3.2.5 Whirling Disease of Salmonids

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A. Name of Disease and Etiological Agent

Whirling disease is a chronic inflammatory disease in salmonid fish caused by the myxozoan parasite *Myxobolus cerebralis* and is characterized primarily by the tight circular movements caused by spinal cord constriction and brain stem compression in infected fish (Rose et al. 2000).

B. Known Geographical Range and Host Species of the Disease

1. Geographical Range

The parasite, *Myxobolus cerebralis* originated in Europe and is now exotic on four other continents: Asia (Russia), Africa (South Africa, Morocco), North America and Oceania (New Zealand). Since the first detection of the whirling disease parasite in the United States, it has been confirmed in hatcheries, ponds or streams in 25 states. By 2009, the parasite was detected in Alaska, Arizona, California, Colorado, Connecticut, Idaho, Maryland, Massachusetts, Michigan, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New York, Ohio, Oregon, Pennsylvania, New Mexico, Utah, Vermont, Virginia, Washington, West Virginia, and Wyoming. Although the parasite has been detected in 25 states, *M. cerebralis* is not necessarily established or widespread in each state. In some states, the parasite has been detected in most major river systems. In other states, the parasite was confirmed in a single hatchery or pond. For example, the parasite's presence in Alaska was confirmed only by the detection and sequencing of *M. cerebralis* DNA; spores were not observed (Arsan et al. 2007). *Myxobolus cerebralis* may be present without causing disease, and detection of the parasite does not necessarily imply fish have or will develop clinical whirling disease.

2. Host Species

Salmonid responses to *M. cerebralis* infection vary among genera, species, strains, and individuals. Exposure to a high parasite dose may result in high prevalence and severity of disease, and high mortality rates in susceptible fish species (recently summarized by Steinback Elwell et al. 2009 and MacConnell and Vincent 2002). Highly susceptible species include rainbow trout *Oncorhynchus mykiss*, cutthroat trout *O. clarkii*, and sockeye salmon *O. nerka*. However, some species of the genus *Oncorhynchus*, including coho *O. kisutch*, chum *O. keta*, and

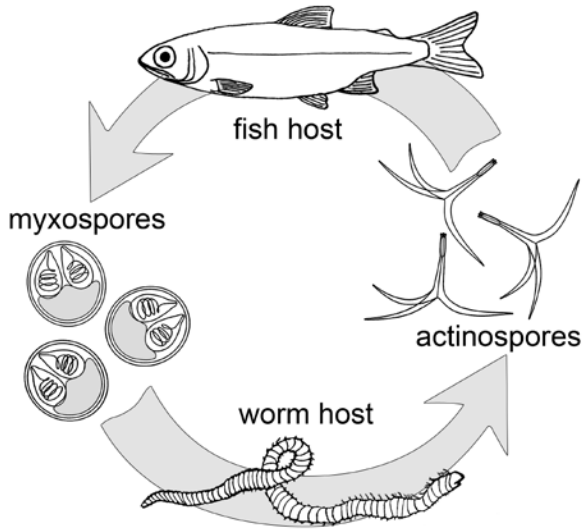
pink salmon *O. gorbuscha*, do not exhibit clinical signs of whirling disease regardless of dose and develop fewer myxospores than rainbow or cutthroat trout at similar exposure levels. Species in the genus *Salmo*, such as brown trout *S. trutta* and Atlantic salmon *S. salar*, can become infected; however, clinical signs are limited and fewer myxospores develop than in other trout species when exposed under similar conditions. Mountain whitefish *Prosopium williamsoni* are susceptible to infection and fry experience high rates of mortality following exposure to high numbers of the parasite. Species of the of the genus *Salvelinus* exhibit variable responses to *M. cerebralis:* brook trout *S. fontinalis* may develop moderate clinical signs of whirling disease when subjected to high doses of the parasite, bull trout *S. confluentus* have shown moderate infection and variable disease signs, and lake trout *S. namaycush* exhibit strong resistance to whirling disease and are unlikely to develop myxospores. Arctic grayling *Thymallus arcticus*, are resistant to infection and do not develop myxospores.

The development and severity of whirling disease depend significantly on the age and size of the salmonid host when exposed to the parasite. Very young fish are most vulnerable to *M. cerebralis*, and susceptibility decreases with age and growth as bone replaces cartilage in the developing fish (Hoffman and Byrne 1974; Halliday 1976, O'Grodnick 1979; Markiw 1991, 1992; El-Matbouli et al. 1992; Ryce et al. 2004, 2005).

C. Epizootiology

The life cycle of *M. cerebralis* is temperature dependent and involves two hosts: salmonid fish and aquatic oligochaete, *Tubifex tubifex* (Figure 1). The cycle in fish and worm is greatly extended in colder water temperatures. Waterborne triactinomyxons are released from *T. tubifex* as early as 74 days after ingesting the myxospore (Hallett and Bartholomew 2012). Triactinomyxons are viable for 6-15 days at water temperatures between 7° and 15°C, during which time they attach to and invade the skin of the fish.

Susceptibility to *M. cerebralis* varies with fish age, size, parasite dose, and environmental factors. Salmonids can become infected at any age from 2 days post hatch (Markiw, 1991), but younger fish are most vulnerable to infection and most prone to disease before cartilage ossification. Resistance is not associated with the level of skeletal ossification but rather other age and size related factors, such as the stage of development of the central nervous system (Ryce *et al*., 2005). Clinical signs of whirling disease may be evident 3 to 8 weeks after infection. Myxospore burden is not proportional to disease severity and both decrease with host age; survivors of long-term infections may only exhibit skeletal deformities (MacConnell and Vincent, 2002). Asymptomatic carriers of the parasite are common and can act as reservoirs for the parasite.



