

## Potential for Dissemination of the Nonnative Salmonid Parasite *Myxobolus cerebralis* in Alaska

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**Abstract.**—*Myxobolus cerebralis*, the myxozoan parasite responsible for whirling disease in salmonids, was first introduced into the United States in 1958 and has since spread across the country, causing severe declines in wild trout populations in the intermountain western United States. The recent detection of the parasite in Alaska is further evidence of the species' capability to invade and colonize new habitat. This study qualitatively assesses the risk of further spread and establishment of *M. cerebralis* in Alaska. We examine four potential routes of dissemination: human movement of fish, natural dispersal by salmonid predators and straying salmon, recreational activities, and commercial seafood processing. Potential for establishment was evaluated by examining water temperatures, spatial and temporal overlap of hosts, and the distribution and genetic composition of the oligochaete host, *Tubifex tubifex*. The most likely pathway of *M. cerebralis* transport in Alaska is human movement of fish by stocking. The extent of *M. cerebralis* infection in Alaskan salmonid populations is unknown, but if the parasite becomes dispersed, conditions are appropriate for establishment and propagation of the parasite life cycle in areas of south-central Alaska. The probability of further establishment is greatest in Ship Creek, where the abundance of susceptible *T. tubifex*, the presence of susceptible rainbow trout *Oncorhynchus mykiss*, and the proximity of this system to the known area of infection make conditions particularly suitable for spread of the parasite.

*Myxobolus cerebralis*, the myxozoan parasite that causes salmonid whirling disease, is exotic to North America and was first detected in the USA in 1958 (Hoffman 1962). It is now reported in 25 states (Bartholomew and Reno 2002; Vermont Department of Fish and Wildlife 2002; Stromberg 2006; Arsan et al. 2007a). Although the pathogen appears to have little impact on fish populations in the eastern states and coastal western states (Modin 1998), it has caused dramatic, rapid population declines in wild rainbow trout *Oncorhynchus mykiss* of the intermountain western United States, particularly Colorado and Montana (Nehring and Walker 1996; Vincent 1996). As salmonids are inextricably linked to the culture and economy of Alaska (Kenai River salmon runs alone generate annual revenues of US\$70 million: Glass et al. 2004), the potential impacts of *M. cerebralis* in the state could be catastrophic, both ecologically and economically.

The first *Myxobolus cerebralis* detection in Alaska

occurred in 2006 (Arsan et al. 2007a) during a study of rainbow trout from an Anchorage hatchery. The prevalence of *M. cerebralis* infection in the hatchery population was low, and the parasite was detected only by molecular methods, as clinical whirling disease was not evident. However, cultured salmonids in Alaska are not routinely monitored for the parasite, and there is limited monitoring of wild salmonids (USFWS 2006). Prior to this detection, the closest *M. cerebralis* enzootic area was the upper Columbia River basin (CRB) in northeastern Oregon, southeastern Washington, and Idaho. The parasite has also been reported in wild and cultured salmonids from the Sakhalin Islands off the east coast of Russia (Bogdanova 1960).

The potential impacts of *M. cerebralis*, in addition to its rapid spread and establishment across the globe, indicate the need to identify pathways of parasite dissemination and to recommend specific measures for halting further spread of the pathogen. This paper uses risk analysis to qualitatively assess the likelihood of future spread of *M. cerebralis* within Alaska and the potential for new introductions. The framework for this type of risk assessment (Bartholomew et al. 2005) was created for use in whirling disease assessment. We use risk analysis as a map (MacDiarmid 2001) for navigating through possible pathways leading to

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Received March 15, 2007; accepted January 25, 2008

Published online August 7, 2008

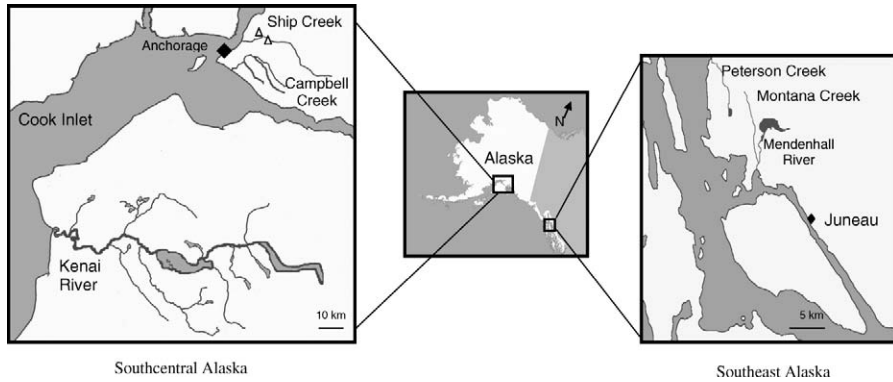


FIGURE 1.—Map of areas at highest risk for *Myxobolus cerebralis* dissemination in southeast and south-central Alaska, indicating sites of rainbow trout hatcheries (triangles).

parasite introduction and establishment and for assessing where to allocate resources to prevent such an occurrence. Our aim is to provide decision makers with tools to evaluate management implications and to eliminate low-probability pathways by using logical scientific arguments.

#### The Parasite Hazard

Tracking the epidemiology of parasites requires knowledge of an organism's life cycle, biophysical properties, and hosts. All of these topics have been reviewed (Bartholomew and Wilson 2002; Gilbert and Granath 2003); therefore, we will focus on those aspects as they pertain to the risk assessment. The life cycle of *M. cerebralis* requires two obligate hosts: a salmonid and the aquatic oligochaete, *Tubifex tubifex* (Wolf and Markiw 1984). In each host, the parasite maintains a unique spore stage. Myxospores develop in the fish host, are released upon the death of the fish, and are ingested by a *T. tubifex* as the worm burrows through sediment (Brinkhurst 1996). The parasite then undergoes reproduction and structural transformation and is released in its triactinomyxon (TAM) stage, which is infectious for the fish host.

