

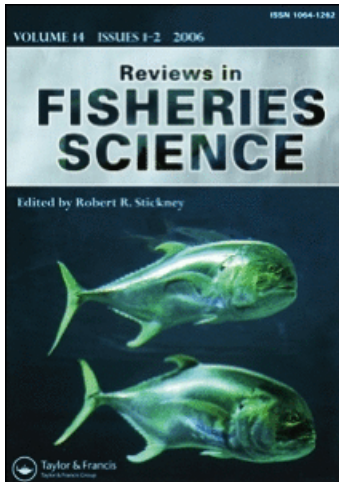
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E. Leyla Arsan ^a; Jerri L. Bartholomew ^b

^a Department of Fisheries and Wildlife, Center for Fish Disease Research, Oregon State University, Corvallis, Oregon, USA ^b Department of Microbiology, Center for Fish Disease Research, Oregon State University, Corvallis, Oregon, USA

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Potential Dispersal of the Non-Native Parasite *Myxobolus cerebralis* in the Willamette River Basin, Oregon: A Qualitative Analysis of Risk

E. LEYLA ARSAN^{1,*} and JERRI L. BARTHOLOMEW²

¹Department of Fisheries and Wildlife, Center for Fish Disease Research, Oregon State University, Corvallis, Oregon, USA

²Department of Microbiology, Center for Fish Disease Research, Oregon State University, Corvallis, Oregon, USA

Myxobolus cerebralis, the myxosporean parasite responsible for whirling disease in salmonids, was first detected in the United States in 1958. It has since spread across the country, causing severe declines in wild trout populations in the intermountain west. This study qualitatively assesses the risk of introduction and establishment of the pathogen into the Willamette River basin, Oregon, by examining potential routes of dissemination and relationships among obligate hosts, the parasite, and the environment. The approach is a synthesis of historical data, literature, and original research. The risk of *M. cerebralis* introduction in the Willamette River basin is addressed as a function of three main elements of dispersal: (1) movement of infected fish by humans, (2) natural dispersal (via migratory birds and stray anadromous salmonids), and (3) recreational activities. Establishment of the parasite is dependent upon several environmental and biological factors, including water temperatures, density, and spatial/temporal overlap of hosts, and the distribution and genetic composition of the oligochaete host, *Tubifex tubifex*. This study finds the probability of introduction of the parasite to vary throughout the Willamette River basin. Areas with greater probability have been identified as the Clackamas and Santiam River subbasins. If the pathogen were introduced, probability of establishment is high in certain areas of the basin as conditions are appropriate for propagation of the parasite lifecycle.

Keywords *Tubifex tubifex*, *Myxobolus cerebralis*, whirling disease, Willamette River, Oregon, stray salmonids, risk assessment

INTRODUCTION

Myxobolus cerebralis, a metazoan fish parasite exotic to North America, was first diagnosed in the USA in 1958 (Hoffman et al., 1962). Thought to have originated from a shipment of frozen infected brown trout (*Salmo trutta*) from Europe, it is now reported from 25 U.S. states and 26 different countries (Bartholomew and Reno, 2002; Vermont Department of Fish and Wildlife, 2002; Stromberg, 2006; Arsan et al., 2007a). The pathogen has been at the forefront of fish health research due to its potential impacts on rainbow trout culture and its implication

in rapid dramatic declines of wild trout populations in Colorado and Montana (Vincent, 1996; Nehring et al., 1998). The ecologic and economic impacts of *M. cerebralis*, in addition to its rapid spread and establishment across the globe, are indicative of the need for specific recommendations to assist fishery managers in halting the spread or effects of the pathogen. These recommendations can be developed for a specific area through the process of risk analysis (MacDiarmid, 2001). This study assesses the risk of introduction and establishment of *M. cerebralis* into the Willamette River basin (WRB), Oregon, a major tributary of the lower Columbia River.

The parasite is enzootic in portions of the upper Columbia River basin (CRB), and has not been demonstrated to cause loss to fish populations in other parts of Oregon. However, its detection in the WRB would effectively stop transport of fish from the area due to state fish health regulations (OAR 635-007-0995; Containment and Treatment of Fish Disease Agents)

*Current address: SWCA Environmental Consultants, Portland, Oregon, USA

Address correspondence to J. L. Bartholomew, Department of Microbiology, Oregon State University, Nash Hall 220, Corvallis, Oregon 97331. E-mail: bartholj@science.oregonstate.edu

and is thus of economic as well as ecologic importance. The framework for this risk assessment (Bartholomew et al., 2005) was created in efforts to tailor such assessments to whirling disease. This article includes a synthesis of historical data as well as new research to fill data gaps. Our aim is to provide decision-makers with tools to assess management implications, to eliminate non-issues by using logical scientific arguments, and to provide guidance on where to allocate resources to prevent introduction of the pathogen.

PARASITE LIFE CYCLE AND BIOPHYSICAL PROPERTIES

Management of parasitic infestations such as *M. cerebralis* are often focused on disruption of the parasite lifecycle, commonly by altering accessibility to one or more of the organism’s hosts. The lifecycle of *M. cerebralis* requires two obligate hosts: a salmonid and the oligochaete worm, *Tubifex tubifex*. There are also two distinct spore stages of the parasite (one particular to each host). The myxospore stage develops in the fish host and is released upon death and decomposition of the fish. These round and durable spores are ingested by *T. tubifex* as the worms burrow through sediment (Brinkhurst, 1996). The parasite then undergoes structural transformation and is released in its actinospore stage as a triactinomyxon (TAM). The TAM attaches to its fish host in the water column, where the parasite sporoplasm is injected through the epidermis of the fish and migrates via the nervous system to the cartilage (El-Matbouli et al., 1995). Each spore stage requires approximately three months to develop and is released from the respective host. The two distinct life stages and hosts of *M. cerebralis* also necessitate the need for a risk assessment model with two distinct segments that address each spore stage (and the respective host) separately.

The oligochaete host, *T. tubifex*, is a hearty and cosmopolitan species capable of withstanding extreme and variable environmental conditions. Such qualities allow the worm to inhabit areas where other species cannot compete, and thus to span across ecosystems as a widespread aquatic invertebrate. These qualities also have made control of this host impractical or unsuccessful (Wagner, 2002). Although *T. tubifex* is the only species of oligochaete capable of acting as a host for *M. cerebralis* (Wolf et al., 1986), its susceptibility to the parasite varies greatly and has been correlated to the specimen’s mitochondrial lineage (Beauchamp et al., 2002). Thus, the potential for *M. cerebralis* establishment depends not only on the abundance of *T. tubifex*, but also on the genetic composition of the population.

