

REVIEW

Whirling disease revisited: pathogenesis, parasite biology and disease intervention

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ABSTRACT: Whirling disease (WD) is an ecologically and economically debilitating disease of rainbow trout *Oncorhynchus mykiss* caused by the actinosporean spores of the parasite *Myxobolus cerebralis*. *M. cerebralis* has a complex, 2-host life cycle alternating between salmonid fish and the oligochaete host *Tubifex tubifex*. The parasite alternates between 2 spore forms as transmission stages: an actinosporean triactinomyxon spore that is produced in the oligochaete host and a myxosporean spore that develops in the salmonid host. Waterborne triactinomyxon spores released from infected *T. tubifex* oligochaetes attach to the salmonid host by polar filament extrusion elicited by chemical (nucleoside) and mechanical (thigmotropy) stimuli — a process which is rapidly followed by active penetration of the sporoplasms into the fish epidermis. Upon penetration, sporoplasms multiply and migrate via peripheral nerves and the central nervous system to reach the cartilage where they form trophozoites which undergo further multiplication and subsequent sporogenesis. *M. cerebralis* myxospores are released into the aquatic environment when infected fish die and autolyse, or when they are consumed and excreted by predators. Myxospores released into the water are ingested by susceptible *T. tubifex* where they develop intercellularly in the intestine over a period of 3 mo through 4 developmental stages to give rise to mature actinospores. In this article, we review our current understanding of WD—the parasite and its alternate hosts, life cycle and development of the parasite in either host, disease distribution, susceptibility and resistance mechanisms in salmonid host and strategies involved in diagnosis, prevention and control of WD.

KEY WORDS: Whirling disease · *Myxobolus cerebralis* · Rainbow trout · Brown trout · Host–parasite interaction

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INTRODUCTION

WD was first discovered in Europe among rainbow trout *Oncorhynchus mykiss* originally imported as eyed eggs from North America for the growing aquaculture industry in the late 1800s (Hofer 1903). The disease is suspected to have originated in Eurasia in

brown trout *Salmo trutta* from where it eventually spread to its current worldwide distribution (Hoffman 1970, 1990). Severe negative economical and ecological impacts have accompanied the infection of salmonids in North America both in cultured and feral trout populations as well as in Europe and other areas where susceptible fish are cultured (Schäperclaus

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1931, Nehring & Walker 1996, Vincent 1996, Hedrick et al. 1998, Bartholomew & Reno 2002). Since the early description of WD among farm-raised rainbow trout in Germany (Hofer 1903), subsequent research in the USA and Europe has provided insight into the characteristics of WD and shed light on disease biology and practical methods to effectively minimize its deleterious effects on salmonids raised in aquaculture facilities (Hoffman & Putz 1969, Hoffman & Hoffman 1972, Hoffman 1975, Halliday 1976, O'Grodnick 1979, Schäperclaus 1931, 1986). Fundamental tenets essential for the understanding of the parasite and WD remained enigmatic for a long time. Hoffman & Putz (1969) clearly demonstrated a lack of direct fish-to-fish transmission, so the discovery of the 2-host life cycle of *M. cerebralis* in the 1980s paved the way towards research regarding the nature and source of the infectious stages for salmonid fish (Markiw & Wolf

1983, Wolf & Markiw 1984, Wolf et al. 1986; corroborated by El-Matbouli & Hoffmann 1989). This led to intensive investigations by fish pathologists and ecologists on the impact of *M. cerebralis* infections on rainbow trout (Vincent 1996, Nehring & Walker 1996, Hedrick et al. 1997, Kent et al. 2001, Hedrick & El-Matbouli 2002). Recent investigations have delved deep into studying the genetic basis of WD resistance in rainbow trout using genome-wide mapping and SNP chip technologies (Baerwald et al. 2008, 2011).

PARASITE *MYXOBOLUS CEREBRALIS*

Among the known species of myxozoan parasites, *M. cerebralis* is one of the most pathogenic for fish (Fig. 1) (Hedrick et al. 1998), hence its life cycle was the first to be investigated and described (Lom 1987).

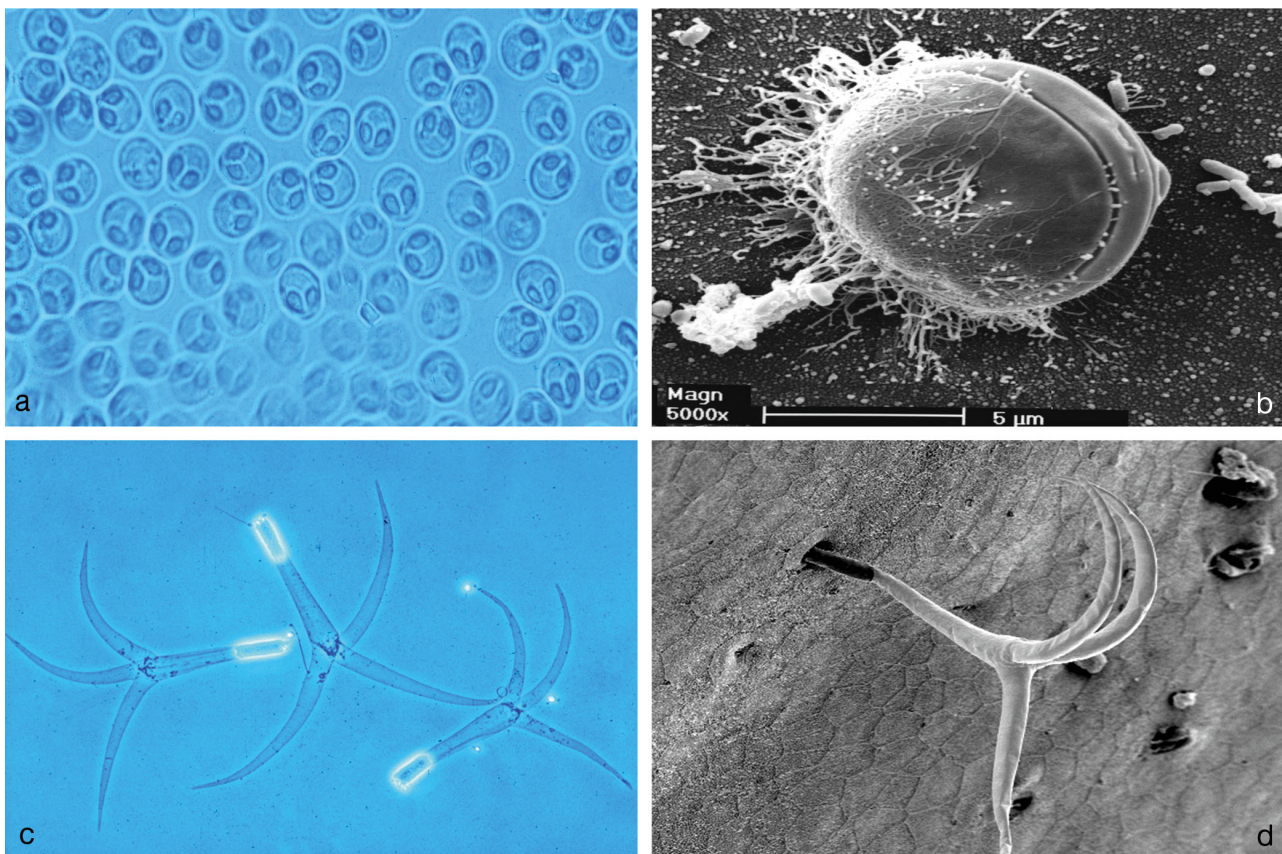


Fig. 1. Fish parasite *Myxobolus cerebralis*. (a) Unstained mature *M. cerebralis* myxospores obtained from rainbow trout displaying whirling disease 120 d post-exposure (p.e.). Each infective myxospore displays 2 intact polar capsules. Light micrograph (LM). (b) Mature *M. cerebralis* myxospore. Scanning electron micrograph (SEM). (c) Unstained mature waterborne triactinomyxon spores of *M. cerebralis* released from *T. tubifex* 120 d p.e. The triactinomyxon spore is larger than the myxospore at approximately 140 µm in style height and 175–200 µm in process length and contains a minimum of 64 spherical sporoplasm cells (sporozoites) (El-Matbouli et al. 1995, El-Matbouli & Hoffmann 1998). The triactinomyxon spores elicit refractile sporoplasm and transparent processes. LM. (d) Mature triactinomyxon penetrating the epidermis of fish host. SEM