The biophysical properties of *M. cerebralis* also affect its potential dissemination, and introduction most likely occurs via the myxospore stage. Myxospores are far more resilient than TAMs and are capable of withstanding environmental extremes (El-Matbouli and Hoffmann 1991) that might occur during transport and dissemination. Of the two obligate hosts of *M. cerebralis*, the fish host is more mobile; therefore, myxospores are more likely to be distributed over a broader area than are TAMs. Indeed, other researchers have speculated that myxozoan colonization on a landscape probably occurs via myxospores (Cone et al. 2006).

Host susceptibility affects both parasite dispersal and establishment. Most Alaskan salmonids except lake trout *Salvelinus namaycush* and arctic grayling *Thymallus arcticus* are susceptible to *M. cerebralis* (MacConnell and Vincent 2002). However, infections result in varying degrees of clinical disease, and rainbow trout generally exhibit the most severe signs of whirling disease (Hedrick et al. 1999a, 1999b; MacConnell and Vincent 2002).

Whereas many salmonid species are susceptible to *M. cerebralis*, only one species of oligochaete, *T. tubifex*, is capable of propagating the pathogen. Moreover, susceptibility of individual *T. tubifex* to *M. cerebralis* varies greatly and has been indirectly correlated with the *T. tubifex* 16S mitochondrial lineage (Beauchamp et al. 2001, 2005). There are at least six cryptic lineages of *T. tubifex*, five of which (I, III, IV, V, and VI) have been reported from North America (Beauchamp et al. 2001; Arsan et al. 2007b). Different *T. tubifex* lineages vary from highly susceptible to *M. cerebralis* (large numbers of TAMs are produced) to unsuitable for the pathogen (infection does not occur).

#### Risk Analysis

Because Alaska is approximately the same size as the continent of Europe (Pagano 2000), we narrowed the focus of this risk assessment to areas of the state where we considered the likelihood for introduction or further dissemination of *M. cerebralis* to be highest: southeast and south-central Alaska (Cook Inlet basin) (Figure 1). These areas have high concentrations of susceptible fish hosts, high angler traffic or a large commercial fishery, the highest concentration of human populations in the state, and high potential organic loading. The areas are also close to the road system and ports and are situated in the migration path of fish from enzootic areas. As a qualitative risk

TABLE 1.—Definitions of risk levels used in an analysis of the risk of further establishment of *Myxobolus cerebralis* in Alaska.

Risk level	Definition
High	The event would be expected to occur
Moderate	There is less than an even chance of the event occurring
Low	The event is unlikely to occur
Negligible	The chance of the event occurring is so small that in practical terms, it can be ignored

assessment, mathematical probabilities were not assigned to score risk. Thus, it is important that the terms are clearly defined; those used in this analysis (Table 1) are based on work by Moutou et al. (2001) and focus on the lower end of the scale to identify low-probability pathways or nonissues.

**Study Sites**

*Southeast Alaska*

Southeast Alaska has a maritime climate with cool winters and wet summers, and stream temperatures are generally warmer than those in the interior of the state. Typical hydrographs for southeast Alaska creeks are influenced by spring snowmelt and autumn rainfall (Milner et al. 1997). River basins of coastal southeast Alaska are generally small due to mountains and ice fields that rise sharply from sea level and create relatively short watersheds flowing into the Pacific Ocean. Two streams near Juneau, Peterson and Montana creeks, were selected for the risk analysis based on the criteria described above. The creeks support various salmonid populations, including steelhead (anadromous rainbow trout), pink salmon *O. gorbuscha*, chum salmon *O. keta*, coho salmon *O. kisutch*, Dolly Varden *Salvelinus malma*, and coastal cutthroat trout *O. clarkii* (Harding and Jones 1992; Chaloner et al. 2004). Detailed site information is provided by Arsan et al. (2007b).

*South-Central Alaska*

In south-central Alaska, the Cook Inlet is home to over half the state’s human population. The area has a transitional climate (National Climate Center 1982) and is the ecotone between the Pacific Northwest rainforest and the northern boreal forest. Hydrographs in the basin are highly predictable and influenced by snowmelt and glacier melt in the summer; typical freshwater inflow into Cook Inlet is 15 times higher in July than in February (Dorava and Milner 2000). This study focuses on Ship and Campbell creeks in the Anchorage area and the Kenai River on the Kenai Peninsula. All three streams host popular sport fisheries because of their abundant fish runs, proximity to major

population centers, and accessibility by roads. The streams also have numerous sources of potential organic loading due to their urban proximity, commercial and industrial activity, streambank degradation by recreational traffic, and large pulses of organic material from spawning salmon runs.

The Kenai River supports populations of rainbow trout, Chinook salmon *O. tshawytscha*, coho salmon, pink salmon, sockeye salmon *O. nerka*, and Dolly Varden and has the largest freshwater sport fishery in Alaska (Hammarstrom 1988). Ship and Campbell creeks support populations of these species as well as chum salmon (Miller and Bosch 2004). Ship Creek is the site of the most popular sport fishery in the Anchorage area and sustains the state’s only two rainbow trout hatcheries, Fort Richardson State Fish Hatchery (FTR) and Elmendorf State Fish Hatchery (ELM). Detections of *M. cerebralis* in Alaska were in rainbow trout from ELM (Arsan et al. 2007a). This hatchery uses untreated surface water from Ship Creek, and effluent from the hatchery flows back into the creek after passage through earthen settling ponds that contain populations of *T. tubifex* (Arsan et al. 2007b).

**Release Assessment**

The release assessment explores potential pathways of pathogen introduction and is focused on the parasite’s myxospore stage. Because *M. cerebralis* has been detected in south-central Alaska, the release assessment provides insight into the possible mode of introduction and the mostly likely route of further dissemination. Four main pathways of *M. cerebralis* introduction were identified: movement of fish by humans, natural dispersal, recreational activities, and commercial seafood processing (Figure 2). Management recommendations are discussed in the conclusions of this paper.