In contrast to the single species of oligochaete capable of acting as a definitive host, numerous salmonid species have varying degrees of susceptibility to *M. cerebralis*. Rainbow trout (*Oncorhynchus mykiss*) have repeatedly shown the highest susceptibility in laboratory and field experiments (Hedrick et al., 1999; MacConnell and Vincent, 2002). Other salmonids in Ore-

gon, except lake trout (*Salvelinus namaycush*), show varying degrees of susceptibility to the parasite (MacConnell and Vincent, 2002; Sollid et al., 2002).

The biophysical properties of *M. cerebralis* also affect its potential dissemination and management. Myxospores, which are more durable than TAMs, have a wide environmental tolerance (El-Matbouli and Hoffmann, 1991) and are more resilient to severe conditions associated with transport and dissemination. Myxospores are also more likely to be distributed over a broader area than TAMs since fish are the more mobile of the two obligate hosts of *M. cerebralis*. Indeed, other researchers have speculated that myxozoan colonization on a landscape would most likely occur via myxospores (Cone et al., 2006).

Study Area

Myxobolus cerebralis was first detected in Oregon in 1986 (Lorz et al., 1989) and is now enzootic in certain tributaries of the upper CRB (Figure 1). Since the 1980s, the parasite has been detected elsewhere in the state in stray adult salmonids originating from the enzootic area (Engelking, 2002), but there

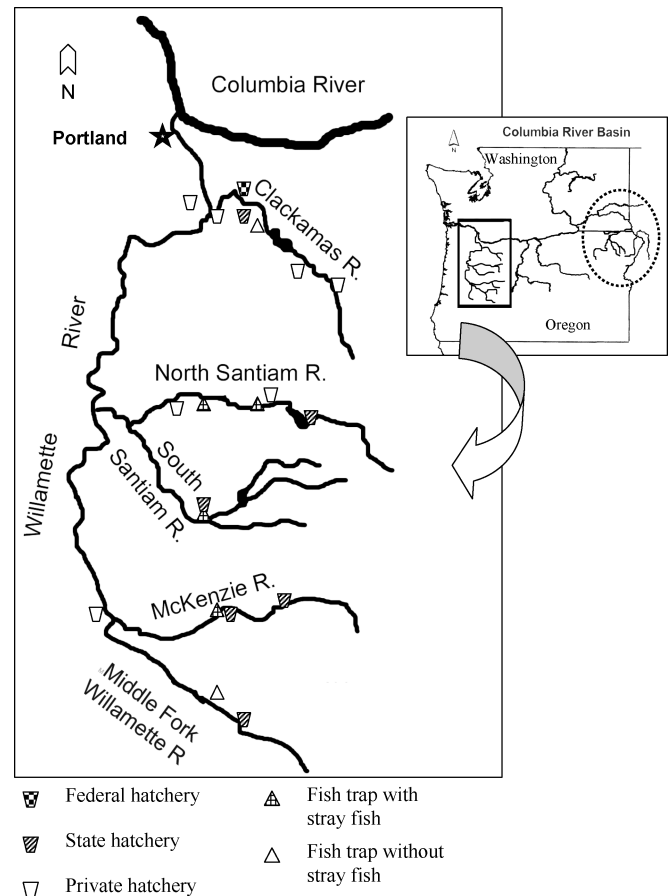


Figure 1 Columbia River basin with Willamette River basin enlarged and *Myxobolus cerebralis* enzootic area depicted by dashed circle. Locations of fish hatcheries and collection sites of adult stray salmonids in the Willamette River basin are noted.

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is no evidence of resulting parasite establishment until 2007 (Zelinski 2009). The parasite was detected in 2001 at a private facility in the lower WRB (Clear Creek). However, infection at the facility was contained (Bartholomew et al., 2007), and *M. cerebralis* is not known to be established in the WRB outside of that facility.

The Willamette River originates in the Cascade Mountains (elevation up to 3,048 m) and flows roughly 300 km to the lower Columbia River (elevation 3 m). The WRB houses 70% of the population of Oregon and is the fastest growing and most economically and agriculturally developed region in the state (USEPA, 2006a, 2006b). The river, which is the 13th largest in terms of discharge in the contiguous U.S. (Urich and Wentz, 1999), flows through Oregon's three largest cities and accounts for 15% of the total flow in the Columbia River (Payne and Baker, 2002). Stream flow reflects precipitation, with the majority (60–85%) of runoff occurring from October to March (Urich and Wentz, 1999). The basin contains the richest native fish fauna in the state (Rathert et al., 1999) and provides a migratory corridor and spawning grounds for a variety of anadromous salmonids.

RISK ASSESSMENT

This study concentrated on four areas of the WRB that we believed to have the highest risk for introduction and establishment of *M. cerebralis*: the Willamette River mainstem, Clackamas River subbasin, McKenzie River, and North and South Santiam Rivers (Figure 1). These areas have high concentrations of susceptible fish hosts, high angler traffic, hatcheries rearing susceptible fish species, private fish-rearing ponds, and potential for high organic loading leading to increased *T. tubifex* habitat.

Definitions of risk levels used in this assessment are high—the event would be expected to occur, moderate—less than an even chance of the event occurring, low—the event is unlikely to occur, and negligible—the chance of the event occurring is so small that it can be ignored.

Validation of Preliminary Assumption

To verify the preliminary assumption that *M. cerebralis* is not present in the WRB, we used data from 20 years of Oregon Department of Fish and Wildlife (ODFW) parasite testing, representing over 12,000 wild and cultured fish (N. Hurtado, ODFW, unpublished data). The pathogen has never been confirmed in fish from the WRB, except those from Clear Creek (2001–2003). The portion of the private facility on Clear Creek surface water was closed in March 2003 (Bartholomew et al., 2007), and the parasite has not been detected in the creek since May 2004, indicating that the parasite had not become widely established in Clear Creek and that closure of the hatchery removed the primary point source of infection for fish.

The United States Fish and Wildlife Service (USFWS) National Wild Fish Health Survey (NWFHS) also conducts limited

testing of wild salmonids in the WRB. The NWFHS reports results of *M. cerebralis* tests for 468 wild fish from 18 locations in the basin (USFWS, 2006); *M. cerebralis* was not detected.

Test results from both the ODFW and NWFHS support the assumption that *M. cerebralis* is not established in the WRB. However, the low sensitivity of parasite detection and confirmation methods (pepsin trypsin digest and histopathology) used before 2001, when sensitive genetic tests such as polymerase chain reaction (PCR) were incorporated into monitoring regimes, may have underestimated *M. cerebralis* presence.