Taxonomy and phylogeny

Originally, Myxozoa were classified as protists but later, several metazoan characteristics were reported (Smothers et al. 1994, Siddall et al. 1995). Genome sequencing and phylogenetic analyses suggest a closer relationship between Myxozoa and Cnidaria (Jiménez-Guri et al. 2007, Holland et al. 2011, Nesnidal et al. 2013, Feng et al. 2014, Shpirer et al. 2014). Myxozoans share morphologic characteristics with cnidarians, including a polymorphic life cycle and organelles (polar capsules and filaments) resembling the stinging nematocysts of cnidarians (Siddall et al. 1995). The hypothesis that Myxozoa are related to Cnidaria through shared recent common ancestry has been validated by the discovery of a diverse array of cnidarian-specific phylogenetically and taxonomically restricted genes in Myxozoa, viz. the nematogalectin, nematogalectin-related and mini-collagen genes, which are critically involved in the maintenance of the nematocyst structure (Holland et al. 2011, Feng et al. 2014, Shpirer et al. 2014). As Cnidaria are the only taxonomic group to possess nematocysts, the identification of these genes in Myxozoa provides an interesting evolutionary link between myxozoan polar capsules and cnidarian nematocysts complementing the phylogenetic studies (Jiménez-Guri et al. 2007, Nesnidal et al. 2013). The investigation of taxon-specific genes for phylogenetic inference within the Metazoa exemplifies how evolutionary relationships of taxons characterized by extreme sequence divergence could be resolved (Holland et al. 2011, Feng et al. 2014, Shpirer et al. 2014).

Life cycle and development

Myxospore development in salmonids

As depicted in Fig. 2, when waterborne triactinomyxon spores (Fig. 1c,d) released from infected *T. tubifex* oligochaetes come into contact with a susceptible salmonid, they attach to the mucous surface (El-Matbouli et al. 1999a). The portals of entry for the infective sporoplasms are the secretory openings of the mucous cells of the epidermis, the gill respiratory epithelium and the buccal cavity (Figs. 1d & 3). Upon attachment, the valve cells open to release the sporoplasm covered by the membranous endospore sheath. The process of attachment of the actinospores and the subsequent penetration of the sporoplasm takes 1 min (Figs. 3 & 4). This facilitates the

penetration of the amoeboid sporoplasm harbouring the 64 infective secondary germ cells into the fish host through the epidermis. The sporoplasm then actively moves by amoeboid movement to penetrate into the epidermal tissue.

Presporogonic and extrasporogonic phase. Within the first 60 min following penetration, the sporoplasm migrates intercellularly in the epidermis and gill epithelium (Fig. 5a,b). This is followed by the subsequent disintegration of the syncytial primary cell containing the secondary germ cells leading to invasion of a host epidermal or gill epithelial cell (El-Matbouli et al. 1999a). Endogenous cleavage of these cells prompts production of an inner secondary cell within an enveloping primary cell (Morris et al. 2006). Rapid synchronous mitosis promotes secondary cell proliferation leading to compression of the host cell nucleus (Fig. 5c,d) between the large parasitic aggregates and the host cell plasmalemma (Daniels et al. 1976, El-Matbouli et al. 1995). Endogenous division of secondary cells then produces cell-doublets with an enveloping cell and inner cell which rupture the primary cell membrane enabling them to enter the host cell cytoplasm. Some cell-doublets are destroyed within the host cell cytoplasm due to host immune response (El-Matbouli et al. 1999a). Inside the host cell cytoplasm, cell-doublets that survive pierce the host cell membrane to enter the extracellular space. New cell-doublets in the extracellular space hereafter penetrate neighboring epithelial cells or migrate deeper into the dermis and subcutis layers to penetrate other host cells in order to resume the early proliferation cycle.

At water temperatures of 15°C and approximately 4 d post-exposure (p.e.), parasite cells migrate intercellularly into and along nervous tissue. As the parasite migrates through the central nervous system, unabated proliferation of cell-doublets continues. From Day 6 to 14, most parasite stages are localized in the spinal cord region of infected fish (Fig. 5e,f); from Days 14 to 24, developmental stages are localized in the cartilage surrounding fish brain tissue (El-Matbouli et al. 1999a).

Sporogonic phase. As illustrated in Fig. 6, a plasmodium develops at the site of sporulation in the non-ossified cartilage tissue in the entire fish skeleton. Numerous internal vegetative nuclei are produced upon growth of the primary cell, followed by subsequent nuclear division. New generative cells are produced as the enveloped cell divides. El-Matbouli et al. (1995) demonstrated that, upon disintegration of the enveloping cell of the plasmodium, each enveloped cell either repeats the cycle produc-

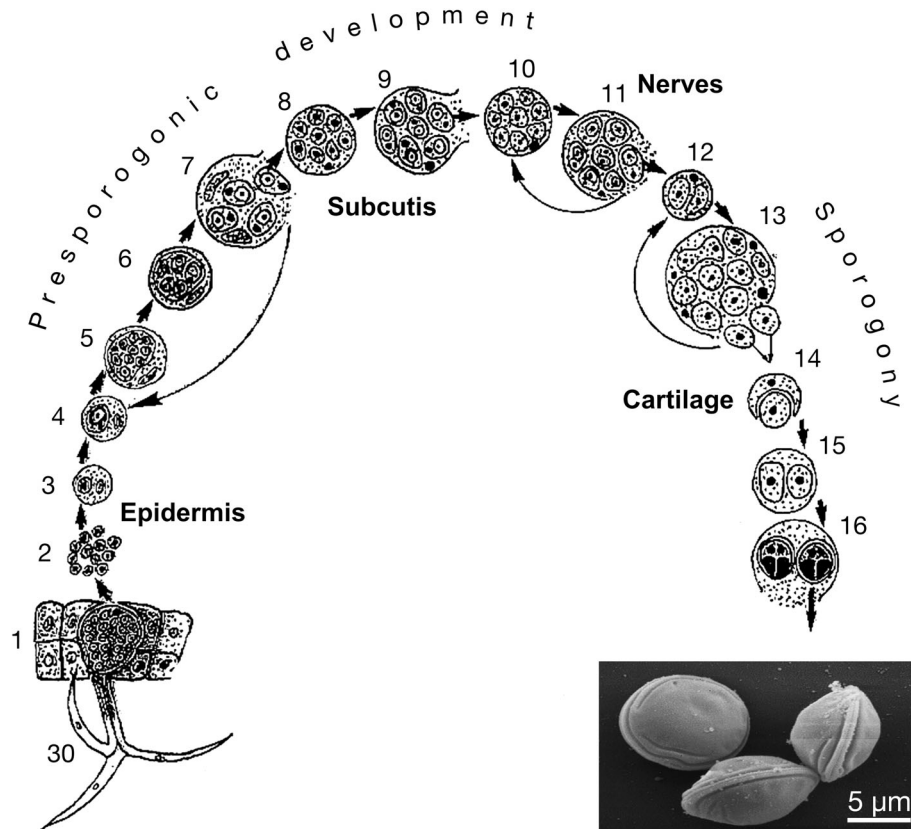


Fig. 2. *Myxobolus cerebralis* life cycle: development of myxospores in fish host. Stages 1 and 2 occur interepithelially in the epidermis, Stages 3–7 occur intraepithelially in the epidermis, Stages 8 and 9 occur intercellularly in the subcutis, Stages 10–12 occur in neural tissue and Stages 12–16 occur in cartilage. Stage 1: compact triactinomyxon sporoplasm between epithelial cells. Stage 2: interepithelial-free sporoplasm cells each of which penetrate an epithelial cell as seen in stage 3. Stage 4: intracellular endogenous cell division of the parasite which produces primary and secondary cells. Stage 5: numerous secondary cells resulting from mitotic division. Stage 6: endogenous division of the secondary cells producing cell doublets. Stage 7: cell doublets rupture the aggregate membrane and pierce the host cell plasmalemma, then penetrate other epithelial cells or migrate deeper into the dermis and subcutis. Stage 8: intercellular growth of the cell doublets in the subcutis through mitosis. Stage 9: endogenous division in the subcutis. Stages 10 and 11 are similar to Stages 8 and 9, but occur in neural tissue. Stage 12: triplet in central nervous system and cartilage. Stage 13: plasmodium in head cartilage. Stage 14: formation of a pansporoblast arising from the unification of a pericyte and sporogonic cells. Stage 15: endogenous division of the sporogonic cell producing 2 sporoblast cells which further divide resulting into 2 *M. cerebralis* spores within the pansporoblast as seen in Stage 16. Inset: Mature myxospores (SEM). (Adapted from El-Matbouli & Hoffmann 1998; used by permission)

ing numerous plasmodia, or unites with another parasitic cell. One becomes the pericyte envelope and the inner one forms the sporogonic cell; this unit generates pansporoblasts forming myxospores (2 myxospores in *M. cerebralis*).

