trout		Fecal mass collected on days:			
	Pre-fed mass	Post-fed mass	Mass change[a]	Treatment	Mass fed	1–4	5–7	8–10	1–10
AWPE	5,783	6,550	767	Uninfected	205.0	12.7	19.1	15.6	47.4
AWPE	5,800	6,800	1,000	Infected	61.7	14.3*	28.8	33.3	76.4
DCCO	1,833	1,850	17	Uninfected	116.0	6.0	6.8	2.5	15.3
DCCO	1,750	1,900	150	Infected	74.0	5.5*	3.7	5.1	14.3
GBHE	1,950	1,983	33	Uninfected	118.5	14.4	16.8	20.0	51.2
GBHE	2,200	2,217	17	Infected	72.4	17.5*	26.2	22.6	66.4

[a] Average increase in bird mass over the course of the experiment.

disinfected between each challenge and bird species, and 1–2 weeks were required for turnaround of the facility.

Six individuals of each bird species were used in the experiment. The additional birds collected were to be used as substitutes in case the experimental birds became sick or injured. During April–June 2006, each of the three species entered separate 10-d feeding trials in the following order: double-crested cormorants, American white pelicans, and great blue herons. Treatments were assigned to each bird randomly, with three birds fed infected rainbow trout and three birds fed disease-free placebos, resulting in known-infected and known-uninfected feces, respectively. To ensure that a sufficient number of myxospores would pass through the avian alimentary canals, we force-fed 10 (infected or uninfected) rainbow trout to each bird on day 1 of each 10-d feeding trial. Mean total fish mass that was force-fed to each American white pelican was 133 g overall (205 g for control birds and 62 g for treated birds), whereas mean mass force-fed to each double-crested cormorant was 95 g overall (116 g for control birds and 74 g for treated birds) and mean mass force-fed to each great blue heron was 95 g overall (118 g for control birds and 72 g for treated birds; Table 1). For all bird species, the treatment and control birds each were force-fed rainbow trout from a different source, and as a result the fish fed to treatment birds were smaller than the fish fed to control birds. On days 2–10 of each feeding trial, all birds were fed a maintenance diet of uninfected rainbow trout.

A sample of fecal material produced during the 10-d acclimation (pretreatment) periods and during the 10-d feeding trials was collected from each individual bird daily using prelabeled, sterile containers and cage-specific tools to prevent cross-contamination. Treatment and control pens were located on opposite sides of the facility, and samples from control pens were collected first each day. Separate boots were used for treatment and control pens, and a foot bath was placed outside each of the treatment pens and the facility doors to prevent movement of myxospores by personnel. All outerwear and boots were kept in the treatment facility and incinerated at the conclusion of the experiment. Because of the sheer numbers of samples collected, we could not test each of them individually for the presence and viability of *M. cerebralis*; therefore, we combined fecal samples for each bird among days as those collected (1) during the pretreatment period; (2) on days 1–4 of the feeding trial; (3) on days 5–7; and (4) on days 8–10 (Table 1). From each of the four combined samples for each bird, a 1-g subsample was analyzed for the presence of *M. cerebralis* DNA by PCR, and a second 1-g subsample was used to determine potential viability of *M. cerebralis*.

Presence of Myxobolus cerebralis in fecal material.—Total DNA from each 1-g fecal sample was extracted by use of Qiagen DNeasy 96 spin columns (Catalog Number 69581; Qiagen, Inc., Valencia, California). Each sample was transferred with a clean spatula to a 15-mL, screw-capped centrifuge tube. All samples were dried in a centrifugal vacuum dryer (Savant AES1010; Savant Instruments, Hicksville, New York) and were microwaved for 1 min at 1,100 W to disrupt myxospores; 10× volume tissue-lysis buffer was then added. After incubation at 55°C for 1 h, total DNA was extracted from the samples by means of the Qiagen rodent-tail protocol. All extracted DNA samples were further purified to remove possible PCR inhibitors by use of GeneReleaser (Catalog Number GR-1; BioVentures, Inc., Murfreesboro, Tennessee) according to the manufacturer's instructions.