RELEASE ASSESSMENT

The release assessment explores potential pathways of pathogen introduction and is focused on the myxospore stage of the parasite. Three main pathways for introduction of *M. cerebralis* into the WRB were identified: movement of fish by humans, natural dispersal, and recreational activities (Figure 2). Shipping and ballast water were not included in the release assessment, as commercial shipping occurs only in the lower portion of the mainstem Willamette River (Port of Portland, river kilometer 19). Additionally, most ballast water entering the WRB is marine and thus would not contain the parasite or appropriate hosts.

Human Movement of Fish

The historical spread of *M. cerebralis* in the U.S. is thought to be a consequence of shipments of infected fish, both commercially and as a result of state and federal stocking programs (Bartholomew and Reno, 2002). All import, export, or transfers of live fish in Oregon require a fish transport permit (Oregon Administrative Rules [OAR] 635-007-0600 and 635-007-0615) that entails an annual health examination and pathogen testing of both the broodstock and the lot of fish that are to be imported (OAR 635-007-0585 and 635-007-0990). All state (6), federal (1), and private (6) trout rearing facilities in the WRB are monitored annually for the parasite (Figure 1).

When buying live fish in Oregon, it is the buyer's responsibility to know the rules and regulations surrounding fish transfers and required permits. Buyers are often unaware of required permits (T. Amandi, ODFW, personal communication), which may result in illegal (even if inadvertent) movements of fish that cannot be traced. While the number of private ponds in the WRB that contain fish is unknown, transport records from a single private facility show that greater than 400 sites in the WRB received fish. Hobby ponds and U-catch facilities are examples of private ponds where fish may be reared and resold or transferred by owners who may be unaware of the need for fish transport permits.

Even legal transports of fish present a risk. For example, although establishment of *M. cerebralis* at the private facility on Clear Creek was contained, the facility legally transferred

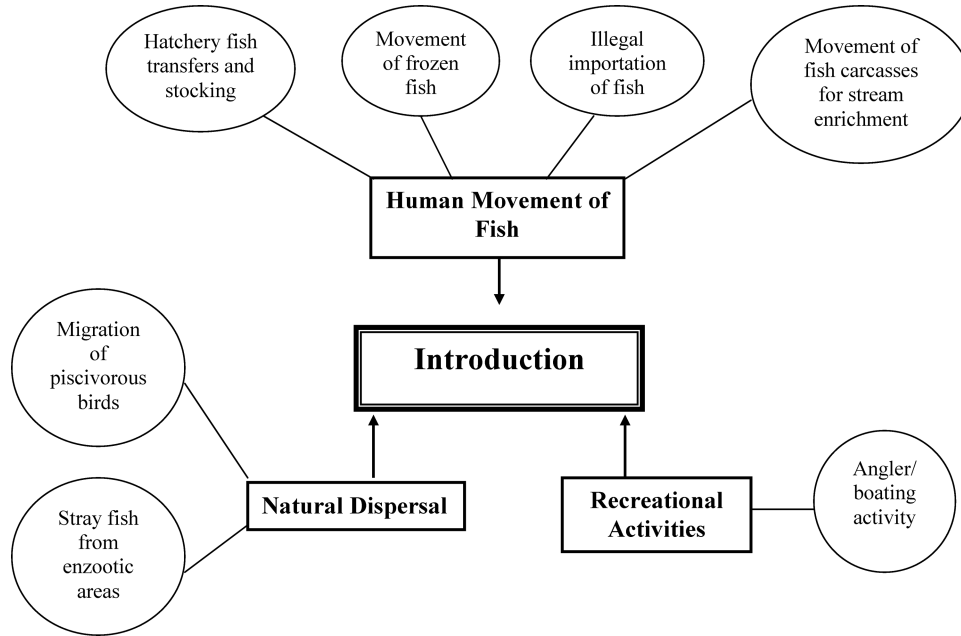


Figure 2 Pathways of potential introduction of *Myxobolus cerebralis* in the Willamette River basin.

potentially infected fish throughout the state of Oregon before detection of the parasite occurred. Over 760 shipments to 430 different locations in the WRB were received between 1999–2000 alone (N. Hurtado, ODFW, personal communication). Three of the facilities in the WRB that received the largest number of fish have been tested for establishment of *M. cerebralis* and were negative (authors’ unpublished data), suggesting that further spread from that facility may be limited. Additionally, some of the buyers that received shipments had recirculating facilities or no-outflow ponds that would limit the spread of the parasite into surrounding water bodies.

There are no laws governing the movement of frozen fish for bait, food, or fish meal markets in Oregon. Because myxospores can survive freezing (El-Matbouli and Hoffmann, 1991), importation of frozen fish still poses a risk for *M. cerebralis* introduction and is thought to be one of the primary routes of original introduction of *M. cerebralis* into the U.S. (Hoffman et al., 1962). However, disease resulting from this initial shipment of frozen fish was likely a consequence of the use of the imports as food for hatchery fish. Hatchery feeding practices have changed considerably since the 1950s, and the likelihood that infected fish would be used for this purpose is low.

It is unlawful to dispose of fish parts or carcasses in Oregon waters (Oregon Department of Fish and Wildlife, 2007). However, current state regulations do not prohibit the use of legally obtained dead salmonids or salmonid parts as bait, though live fish may not be used.

Movement of fish carcasses for stream enrichment is another potential route for introducing myxospores. Although this practice is “restricted to the originating river basin” (OAR 635-007-1000), fish of the same broodstock but from different rearing basins can be transferred among basins.

Assessment of Risk

Human movement of fish represents a high risk for parasite introduction for the following reasons:

1. The regulations pertaining to live fish transport are ambiguous and are not easily enforced or widely known;
2. Introduction (though locally contained) has already occurred;
3. Shipments of infected fish have already been made within the WRB;
4. Shipments of infected fish could introduce a large number of myxospores; and
5. Historically, many fish pathogens and non-native species introductions have been a result of human movement of organisms.

Dispersal via Recreation

Willamette River tributaries have sport fisheries that attract anglers from across the state and region, and many of these anglers use equipment that has been used in other river systems. Though myxospores are commonly regarded as able to withstand desiccation, data on myxospore viability after drying (Schäuperclaus, 1931; Hoffman and O’Grodnick, 1977) were collected before the determination of the *M. cerebralis* lifecycle and are anecdotal at best. In a recent laboratory study, waders with removable felt soles were demonstrated to transport myxospores and TAMs (Gates et al., 2007; P. Reno, Oregon State University, personal communication). However, spore viability decreased after drying soles for 8–24 hr, and, after drying

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for 7 days, spores were no longer able to cause infection in appropriate hosts. These findings support a recent study that demonstrated that myxospores do not remain viable after drying for 24 hr (Hedrick et al., 2008). Myxospores trapped in the moisture of a felt sole would likely remain wet and thus viable for longer than in a less absorbent material. Thus, time required to dry and disinfect waders will vary with environmental conditions. Because of their fragility, it is unlikely that TAM stages could be transported large distances by this route. However, infected *T. tubifex* adhered to a felt sole may provide a suitable environment for TAMs to remain viable.