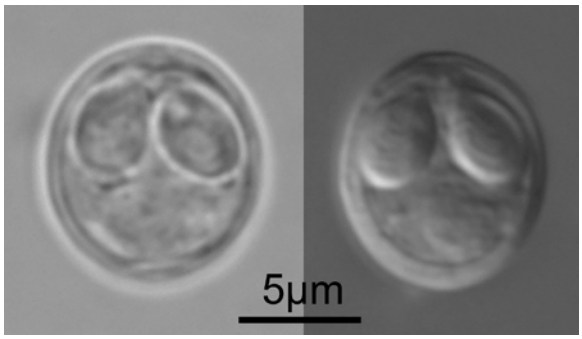


Figure 1. A: life cycle of *Myxobolus cerebralis* showing salmonid and tubificid hosts, with alternating myxospore and triactinomyxon stages. B: bright field (L) and Nomarski interference contrast microscopy of myxospore. Graphics: Stephen Atkinson, Oregon State University.

D. Disease Signs

1. **Behavioral Signs**
   Tail-chasing or "whirling" behavior, for which whirling disease is named, results from spinal cord constriction and brain stem compression (Rose et al. 2000). Heavily infected fish cannot maintain equilibrium and swim erratically, often until exhausted.

2. **Gross Signs**
   Clinical disease signs include blacktail and subtle to striking skeletal deformities (Figure 2). A blackened tail is caused by pressure on nerves that control pigmentation. Permanent deformities of the head, spine, and operculum are caused by cartilage damage, associated inflammation, and interference with normal bone development (Hedrick et al. 1999a, MacConnell and Vincent 2002). Commonly observed deformities include shortened operculum, indented skull, crooked spine and shortened nose. During the active phase of infection growth rates may be depressed.

Figure 2. Severe black tail (darkened pigmentation) and skeletal deformities and in rainbow trout naturally infected with *Myxobolus cerebralis*. Photos: S. Hallett and J. Bartholomew.

3. Microscopic Signs

When parasite developmental stages reach their final tissue destination, trophozoites digest the cartilage as they multiply and mature into myxospores. Tissue sections show a range of microscopic signs from discrete foci of parasites and cartilage degeneration (Figure 3) to widespread cartilage necrosis, numerous parasites, granulomatous inflammation, and involvement of surrounding tissues (Figure 4a, b). In adult fish, spores are found in isolated pockets in bone and are rarely associated with inflammatory lesions (Figure 5).

The principal location of parasitic lesions varies among salmonid species. Lesions in more-resistant brown trout are smaller than in highly susceptible rainbow trout, contain fewer parasite stages and have fewer associated leukocytes but more multinucleated giant cells (Baldwin et al. 2000).

E. Disease Diagnostic Procedures

Diagnosis of *M. cerebralis* depends upon the detection and identification of prespore or sporogonic stages of the parasite. The myxospores are often asymmetrical, broadly oval in frontal view, broadly lenticular in side view with length 8.7 µm, width 8.2 µm and thickness 6.3 µm (Lom and Hoffman, 1971). Two hard valve cells encapsulate a binucleate sporoplasm and two polar capsules which each house a solid extrudible polar filament with five to six coils (Figure 5). Trophozoites are multinucleate and much larger than spores (Figure 6).

Spore extraction procedures involve processing fish heads and may result in the observation of other *Myxobolus* spp., including unnamed species, found in cranial tissues and of similar morphology. *M. squamalis* found in scales is similar in size to *M. cerebralis* but has two distinctive ridges on either side of the suture. Several species are found in the central nervous system of salmonids: *M. kisutchi,* is similar in size to *M. cerebralis; M. neurobius* is oval in shape and larger (13.4 to 14 µm long); and *M. arcticus* is pyriform and large (14.3 x 16.5 µm). See Figure 5.2 under Section 2, 5.2 *M. cerebralis* (Whirling Disease). Methods for measuring myxosporean spores are described by Hoffman (1999).

Detailed procedures for screening and confirmatory tests are provided in Section 2, 5.2.A "Screening Test."

1. Sampling Criteria

The greatest numbers of spores are found in young fish five months after parasite exposure (when water temperature is >10°C) and spore numbers then decline throughout the life of the fish. Optimal samples for the detection of *M. cerebralis* are collected from fingerlings or yearlings of the most susceptible species. Spore development is temperature dependent. A minimum of 90 days is required at 12 to 13°C and up to 11 months at 0 to 7°C for sporogenesis to occur.

Entire heads are removed from fingerlings and yearlings. A wedge or core sample is removed from fish too large to sample the entire head (see Section 2, 2.2.E.4 "Collection of Specimens for the Detection of *Myxobolus cerebralis* evaluation"). Processing cranial cartilage may not yield spores from all *M. cerebralis*-infected salmonids. Negative results may be obtained from bull trout and mountain whitefish cranial cartilage when cartilage from the caudal peduncle contains *M. cerebralis* spores.