*Human Movement of Fish*

Since the mid-1970s, the state of Alaska has adopted strict laws prohibiting the import of live, nonornamental fish (Alaska Administrative Code [AAC] 5:41.070), thus reducing the likelihood of inadvertent pathogen introduction as a result of aquaculture or resource management programs. Although there is little data regarding the number and distribution of private fish ponds, instances of illegal salmonid importation from outside the state for private use are also likely to be low considering the general lack of accessibility and proximity to ports, legal complications, and climatic limitations for private fish pond operations in Alaska.

State regulations (AAC 5:41.005) also prohibit within-state transport, possession, or release of any live fish or fish eggs without a permit. The frequency

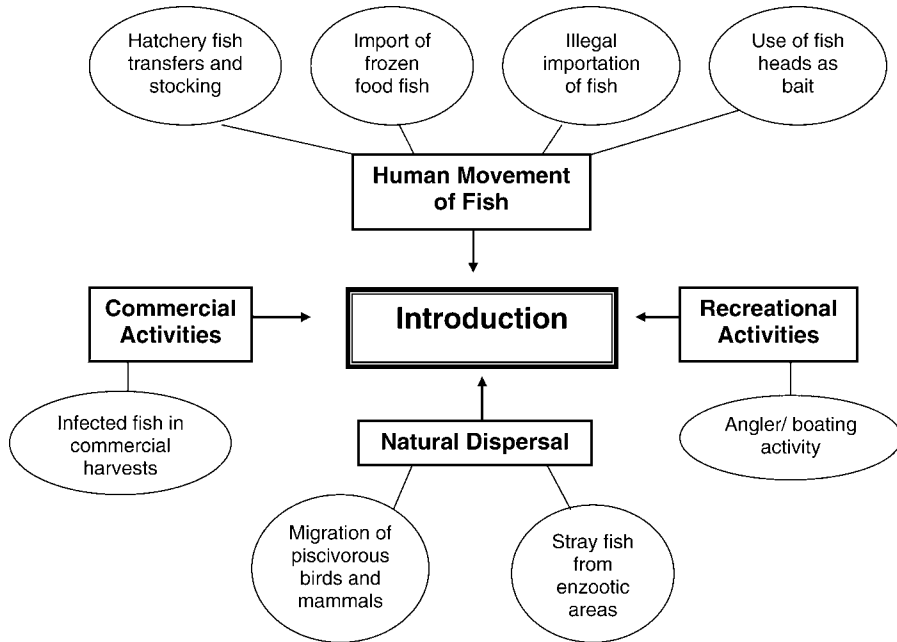


FIGURE 2.—Model of potential *Myxobolus cerebralis* introduction in Alaska. Four main pathways of dissemination (human movement of fish, commercial fishing activities, recreational activities, and natural dispersal) are shown, and specific activities are listed.

of unregulated movement of salmonids is difficult to estimate; however, illegal transfer (stocking) of fish within the state does occur, as evidenced by the presence of northern pike *Esox lucius* in the upper Cook Inlet basin and Kenai Peninsula (ADFG, no date).

Importation of frozen food fish is legal, and imported, frozen whole rainbow trout are common supermarket items in Alaska (T. Meyers, Alaska Department of Fish and Game [ADFG], personal communication). Importation of frozen fish is speculated to be the original pathway for introduction of *M. cerebralis* into the USA (Hoffman 1962), and at least one study demonstrated that *M. cerebralis* myxospores can survive freezing (El-Matbouli and Hoffmann 1991). However, a recent study on survival of myxospores in frozen fish heads found that the parasite is not viable after freezing for 1 week at  $-20^{\circ}\text{C}$  or  $-80^{\circ}\text{C}$  (R. Hedrick, University of California–Davis, personal communication). Although parasite viability between temperatures of  $0^{\circ}\text{C}$  to  $-20^{\circ}\text{C}$  is unknown, processors typically freeze seafood by rapidly chilling fish to  $-40^{\circ}\text{C}$  to prevent ice crystal damage in meat (Simply Seafood 2006). Frozen fish are then recommended to be stored at  $18^{\circ}\text{C}$  or below (U.S. Department of Commerce 2007).

Another potential pathway for within-state dissemination is the use of game fish (fresh or frozen) as bait. Heads, tails, fins, and viscera of legally caught game fish may be used as bait (AAC 5:75.026), but live fish may not. Fish heads, where parasite concentrations would be highest, could therefore be dispersed throughout the state. However, in Alaska, the use of fish heads as bait is more likely practiced in saltwater fisheries (sharks, Pacific halibut *Hippoglossus stenolepis*, etc.) than in freshwater fisheries.

#### Assessment of Risk from Human Movement of Fish

Although human movement of fish presents a low risk for new introductions of *M. cerebralis* to Alaska, it is the most likely pathway for parasite dissemination within the state. Prior to parasite detection, potentially infected fish were transplanted throughout south-central and interior Alaska. Thus, some degree of parasite dissemination may have already occurred. Many of these stocked locations were no-outlet lakes, which would limit the spread of the parasite, but some fish were also stocked in open stream systems. The estimated prevalence of infection in production rainbow trout from ELM in 2006 was 25% (Arsan et al. 2007a); therefore, among a stocked group of 10,000 fish, 2,500 fish potentially have some degree of

TABLE 2.—Summary of the risk of *Myxobolus cerebralis* introduction and dissemination within Alaska.

Pathway of parasite dissemination	Risk of introduction from outside Alaska	Risk of dissemination within Alaska	Risk of dissemination with limited stocking <sup>a</sup>
Human movement of fish	Low	High	Low–moderate
Commercial seafood processing	Negligible–low	Negligible–low <sup>b</sup>	Negligible–low <sup>b</sup>
Recreation	Low–moderate	Low–moderate <sup>b</sup>	Low–moderate <sup>b</sup>
Natural dispersal by predators	Negligible	Low <sup>b</sup>	Low <sup>b</sup>
Natural dispersal by stray salmonids	Negligible	Low <sup>b</sup>	Negligible <sup>c</sup>
Overall risk level	Low	High	Low–moderate

<sup>a</sup> Scenario in which (1) stocking is limited to no-outlet lakes where native susceptible resident salmonid species are absent or to seawater net-pens for terminal commercial and sport fisheries and (2) use of fish heads as bait is restricted to saltwater fisheries.