Actinospore development

The actinosporean stages of *M. cerebralis* are produced after myxospores are released from the salmonid host and become ingested by *T. tubifex* (Fig. 7a). Within the gut lumen of the oligochaete, myxospores (Fig. 7c) extrude their polar filaments in order to attach to the gut epithelium. Myxospore

valves open to enable emission and penetration of the binucleated sporoplasm between the gut epithelial cells. The following description of their development is based on the observations made by El-Matbouli & Hoffmann (1998).

Schizogony (Stages 3 and 4). Subsequent multiple divisions of both nuclei of the sporoplasm produce numerous uninucleate cells which migrate intercellularly through the gut epithelia of the worm (Fig. 8). Some of these stages undergo further nuclear and cellular divisions forming additional multinucleate and uninucleate cells.

Gametogony (Stages 5 to 10). In the binucleate stage (Fig. 8), nuclei divide to form a cell with 4 nuclei which further divides to form an early pansporocyst

with 4 cells: 2 enveloping somatic cells and 2 generative cells, termed α and β . Three mitotic divisions of the 2 generative cells yield 16 diploid gametocytes (8α and 8β) which undergo one meiotic division to produce 16 haploid gametocytes and 16 polar bodies. Each gametocyte from line α unites with one from line β to produce 8 zygotes. Meanwhile, the somatic cells divide twice to produce 8 enveloping cells. This is the only phase in the life cycle

Fig. 3. Interaction between *Myxobolus cerebralis* triactinomyxon spores and the host fish epidermis. The triactinomyxon spores locate the secretory openings of the fish epidermal mucous cells, using them as entry portals for the sporoplasm cells. SEM

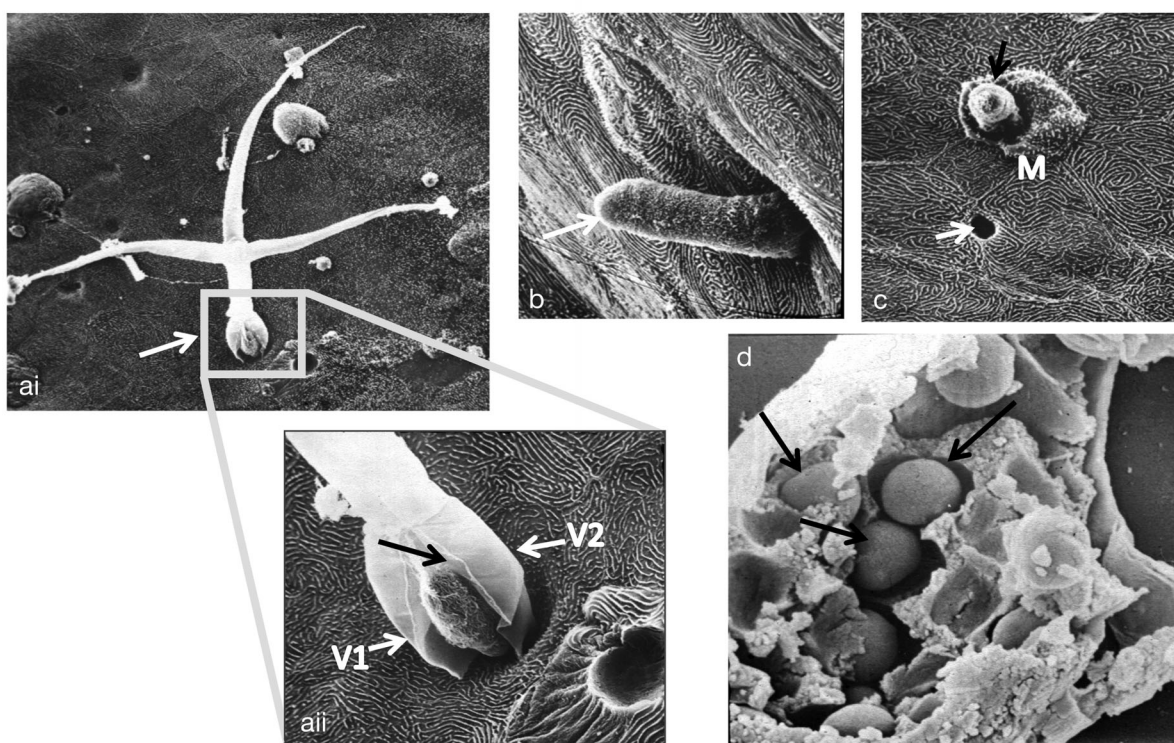
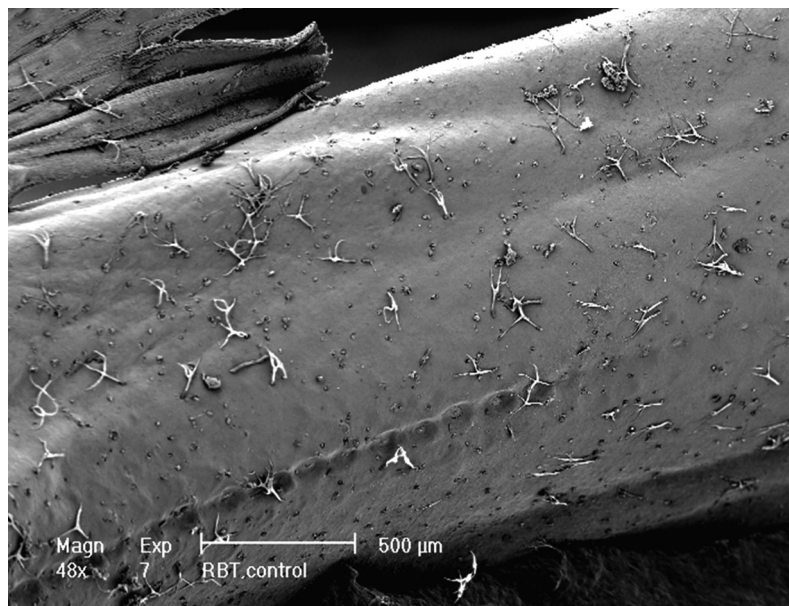


Fig. 4. Interaction between *Myxobolus cerebralis* triactinomyxon spore and the fish host. SEM. (a-i) Triactinomyxon spore (white arrow) attached to the secretory opening of a mucous cell in the dorsal fin of the fish host, 1 min post-exposure (p.e.); (a-ii) higher magnification of a part of Fig. 4a-i showing penetration by triactinomyxon sporoplasm through the secretory opening of a mucous cell. One of the suture edges between 2 valves (V1 and V2; white arrows) is open. Note fibrous structure surrounding sporoplasm (black arrow). (b) Penetration of a triactinomyxon sporoplasm cell (white arrow) on the dorsal fin of fish host after the shell valves have fallen away, 2 min p.e. (c) Deeper penetrated triactinomyxon (black arrow) in the secretory opening of a mucous cell (M: mucus). White arrow: secretory opening of another mucous cell, 4 min p.e. (d) Broken style in region of sporoplasm. Note the sporoplasm cells (black arrows) which are embedded in the cytoplasm of the sporoplasm somatic cell (Adapted from El-Matbouli et al. 1999b; used by permission)