Examination for the presence of *M. cerebralis* DNA was done by PCR to detect the heat-shock protein 70 gene (*Hsp70*; Epp et al. 2002). Details of the *Hsp70* PCR protocol are as follows. The forward primer (PM76) sequence was 5′-GGG CTG AAC GTC CTT CGC ATA-3′; the reverse primer (PM77) sequence

TABLE 2.—Examination of 1-g samples of double-crested cormorant fecal material spiked with known concentrations of myxospores to determine the minimum detection limit of the *Myxobolus cerebralis* heat-shock protein gene fragment (*Hsp70*) by polymerase chain reaction (*Hsp70* scoring: ++ = strong positive signal; — = no signal or below limit of detection).

Treatment (number of myxospores)	*Hsp70* results
25,000	++
10,000	++
1,000	++
500	++
250	++
100	—
50	—
25	++
10	—
5	—
0	—

was 5′-GAG TGG ATC CAC CGA CAA GG-3′. The reaction components were 2 μL of 10× GeneAmp II core buffer (Applied Biosystems, Inc., Foster City, California), 3.5-mM Mg^{2+}, 800 μM for each deoxynucleotide triphosphate (New England BioLabs, Ipswich, Massachusetts), 400 nM each for the forward and reverse primers, 1 unit of AmpliTaq Gold (Applied Biosystems), and 2 μL of template DNA, with a total reaction volume of 20 μL. The thermal cycling parameters were 95°C for 5 min; 45 cycles of 94°C for 30 s, 61°C for 90 s, and 72°C for 150 s; and storage incubation at 15°C. The expected *M. cerebralis* amplicon size was 534 base pairs.

Validation of the *Hsp70* method for use with fecal samples was required, as the current and standard procedures recommended by the American Fisheries Society's Fish Health Section for *M. cerebralis* DNA examination (Andree et al. 1998) apply only to cranial cartilage (half head, wedge sample, or core sample; see AFS-FHS 2007). We determined the minimum detection limit of *M. cerebralis* DNA by analyzing a series of double-crested cormorant fecal samples (1 g each) spiked with 5–25,000 myxospores. Concentrations of 0, 5, 10, 50, and 100 myxospores/g of fecal material showed no signal for *M. cerebralis* DNA (Table 2). However, concentrations of 25, 250, 500, 1,000, 10,000, and 25,000 myxospores/g of fecal material provided a strong positive signal for *M. cerebralis* DNA. From this analysis, we concluded that the minimum detection limit for our PCR procedure was approximately 250 myxospores/g of fecal material. Therefore, any fecal sample not resulting in the molecular marker being positive for *M. cerebralis* DNA may in fact have contained myxospores but at a concentration less than 250 myxospores/g of fecal material. Furthermore, all PCRs included *M. cerebralis* DNA-positive, DNA-negative, and no-DNA (H_2O only) controls.

Viability of Myxobolus cerebralis in fecal material.—Fifty *T. tubifex* (tubificids; Argentina origin, 16S mitochondrial DNA lineage III; Rasmussen et al. 2008) were placed in each of 30 plastic containers along with 40 g of sediment (masonry sand) and were maintained in incubators at 15°C with aeration and a photoperiod of 12 h light : 12 h dark (Stevens et al. 2001; Kerans et al. 2004; Rasmussen et al. 2008). Water was changed weekly, and tubificids were fed one-quarter of a *Spirulina* tablet every 3 weeks.

Fecal material (1 g) collected from each bird during days 1–4 of the feeding trials were added to separate tubificid containers, resulting in three containers receiving uninfected (PCR-negative) feces and three containers receiving infected (PCR-positive) feces for each bird species (i.e., 18 containers total). As part of the experimental design, tubificids also received feces spiked with or without known concentrations of *M. cerebralis* myxospores (as controls). The controls were created by initially adding 1 g of sheep manure to each container. Two positive controls per bird species (6 total positive controls) were created by adding a solution of myxospores to the containers such that there were 500 myxospores/tubificid. A spore-free emulsion of equivalent volume was added to containers to create two negative controls per bird species (6 total negative controls).

Scanning the tubificid containers for TAMs began approximately 90 d after dosing with fecal material. Known-uninfected and known-infected containers were filtered at separate times—and usually on separate days—to avoid the potential for cross-contamination. Water was removed from each sample container by pouring the samples through a 20-μm-mesh sieve into a glass beaker. The sieve was then rinsed into a centrifuge tube such that the remaining liquid totaled 10 mL. A volume of 55 μL was dispensed onto a microscope slide with a pipette. Four slides were used for each filtration, and each was then scanned for the presence of TAMs using a phase-contrast microscope (Stevens et al. 2001; Kerans et al. 2004; Rasmussen et al. 2008).