<sup>b</sup> This risk level is conditional upon infection prevalence and severity remaining low.

<sup>c</sup> This risk level is conditional upon the parasite’s failure to establish outside of the Elmendorf State Fish Hatchery, where *M. cerebralis* was first detected in Alaska.

infection. Typically, 2,000–10,000 fish are transplanted per site in south-central Alaska (ADFG 2006b). Thus, without management action, the probability of *M. cerebralis* being spread by human movement of fish is high. In contrast, if (1) stocking of ELM fish is limited to no-outlet lakes where susceptible native resident fish are absent or to seawater net-pens for terminal commercial and sport fisheries and (2) anglers are prohibited from using fish heads as bait in freshwater, the probability of further parasite dissemination by human movement of fish decreases (Table 2).

*Commercial Seafood Processing*

Fish from *M. cerebralis* enzootic areas of the upper CRB are regularly caught in commercial fisheries in Alaska (ADFG 2006a; Pacific States Marine Fisheries Commission 2006). *Myxobolus cerebralis* could potentially be introduced if effluent from seafood processors is discharged into freshwater or if fish solids released into marine waters are ingested by scavengers and dispersed inland (see Natural Dispersal by Predators section below).

Authorized seafood processors in Alaska individually discharge from 13,608 kg to over 4.5 million kg of waste solids annually, and shore-based fish processors are required to grind solid waste to 1.27 cm or less prior to discharge (USEPA, no date[a]). These smaller pieces, including cartilage in which myxospores would be concentrated, would be less attractive to the scavengers that could distribute the parasite throughout freshwater ecosystems. Although processors with National Pollutant Discharge Elimination System waivers are allowed to discharge into freshwater rivers, very few of these waivers are granted and none are held by processors in the Cook Inlet basin or in southeast Alaska (USEPA 2006).

Alternatively, solid seafood processing wastes (including fish heads) are taken to by-product reduction facilities for reduction to fish meal or other secondary

products (USEPA, no date[b]). All fish meal is brought to 100–600°C during processing (USEPA 1995); these temperatures would destroy *M. cerebralis* myxospores.

Seafood processors discharging less than 454 kg of seafood waste per day and less than 13,608 kg per calendar year are not required to have a discharge permit. This limit was imposed to allow subsistence and direct market processors (processors receiving seafood that requires minimal further processing) to discharge without a permit. These processors are not included in this risk assessment, as information regarding their effluent discharges is unavailable.

*Assessment of Risk from Commercial Seafood Processing*

The likelihood of introducing *M. cerebralis* into Alaskan freshwaters via processing of infected CRB salmonids by means of permitted commercial seafood practices is negligible. None of the permitted processing plants in the study area discharge into freshwater, and discharges into marine waters are made less attractive to scavengers by grinding effluent to a small size. Scavengers are also more likely to be attracted to tissue than to cartilage, which has the greatest parasite loads. Because of the data gaps regarding effluents from unpermitted processors, the risk of new introductions of *M. cerebralis* by seafood processing was designated as negligible to low. If parasite establishment in Alaska occurs beyond ELM and if anadromous species become infected, the risk of dissemination by unpermitted fish processors will increase.

*Dispersal via Recreation*

Alaska has a world-class sport fishery that attracts anglers from across the country and the globe; many of these anglers use equipment in Alaska that has been used in other river systems. River systems that are most likely to experience introduction of *M. cerebralis* via recreational activity are those with popular sport

fisheries. The Kenai River has the state's largest freshwater sport fishery (Hammarstrom 1988). Ship Creek hosts the most popular sport fishery in the Anchorage area (Miller and Bosch 2004), and Peterson Creek has the only recreational steelhead fishery on the Juneau road system (Harding and Jones 1992).

A recent survey of anglers in Montana (Gates et al. 2006) reported that 40% of anglers do not clean their equipment between uses. Thus, anglers could introduce *M. cerebralis* by inadvertently transporting the parasite on the soles of their waders. Though anecdotal data collected prior to the determination of the *M. cerebralis* life cycle (Schäuperclaus 1931; Hoffman and O'Grodnick 1977) suggest that myxospores remain viable after drying, recent studies challenge this. One study demonstrated that myxospores on a nonpermeable surface do not remain viable after drying for 24 h (Hedrick 2008). Another study demonstrated that although waders with removable felt soles could transport myxospores and TAMs, spores were less viable after the soles were dried separately for 8–24 h and infectious parasites were no longer transmitted after 7 d of drying (P. Reno, Oregon State University, personal communication). Because of their fragility, individual TAMs are less likely to be transported by this route, although infected *T. tubifex* that are adhered to a felt sole (as documented in the latter study) could provide a suitable environment for TAMs to remain viable. Additionally, the duration of drying required to disinfect waders will vary by environmental conditions and wader material.

The likelihood of *M. cerebralis* transfer by a single angler or within a single angling day may be low, but when all angler-days in a year are considered, the likelihood increases. In 2001, there were 421,000 anglers fishing in state waters; 239,000 of these anglers were not residents of Alaska (USFWS and U.S. Census Bureau 2001) and may have used gear from outside the state. The number of anglers in Alaska and the potential for parasite transport continues to grow, increasing 36% from 1991 to 2001.

Recreational activities can also indirectly influence the risk of *M. cerebralis* introduction by enhancing *T. tubifex* habitat. Recreational foot traffic (such as angling from the bank) can damage vegetation and increase streambank erosion, causing more sediment to enter surrounding waters. Erosion is further compounded by boat wakes (Liepitz 1994).