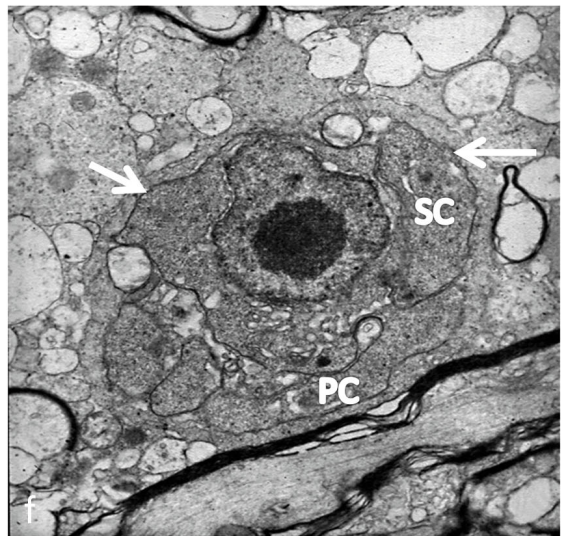
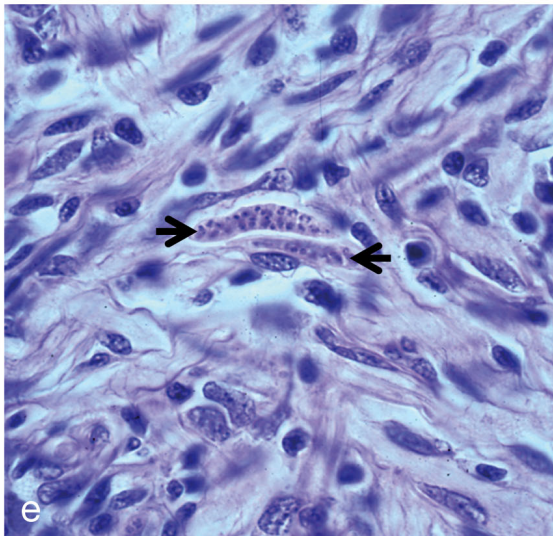
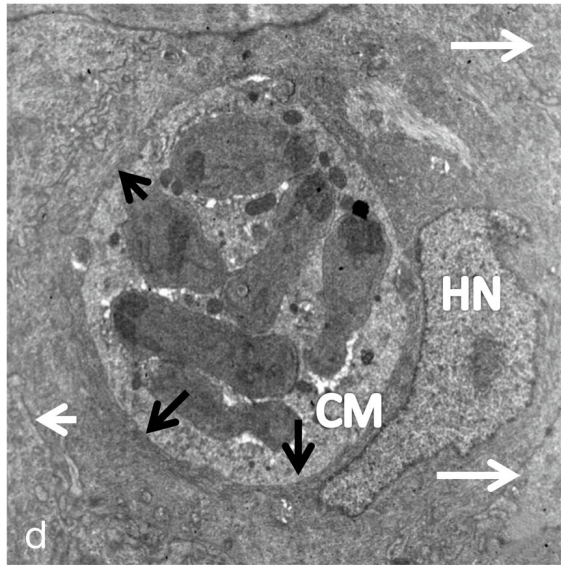
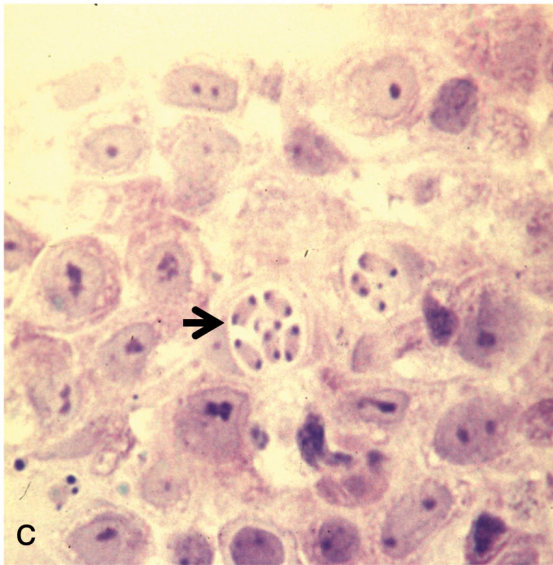
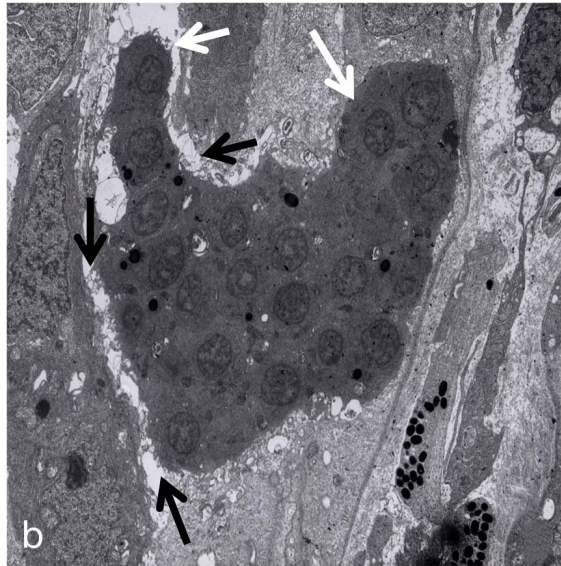
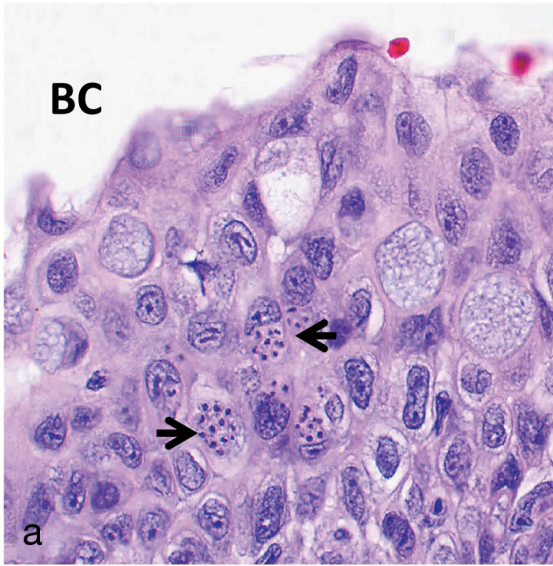


Fig. 5. *Myxobolus cerebralis* presporogonic development. (a) Buccal cavity mucosa (BC) with numerous intracellular aggregates of darkly stained sporoplasm (black arrows), 1 h p.e. LM, Giemsa. (b) Interepithelial localized aggregate of triactinomyxon-sporoplasm forming pseudopodia-like projections (white arrows), 1 h p.e.; note lesions of epidermis (black arrows). SEM. (c) Part of caudal fin with an infected epidermal cell (black arrow), 2 h p.e. All sporoplasm cells are in telophase of mitosis. LM, Giemsa. (d) Dividing sporoplasm cells, 2 h p.e. Cytoplasmic matrix (CM) of aggregate contains numerous mitochondria and is less dense than that of host cell. Note membrane (black arrows) surrounding sporoplasm cells, and displacement of host cell nucleus (HN). Host cell plasmalemma (white arrows) is also evident. SEM. (e) Aggregates of parasitic stages (black arrows) between nerve fibres and spinal cord, 14 d p.e. LM, Giemsa. (f) Cell doublet consisting of primary cell (PC) and secondary cell (SC) between nerve fibres of spinal cord, 14 d p.e. Note pseudopodia-like cytoplasmic protrusions (white arrows) of the enveloped secondary cell. SEM. (Adapted from El-Matbouli et al. 1995; used by permission)