Results

Avian Piscivore Feeding Trials

Birds adjusted remarkably well to their captive settings and rainbow trout diets, and nearly all birds gained body mass over the course of the experimental trials; mass gained during the experiment for several birds exceeded 15% (Table 1). Mean fecal mass collected ranged from 14 to 76 g. Fecal mass for double-crested cormorants was relatively low by

TABLE 3.—Days that *Tubifex tubifex* containers were scanned for *Myxobolus cerebralis* triactinomyxons (TAMs) after initial exposure of *T. tubifex* to feces obtained from birds fed *M. cerebralis*-infected and uninfected rainbow trout ($n = 3$ containers/treatment for each bird species). Controls (each $n = 2$) were created using sheep manure with (positive) or without (negative) myxospores added. Number in parentheses is the scan day when TAM production by *T. tubifex* was first observed. See Table 1 caption for explanation of bird species codes.

	Scan days			Trout treatment		Control	
Bird species	Start	End	Duration	Infected	Uninfected	Positive	Negative
AWPE	71	132	62			TAM (115)	-
DCCO	89	138	50			TAM (89)	-
GBHE	92	147	56	TAM (115)		TAM (135)	-

comparison because they used the water tanks extensively, making much of their fecal mass unattainable.

Presence of Myxobolus cerebralis in Fecal Material

Total DNA was extracted from seventy-two 1-g fecal subsamples (pretreatment and days 1–4, 5–7, and 8–10; six individuals for each of the three species) and from *M. cerebralis*-positive and negative controls. Feces from all birds (18 total) collected during the pretrial period all tested negative for the presence of *M. cerebralis* DNA. However, fecal samples from all (100%) of the nine birds fed infected rainbow trout and collected during days 1–4 of each feeding trial displayed a strong positive signal for the *M. cerebralis* DNA molecular marker, while none (0%) of the samples collected during days 1–4 from the nine birds that were fed uninfected placebos (controls) provided any *M. cerebralis* DNA signal (Table 1). None (0%) of the fecal samples from treatment and control cages collected during days 5–7 or days 8–10 showed evidence of containing *M. cerebralis* DNA.

Viability of Myxobolus cerebralis in Fecal Material

Containers holding *T. tubifex* in the laboratory were scanned for TAMs beginning at 71–92 d and ending at 132–147 d after treatment or control exposures (Table 3). The TAMs appeared in *M. cerebralis*-positive controls created for American white pelicans, double-crested cormorants, and great blue herons 89–135 d after exposure to known concentrations of myxospores (500 myxospores/tubificid). No TAMs were observed in any of the negative controls from any of the bird species.

Because fecal material from all birds that were fed infected rainbow trout tested positive (by PCR) for the presence of *M. cerebralis* DNA (Table 1) and because TAMs were observed in positive controls, we were able to examine the potential viability of *M. cerebralis* in fecal samples from all three bird species. Tubificids exposed to feces from great blue herons that were fed infected rainbow trout produced TAMs at 115 d after exposure (Table 3). However, during the days they were scanned, TAMs were not observed in containers with tubificids exposed to feces from American white pelicans or double-crested cormorants that were fed infected rainbow trout. Also, TAMs were not observed in containers with tubificids exposed to uninfected fecal samples from any of the bird species.

Discussion

Myxobolus cerebralis DNA was found in the feces of all nine birds (three species) that were fed infected rainbow trout. In addition, the infected feces from great blue herons induced TAM production from *T. tubifex* held in laboratory cultures, confirming their ability to disseminate *M. cerebralis* among aquatic habitats in the wild. Results of our experiments were more equivocal, however, for American white pelicans and double-crested cormorants as *M. cerebralis* DNA was found in their feces but we were not able to locate TAMs in containers with tubificids exposed to these feces. It remains unclear whether these results represent an effect (degradation or deactivation) produced by the GI tracts of American white pelicans and double-crested cormorants, thus causing a loss of myxospore viability, or whether they are merely an artifact of our study protocols. Given that TAMs were produced by tubificids in all positive-control containers spiked with known numbers of myxospores and by tubificids exposed to feces from great blue herons that were fed *M. cerebralis*-infected rainbow trout, it is possible that any further effects of the parasite were reduced or negated in American white pelicans and double-crested cormorants. Only through additional refinement of methods (e.g., PCR of fecal samples or TAM scanning of tubificid cultures) will the ability (or lack thereof) of American white pelicans and double-crested cormorants to disseminate *M. cerebralis* be confirmed.