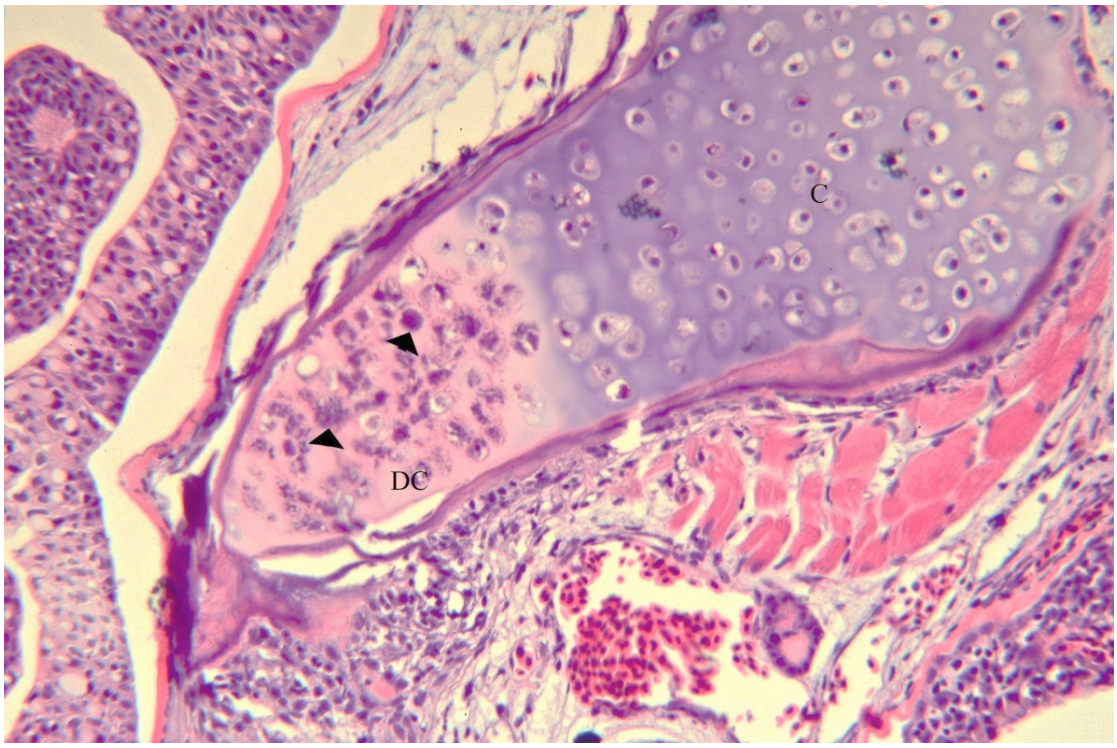


Figure 3. Focal lesion in a rainbow trout showing *Myxobolus cerebralis* trophozoites (arrowheads), degenerate cartilage (DC) and normal cartilage (C) in hematoxylin and eosin (H&E) stained section. Photo: E. MacConnell.

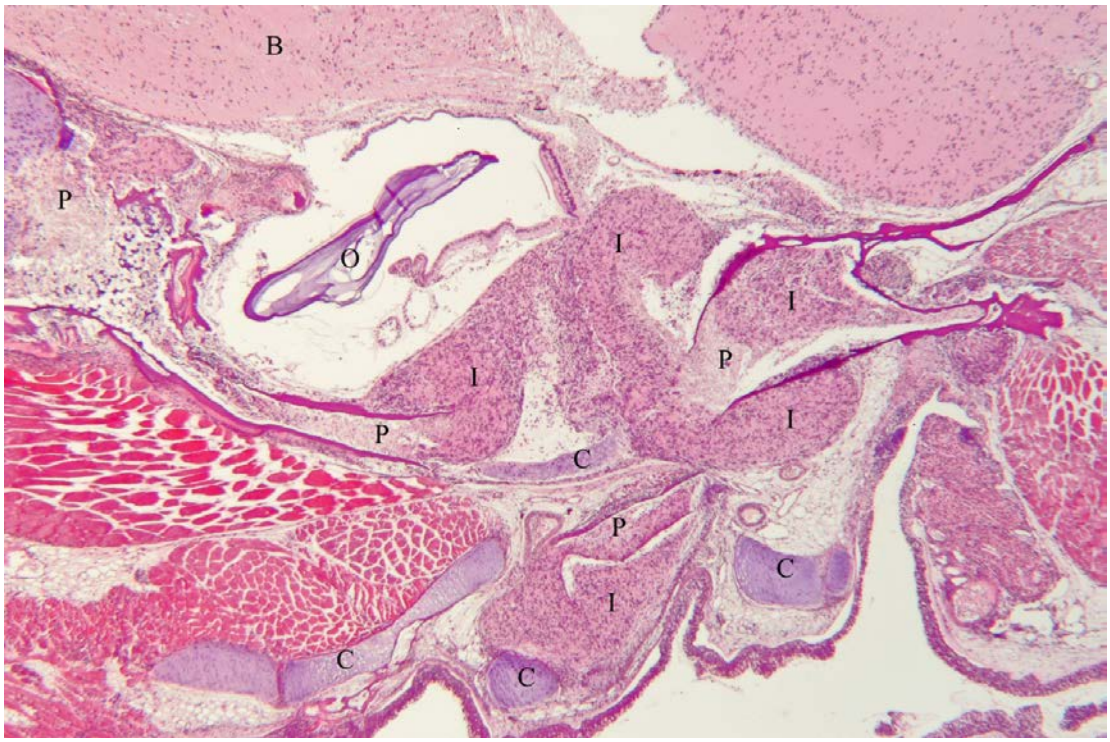


Figure 4a. Widespread parasite (P) infection in upper jaw and cranial cartilage (C) with extensive granulomatous inflammation (I) and involvement of surrounding tissues in head of rainbow trout. Brain (B) and otolith (O). H&E stain. Photo: E. MacConnell.