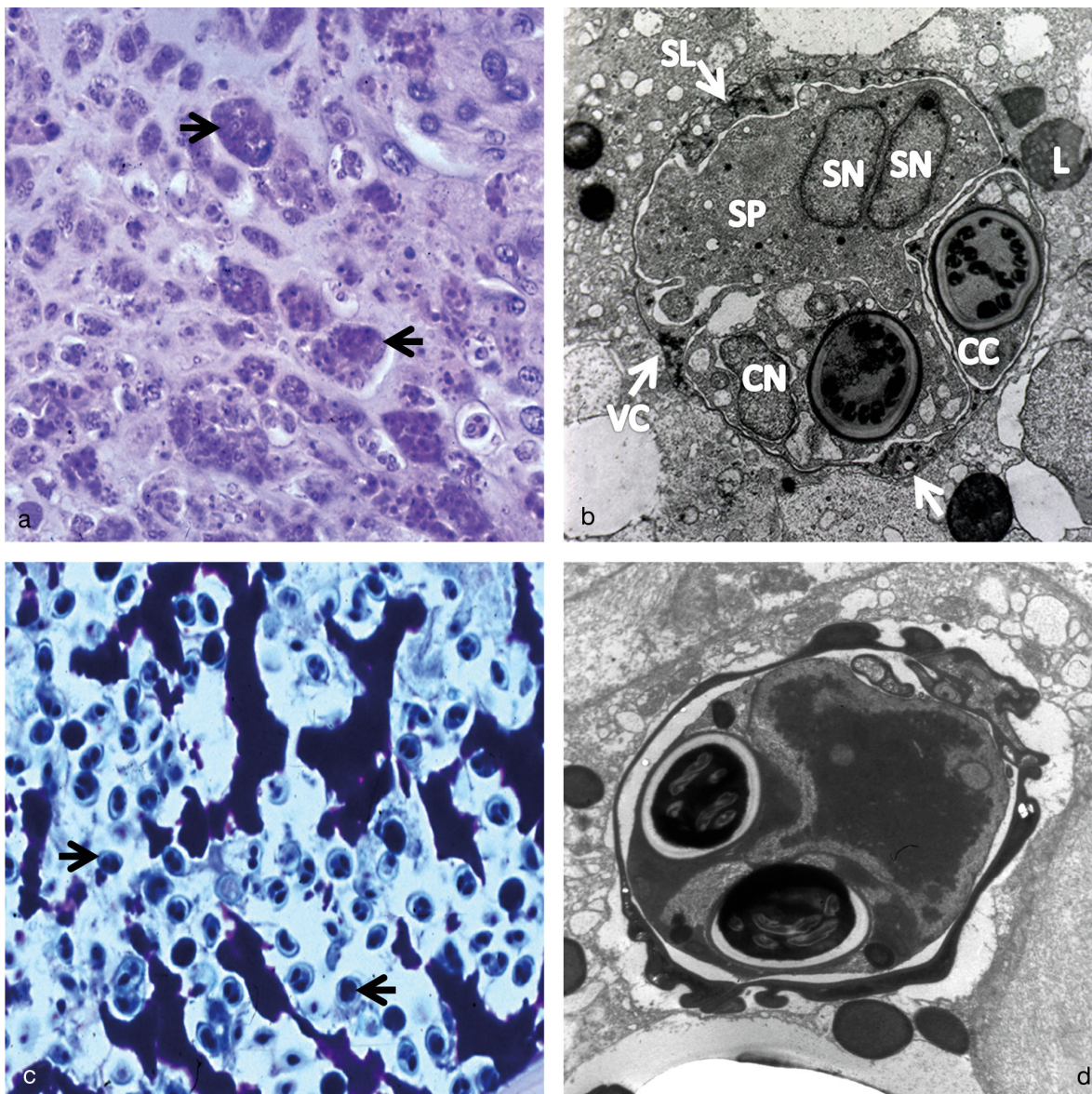


Fig. 6. *Myxobolus cerebralis* sporogonic development. (a) Aggregates of parasitic stages (black arrows) in the cartilage, 12 wk p.e. LM, Giemsa. (b) Maturing sporoblast in the head cartilage. SP: Sporoplasm with 2 nuclei (SN) and characteristic sporoplasmosomes (arrows); CC: a capsulogenic cell with polar capsules; CN: capsulogenic cell nucleus; VC: 2 valvogenic cells; SL: sutural line; L: lipid droplet in surrounding cartilage. SEM. (c) Aggregates of parasitic stages (black arrows) in the cartilage, 18 wk p.e. LM, Giemsa. (d) Longitudinal section through a mature spore, 18 wk p.e. SEM. (Adapted from El-Matbouli et al. 1995; used by permission)

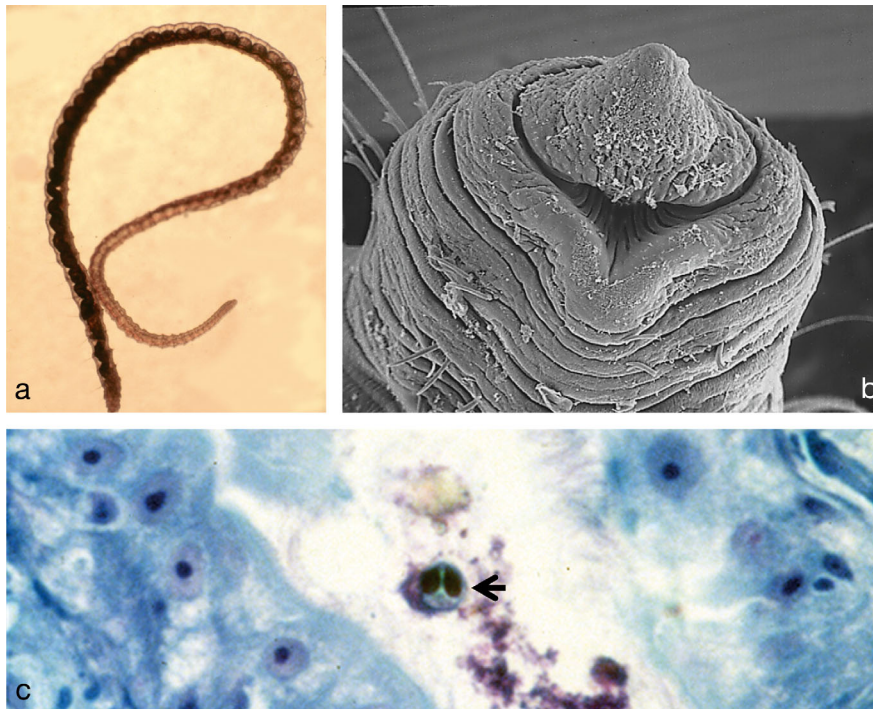


Fig. 7. Interaction between *Myxobolus cerebralis* myxospore and *Tubifex tubifex*. (a) Unstained single *T. tubifex* oligochaete. *T. tubifex* oligochaetes typically average 10–30 mm in length. LM. (b) Oral cavity through which *T. tubifex* oligochaetes ingest free *M. cerebralis* myxospores from the sediment. SEM. (c) Mature *M. cerebralis* spore (black arrow) in *T. tubifex* intestine, where myxospores interact with the intestinal epithelial cells and extrude the polar filament releasing the sporoplasm, which then undergoes intercellular development within the intestine to the triactinomyxon stage via schizogony, gametogony and sporogony (El-Matbouli & Hoffmann 1998). LM, Giemsa. (Adapted from El-Matbouli et al. 1999b; used by permission)

of *M. cerebralis* in which meiosis occurs, which thus can be referred to as a sexual process.

Sporogony (Stages 11 to 15). As a result of gametogamy, each pansporocyst consists of 8 somatic cells surrounding 8 zygotes (Fig. 8). Every zygote subsequently undergoes 2 mitotic divisions to produce a 4-cell stage. Three cells are located peripherally and divide to form 3 capsulogenic and 3 valvogenic cells, while the fourth cell, centrally located, undergoes numerous mitotic divisions to form the sporoplasm of the actinosporean stage with its numerous secondary cells. Subsequently, the capsulogenic cells and the sporoplasm are enclosed within a proteinaceous membrane shell composed of 3 discrete valves. The valvogenic cells which are distal from the apically located sporoplasm (i.e. below the capsulogenic cells), extend into folded membranes that inflate to form the shell valves once released into the water body. This final stage with pansporocysts containing 8 folded actinosporean spores is observed 90 d p.e. to *M. cerebralis* at a water temperature of 16°C.

Actinospore viability. Kallert & El-Matbouli (2008) showed that *M. cerebralis* actinospores have a short

effective life span compared to those from other fish infecting myxozoans such as *Henneguya nuesslini* and *Myxobolus pseudodispar*. Storage at lower temperatures resulted in longer lasting viability and reactivity patterns of *M. cerebralis* actinospores (Kallert & El-Matbouli 2008).