Proliferation within Habitats

The fact that predators choose sick or injured prey is well established and has been documented for depredation by great blue herons at aquaculture facilities

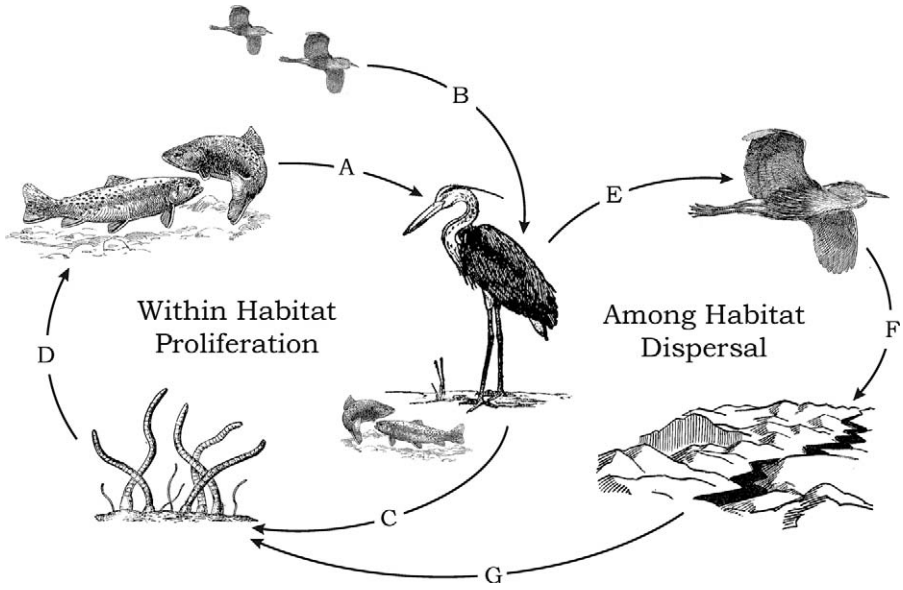

FIGURE 1.—Conceptual model of a hypothesized mechanism promoting within-habitat proliferation and among-habitat dispersal of *Myxobolus cerebralis*. Great blue herons successfully prey on diseased trout (A) and attract additional birds to the area (B). Birds quickly digest the diseased trout, concentrate *M. cerebralis* myxospores, and release them into shallow-water habitats along with other fecal material (C). *Tubifex tubifex* abundance and growth are enhanced by nutrient enrichment, and the probability of myxospore consumption is increased. The result is that greater numbers of triactinomyxons are released into the water column, thereby infecting juvenile trout (D). When the great blue herons seek new habitats (E), they can disseminate *M. cerebralis* myxospores with the potential to remain viable for decades in the sediments (F). When *M. cerebralis* myxospores are consumed by *T. tubifex* and if this occurs in the presence of susceptible trout, proliferation within the new habitat may occur (G).

(Glahn et al. 2000, 2002; Dorr and Taylor 2003). As piscivorous avifauna are often opportunistic (Findholt and Anderson 1995; Nettleship and Duffy 1995) in natural settings, the sick prey they select may be *M. cerebralis*-infected trout; in the case of great blue herons, the selection of infected fish would result in a proliferation of the parasite (Figure 1). Within an aquatic habitat harboring *M. cerebralis*, the onset of whirling behavior in infected trout fry reduces their ability to avoid predation (El-Matbouli et al. 1992). Predators recognize this vulnerability, and in the case of colonial nesting birds such as the great blue heron and the other species we examined, the information learned may be exchanged with other birds within the nesting colony (Krebs 1974). The birds are attracted to feeding areas by the presence of other birds (Krebs 1974; Anderson 1991). These behaviors result in flocks building up where feeding conditions are good.