#### *Assessment of Risk from Recreation-Mediated Dispersal*

The likelihood of new *M. cerebralis* introductions by recreational activity can be conservatively estimated as low to moderate. The likelihood of within-state transfer

of the parasite is also low if prevalence and severity of infection remain low (Table 2) and if management actions are taken to limit stocking of infected sport fish. However, the cumulative and long-term effects of angler and recreational activities in heavily used areas could be much greater than the likelihood of introduction (or further spread) in the short term or by a single event.

#### *Natural Dispersal by Predators*

The ability of piscivorous birds to pass viable *M. cerebralis* myxospores has been examined in several studies (Taylor and Lott 1978; El-Matbouli and Hoffmann 1991). Because *M. cerebralis* survives passage through the guts of birds, long food retention times would lengthen the distance over which the pathogen could be dispersed. However, numerous events must align in order for parasite introduction to occur.

Alaska is a migratory destination for thousands of birds worldwide. Although the likelihood of a bird releasing viable myxospores over a water body remains unknown, deposition near water may be sufficient for transport of the parasite if spores are rapidly washed into the river by high water or precipitation. The period of viability for myxospores deposited in bird feces is unknown but probably varies with environmental conditions.

Since *M. cerebralis* manifests in cartilage of fish, it is likely that birds would regurgitate the parasite in pellets. Small fish are more likely to be swallowed whole and thus present the highest risk of *M. cerebralis* dispersal. Double-crested cormorants *Phalacrocorax auritus* have a simple gut structure and were shown to egest bones, pieces of fish, and solid markers 1–2 d after ingestion (Brugger 1993). In contrast, the passage time of rainbow trout through bald eagles *Haliaeetus leucocephalus*, which have a more complex gut morphology, is approximately 62 h (F. Barrows, U.S. Fish and Wildlife Service, personal communication). Bald eagles may also store food in the crop (a pouch in the esophagus) and digest the contents over several days (Buehler 2000). Spores could therefore be excreted 2–3 d after a bald eagle eats an infected fish.

Raptors and large waterbirds have some of the fastest known migration speeds among birds; bald eagles travel 201 km/d in migration (Kerlinger 1995), and ospreys *Pandion haliaetus* travel 108–431 km/d (Hake et al. 2001; Alerstam 2003). The nearest *M. cerebralis* enzootic area outside Alaska (upper CRB) is approximately 1,800 km from southeast Alaska and 2,750 km from south-central Alaska. An osprey would have to retain food material for 4.2–16.7 d to transport

spores to Juneau and 6.4–25.5 d to transport spores to Anchorage.

The risk of *M. cerebralis* dispersal by other fish-eating species, such as American black bears *Ursus americanus*, brown bears *U. arctos*, and river otters *Lutra canadensis*, is unknown. Only one study has examined parasite survival after passage through the guts of mammals (El-Matbouli et al. 2005). The pathogen did not survive passage through the guts of mice *Mus musculus*; however, spore viability after gut passage may differ between mice and larger mammals with more complex gut morphologies.

#### *Assessment of Risk from Dispersal by Predators*

The likelihood of new parasite introduction via bird transport from the CRB is negligible. The likelihood of within-state transfer by bird or mammal transport is low because of the low infection prevalence, the apparently limited establishment in the state, and the fact that numerous events must align for dissemination to occur. This risk could change if the prevalence or severity of *M. cerebralis* infection increases.

#### *Natural Dispersal by Stray Anadromous Salmon*

Anadromous salmonids may stray into nonnatal streams during their return migration to spawning grounds, thereby potentially introducing new pathogens. For example, introduction of *M. cerebralis* as a result of straying salmonids has been documented in the Deschutes River, Oregon (mid-CRB; Engelking 2002).

Though CRB fish are commonly harvested in commercial marine fisheries off the coast of Alaska, little data are available on salmon straying into freshwater systems of Alaska. There is only one such record in state and regional databases: in 2001, a Chinook salmon from Marion Forks Hatchery on the North Santiam River, Oregon (lower CRB; non-zootic for *M. cerebralis*), was recovered in the Copper River of south-central Alaska (ADFG 2006a; RMIS 2006). Because wild fish do not receive marks or tags that could be used to identify strays, no data (current or historical) are available on the straying rates of wild CRB salmon into Alaska, yet these fish are potential carriers of *M. cerebralis*.

#### *Assessment of Risk from Dispersal by Stray Salmon*

Based on the available data, the likelihood of *M. cerebralis* dispersal by straying anadromous hatchery salmon from the CRB is negligible. However, the limited data represent a gap in this risk analysis, and the presence of the parasite at ELM on Ship Creek could provide a local source of dissemination. It is unknown whether *M. cerebralis* is established in Ship

Creek; however, if naturally reproducing fish become infected, the potential for further parasite dispersal will increase. Since rainbow trout are typically resident and do not make long migrations (Morrow 1980), the spread of the parasite is likely to be local. Other susceptible anadromous fish from Ship Creek could disperse the parasite, but *M. cerebralis* has not been documented in anadromous fish in Alaska.

#### *Summary of the Release Assessment*

The likelihood of re-introduction and within-state dissemination of *M. cerebralis* in Alaska is summarized in Table 2. The most likely pathway for new introductions into the state is recreation, which is conservatively assessed as having a low to moderate risk due to the number of anglers in the state and the potential cumulative and long-term effects of angler and recreational activities in areas of heavy usage.

While the overall likelihood of new introductions is low, the likelihood of further transport within the state is high if no management actions are taken. The pathway with the greatest likelihood for parasite transfer is stocking of infected rainbow trout, as this would repeatedly introduce large numbers of potentially infected fish. However, if stocking is limited to no-outlet lakes that lack native susceptible resident salmonids or to seawater net-pens (i.e., for terminal commercial and sport fisheries), the likelihood of further spread of *M. cerebralis* from these sites would be low to moderate. Significant data gaps exist for dissemination by illegal stocking, nonpermitted fish processing operations, and use of fish heads as bait; the likelihood of further movement by these routes will increase if the parasite establishes outside ELM. The risk posed by using fish heads as bait could be reduced by prohibiting the practice in freshwater or prohibiting the use of fish from ELM-stocked areas. Because of the low prevalence of infection in the state, the likelihood of parasite transport by pathways other than human movement of fish is likely to remain low unless prevalence or severity of infection increases.