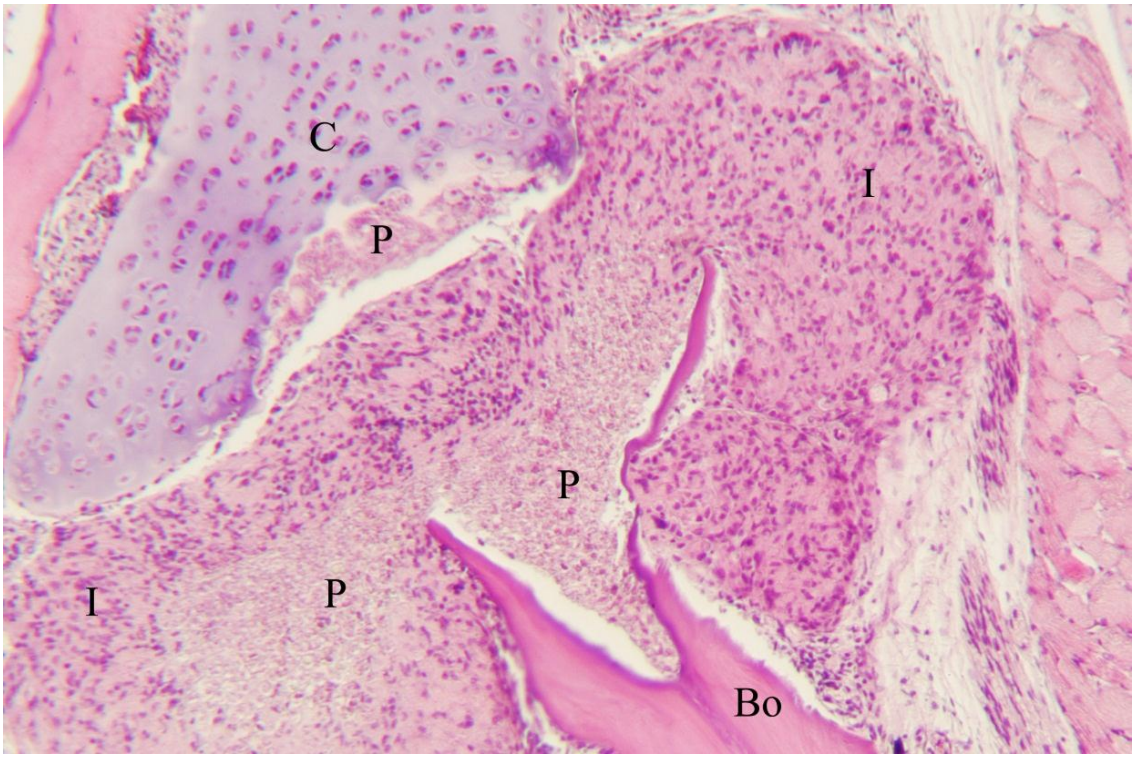


Figure 4b. Higher magnification of cranial lesion showing numerous parasites (P), granulomatous inflammation (I), displacement of bone (Bo) and normal cartilage (C). H&E stain. Photo: E. MacConnell.

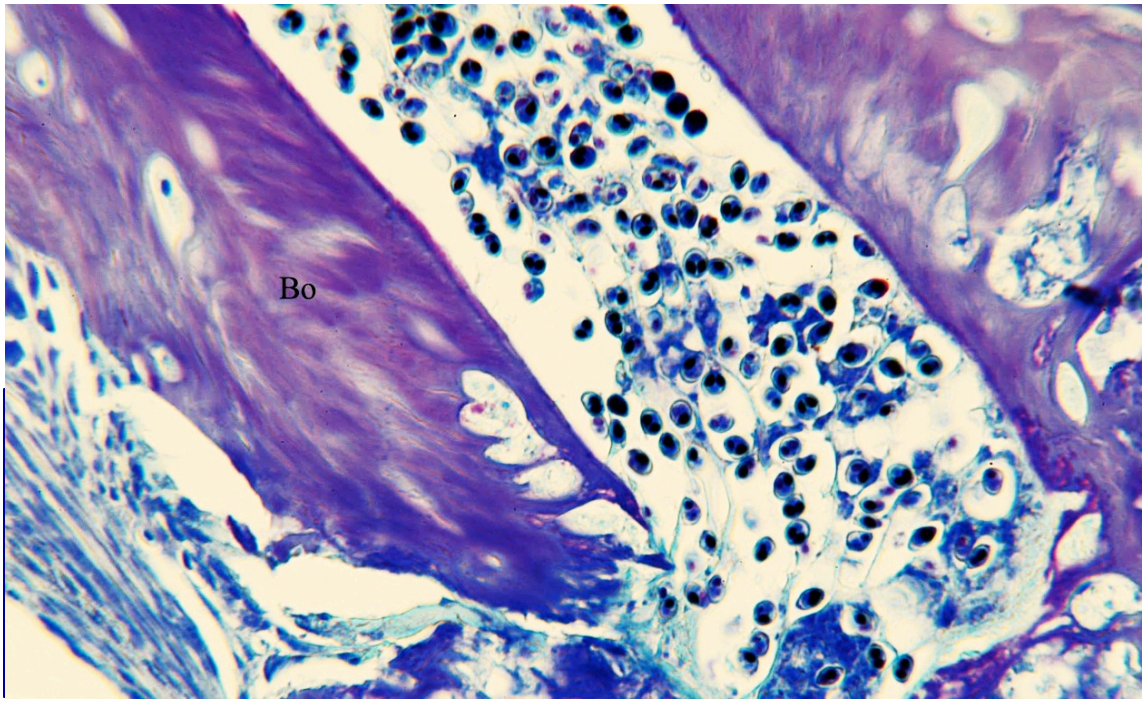


Figure 5. Numerous *Myxobolus cerebralis* spores surrounded by bone (Bo) in Giemsa stained tissue section. Inset: spherical spores with pyriform polar capsules. Photo: E. MacConnell.