Piscivorous avifauna frequent aquatic habitats that are suitable for supporting *M. cerebralis*. Great blue herons prefer still or slow-moving waters of side channels and backwater marshes for (typically) solitary feeding (Parker 1977; Warren 1979; Gebauer and Moul 2001), whereas American white pelicans and double-crested cormorants feed in well-coordinated groups (cooperative herding; Anderson 1991), often driving fish into shallow areas or targeting fish stranded in isolated pools (Dowd and Flake 1985). In addition, many birds rest or roost on or near the shallow-water feeding areas (O'Malley and Evans 1984; Butler 1992). Localized changes in foraging areas occur from day to day (Thompson 1979; Kaeding 2002).

Areas with low water velocity retain fine sediments, which support tubificids (Lestochova 2004; Anlauf and Moffitt 2008) and harbor *M. cerebralis* myxospores (Lemmon and Kerans 2001). As waterbirds defecate in these areas, there is enrichment of organic materials and other nutrients, including phosphorus and nitrogen (Baxter and Fairweather 1994; Hahn et al. 2007), which allows for high tubificid densities (Lazim and Learner 1987; Kaeser and Sharpe 2006). These conditions support a high risk of whirling disease infection in both the salmonid (Hiner and Moffitt 2001; Murcia 2008) and tubificid hosts (Krueger et al. 2006; Hallett and Bartholomew 2008). This implies that the concentration and release of *M. cerebralis* myxospores by great blue herons in these same locations form the basis of a positive feedback loop in which the proliferation of *M. cerebralis* is supported (Figure 1). The cycle would continue until the trout population has