While the probability of transporting *M. cerebralis* in a single event (i.e., a single angler or single angling day) by recreational activities may be low, when all angler days in a year are considered, the probability increases. The Middle Fork of the Willamette River and South Santiam River saw 22,501 and 17,937 anglers, respectively, in 1975 (Moring, 1985). These areas, along with the Clackamas River (the closest major tributary and sport fishery to Portland) would be most likely to experience introduction of the parasite via recreational activity. Though the number of anglers traveling between the enzootic area and the WRB is unknown, Portland has the greatest number of anglers in the state (U.S. Department of Interior, 2001) and thus an increased potential for parasite transport.

Boat bilge and engine cooling water represent another route by which recreational activities could introduce *M. cerebralis*. TAMs would be more likely than myxospores to be suspended in the water column and be retained in bilge and engine cooling water. Survival of TAMs is temperature dependent, with spores surviving for 5–7 days at 7°C and only 2 days at 20°C (Markiw, 1992b). Thus, TAMs are unlikely to survive multiple days of transport or high summer temperatures that would alter the condition of small amounts of bilge water. However, water caught in the covered intake of the engine cooling system does not experience elevated temperatures and has been shown to transport live larval zebra mussels (Johnson et al., 2001). While the volume of spores transported per boat is likely to be low, when considering all boats recreating in a year, the potential for *M. cerebralis* transfer increases. In 2002, 330 boats registered in the enzootic area were used in the WRB (Oregon State Marine Board, 2002); conversely, in 2005, 3,380 boats registered in the WRB were used in the enzootic area (Oregon State Marine Board, 2005).

Assessment of Risk

A conservative estimate of recreational activities as a pathway for transfer of *M. cerebralis* to the WRB is that it represents a moderate risk. Numbers of viable spores introduced in a single recreational event are expected to be low. However, laboratory studies have demonstrated that between one and ten triactinomyxons can cause infection in a susceptible fish (Hedrick et al., 1999) and that the infectious dose for worms is between one and five myxospores (Kerans and Zale, 2002). Thus, recreational ac-

tivities could introduce *M. cerebralis* stages infectious for either host, particularly in high-use areas or over time. Recreational activity is also one of the few pathways where management steps can be taken to reduce the risk of introduction.

Natural Dispersal: Birds

The possibility of *M. cerebralis* dissemination by birds has been considered in several studies (Taylor and Lott, 1978; El-Matbouli and Hoffmann, 1991). However, these studies did not consider the numerous events that must align for *M. cerebralis* to be spread via bird transport. Below, we outline the chain of events required for parasite introduction.

1. Bird Preys on Infected Fish. The CRB estuary houses the largest colony of Caspian terns (*Sterna caspia*) in North America and the second largest colony of double-crested cormorants (*Phalacrocorax auritus*)—11,223 and 7,501 birds, respectively, in 1998 (Collis et al., 2002). A smaller Caspian tern colony is located at Crescent Island, just below the confluence of the Snake and Columbia Rivers and near the enzootic area. Birds from these colonies eat juvenile salmonids as their primary food source, with steelhead smolts being the most vulnerable to predation (Collis et al., 2001). This species is also among the most susceptible to *M. cerebralis* (MacConnell and Vincent, 2002).

While prevalence of *M. cerebralis* in juvenile salmonids in the CRB is unknown, both the estuary and the enzootic area present a risk for pathogen dispersal. Though the proportion of infected fish is higher in the enzootic area, the estuary houses larger piscivorous bird colonies, and timing of seasonal dispersal from these colonies coincides with peak out-migration of susceptible salmonids. Numerous other piscivorous bird species also inhabit the CRB and may present risks for *M. cerebralis* dispersal.

2. Myxospores Survive Passage through Gut of Piscivorous Bird. *Myxobolus cerebralis* myxospores have been demonstrated to survive passage through the guts of piscivorous birds and fish (El-Matbouli and Hoffmann, 1991).

3. Bird Enters WRB. Banding data on the dispersal of birds from CRB breeding colonies are limited, thus flight paths mentioned here are speculative based on expert opinion (D. Roby, USGS-Oregon Cooperative Fish and Wildlife Research Unit, personal communication). Caspian terns at Crescent Island have been banded and resighted 1–2 days later in the Columbia River estuary. Although these birds do not nest in the WRB, they do forage there during migration, and terns from breeding colonies in eastern Washington may use this route post-nesting. Migration and seasonal movements of ospreys and sub-adult bald eagles could disperse both species from the enzootic area to the WRB.

4. Bird Retains Infected Fish in Gut for Flight to WRB. As *M. cerebralis* manifests in cartilage of fish, it is likely that birds would egest (regurgitate) the parasite in pellets. Double-crested cormorants have a simple gut structure and egest bones and pieces of fish 1–2 days following ingestion (Brugger, 1993). In

contrast, the passage time of rainbow trout through the guts of bald eagles (which have a more complex gut morphology) is roughly 62 hr (F. Barrows, USFWS, personal communication). Thus, spores could be excreted 2–3 days after eating an infected fish.

Raptors have some of the fastest known migration speeds among birds. Migrating bald eagles can travel 201 km/day (Kerlinger, 1995), and ospreys 108–431 km/days (Hake et al., 2001; Alerstam, 2003). As the nearest enzootic area is approximately 450–500 km from the WRB, an osprey would have to retain food material for 1–4.2 days and bald eagles for 2.2–2.5 days to transport spores to the WRB.

5. Bird Releases Viable Spores Over Water Body. The probability of such an occurrence is unknown, and is likely to vary by species. For example, waterbirds (waders, waterfowl, gulls, etc.) characteristically spend more time over water than raptors. It is unknown how long myxospores in bird feces or pellets would remain moist and thus viable if deposited on land. Hence, if spores are deposited near a water body and are rapidly washed into it by high water or precipitation, dissemination of the parasite could occur.

Assessment of Risk

Although the likelihood of parasite introduction by a single bird may be low, if the event were to occur, a considerable number of spores could be released because many piscivores swallow their prey whole. As many as 1.7×10^6 myxospores have been reported from experimentally challenged rainbow trout, though such numbers are variable (Markiw, 1992b; Sollid et al., 2002). Therefore, even a single introduction could have large consequences. Still, numerous events must align in order for birds to transport *M. cerebralis* long distances, and thus the probability of birds transporting the pathogen to the WRB is low. The cumulative risks when considering the large numbers of birds traveling through the WRB in a year would be higher.