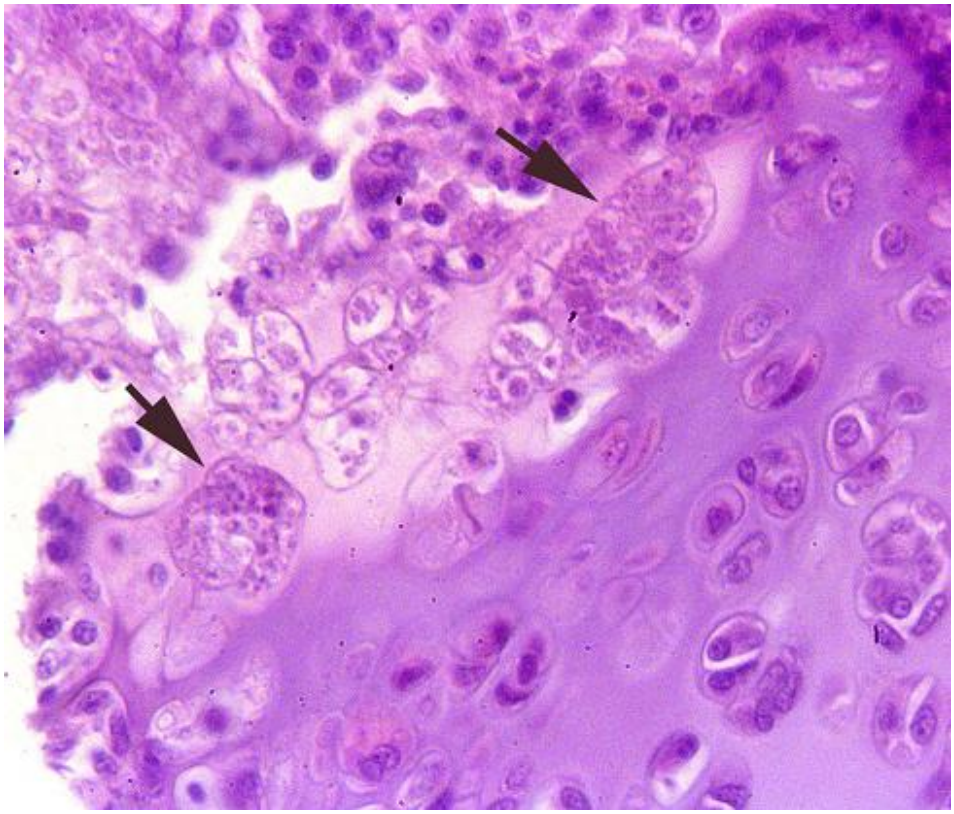


Figure 6. Trophozoite stages (arrows) of *Myxobolus cerebralis* in cartilage (H&E). Photo: E. MacConnell.

2. Presumptive Diagnosis

a. Clinical Signs
Including blackened tail, whirling behavior, and skeletal deformities. Commonly observed deformities include shortened operculum, indented skull, crooked spine and shortened nose.

b. Spore Extraction
Pepsin-trypsin digest (PTD) (Markiw and Wolf 1974a,b). Refer to Section 2, 5.2.A "Screening Test" for specific assay procedures.

Note: Spore extraction methods will not detect presporogonic stages of the parasite. Fish (e.g., rainbow trout) exposed at young ages to *M. cerebralis* may develop clinical signs of whirling disease prior to the development of spores.

3. Confirmatory Diagnosis

a. Histological Confirmation
Confirmation of *M. cerebralis* requires the observation of presporogonic and spore parasite stages located in cartilage tissue surrounded by bone (Figure 5). Mature spores are unstained in hematoxylin and eosin (H&E) stained tissue sections. Polar capsules of the spore stain darkly and are clearly visible in Giemsa or methylene blue stained sections. Refer to Section 2, 5.2.B.1 "Histopathology" for specific procedures.

b. Polymerase Chain Reaction (Andree et al. 1998)
Confirmation of *M. cerebralis* is based on the amplification of parasite DNA using the nested polymerase chain reaction (PCR) assay. Refer to Section 2, 5.2.B.2 "PCR" for specific nested PCR assay procedures.

F. Procedures for Detecting Subclinical Infections

1. Pepsin-Trypsin Digest (PTD) (Markiw and Wolf 1974a,b)
Refer to Section 2, 5.2.A "Screening Test" for specific assay procedures.

2. Polymerase Chain Reaction (PCR) (Andree et al. 1998; Schisler et al. 2001)
The highly sensitive PCR assays should be used to detect early or light infections.