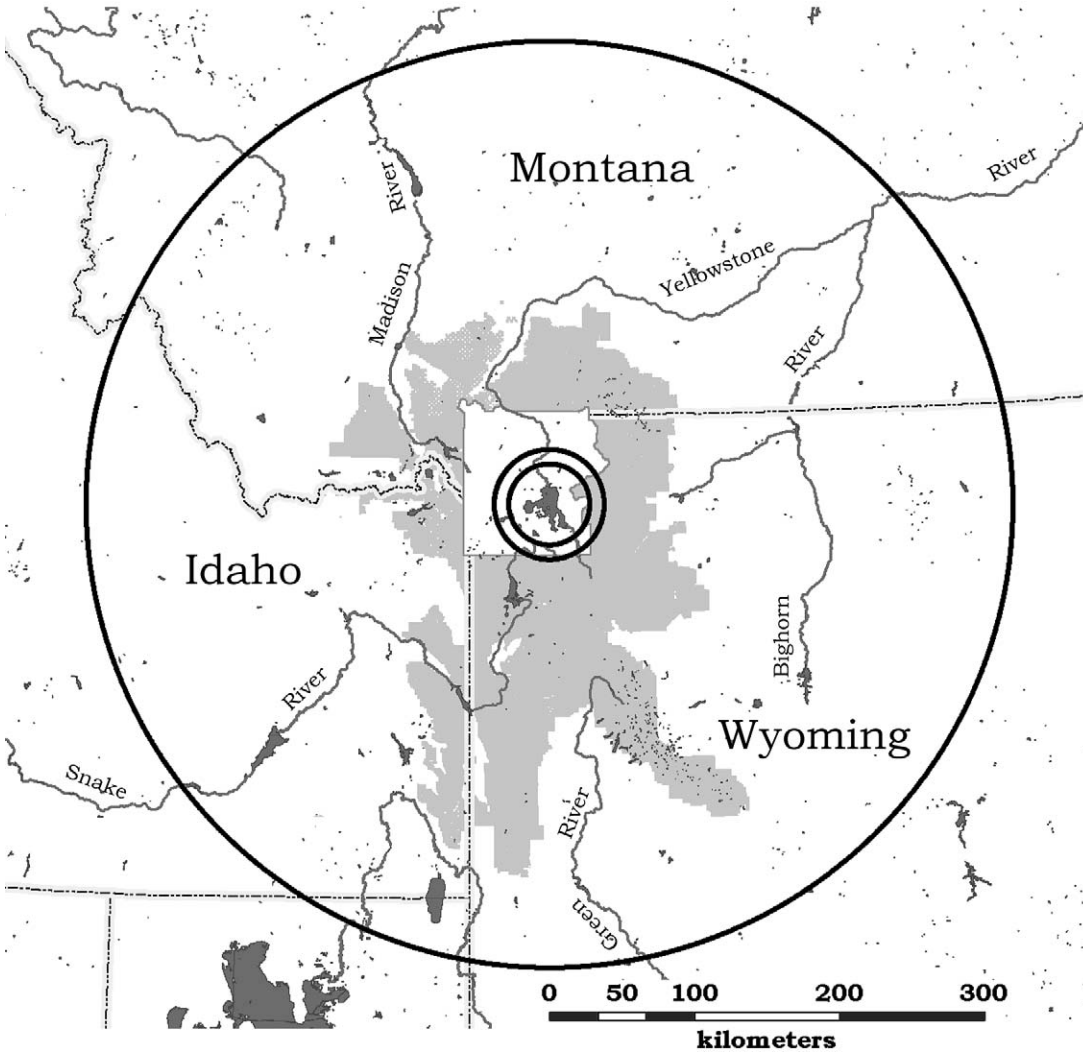

FIGURE 2.—Yellowstone National Park within the Greater Yellowstone Ecosystem (shaded gray) and major river systems in Idaho, Montana, and Wyoming. Circles represent the potential extent of dispersal from Yellowstone Lake over 1–2 d by great blue herons (inner circle), double-crested cormorants (middle circle), and American white pelicans (outer circle).

collapsed, the great blue herons seek new foraging habitats within or among aquatic systems, or both.

Dispersal among Habitats

During the time it takes for digestion and evacuation of prey, piscivorous birds have the ability to move long distances. In our study, all fecal samples collected during the first 4 d after birds were fed infected rainbow trout tested positive for the presence of *M. cerebralis* by PCR, and the parasite was not confirmed from samples collected later in the trials (Table 1). These findings support the results of Barrows et al. (1999), who demonstrated that evacuation rates of trout fed to American white pelicans were 2–3 d; to some degree, such evacuation rates may limit the extent to which great blue herons could disperse *M. cerebralis* from Yellowstone Lake (Figure 2). Daily movements by great blue herons documented elsewhere averaged 0.7–6.5 km between nesting colonies and foraging areas (Thompson 1979; Custer et al. 2004; Tiller 2005), but individual flights extending as much as 24 km have been observed for great blue herons (Dowd and Flake 1985). Individual flight distances as much as 40 km from the colony to the first feeding area have been observed for double-crested cormorants (Custer and Bunck 1992). In contrast, however, American

white pelicans, which typically move in large flocks, may forage more than 300 km from the colony over just 1 or 2 d (Lingle and Sloan 1980). Given these typical travel distances, the potential of piscivorous birds to move among multiple watersheds within the Greater Yellowstone Ecosystem is very high and includes the potential to travel across continental divides (Figure 2). All American white pelican colonies in Montana, for example, are known to have some members that migrate both east and west of the Continental Divide (Hendricks and Johnson 2002).

The Role of Temperature

Temperature may play an overarching role in the degree to which *M. cerebralis* is proliferated and dispersed by piscivorous avifauna because it is one of the most important abiotic factors influencing *M. cerebralis* transmission by tubificids (El-Matbouli et al. 1999), trout spawning and fry emergence (Gresswell and Varley 1988), and colonial waterbird nesting and feeding behaviors (McEneaney 2002). The growth rates of tubificids are highest in the warm waters of summer, and they consume more sediment during this period than at other times of the year, resulting in a greater likelihood of ingesting myxospores. In addition, higher temperatures are known to induce TAM release by tubificids, a phenomenon shown both in the laboratory (Blazer et al. 2003; Kerans et al. 2005) and in the field via exposure of trout in sentinel cages (Baldwin et al. 2000). In the Yellowstone Lake system, emergence of wild-reared Yellowstone cutthroat trout fry occurs when the prevalence and severity of *M. cerebralis* infection risk are high (Koel et al. 2006; Murcia 2008). Of interest here is how this summer period also coincides with increased avian feeding activity resulting from fledglings requiring additional food resources as they mature. Seasonal weather patterns and resulting stream temperature regimes are probably the primary drivers regulating proliferation and dispersal of *M. cerebralis* by piscivorous avifauna (Figure 1).