DISTRIBUTION OF WD

Hoffman (1970) suggested that *M. cerebralis* originally covered a geographic region encompassing Central Europe to Northern Asia, basing that conclusion on the resistance of native brown trout to WD. Hoffman (1970) hypothesized that resistance to WD is specific and acquired through co-evolutionary processes. This attributed the spread of WD in Europe to unrestricted transfers of live rainbow trout throughout Europe. By the late 1950s, *M. cerebralis* had spread towards the Sakhalin islands off the coast of eastern Russia (Bogdanova 1960, 1966, 1968). This was followed by subsequent detection of *M. cerebralis* in Korea (Tinkina 1962) and Japan (Halliday

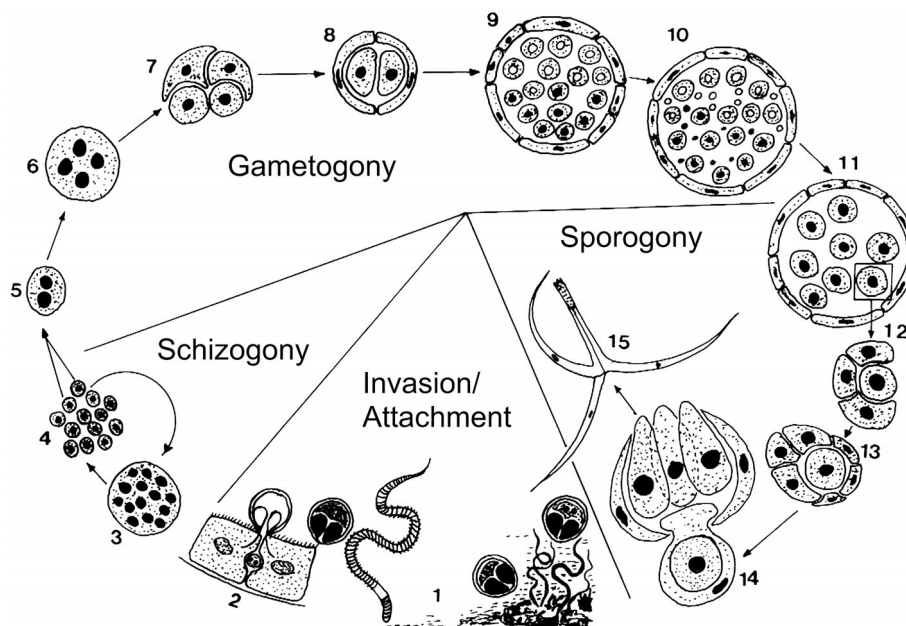


Fig. 8. *Myxobolus cerebralis* life cycle: development of myxospore in oligochaete host *Tubifex tubifex*. Stage 1: ingestion of *M. cerebralis* by *T. tubifex*. Stage 2: extrusion of the polar filaments and anchorage of *M. cerebralis* spore into gut epithelium. After shell valves open, binucleate sporoplasm escapes and penetrates between epithelial cells. Stage 3: interepithelial schizogonic multiplication of binucleate sporoplasm. Stage 4: uninucleate 1-cell stages. Stage 5: plasmogamy of 2 uninucleate cells to produce one binucleate cell-stage. Stage 6: mitotic division of both nuclei to produce 4-nuclei stage. Stage 7: formation of 4-cell stage by plasmotomy; 2 cells begin to envelop the other 2 cells. Stage 8: formation of early pansporocyst with 2 somatic and 2 generative cells. Stage 9: following 3 mitotic divisions of both generative cells and 2 mitotic divisions of the somatic cells, 16 gametocytes (8 α and 8 β) enveloped by 8 somatic cells are formed. Stage 10: following meiotic division of the 16 diploid gametocytes, 16 haploid gametocytes and 16 polar bodies result. Stage 11: production of 8 zygotes after copulation of each pair of α - and β -gametes. Stage 12: sporoblast formation after 2 mitotic divisions of zygote, 3 pyramidally arranged cells and one inner cell are formed. Stage 13: following mitotic division of the 3 peripheral cells, 3 capsulogenic and 3 valvogenic cells are produced. Stage 14: the valvogenic cells extend around the capsulogenic cells, while internal cleavage of the developing sporoplasm cell produces one generative cell enveloped by one somatic cell. The sporoplasm remains naked in the pansporocyst until reaching the final number of germs through repeating mitotic divisions. Stage 15: inflated mature triactinomyxon spore. (Adapted from El-Matbouli & Hoffmann 1998; used by permission)

1976). Reports of WD outbreaks were also reported from Ecuador (Meschkat 1971). Following the introduction of WD to the USA, an intensive research program largely led by the US Fish and Wildlife Service was initiated to develop means to detect and control the disease (Hoffman 1975, Hoffman & Markiw 1977, Hoffman & Hoffman 1972, Hoffman & Putz 1969). In addition, the Whirling Disease Foundation (WDF) was established in 1995, playing a very important role towards raising funds needed for solutions to the damage caused by WD to wild trout populations. The first confirmed detection of *M. cerebralis* in the USA occurred at the Benner Spring Fish Research Station, Pennsylvania, in 1958 (Hoffman et al. 1962). In addition, examination of archived fish cranial tissue samples revealed that *M. cerebralis* was present in 1957 at the Verdi Fish Hatchery on the Truckee River in Nevada (Taylor et al. 1973). Since these early detections of *M. cerebralis* in the USA, WD has been con-

firmed in hatcheries, ponds and streams in numerous states including Arizona, California, Colorado, Connecticut, Idaho, Maryland, Massachusetts, Michigan, Montana, Nebraska, Nevada, New Hampshire, New Jersey, New Mexico, New York, Ohio, Oregon, Pennsylvania, Utah, Vermont, Virginia, Washington, West Virginia and Wyoming (Nehring & Walker 1996, Vincent 1996). However, *M. cerebralis* is not necessarily established or widespread in each state: for example, the parasite's presence in Alaska was confirmed only by the detection and sequencing of *M. cerebralis* DNA, but myxospores were not observed in fish (Arasan et al. 2007). *M. cerebralis* is often present in the absence of external signs and thus detection of the parasite does not necessarily imply that fish develop clinical forms of WD (Figs. 9 & 10). Between 1957 and 1990, *M. cerebralis* was detected in many aquaculture facilities across the USA with only a few reports of WD in wild habitats (Yoder 1972, Hoffman

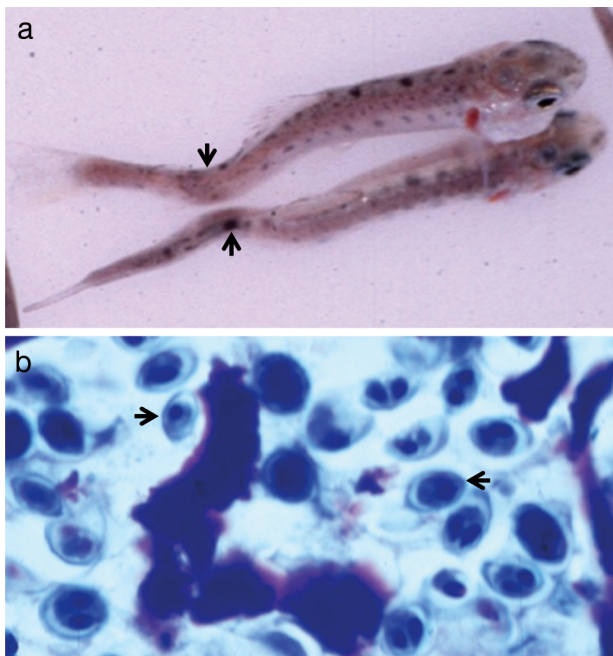


Fig. 9. Etiology of whirling disease (*Myxobolus cerebralis*). (a) Two rainbow trout fry during acute phase; characteristic symptoms include destruction of ossification, skeletal deformation, and blackening of the caudal part of the fish body (arrows). (b) Aggregation of *M. cerebralis* parasitic stages (black arrows) accompanied by digestion and destruction of ossification by the trophozoites leading to skeletal deformities

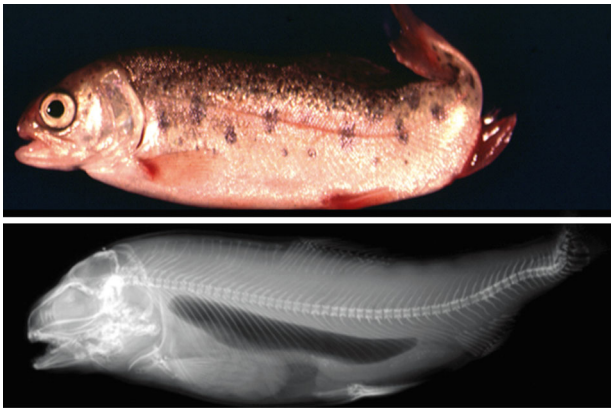


Fig. 10. Etiology of whirling disease (WD). Representative X-ray image displaying characteristic skeletal deformity

1990, Modin 1998, Bartholomew & Reno 2002). It was initially thought that WD would become a manageable problem and it was not expected to cause widespread disease or population losses among wild salmonids (Modin 1998). However, this view was challenged in the mid 1990s when dramatic trout population declines in Colorado and Montana were attributed to WD (Nehring & Walker 1996, Vincent

1996). In response to these observations, coordinated, multiagency research efforts were initiated and subsequent studies have contributed to significant advances in our understanding of the epidemiology, biology and management principles for controlling WD.