#### **Exposure Assessment**

The exposure assessment explores the risk of parasite establishment and focuses on the TAM stage of *M. cerebralis* and its oligochaete host. Establishment of *M. cerebralis* has already occurred in south-central Alaska at ELM on Ship Creek. Whether establishment has also occurred outside the hatchery remains unknown.

Establishment of *M. cerebralis* is dependent upon environmental and biological factors, including distribution and genetic composition of *T. tubifex* populations, water temperatures, and spatial temporal overlap

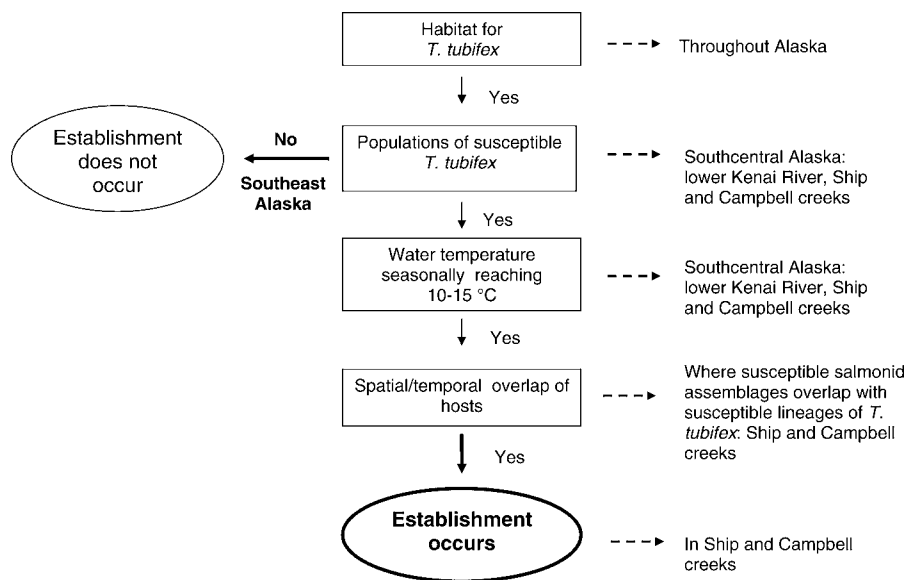


FIGURE 3.—Scenario tree depicting the risk of *Myxobolus cerebralis* establishment in southeast and south-central Alaska. Requirements for establishment (e.g., suitable habitat for the oligochaete host, *Tubifex tubifex*) and areas that meet each requirement are displayed.

of hosts. Each of these factors determines the outcome of the exposure assessment, as depicted by the scenario tree in Figure 3.

#### *Tubifex tubifex* Habitat and Populations

Habitat for *T. tubifex* is defined as areas with fine sediment, low flow, and organic matter (Brinkhurst 1996). Suitable habitat for *T. tubifex* was found throughout the study area.

A limited survey was conducted to ascertain relative abundance of *T. tubifex* in selected streams; details of the survey are described by Arsan et al. (2007b). Over 2,700 oligochaetes were collected from southeast Alaskan sample sites; however, morphological and genetic analysis demonstrated that none of the worms examined were *T. tubifex*. Inability to detect *T. tubifex* at these sites could have resulted from the limited sample size. Collections focused on areas of Alaska that we considered to have the highest likelihood for *M. cerebralis* introduction or further dissemination based on the risk assessment criteria. Areas that fit these criteria in southeast Alaska were few; thus, the number of sites sampled was low. In addition, the typical physical nature of streams in this region (short, low order, and steep) may limit availability of appropriate habitat. In contrast to southeast Alaska, *T. tubifex* were commonly found at south-central Alaska sites. Sites with the highest numbers (1,768 *T. tubifex* among 3,024 total worms) were in the lower Kenai River,

particularly Centennial and Eagle Rock boat landings; these areas have heavy recreational use and high sedimentation and organic loading (primarily from decaying salmon carcasses). *Tubifex tubifex* were also identified as occurring in Ship and Campbell creeks but were not found in the upper or middle Kenai River.

Oligochaetes gathered during the *T. tubifex* survey were held in water that was screened for *M. cerebralis* actinospores. No *M. cerebralis* TAMs were observed in samples from any of the surveyed sites. However, actinospores of several other myxozoans were detected (Arsan et al. 2007b), confirming that other myxozoan life cycles have established in both southeast and south-central Alaska.

#### *Tubifex tubifex* Susceptibility

*Tubifex tubifex* mitochondrial lineages I, III, IV, and VI were represented in the samples collected at our study sites. Parasite exposure experiments (described fully by Arsan et al. 2007b, and abbreviated here) demonstrated that three of these lineages (I, IV, and VI) did not support the *M. cerebralis* life cycle. Among the *T. tubifex* lineages detected in Alaska, lineage III was the only lineage that propagated the parasite. The presence of nonsusceptible lineages I, IV, and VI could translate to a reduced exposure risk for Alaskan salmonids in areas where these lineages dominate the *T. tubifex* populations. Lineage I predominated (71–86%) at sites on Ship and Campbell creeks, whereas



lineage VI predominated (69%) at sites on the lower Kenai River. Lineage III was present in low numbers (7–21%) throughout the Cook Inlet basin at seven of the nine sites where *T. tubifex* were collected; however, it was not detected at any of the hatchery sites.

Populations of lineage III from other geographic regions are highly susceptible to *M. cerebralis* (DuBey et al. 2005; Beauchamp et al. 2006; Arsan et al. 2007b), and detection of the lineage in Alaska is cause for concern. Though this lineage constituted approximately 14–21% of worms sampled in Ship and Campbell creeks and only 7% of those sampled in the lower Kenai River, lineage III can become infected with *M. cerebralis* and can release TAMs even when found in proportions as low 3% of the total population (Arsan et al. 2007b).