Management Implications

Piscivorous birds are extremely opportunistic, and their numbers vary depending on the seasonal and annual availability of their prey. The fact that great blue herons are capable of disseminating *M. cerebralis* has implications for managers across a wide range of disciplines within and beyond the Greater Yellowstone Ecosystem. In particular, management activities that tend to concentrate trout, great blue herons, or both, may increase the probability of disease proliferation and dispersal. Rivers that are stocked to support recreational fisheries or reaches below dam tailraces are prime examples of areas where trout and bird concentrations have been artificially increased. Stocking of trout followed by increased bird piscivory increases prevalence of parasites, as was demonstrated at High Rock Lake, Manitoba, with nematodes *Contracaecum* spp. (Dick et al. 1987). In the Greater Yellowstone Ecosystem, if birds from Yellowstone Lake are attracted to waters elsewhere, the potential number of myxospores dispersed could be high. Estimates of *M. cerebralis* prevalence and severity in Yellowstone cutthroat trout of the Yellowstone Lake system (Koel et al. 2006) and daily rates of Yellowstone cutthroat trout consumption by birds (Major et al. 2003) would suggest that the roughly 550 American white pelicans, 110 double-crested cormorants, and 20 great blue herons using the lake (McEneaney 2007) could potentially move more than 1×10^9 myxospores during a 100-d breeding season. The recent decline of Yellowstone cutthroat trout in Yellowstone Lake (Koel et al. 2005) has already affected nesting and foraging of avian piscivores in the area. Once dispersed, parasite establishment and proliferation depend on several other factors, including habitat suitability, tubificid abundance and susceptibility, temperature, and ultimately the viability of the myxospore (which we did not demonstrate for American white pelicans or double-crested cormorants).

Although we were unable to demonstrate viability of *M. cerebralis* myxospores after their passage through the GI tracts of American white pelicans and double-crested cormorants, this negative test should not be interpreted as proof of absence given our study design (one set of experiments with relatively few replications). It remains unclear what physiological differences may exist among digestive processes in American white pelicans, double-crested cormorants, and great blue herons that would lead to differences in *M. cerebralis* viability in feces. Future research should examine a potential benefit of American white pelican and double-crested cormorant consumption of *M. cerebralis*-infected trout. Verification would further emphasize the important role these species can play in maintaining a healthy, naturally functioning aquatic ecosystem.

Conclusions

In the case of *M. cerebralis*, much has been learned regarding the species' biology and distribution across the United States since 1956, when it was initially diagnosed in brook trout *S. fontinalis* at a fish research station in Pennsylvania (Hoffmann 1961; Bartholomew and Reno 2002; Gilbert and Granath 2003). In particular, recently developed molecular methods (Andree et al. 1998; Epp et al. 2002) have resulted in an

advanced understanding of *M. cerebralis* prevalence and severity and the spatial and temporal variation in these variables among trout species (Hedrick et al. 1999; Vincent 2002), tubificid strains (Kerans et al. 2004), and habitats (Kerans and Zale 2002). Many *M. cerebralis* introductions to waters across the Intermountain West have been traced to movement of fish or fish parts (Bartholomew and Reno 2002). Until now, however, there had been only speculation about other dispersal vectors of *M. cerebralis* myxospores (Meyers et al. 1970; Kent et al. 2001; Bartholomew et al. 2005), particularly by other fish or wildlife species that specifically prey on trout that are susceptible to whirling disease.

Before this study, it was uncertain how *M. cerebralis* dispersed to Yellowstone Lake (Koel et al. 2006). We have identified great blue herons as one likely mode of dispersal, which has implications for managing the current problem with whirling disease in Yellowstone Lake and potentially for other diseases that could arrive to the region in the future (e.g., viral hemorrhagic septicemia; Vitousek et al. 1997; Harvell et al. 2002). Even though the lands and waters of Yellowstone National Park are highly protected, additional introductions may be inevitable. The piscivorous birds we studied are all species of special conservation concern and are natural, ecologically significant components of the Greater Yellowstone Ecosystem. The fact that great blue herons disseminate *M. cerebralis* probably will not result in changes to the way they are viewed and managed within the park. However, because disturbance predisposes invasions (Hulme 2006) and because whirling disease risk is strongly influenced by stream biological integrity and anthropogenic perturbations (McGinnis 2007), we suggest that management practices that do not degrade aquatic habitats or encourage unnatural concentrations of fish and birds will be an important means of reducing the risk of disease transmissions within the Greater Yellowstone Ecosystem in the coming years.

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