Natural Dispersal: Stray Anadromous Salmonids

Though anadromous salmonids may stray into non-natal streams during their return migration to spawning grounds, the potential for such behavior to disseminate pathogens is often overlooked. Introduction of *M. cerebralis* as a result of straying salmonids has been documented in the Deschutes River, Oregon (mid CRB) (Engelking, 2002).

Stray fish are typically documented by examination of fin clips or evidence of a coded wire tag (CWT). As any single fin clip pattern is commonly used by more than one hatchery in the Pacific Northwest in a given year, fin clips reveal only the “stray” status of a fish and a rough list of potential origins. A CWT or fin clip unique to a certain hatchery is required for identification of exact hatchery of origin.

Little data (current or historical) are available on the stray rates of cultured or wild salmonids in the WRB. A search of

the Regional Mark Information System (RMIS) database shows only 16 recoveries of stray salmonids in the WRB between 1977–2003 (RMIS, 2006). Four of the hatcheries of origin of these fish, representing 81% of the historical strays, are within the *M. cerebralis* enzootic area.

As the frequency of adult salmonids straying into the WRB was identified as a significant data gap for this risk assessment, we conducted a study to investigate the frequency and prevalence of infection of straying salmonids in the WRB.

Methods for Investigation of Frequency and Infection of Stray Fish in the WRB

From 2004 to 2005, ODFW personnel at all adult salmonid collection facilities in the WRB (Figure 1) monitored returning adults for marks or tags that would indicate they were strays from other river basins. Traps were located on the McKenzie, Clackamas, Middle Fork Willamette, and North and South Santiam Rivers.

Collected fish were killed, checked for coded wire tags, the fins or maxillary marks recorded, and the heads removed and frozen for *M. cerebralis* testing. The RMIS and ODFW databases were queried to determine what hatcheries used marks corresponding to those of the stray fish. Additional efforts were made by the National Marine Fisheries Service (NMFS, Conservation Biology Division, DNA Laboratory, Seattle, WA) to genetically identify the strays using microsatellite markers.

To determine *M. cerebralis* infection in strays, core samples were taken from the cranium using an electric hand drill with a 2.5-cm diameter coring bit. Cores were frozen individually and processed by pepsin-trypsin digest (PTD) (USFWS and AFS-FHS, 2003). The defleshed cores were blended with a commercial Waring blender for 30 sec to accelerate digestion in pepsin solution; 0.05% trypsin in Rinaldini’s saline solution was used during the trypsin digestion phase. The final pellet of digest was suspended in 1 ml of phosphate-buffered saline or 70% ethanol and observed microscopically for myxospores by examining wet mounts of undiluted and diluted (1:5 and 1:10) digest at 250× and 400× magnification. The PTD product was tested by either a polymerase chain reaction (PCR) assay (in 2004) (Andree et al., 1998), or a quantitative PCR (in 2005) (Kelley et al., 2004).

Results and Discussion of Stray Fish Studies in the WRB

In contrast to the low number of reported stray salmonids from 1977–2003, 129 adult summer steelhead with fin clips identifying them as strays were recovered at traps in the WRB during 2004–2005. Table 1 lists their locations, numbers, and marks observed. The majority of strays were collected in the Santiam River sub-basin (97%), possibly because the area releases more steelhead than other WRB tributaries (ODFW, unpublished data). If strays follow the masses of fish migrating

Table 1 Summary of adult summer steelhead strays found at Oregon Department of Fish and Wildlife adult collection facilities in the Willamette River basin in 2004 and 2005

Observed marks*	Recovery river (no. fish)	Total collected	Percent of total collected	Potential origin
ADLM	Mckenzie (2)	2	1.5%	Seymour R, BC; Chehalis R, BC; Deschutes R; Umpqua R N Fk
ADRM	Mckenzie (2)	2	1.5%	Chehalis R, BC; Capilano R, BC; Hood R E Fk; Deschutes R
ADLV	S Santiam (36)	45	35%	Wallowa R; Umatilla R; Clearwater R; Grande Ronde R; Salmon R; Pahsimeroi R; Cowlitz R; Lewis R; Big Sheep Cr; Big Canyon Hatchery
	N Santiam [†] (6)			
	N Santiam M [‡] (3)			
ADRV	S Santiam (23)	27	21%	Columbia R; Wilson R; Cedar Cr; Deschutes R
	N Santiam [†] (4)			
ADLVRV	S Santiam (26)	45	35%	Unknown
	N Santiam [†] (1)			
	N Santiam M [‡] (18)			
Other marks/tags	S Santiam (8)	8	6%	Unknown
Total	N/A	129	100%	

*Fin clips: AD = adipose; LV = left ventral; RV = right ventral; LM = left maxillary; RM = right maxillary.

[†]NS = North Santiam River at Lower Bennett Trap.

[‡]NSM = North Santiam River at Minto Pond.

Bold letters connote facilities in the *Myxobolus cerebralis* enzootic area.

upriver, they would be more likely to arrive in the Santiam River sub-basin. Also, mis-marked fish (i.e., not strays) would be more probable in an area where more fish are clipped. No stray fish were obtained from traps on either the Clackamas or Middle Fork Willamette Rivers.

Fin marks recorded from WRB strays were used by a number of hatcheries releasing summer steelhead in the Pacific Northwest during 1999–2002 (RMIS, 2006; ODFW, Fish Liberation Database, unpublished data). Of the 19 facilities, seven (37%) are in the *M. cerebralis* enzootic area (Table 1). Interestingly, ADLVRV (adipose, left ventral, right ventral), one of the most prominent markings (31%), was not recorded in RMIS or any of the federal, state, or regional databases from Oregon or surrounding states. Efforts to genetically identify the WRB strays did not reveal a hatchery of origin (Melanie Paquin, NMFS, personal communication); however, the high level of genetic differentiation between the WRB strays and Snake River basin hatchery summer steelhead indicate that these fish likely originated outside the enzootic region.

Stray Chinook salmon were not encountered in this study, which could be explained by a decreased rate of straying compared with steelhead, or by the lack of distinctive marks. Chinook salmon rarely receive marks other than an adipose fin clip, and determination of hatchery of origin would require a CWT. However, Chinook salmon also represent a lower risk for *M. cerebralis* introduction because of their decreased susceptibility to the parasite (Sollid et al., 2003).