SALMONID HOSTS

Susceptibility and resistance

As indicated in Table 1, *M. cerebralis* is able to infect several species of salmonids (O'Grodnick 1979, Hoffman 1990, Hedrick et al. 1999a,b, MacConnell & Vincent 2002). Among species that do exhibit clinical symptoms of WD, rainbow trout *O. mykiss* exhibits the worst pathology (O'Grodnick 1979, Hedrick et al. 1999a,b). One year-old rainbow trout exposed to approximately 20 000 triactinomyxon spores fish⁻¹ produced 10× more myxospores than 3 yr old fish exposed to approximately 100 000 spores fish⁻¹. Other species of salmonids appear to be more resistant to WD, such as brown trout *S. trutta* where infections are usually asymptomatic; there is no report of brown trout exhibiting whirling behavior. The coho salmon *Oncorhynchus kisutch* native to western North America is also resistant to WD (O'Grodnick 1979). In contrast, the European Danube salmon *Hucho hucho* is highly susceptible to WD (El-Matbouli et al. 1992). *H. hucho* has very low reproduction rates in the wild and has been stocked for almost 100 yr, which may have hampered effective co-evolution. The mechanism that conveys the varying levels of resistance observed in salmonid species remains unknown and it appears that different species may limit the disease by different mechanisms. With regard to other salmonid species that are susceptible to infection, for example the bull trout *Salvelinus confluentus*, parasite development was observed in the skin and in the nerves, while lesions and concomitant parasite numbers in the cartilage were eventually resolved over time (Hedrick et al. 1999a,b). This species rarely exhibits clinical signs of WD. Arctic grayling *Thymallus arcticus* appears to be completely refractory to infection. In laboratory exposures of Arctic grayling to actinospores of *M. cerebralis*, no developmental stage of the parasite was observed in the nerves or skin at 35 d p.e., indicating that this species may be able to inhibit development of early parasite stages (Hedrick et al. 1999a,b). Some reports from Montana found strains of rainbow trout naturally resistant to *M. cerebralis*, such as the

Harrison Lake strain (Hedrick et al. 1998, 2001b, 2003). A number of review articles list Atlantic salmon *Salmo salar* as a susceptible species in feral populations and fish hatcheries in Europe, Asia and the Far East, but not in the USA (El-Matbouli et al. 1992, Halliday 1976). However, there is little information regarding the susceptibility of Atlantic salmon to *M. cerebralis*. Blazer et al. (2004) compared the susceptibility of Canadian lake trout *Salvelinus namaycush*, rainbow trout and Atlantic salmon to *M. cerebralis* in controlled laboratory exposures and found that rainbow trout were highly susceptible to *M. cerebralis* at low and high exposure doses, and was the only species among the 3 tested to show whirling behavior and black tails. Atlantic salmon were less susceptible but did show associated cartilage damage at both exposure doses. Canadian lake trout showed no clinical signs of infection and were negative for spores at both exposure doses, exhibiting no histopathological signs of *M. cerebralis* infection. The inflammatory responses of rainbow trout and Atlantic salmon differed and this may account for some of the differences in susceptibility. The fact that Atlantic salmon can be infected and myxospores can develop without overt clinical indications shows that this species represents a suitable host for *M. cerebralis* transmission (Blazer et al. 2004). However there is no evidence to suggest that the spores built in the cartilage of Atlantic salmon are viable.

Mechanisms of susceptibility and resistance

With respect to the mechanisms of resistance to *M. cerebralis* in rainbow trout, some evidence indicate the involvement of transforming growth factor-beta (Severin & El-Matbouli 2007), metallothionein B (Baerwald et al. 2008) and immune-regulatory genes, arginase-2 and inducible-nitric oxide synthase (Severin et al. 2010). Gene expression profiling using microarrays is an extremely high-throughput method to uncover specific genes and pathways involved in a disease phenotype without the bias of a candidate gene approach. Baerwald et al. (2008) showed that for both resistant German (GR) and susceptible Trout Lodge (TL) rainbow trout strains, response to infection is linked with the interferon system. Ubiquitin-

Table 1. Comparative susceptibility to whirling disease (WD) among salmonid hosts of *Myxobolus cerebralis*, ascertained via laboratory or natural exposure to *M. cerebralis* at vulnerable life stages. 0 = resistant, no spores develop; 1 = partial resistance, clinical disease rare and develops only when exposed to very high parasite doses; 2 = susceptible, clinical disease common at high parasite doses or when very young, but greater resistance to disease at low doses; 3 = highly susceptible, clinical disease common. (?) susceptibility is unclear (conflicting reports or insufficient data) (adapted from MacConnell & Vincent 2002; used by permission)

Genus	Species or subspecies	Common name	Susceptibility
<i>Oncorhynchus</i>	<i>mykiss</i>	Rainbow trout	3
	<i>mykiss</i>	Steelhead trout	3
	<i>clarki</i>	Cutthroat trout	2–3
	<i>c. bouvieri</i>	Yellowstone cutthroat	3
	<i>c. lewisi</i>	Westslope cutthroat	2
	<i>c. pleuriticus</i>	Colorado River cutthroat	2
	<i>c. virginialis</i>	Rio Grande cutthroat	2
	<i>c. stomias</i>	Greenback cutthroat	2
	<i>tshawytscha</i>	Chinook salmon	2
	<i>nerka</i>	Sockeye salmon	3
	<i>keta</i>	Chum salmon	1 (?)
	<i>gorbuscha</i>	Pink salmon	1 (?)
	<i>masu</i>	Cherry salmon	1 (?)
	<i>kisutch</i>	Coho salmon	1
<i>Salvelinus</i>	<i>fontinalis</i>	Brook trout	2
	<i>malma</i>	Dolly Varden	1 (?)
	<i>confluentus</i>	Bull trout	1
	<i>namaycush</i>	Lake trout	0
<i>Salmo</i>	<i>salar</i>	Atlantic salmon	2 (?)
	<i>trutta</i>	Brown trout	1
<i>Prosopium</i>	<i>williamsoni</i>	Mountain whitefish	2
<i>Thymallus</i>	<i>thymallus</i>	European grayling	2 (?)
	<i>arcticus</i>	Arctic grayling	0
<i>Hucho</i>	<i>hucho</i>	Danube salmon	3

like protein 1 was up-regulated over 100-fold and interferon regulating factor 1 was up-regulated over 15-fold following pathogen exposure for both strains. Expression of metallothionein B, which has known roles in inflammation and immune response, was up-regulated over 5-fold in the resistant GR strain but was unchanged in the susceptible TL strain following pathogen exposure. The metallothionein B gene (*metB*) was mapped to the rainbow trout chromosome Omy2 (Phillips et al. 2006). Similar responses to infection may occur in both resistant and susceptible trout strains in WD. Baerwald et al. (2011) identified a single quantitative trait locus (QTL) on rainbow trout chromosome Omy9 and suggested that a single major QTL might be responsible for the resistance of the GR strain to WD. However, it must be considered that multiple resistance-loci may be present within this identified QTL region. Nichols et al. (2003) found

a significant QTL region on chromosome Omy9 in relation to another myxosporean parasite, *C. shasta*. It is unlikely, however, that a single gene is causing resistance to both parasites given that salmonid strains resistant to *C. shasta* are susceptible to *M. cerebralis* (Hedrick et al. 2001a).

Resistance to disease in animals is often a complex, polygenic trait that results from a series of complex interactions among the host, pathogen and environment (Snieszko 1974, Hedrick et al. 1998) as observed in rainbow trout resistant to *C. shasta* (Hedrick et al. 2001a). In laboratory experiments, *C. shasta*-resistant rainbow trout strains were highly susceptible to *M. cerebralis*, suggesting different mechanisms of resistance for the 2 parasites (Hedrick et al. 2001b). Until specific genetic and immune mechanisms of resistance are known, we must rely on multiple approaches to explore how resistance is inherited including selective breeding of disease-resistant populations and by using quantitative genetic experiments to measure heritability within fish brood stocks. The establishment of *M. cerebralis*-resistant rainbow trout lineages is still in progress, but quantitative genetic approaches and selective breeding programs have been used to change population characteristics in several other salmonid species (Fetherman et al. 2011, 2014). Statistical and experimental techniques for estimating heritability are often used in selective aquaculture breeding programs (Becker 1992, Falconer & MacKay 1996, Lynch & Walsh 1998). Fetherman et al. (2012) used a quantitative genetics approach to estimate broad sense heritability (h^2_b) of myxospore count of infected trout as a measure of susceptibility to *M. cerebralis*. The results showed that resistance to *M. cerebralis* is a heritable trait and would respond to selection.