#### Seasonal Water Temperature

Water temperatures below 10°C retard spore formation (El-Matbouli et al. 1999; Baldwin et al. 2000) and could delay TAM development and release at high latitudes. However, *M. cerebralis* has been found to persist and to cause reduced juvenile rainbow trout recruitment even in cold, oligotrophic, sediment-poor, high-gradient streams (Allen and Bergersen 2002) and at elevations as high as 3,300 m in Colorado (Nehring and Thompson 2002). Temperatures in such areas are similar to, and perhaps slightly warmer than, those at our study sites in Alaska.

Streams in south-central Alaska are generally cool and typically exhibit a total of 1,780 degree-days annually (Oswood 1997). In comparison, the Madison River in Montana (enzootic for *M. cerebralis*) has roughly 2,650 degree-days annually (USGS 2005). The mean summer (June–August) water temperature in the lower Kenai River during 1999–2001 was 11°C (USGS 2005); the average continuous period in which temperature exceeded 10°C was 79 d/year, and 1,801 degree-days were accumulated annually. Campbell Creek had a mean summer temperature of 10°C during 2000–2001, and temperature exceeded 10°C for a period of only 50 d/year (USGS 2002).

*T. tubifex* lineage III from Alaska required 1,382–1,536 degree-days for *M. cerebralis* to develop (Arsan et al. 2007b). Therefore, water temperatures in south-central Alaska are sufficient for parasite development and propagation, although complete life cycle duration may be longer than that seen in warmer climates.

Future climate trends could also influence parasite development; water temperatures in the Cook Inlet basin are likely to increase 3°C between 2001 and 2011 (Kyle and Brabets 2001). If such an increase occurs, water temperatures in south-central Alaska will be similar to that of the Madison River.

#### Spatial and Temporal Overlap of Hosts

For the parasite to establish after introduction of myxospores, spatial overlap of parasite and host must occur twice: once between myxospores and *T. tubifex* and subsequently between salmonids and TAMs.

Varied patterns of seasonality have been associated with TAM release; releases of this stage occurred during the spring warming and fall cooling periods in Montana (Gilbert and Granath 2003), during fall through winter in high-altitude areas of Colorado (Nehring and Thompson 2002), and from summer to early fall in other areas of Colorado (Thompson and Nehring 2000; Allen and Bergersen 2002). Seasonality of TAM releases is believed to be related to water temperature and the availability of myxospores as influenced by fish stocking schedules. We speculate that high-latitude Alaskan water temperatures would be similar to (if slightly cooler than) high-altitude water temperatures in Colorado and that seasonal TAM release in Alaska would be comparable to TAM releases in these areas.

Breakdown of cartilage tissue and release of myxospores is likely to occur gradually in slow-moving or cold waters (Hallett and Bartholomew 2008). Once infected, *T. tubifex* can remain persistently infected throughout their life span (Gilbert and Granath 2001), and TAM release occurs when water temperatures are appropriate. Hatchery and wild salmonids in south-central Alaska hatch primarily during December–August (ADFG 2003; Quinn 2005), and fish would be most susceptible to infection during the first few weeks posthatch. In rainbow trout (and to a lesser extent, steelhead), the period of greatest susceptibility is from 0 to 9 weeks posthatch (up to 756 degree-days at 12°C; Ryce et al. 2004). However, rainbow trout hatched in June would exhibit resistance to the parasite by September. On this schedule, young rainbow trout may avoid peak TAM release from oligochaetes if peak release begins in September. Thus, it may be possible for the parasite to proliferate at cold temperatures and yet have little impact on rainbow trout populations (Kerans et al. 2005).

Salmonid species composition will also affect the outcome of introduction. Generally, areas with the highest *T. tubifex* abundance, like the lower Kenai River, will have a higher likelihood of spatial–temporal overlap between hosts and parasite and thus a higher likelihood of *M. cerebralis* establishment. However, the contribution of rainbow trout to the juvenile salmonid assemblage in this area is 1% or less (Bendock and Bingham 1988; King and Breakfield 1998, 2002), whereas Chinook salmon and sockeye salmon contribute the greatest percentages. Chinook

salmon are susceptible to *M. cerebralis*, but they acquire resistance to the parasite more quickly and are less susceptible than rainbow trout (MacConnell and Vincent 2002; Sollid et al. 2003). Sockeye salmon susceptibility to *M. cerebralis* is lower than that of rainbow trout or steelhead but greater than that of Chinook salmon (O'Grodnick 1979; Sollid et al. 2002). Thus, the likelihood of spatial overlap of highly susceptible hosts in this area is low. Chinook salmon and coho salmon are believed to make up over 80% of the juvenile salmonid assemblage in Ship Creek (D. Bosch, ADFG Sport Fish Division, personal communication), but the percentage of rainbow trout is unknown.

#### *Summary of the Exposure Assessment*

The probability of further establishment of *M. cerebralis* in south-central Alaska is variable among locations (Figure 3). Susceptible *T. tubifex* distribution, water temperature, and juvenile salmonid species composition would be determining factors for establishment. Thus, the probability of establishment in the upper Kenai River is low due to a lack of oligochaete hosts. The lower Kenai River maintains a high abundance of *T. tubifex*, but the low abundance of juvenile rainbow trout decreases the risk of *M. cerebralis* establishment there. Ship and Campbell creeks have appropriate environmental and biological conditions and remain the most likely areas of parasite establishment (Figure 3). However, although these creeks support populations of *T. tubifex*, abundance and susceptibility of the worms appear to be low. Susceptibility is limited to lineage III, which was in low abundance (7–21%) in the Cook Inlet basin (Arsan et al. 2007b). Thus, although conditions are permissible for *M. cerebralis* establishment in south-central Alaska, their suboptimality may suppress infection rates and prevent disease from becoming apparent. Changes to physical or environmental conditions, such as climate change, may alter the probability of parasite establishment in the state.