G. Additional Procedures for Detecting *Myxobolus cerebralis* (not for Inspection Purposes)

1. Plankton Centrifuge Method (O'Grodnick. 1975).
This method is useful for concentration and quantification of myxospores. Only the myxospore stage of the parasite is detected and it may be difficult to distinguish between similar-appearing myxozoan species.

a. Deflesh heads and/or whole fish. Process remaining cartilage in 20 g batches.

b. Homogenize each batch of cartilage in 175 to 200 mL of water for 3 to 5 minutes in a high-speed blender. Blend the sample completely.

c. Strain blended sample through a new or disinfected urinary calculi filter. Rinse any remaining sample with water and save all washings. Disinfect and discard tissues.

d. Add the entire filtrate to a separatory funnel located directly above the plankton centrifuge (026WA106 plankton centrifuge, Kahl Scientific Instrument Corp., PO Box 1166, El Cajon, CA 92022, or equivalent). Turn on the plankton centrifuge and adjust the separatory funnel to a slow and steady flow into the plankton centrifuge cup.

e. Run the centrifuge until the separatory funnel has been completely emptied including three rinses with water.

f. Scrape the sides of the plankton centrifuge cup with a rubber spatula to liberate spores. Rinse the spatula. Transfer the sample to labeled tubes. Samples can be stored in a solution of 10% neutral buffered formalin until examination.

g. Shake the sample well. Place a drop of the suspension: 1) under coverslip on both sides of hemocytometer and search grids for *M. cerebralis* spores; or 2) on microscope slide, add a cover glass and systematically search for spores. If the suspension is thick, a drop of water may be added.

2. *In situ* Hybridization (Antonio et al. 1998)

Digoxigenin labeled primers, specific for *M. cerebralis*, bind to the parasite and are visualized in tissue sections using standard immunohistochemical procedures (Figure 7). By detecting parasite DNA, this assay identifies all stages of the parasite in either host. Because it is more expensive and time intensive than other methods it is primarily a research tool. For this assay, tissues should be fixed in neutral buffered formalin without decalcification. Refer to Antonio et al. (1998) for the full procedure.

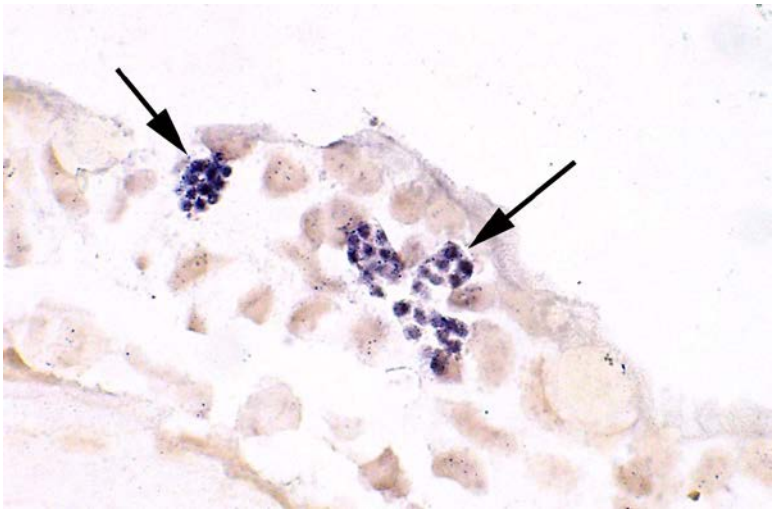


Figure 7. Sporoplasm cells of *M.yxobolus cerebralis* in the epidermis of rainbow trout visualized by in situ hybridization (ISH) using labeled probes to the parasite 18S rRNA gene. Arrows indicate aggregates of the sporoplasm cells 2 h following waterborne exposures to the triactinomyxon stages of the parasite (photo courtesy R. P. Hedrick and D. Baxa, University of California, Davis).

3. Polymerase Chain Reaction (PCR) Single Round Assay (Baldwin and Mykleburst 2002; Schisler et al. 2001)

One set of primers can be used in a single round of amplification to detect *M. cerebralis* DNA. The PCR assay described and tested in Colorado by Schisler et al. (2001) utilizes a primer set that has a slight sequence variation from Andree et al. (1998) in a single round format. Refer to Section 2, 5.2.B.2 “PCR” for PCR assay procedures.

a. Modified PCR Procedure
Follow procedures in Section 2, 5.2.A thru 5.2.C.

i. Amplification of *M. cerebralis* DNA (Baldwin and Mykleburst 2002).
PCR master mix:

PCR buffer (300 mM Tris, 75 mM ammonium sulfate, pH 9.0)	
MgCl	2.5 mM
dNTPs	400 uM
Tr5-16 Primer	20 pmol
Tr3-17 Primer	20 pmol
Taq Polymerase	2 U μL^{-1}

Primers (Andree et al. 1998):

Tr5-16	(5'-GCATTGGTTTACGCTGATGTAGCGA-3')
Tr3-17	(5'-GGCACACTACTCCAACACTGAATTTG-3')

Standard reaction volume: 50 µL (45 µL master mix and 5 µL DNA template)

ii. Perform amplification in thermocycler. Denature at 95°C for five minutes.
Cycle:

95°C for 1 minute.
65°C for 2.5 minutes.
72°C for 1.5 minutes.

Repeat cycle 35 times. Conclude with 72°C for 10 minutes.

iii. Visualization of PCR Product Gel Electrophoresis
Prepare agarose gel. Dilute 10 µL PCR product with 2 µL loading dye. Load 10 µL into each well. Run for approximately one hour at 140 V.

4. Quantitative Polymerase Chain Reaction (QPCR) Assay
Quantitative PCR detects the presence of *M. cerebralis* and determines the quantity of parasite present. For protocol details see Kelley et al. (2004).