Overall, numbers of strays collected in the WRB in 2004–2005, while lower than those of mid CRB tributaries (ODFW, unpublished data), are far higher than indicated by database records. It is likely that this increase in number can, at least in part, be attributed to a concerted effort by ODFW to train personnel to recognize stray fish. It is also possible that the years 2004–2005 were abnormal. As there are few historical data, we cannot determine a “normal” stray rate per year. However,

stray rates of 0–20% are not uncommon for hatchery salmonids (Quinn, 1997). Actual numbers of strays in the WRB are likely to be higher than seen in this study as all stray wild fish as well as unmarked or single-marked (only adipose clip) hatchery fish were not recorded as out-of-basin fish. Another source of error in estimating straying frequency is that collections occurred only at fish traps; fish spawning in the river and tributaries were not counted. It is also possible that some “strays” were actually mis-marked fish from WRB hatcheries and were indeed not strays.

Spores of a *Myxobolus* spp. were detected in three fish. All samples were negative by PCR, suggesting that the spores detected were of another *Myxobolus* species.

Assessment of Risk

Although the straying frequency for steelhead was higher than expected, none of the strays had detectable *M. cerebralis* infection and therefore are not likely to have originated in the enzootic area. If we assume this stray rate and origin are representative of strays in other years, the likelihood of introducing *M. cerebralis* as a result of stray adult salmonids is low. However, if it were to occur, its probability is higher in the Santiam River sub-basin, as the majority of stray fish were recovered in this area.

Summary of the Release Assessment

The risk of introduction of *M. cerebralis* into the WRB is summarized as:

- Dispersal via human movement of fish: high
- Dispersal via recreation: moderate
- Dispersal via natural dispersal (birds and stray anadromous salmonids): low

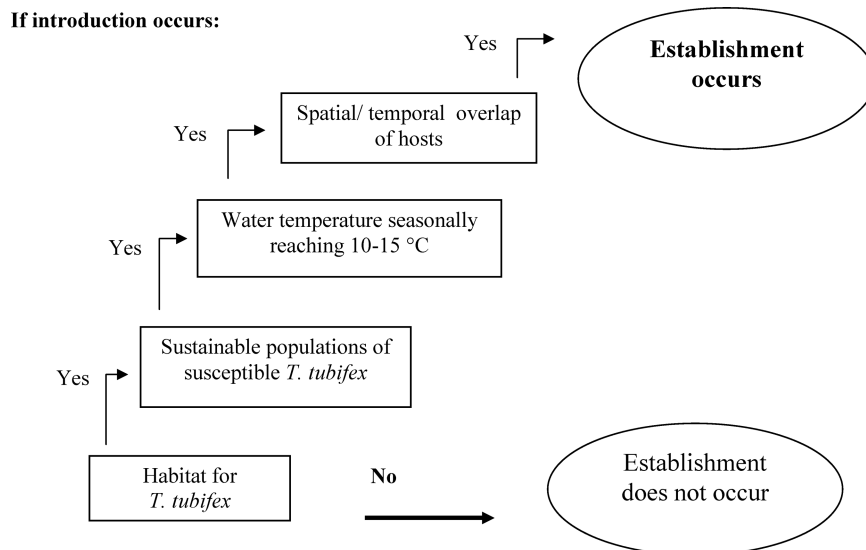


Figure 3 Scenario tree of the risk of establishment of *Myxobolus cerebralis* in the Willamette River basin.

- Overall risk of introduction: moderate (though long-term cumulative risk is higher)

Areas with the highest risk for *M. cerebralis* introduction are the Clackamas and Santiam River sub-basins. The Clackamas River has already experienced an introduction of the parasite, has the largest concentration of hatcheries (state, federal, and private), has a popular sport fishery, and is the closest major tributary to the enormous piscivorous bird populations in the Columbia River estuary. The Santiam sub-basin has a popular sport fishery, received the highest number of stray fish in the WRB, and has the second largest concentration of hatcheries in the WRB.

Exposure Assessment

The exposure assessment explores the risk of parasite establishment and is focused on the TAM stage of *M. cerebralis* and its oligochaete host. Establishment of *M. cerebralis* is dependent upon several environmental and biological factors (depicted by the scenario tree in Figure 3), including water temperatures, spatial temporal overlap of hosts, and the distribution and genetic composition of *T. tubifex* populations.

Habitat for *T. tubifex*

Tubifex tubifex is commonly associated with areas of fine sediment, low flow, and organic matter (Brinkhurst, 1996). These areas were found throughout the WRB.

Sustainable Populations of Susceptible *T. tubifex*

Bartholomew et al. (2007) documented only a single specimen of *T. tubifex* in a survey of Clear Creek, Clackamas River

sub-basin. In contrast, a private hatchery adjoining the creek had dense populations of the species. A literature review revealed little data regarding *T. tubifex* in the WRB. The prevalence and susceptibility of the organism were identified as significant data gaps, and a *T. tubifex* survey was conducted.

T. tubifex Survey Methods

A survey was used to determine relative abundance and mitochondrial lineages of *T. tubifex* in selected streams in the study area. Areas most likely to contain the organisms were targeted for sampling, i.e., those with low flow, fine sediments, and accumulations of organic material. Thirty-three sample sites with these attributes as well as with temperatures appropriate for parasite propagation, populations of susceptible salmonids, a large sport fishery, documented stray fish, and accessibility by road were chosen (Figure 4). Ten sites were located in the mainstem Willamette River, 12 in the South Santiam River, 4 in the North Santiam River, and 7 in the McKenzie River. Clear Creek in the Clackamas River system has previously been surveyed (Bartholomew et al., 2007), and no further collections were made in that system.

Oligochaetes in sediment were collected by a 19-L bucket and a 500- μm sieve or by a 500- μm kicknet, maintained, sorted, and presumptively characterized as *T. tubifex* following methods described in Arsan et al. (2007b). A subsample of 20 worms morphologically identified as *T. tubifex* were then genetically analyzed to confirm their identity (Hallett et al., 2005) and to determine lineage (Sturmbauer et al., 1999; Beauchamp et al., 2001). In preparation for genetic analysis, worms were digested with 95- μL ATL buffer (QIAGEN) and 5- μL proteinase K at 55°C, boiled for 5 min, and diluted 1:101 with buffer AE (QIAGEN) and then stored frozen.