The likelihood of establishment in the surveyed areas of southeast Alaska is considered negligible. Drainages in this region have frequent flushing action that may prevent the invertebrate host from becoming significantly abundant, as was suggested by Modin (1998). In addition, no *T. tubifex* were detected in southeast Alaska; however, oligochaete surveys were limited (Arsan et al. 2007b).

### **Conclusions and Risk Management**

#### *Risk of New Introductions*

The probability of new introductions of *M. cerebralis* into Alaska is low, and the most likely pathway

is recreational and angler activity. Thus, areas that are most likely to first experience introduction are high-use sport fisheries, such as those of the Kenai River and Ship Creek. If a new introduction occurs, the probability of parasite establishment is moderate, particularly in systems like Ship Creek, which has permissive temperatures, a susceptible lineage of *T. tubifex*, and potential rainbow trout host populations.

Conditions in Ship Creek are permissive for *M. cerebralis* development but they are not optimal; thus, parasite development and establishment may be hindered. For example, water temperatures are acceptable for parasite development but are low enough to abate rapid proliferation. Susceptible *T. tubifex* are present in the creek, but the overall *T. tubifex* population consists primarily of nonsusceptible strains, again lowering the risk of rapid parasite proliferation. Lastly, fish species composition in south-central Alaskan creeks may also help to lower the risk of parasite establishment, as less-susceptible Chinook salmon tend to predominate in areas where susceptible *T. tubifex* were collected.

Policies that prevent importation of live salmonids into Alaska have been the most effective tool for limiting introduction. Existing regulations discourage establishment of private ponds, which are believed to contribute to spread of the parasite in areas of the contiguous USA (B. Nehring, Colorado Division of Wildlife, personal communication). We also recommend (1) maintenance of the state's policy requiring disposal of all seafood processor effluent into marine waters and (2) restriction of effluent waivers in areas considered to be high risk (Cook Inlet basin). Although the potential for introduction by anglers is moderate, the risks could be further reduced, especially in light of recent data on the vulnerability of myxospores to desiccation. To further reduce the angler-mediated introduction risk, we urge the state to allocate resources to angler education and further research on the effects of angler activity on dispersal of *M. cerebralis* (and other aquatic nuisance species). Education could be accomplished with signage at boat ramps, parking areas, or other access points; brochures distributed upon purchase of fishing licenses; and an informational web page recommending that anglers clean and thoroughly dry their gear before and after entering Alaskan waters.

#### *Risk of Further Dissemination within Alaska*

The probability of further transport of *M. cerebralis* within the state is high due to the presence of the parasite at ELM. The pathway presenting the greatest risk for within-state parasite transfer is human movement of fish. Because infection has only been

detected at a low prevalence in hatchery rainbow trout, the likelihood of parasite transport by other pathways is likely to remain low unless prevalence or severity of infection increases. Although the probability of *M. cerebralis* establishment in southeast Alaska is considered negligible due to the lack of suitable invertebrate hosts, only a few sites were surveyed. To gain more confidence in this assessment, additional sites should be surveyed for susceptible lineages of *T. tubifex*. In contrast, Ship and Campbell creeks in south-central Alaska have appropriate environmental and biological conditions and remain the most likely areas of parasite establishment.

It is unknown how long *M. cerebralis* has been present in Alaska, but previous monitoring using the pepsin–trypsin digest method as an initial screening test would probably have missed a low infection prevalence. Not only did all prior monitoring efforts in Alaska use pepsin–trypsin digest, but testing was nontargeted and did not focus on areas at risk for *M. cerebralis* introduction or on highly susceptible species. Molecular tests such as polymerase chain reaction are approximately 10-fold more sensitive than pepsin–trypsin digest (Andree et al. 2002) and could have detected the parasite. For management purposes, it may only be necessary to detect infections that cause negative impacts on fish populations or that are considered “significant” infections; both pepsin–trypsin digest and histology are adequate for such cases. However, if the criterion for a significant infection is presence of the parasite, then use of more sensitive assays is necessary.

Testing of sentinel rainbow trout fry in Ship Creek could determine whether the parasite has become established outside the hatchery; these methods have been used in similar situations after isolated parasite introduction (Bartholomew et al. 2007). Given the low prevalence and severity of infection in ELM fish, examination of other salmonid species or monitoring of Ship Creek water for TAMs would probably prove ineffective. Regular monitoring for *M. cerebralis* in cultured salmonids and regular testing of sentinel rainbow trout held in the hatchery inflow would provide baseline data for identifying changes in infection prevalence or severity. Similarly, monitoring of wild salmonids should focus on the most susceptible species and the areas of highest risk for parasite introduction and establishment.

Locations where potentially infected rainbow trout have been stocked should also be monitored by testing of sentinel or resident rainbow trout. To evaluate the likelihood of establishment in these areas, oligochaete populations should be surveyed for presence and lineage composition.

Furthermore, we recommend that the state prohibit the use of fish heads as bait in freshwater. Allotment of resources toward angler education would further benefit this action.

The risk of *M. cerebralis* dissemination in Alaska is not static and will vary with changes in environmental or physical conditions that affect parasite proliferation and development, such as climate change or land use modifications. The risk assessment should be as dynamic as the conditions it addresses, and this study provides a framework for re-evaluating the risk of *M. cerebralis* dispersal in Alaska.

### Acknowledgments

This work was supported in part by the U.S. Fish and Wildlife Service, the U.S. Geological Survey, and the Whirling Disease Foundation. We are grateful to the following members of the Center for Fish Disease Research at Oregon State University: Sascha Hallett and Stephen Atkinson provided critical consultation on oligochaete sampling, identification, and genetic assays; Don Stevens assisted with worm identification; and Harriet Lorz maintained oligochaete cultures. We also thank the following ADFG personnel: Tammy Burton provided live worm samples and field support; Tim McKinley assisted in the field; and Ted Meyers provided essential consultation on study design and background and contributed field assistance. We are thankful to Alaska Wildland Adventures for providing equipment and field support on the upper Kenai River.

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