5. Non-Lethal Sampling Techniques
Non-lethal sampling techniques for PCR analyses have been attempted using fin tissue, opercula, gill filaments, and skin scrapes (Toner et al. 2004). Examination of gill filaments for *M. cerebralis* by PCR suggests that positive results matched positive histological results, and sampling of that tissue produced no negative effects upon the fish (Schill et al. 1999). PCR analysis of gill filament samples was more sensitive for detection of *M. cerebralis* compared to samples from the caudal fin or operculum, but was less sensitive than lethal methods (Sandell et al. 2000). Although non-lethal sampling locations are preferred over lethal techniques for obvious reasons, PCR results using these sampling techniques have been inconsistent and are not validated for use in inspections.

H. Procedures for Determining Prior Exposure to the Etiological Agent

See above.

I. Procedures for Transportation and Storage of Samples to Ensure Maximum Viability and Survival of the Etiological Agent

Samples collected from apparently normal, moribund, or dead fish should be packed on ice for shipment. For histological examination, fresh tissue samples should be preserved in fixative containing formalin (e.g., 10% neutral buffered formalin, Davidson's).

References

Andree, K. B., E. MacConnell, and R. P. Hedrick. 1998. A polymerase chain reaction test for detection of *Myxobolus cerebralis*, the causative agent of salmonid whirling disease in fish, and a comparison to existing detection techniques. Diseases of Aquatic Organisms 34:145-154.

Andree, K. B., R. P. Hedrick, and E. MacConnell. 2002. A Review of the Approaches to Detect *Myxobolus cerebralis*, the Cause of Salmonid Whirling Disease. Symposium number 29 i*n* J. L. Bartholomew, and J. C. Wilson, editors. Whirling Disease Reviews and Current Topics. American Fisheries Society and the Whirling Disease Foundation.

Antonio, D. B., K. B. Andree, T. S. McDowell, and R. P. Hedrick. 1998. Detection of *Myxobolus cerebralis* in rainbow trout and oligochaete tissues using a nonradioactive *in situ* hybridization (ISH) protocol. Journal of Aquatic Animal Health 10:338-347.

Arsan, E. L., S. D. Atkinson, S. L. Hallett, T. Meyers, and J. L. Bartholomew. 2007. Expanded geographical distribution of *Myxobolus cerebralis*: First detections from Alaska. Journal of Fish Diseases 30:483-491.

Baldwin, T. J., E. R. Vincent, R. M. Silflow, and D. Stanek. 2000. *Myxobolus cerebralis* infection in rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) exposed under natural stream conditions. Journal of Veterinary Diagnostic Investigation 12:312-321.

Baldwin, T. J., and K. A. Mykleburst. 2002. Validation of a single round polymerase chain reaction assay for identification of *Myxobolus cerebralis* myxospores. Diseases of Aquatic Organisms 49:185-190.

El-Matbouli, M., T. Fischer-Scherl, and R. W. Hoffmann. 1992. Present knowledge of the life cycle, taxonomy, pathology, and therapy of some *Myxosporea* spp. important for freshwater fish. Annual Review of Fish Diseases 3:367-402.

Hallett, S. L., and J. L. Bartholomew. 2012. *Myxobolus cerebralis* and *Ceratomyxa shasta*. *in* Fish Parasites: Pathobiology and Protection. P. T. K. Woo and K. Buckmann eds. CAB International, Oxfordshire, U.K. In Press

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: The causative organism of whirling disease of salmonids. Journal of Fish Biology 9:339-357.

Hedrick, R. P., and M. El-Matbouli. 2002. Recent Advances with Taxonomy, Life Cycle, and Development of *Myxobolus cerebralis* in the Fish and Oligochaete Hosts. Symposium number 29

in J. L. Bartholomew, and J. C. Wilson, editors. Whirling Disease Reviews and Current Topics. American Fisheries Society and the Whirling Disease Foundation.

Hedrick, R. P., T. S. McDowell, M. Gay, G. D. Marty, M. P. Georgiadis, and E. MacConnell. 1999a. Comparative susceptibility of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) to *Myxobolus cerebralis* the cause of salmonid whirling disease. Diseases of Aquatic Organisms 37:173-183.

Hedrick, R. P., T. S. McDowell, K. Mukkatira, M. P. Georgiadis, and E. MacConnell. 1999b. Susceptibility of selected inland salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. Journal of Aquatic Animal Health 11:330-339.

Hedrick, R. P., T. S. McDowell, K. Mukkatira, M. P. Georgiadis, and E. MacConnell. 2001. Susceptibility of three species of anadromous salmonids to experimentally induced infections with *Myxobolus cerebralis*, the causative agent of whirling disease. Journal of Aquatic Animal Health 13:43-50.

Hoffman, G. L. 1999. Parasites of North American Freshwater Fishes, Second Edition. Comstock Publishing Associates, Ithaca NY. 539 pp.

Hoffman, G. L., and C. J. Byrne. 1974. Fish age as related to susceptibility to *Myxosoma cerebralis*, cause of whirling disease. The Progressive Fish-Culturist 36:151.

Kelley, G. O., F. J. Zagmutt-Vergara, C. M. Leutenegger, K. A. Myklebust, M. A. Adkison, T. S. McDowell, G. D. Marty, A. L. Kahler, A. L. Bush, I. A. Gardner, and R. P. Hedrick. 2004. Evaluation of five diagnostic methods of the detection and quantification of *Myxobolus cerebralis*. Journal of Veterinary Diagnostic Investigation 16:202-211.