Water in which samples were transported was retained, passed through a 20- μm mesh filter, and inspected

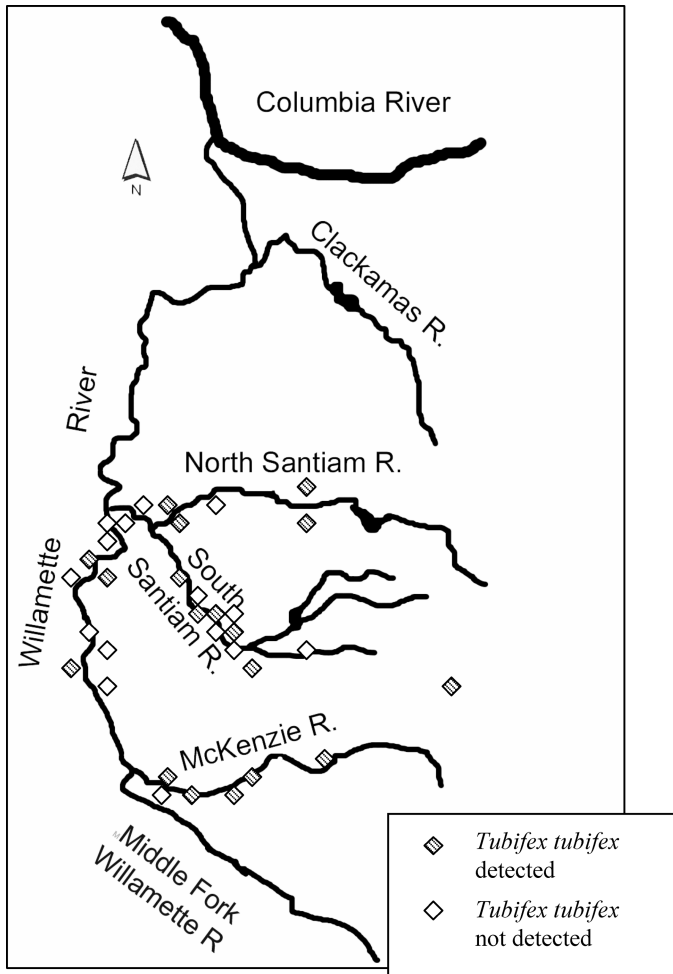


Figure 4 Locations of survey sites for *Tubifex tubifex* in the Willamette River basin.

microscopically to determine if actinospores were present. Actinospores were identified to group and species when possible.

Results of *T. tubifex* Survey

Over 5,300 oligochaetes were collected from 33 sites, with *T. tubifex* identified from 19 sites (Figure 4) and accounting for approximately 10% of the total oligochaetes collected (Table 2). Other oligochaetes morphologically identified from the WRB

include *T. ignotus*, *Limnodrilus hoffmeisteri*, *Lumbriculus variegatus*, *Kincaidiana hexatheca*, *Aulodrilus plurisetia*, *Dero digitata*, *Spirosperma nikolski*, *Ophidonais serpentina*, *Chaetogaster limnaei*, and *Pristonella jenkiniae*.

The largest numbers of *T. tubifex* were observed from the South Santiam and McKenzie Rivers, particularly at sites that receive heavy recreational use and had areas with deep sediments and organic enrichment due to both decaying salmonid carcasses and overhanging vegetation.

While this study presents some of the few data on WRB *T. tubifex* populations, our surveys were semi-quantitative and limited by access. Accessibility to sample sites on the North Santiam River was restricted to areas accessible by road. As only four of these sites had appropriate *T. tubifex* habitat, data from that river are limited.

Susceptibility of *T. tubifex* to *M. cerebralis* was inferred from results of the lineage analysis. Different lineages vary in susceptibility, with populations composed of lineages I and III susceptible to the parasite, and those of lineages IV, V, and VI considered resistant (Beauchamp et al., 2002; DuBey et al., 2005; Arsan et al., 2007b). All five *T. tubifex* lineages reported from North America were found in the WRB (Table 2), including lineage IV, which had previously been described only from Europe and Alaska (Sturmbauer et al., 1999; Arsan et al., 2007b).

The presence of resistant lineages IV, V, and VI *T. tubifex* could have significant beneficial implications for WRB salmonids in areas where these lineages dominate *Tubifex* populations. Together, these lineages comprised approximately 88% of the *T. tubifex* population in the mainstem Willamette River and 24–54% in river tributaries. Other researchers have found lineage susceptibility to correspond to severity of infection in fish (Beauchamp et al., 2005). This suggests that when lineages IV, V, and VI are in high abundance, the effects of *M. cerebralis* may be diminished if the parasite was introduced and established in the WRB.

Lineage III is the most susceptible to *M. cerebralis* (Beauchamp et al., 2002; DuBey et al., 2005; Arsan et al., 2007b), and its detection in the WRB is cause for concern. This lineage comprised approximately 12–56% of *T. tubifex* populations, with the greatest numbers in the North Santiam River and fewest in the mainstem of the Willamette River (Table 2).

Table 2 *Tubifex tubifex* survey sites and corresponding abundance and mitochondrial lineages

River	Sites sampled	Sites with <i>Tubifex tubifex</i>	No. total worms*	No. <i>Tubifex tubifex</i> †	<i>Tubifex tubifex</i> assayed	Lineages
Mainstem Willamette	10	3	414	8	27	III (12%), VI (88%)
North Santiam	4	2	462	53	45	I (20%), III (56%), IV (12%), V (8%), VI (4%)
South Santiam	12	8	2532	235	132	I (13%), III (45%), V (1%), VI (41%)
McKenzie	7	6	1939	242	126	I (3%), III (43%), IV (2%), VI (52%)
Total	33	19	5347	538	330	I, III, IV, V, VI

*Total worms; 3 subsamples of 30 ml each per sample processed.

†Total *Tubifex tubifex*; 3 subsamples of 30 ml each per sample processed. Number reflects total number morphologically identified as *T. tubifex* subtracted by the proportion of false positives (worms genetically identified as another species).

However, even in proportions as low 3% of the total population, lineage III *T. tubifex* become infected with *M. cerebralis* and release TAMs (Arsan et al., 2007b). When populations of *T. tubifex* from the North Santiam, McKenzie, and Middle Fork Willamette Rivers were experimentally exposed to the parasite, only lineage III became infected (S. Hallett, Center for Fish Disease Research, personal communication).

No *M. cerebralis* TAMs were observed in filtrates from any of the sites sampled. However, actinospores of other myxozoans were observed and were genetically identified as *Myxobilatus gasterostei* and other *Myxobolus* spp. (S. Atkinson, Center for Fish Disease Research, unpublished data).

Water Temperature Seasonally Reaching 10–15°C

Water temperature influences parasite development in both hosts (Hedrick and El-Matbouli, 2002). In the oligochaete, 10–15°C is optimal for development and release of the TAM stage (El-Matbouli et al., 1999). Temperatures below 10°C delay TAM development, and those above 20°C hinder parasite growth and can be lethal to the TAM stage. Highest prevalence of infection in fish occurs when average daily water temperatures are between 11°C and 14°C (Baldwin et al., 2000).

Average summer (May–September) water temperatures for WRB sites where data were available ranged from 11.5–16.5°C during 2001–2005 (USGS, 2006), with an average of 142–204 days/year continuously >10°C. In major tributaries, temperatures are within the optimum range for parasite development from roughly May to October. The mainstem Willamette River has higher water temperatures and could temporally support parasite development for over half the year. However, there are locations where temperatures may be too warm for TAM development and survival during some parts of the summer.

Spatial/Temporal Overlap of Hosts

For the parasite to establish after introduction of myxospores, obligatory spatial overlap of myxospores and *T. tubifex*, with subsequent spatial overlap of salmonids and TAMs, would be required.

If an introduction of myxospores occurred, the spores could remain viable in river sediments for at least five months (El-Matbouli and Hoffmann, 1991), with anecdotal evidence suggesting they may survive up to 30 years (Halliday, 1976). Furthermore, worms can remain persistently infected throughout their lifespan, which can be at least 3 years (Gilbert and Granath, 2001, 2003), with TAM release occurring when water temperatures are appropriate. However, the number of TAMs to which fish would be exposed would be diluted during the winter by seasonal high water flows typical of western Oregon streams. Thus, peak infections (in both fish and oligochaetes) would likely occur seasonally in response to temperature and water flows.

The majority of young salmonids in the WRB emerge April to October, coinciding with the period when temperatures are conducive for complete parasite lifecycle proliferation and when flows are lowest. Younger fish are more susceptible to the parasite, with rainbow trout obtaining some resistance at 9 weeks post-hatch (Ryce et al., 2004). Chinook salmon require less time to become resistant to the pathogen (0–3 weeks post-hatch) (Sollid et al., 2003). Thus, the progeny of late fall spawners that emerge once water temperatures have cooled and flows have increased could have a lower risk of infection of *M. cerebralis* by avoiding peak TAM release periods earlier in the season.

Summary of the Exposure Assessment

The risk of establishment of *M. cerebralis* in certain locations in the WRB is high, as all of the following exist: habitat for *T. tubifex*, populations of susceptible *T. tubifex*, seasonal water temperatures of 10–15°C, and spatial temporal overlap of hosts. If introduced, conditions are appropriate for the parasite lifecycle to proliferate. Tributaries to the mainstem Willamette River have the highest risk of establishment since these areas also have the greatest numbers of susceptible *T. tubifex*. However, the abundance of resistant strains of *T. tubifex* could mitigate the effects of *M. cerebralis* if introduced.

CONCLUSIONS AND RISK MANAGEMENT

Human movement of fish is the most likely route for introduction of *M. cerebralis* into the WRB. The ODFW has taken steps to decrease this risk by increasing the frequency of inspections of fish transferred from public and private facilities and by working cooperatively with fish sellers regarding fish transfer regulations and permit acquisition. However, although rules, regulations, and policies exist, they are not well known to the general public in regards to transfers of live salmonids. These could be articulated more clearly and made more prominent by emphasizing policies to the buyers of fish (mostly private pond owners) in the form of a brochure or website. State managers are concerned with the current fishing regulations allowing use of dead salmonids for bait and are considering further restrictions to limit pathogen spread. We suggest that these be implemented as soon as possible, with explanation of the reason for these changes. Additionally, efforts should be made to restrict carcass planting for stream enrichment to streams where fish were reared, regardless of broodstock. Alternatively, carcasses could be de-headed and frozen individually, with outplanting of the remaining carcass occurring after fish health testing has been conducted. This method is already in place at several hatcheries in the Pacific Northwest (S. Gutenberger, USFWS, personal communication).

Recreation and angler activity were evaluated as the second most likely route for introduction of *M. cerebralis* into the WRB. Management steps can be taken to reduce this risk of

introduction by allotting resources to angler and boater education. This could be done using a combination of brochures and signage at boat ramps and other river access areas, describing how to prevent spread of aquatic nuisance species.

The role of infected anadromous fish in disseminating *M. cerebralis* was difficult to evaluate because stray fish have not always been identified at fish traps, marks are commonly used by multiple hatcheries in the CRB, and databases within the CRB are not coordinated. While a designated clip used uniquely by enzootic-area hatcheries would allow more thorough investigations of stray fish and their potential to disperse pathogens, the practice may not be feasible. Marks are typically used to differentiate lots within a hatchery, not a geographic area. The use of a CWT would alleviate the need for a distinctive clip, but would require more time and resources. Results of this study also suggest that stray fish are not routinely identified, and thus historic stray rates in the WRB have been underestimated. To improve detection of strays, fish trap handlers should be instructed on how to identify fish with markings indicating they are from out of basin, and to remove these fish from the population.

In contrast to introduction, few steps can be taken to reduce the likelihood of establishment of *M. cerebralis*. Though measures for eliminating the oligochaete host are impractical or have proven unsuccessful (Wagner, 2002), management of land use to reduce sedimentation and organic enrichment, and to maintain cool water temperatures, would help limit habitat for *T. tubifex* and lower the future risk of establishment. The presence of resistant lineages of *T. tubifex* may help to diminish negative effects of *M. cerebralis* in areas where these strains dominate, such as the mainstem of the Willamette River. However, data on the distribution of *T. tubifex* are limited, and surveys in this study were narrow in scope. Additional survey work would more clearly delineate areas of high risk.

Similarly, little can be done once parasite establishment has occurred, as there are no vaccines or effective treatments against *M. cerebralis*. This makes the management of potential parasite introduction more imperative. Steps to control the pathogen should start by focusing resources on areas where risk of introduction and establishment are greatest: the Santiam River sub-basin. This sub-basin has the second largest concentration of hatcheries (state and private) in the WRB, a popular sport fishery, close proximity to the enormous piscivorous bird populations in the Columbia River estuary, the highest number of documented stray fish in the WRB, and the greatest numbers of susceptible *T. tubifex*. The risk of introduction of the parasite into the Clackamas River sub-basin is also high. This sub-basin has already experienced an introduction, has the largest concentration of hatcheries in the WRB, and the closest proximity to piscivorous bird populations in the Columbia River estuary. Yet, the risk of parasite establishment in this system appears lower (limited oligochaete surveys in the sub-basin indicate few *T. tubifex* inhabit the tributary where introduction previously occurred (Bartholomew et al., 2007)).

It must also be considered that risk levels associated with parasite introduction and establishment may vary with changes

to physical or environmental conditions, such as climate change or land-use modifications. Land-use practices that enhance *T. tubifex* habitat by increasing sedimentation or organic enrichment, or by decreasing flow, may increase the risk of *M. cerebralis* establishment.

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