Lom, J., and G. L. Hoffman. 1971. Morphology of the spores of *Myxosoma cerebralis* (Höfer, 1903) and *M. cartilaginis* (Hoffman, Putz, and Dunbar, 1965). Journal of Parasitology 56:1302-1308.

Lorz, H. V., and A. Amandi. 1994. Whirling Disease of Salmonids. Chapter VI, Parasitic Diseases of Fishes *in* J. Thoesen, editor. Suggested Procedures for the Detection and Identification of Certain Finfish and Shellfish Pathogens. 4th Edition, Version 1. Fish Health Section, American Fisheries Society. Bethesda, Maryland.

Luna, L. G., editor. 1968. Manual of Histologic Staining Methods of the Armed Forces Institute of Pathology. 3rd Edition. McGraw-Hill Book Company, New York. 258 pp.

MacConnell, E., and E. R. Vincent. 2002. The Effects of *Myxobolus cerebralis* on the Salmonid Host. Symposium number 29 *in* J. L. Bartholomew, and J. C. Wilson, editors. Whirling Disease Reviews and Current Topics. American Fisheries Society and the Whirling Disease Foundation.

MacLean, D. G. 1971. A simplified procedure for detecting *Myxosoma cerebralis* (whirling disease) spores in large lots of fish. The Progressive Fish-Culturist 33:203.

Markiw, M. E. 1992. Salmonid Whirling Disease. Fish and Wildlife Leaflet 17. U. S. Fish and Wildlife Service. Washington, D.C. 11 pp.

Markiw, M. E., and K. Wolf. 1974a. *Myxosoma cerebralis:* isolation and concentration from fish skeletal elements -sequential enzymatic digestions and purification by differential centrifugation. Journal of the Fisheries Research Board of Canada 31:15-20.

Markiw, M. E., and K. Wolf. 1974b. *Myxosoma cerebralis:* comparative sensitivity of spore detection methods. Journal of the Fisheries Research Board of Canada 31:1597-1600.

Markiw, M. E., and K. Wolf. 1983. *Myxosoma cerebralis* (Myxozoa: Myxosporea) etiological agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. Journal of Protozoology 30:561-564.

Markiw, M. E. 1991. Whirling disease: Earliest susceptible age of rainbow trout to the triactinomyxid of *Myxobolus cerebralis*. Aquaculture 92:1-6.

Markiw, M. E. 1992. Experimentally induced whirling disease. I. Dose response of fry and adults of rainbow trout exposed to the triactinomyxon stage of *Myxobolus cerebralis*. Journal of Aquatic Animal Health 4:40-43.

O' Grodnick, J. J. 1975. Whirling disease (Myxosoma: cerebralis) spore concentration using the continuous plankton centrifuge. Journal of Wildlife Diseases 11:54-57.

O' Grodnick, J. J. 1979. Susceptibility of various salmonids to whirling disease (Myxosoma: *cerebralis).* Transactions of the American Fisheries Society 108:187-190.

Rose, J. D., G. S. Marrs, C. Lewis, and G. Schisler. 2000. Whirling disease behavior and its relation to pathology of brain stem and spinal cord in rainbow trout. Journal of Aquatic Animal Health 12:107-118.

Ryce, E. K. N., A. V. Zale, and E. MacConnell. 2004. Effects of fish age and development of whirling parasite dose on the disease in rainbow trout. Diseases of Aquatic Organisms 59:225-233.

Ryce, E. K. N., A. V. Zale, E. MacConnell, and M. Nelson. 2005. Effects of fish age versus size on the development of whirling disease in rainbow trout. Diseases of Aquatic Organisms 63:69-76.

Sandell, T. A., H. Lorz and J. L. Bartholomew. 2000. An evaluation of three tissues for non-lethal, PCR-based detection of *Myxobolus cerebralis*. Pages 54-56 *in* Proceedings of the 6th Annual Whirling Disease Symposium, Coeur d'Alene, Idaho, Whirling Disease Foundation, Bozeman, Montana.

Schill, B., T. Waldrop, C. Densmore and V. Blazer. 1999. Non-lethal sampling for detection of *Myxobolus cerebralis* in asymptomatic rainbow trout. Pages 208-209 *in* Proceedings of the 6th Annual Whirling Disease Symposium, Coeur d'Alene, Idaho, Whirling Disease Foundation, Bozeman, Montana.

Schisler, G. J., E. P. Bergersen, P. G. Walker, J. Wood, and J. Epp. 2001. Comparison of single-round polymerase chain reaction (PCR) and pepsin-trypsin digest (PTD) methods for detection of *Myxobolus cerebralis*. Diseases of Aquatic Organisms 45:109-114.

Steinback Elwell, L. C., K. E. Stromberg, E. K. N. Ryce, and J. L. Bartholomew. 2009. Whirling Disease in the United States: A summary of progress in research and management. Whirling Disease Initiative and Trout Unlimited, Bozeman, MT.

Toner, M., E. MacConnell, C. Hudson, and L. Beck. 2004. Evaluation of non-lethal techniques for the detection of *Myxobolus cerebralis* in naturally exposed rainbow trout. Pages 34-35 *in* Proceedings of the 10th Annual Whirling Disease Symposium, Salt Lake City, Utah. Whirling Disease Foundation, Bozeman, Montana.

Wolf, K., and M. E. Markiw. 1979. *Myxosoma cerebralis* a method for staining spores and other stages with silver nitrate. Journal of the Fisheries Research Board of Canada 36:88-89.

Wolf, K., and M. E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: new discoveries show alternation of invertebrate and vertebrate hosts. Science 225:1449-1452.