Differences in susceptibility offer a unique opportunity to examine the role of host factors determining salmonid resistance to WD. Hedrick et al. (2003) compared the relative susceptibility of the GR rainbow trout strain with a strain of rainbow trout from North America TL in a controlled laboratory exposure and found that the GR strain was more resistant than the TL strain with respect to onset and severity of clinical signs, risk and severity of infections as well as production of mature myxospores. The differences in response of these 2 rainbow trout strains to experimental infections with *M. cerebralis* in laboratory trials combined with empirical evidence collected from the field provide the most convincing evidence that some strains of rainbow trout are more resistant to WD. As previously mentioned, only the Harrison Lake rainbow trout strain from Montana is naturally

resistant to *M. cerebralis* (Hedrick et al. 1998, 2001b, 2003). Studies available to date have failed to identify more strains of rainbow trout from North America with significant resistance to WD (O'Grodnick 1979, Markiw 1992, Hedrick et al. 1998, 1999a,b, 2001b, Thompson et al. 1999, Densmore et al. 2001, MacConnell & Vincent 2002), which may be due to a shorter period of co-evolution of host and parasite. The parasite was presumably introduced to the east and west coasts of the USA during the early 1950s and mid-1960s, with frozen rainbow trout from Europe (Hoffman 1970, 1990, Modin, 1998). Therefore, rainbow trout in the USA had only 50 yr of putative contact with the parasite. Rainbow trout surviving under hatchery conditions have subsequently been used as brood stocks (Schäperclaus 1986). One has to bear in mind that differences in virulence of the parasite in Europe and in the USA may explain the absence of WD outbreaks in recent years among wild and cultured rainbow trout stocks from Germany. Several studies suggest that alterations in host resistance but not geographically different parasite isolates are the principal factors responsible for the lack of WD among rainbow trout from certain German brood stocks.

Differences in *M. cerebralis* development have been observed in GR compared to TL trout (M. Addison et al. unpubl.). Parasites were followed by qPCR through the 3 principal tissue compartments (epidermis, nerves and cartilage) and clear differences in the patterns of development were observed consistent with the greater resistance of the GR strain. Studies comparing brown trout with rainbow trout have shown quantitative and qualitative differences in response to *M. cerebralis* infection in both field and laboratory trials (Hedrick et al. 1999b, Baldwin et al. 2000). Lesions in rainbow trout were located in the cranial (ventral calvarium), gill arch cartilage, vertebrae, jaws, opercula and fin rays. In contrast, in the brown trout, incidence of lesions in the cranial and vertebral tissues was rare while most severe lesions were found in gill arches.

Salmonid immune response to WD

M. cerebralis proliferative stages in the salmonid host effectively evade the host immune system during development and migration through the host nervous system and intracellular development. In the initial host compartment (epidermis and dermis) there are no obvious host cellular associations with the sporoplasm cells as they begin to divide and

spread to adjacent epidermal cells (El-Matbouli et al. 1995). A number of the parasites spontaneously degenerate, perhaps due to a humoral response in the fish skin. Those parasite stages not reaching the peripheral nerves within 2 to 4 d are no longer evident (El-Matbouli et al. 1995). These initial interactions within the skin may be critical in determining why certain salmonid species are more resistant than others. During development of *M. cerebralis* in trout epidermis, some of the parasites are killed; parasitic stages in the subcutis were found to be surrounded by host immune cells such as macrophages (El-Matbouli et al. 1995). At this time, macrophages could be observed at the periphery of lesions in the cartilage (El-Matbouli et al. 1995). Host response varies greatly, depending on the fish species infected and the water temperature. It seems that only late during development and upon significant cartilage damage, do host cellular immune responses become active.

As with most myxozoan parasites, the immune response of rainbow trout following infection with *M. cerebralis* is poorly understood, although a strong acquired immunity to reinfection develops in rainbow trout having survived WD (Hedrick et al. 1998). Resistance to reinfection was shown to develop shortly after but not prior to the appearance of presporogonic stages in the cartilage of rainbow trout (Hedrick et al. 1998). Currently, there are no vaccines in use nor have any been developed to protect fish from *M. cerebralis* infections. In the absence of vaccines or suitable therapeutic approaches, the primary method to control WD among hatchery populations of salmonid fish is to avoid contact with the parasite at least in young of the year fry (Hoffman 1990). However, work with UV-treated parasites (see 'Parasite detection and disease intervention') does provide evidence that a 'putative vaccine' can confer protection (Hoffman 1975, Hedrick et al. 2000, 2007, 2008, 2012).

MYXOBOLUS CEREBRALIS TRANSMISSION STAGES

The transmission success of *M. cerebralis* has been attributed largely to the resistance of the myxospore stage to harsh environmental conditions. Numerous studies have examined the survival of *M. cerebralis* myxospores upon freezing, drying and various disinfectants (Hoffman & Putz 1969, Hoffman & Hoffman 1972, Hoffman & Markiw 1977, O'Grodnick 1979, Wolf & Markiw 1984, Hedrick et al. 2008). The myxo-

sporean spore stages that form in the deeper skeletal tissues of infected fish have hardened shell valves to resist rigorous or changing environmental constraints; spores retain infectivity after freezing, low pH exposure and resistance to enzymatic degradation during passage through the alimentary tract of fish-eating birds or other predators (El-Matbouli et al. 1992). These features of the rigid myxospore stage increase their survival rate in stream sediments until ingested by the oligochaete host. The period that the actinospores are viable is important as their dispersal via water from infected oligochaetes determines in part their range of distribution for transmission to the susceptible fish host.

Although *M. cerebralis* actinospores are able to infect rainbow trout as young as 2 d old, eggs of salmonids do not appear to be susceptible to infection (Markiw 1991). The triggers for the extrusion of polar filaments are a combination of mechanostimulation and a chemical host cue is located in the fish surface mucus. Kallert et al. (2010) isolated stimulating fractions and employed a sequential exclusion approach to identify the active components from natural trout mucus substrate which prompt *M. cerebralis* actinospores to react by polar filament discharge and subsequent sporoplasm emergence. Nucleosides inosine and guanosine were discovered to elicit polar filament discharge and activation of sporoplasm emission, representing the natural chemical stimuli necessary for the rapid host recognition by at least some myxosporean actinospores including *M. cerebralis* (Kallert et al. 2005, 2007, 2009, 2011, 2012). However, *M. cerebralis* actinospores are not able to attach and release sporoplasms into the epidermis of anesthetized fish or fish parts as fins (R.P. Hedrick et al. unpubl. obs.).

OLIGOCHAETE HOST *TUBIFEX TUBIFEX*

T. tubifex is the only species of aquatic oligochaetes known to be a suitable host for *M. cerebralis* (Gilbert & Granath 2002). *Limnodrilus hoffmeisteri*, *Ilyodrilus templetoni*, *Quistadrilus multisetosus* (Wolf et al. 1986), *Dero* spp., *Stylaria* spp., *Aeolomsoma* spp. (Markiw & Wolf 1983), and *Tubifex ignotus* (El-Matbouli & Hoffman 1989) are not susceptible hosts for *M. cerebralis*. Although no worldwide survey for *T. tubifex* has been conducted, it is widely believed that these oligochaetes are cosmopolitan and the distribution of *M. cerebralis* over several continents (Hoffman 1970, 1990, Bartholomew & Reno 2002) supports this view. *T. tubifex* also have the unique

ability to survive drought and food shortages by secreting a protective cyst and lowering their metabolic rate. Cysts containing live *T. tubifex* have been recovered from a cattle pond after 5 mo of drought and oligochaetes in the laboratory survived 6 mo of starvation in cysts (Anlauf 1990). Food shortage, which often occurs as a result of drought, is believed to be the main factor in cyst formation since addition of food is the only means by which oligochaetes exit the cysts (Anlauf 1990). Some researchers have speculated that *T. tubifex* can be dispersed to new locations in these cysts. If oligochaetes infected with *M. cerebralis* could use this type of dispersal mechanism, it could have significant impacts on epidemiology and efforts to control the spread of WD. Similar to other oligochaetes, *T. tubifex* is hermaphroditic; it usually breeds sexually but has the ability to reproduce asexually by parthenogenesis (Poddubnaya 1984). Fertilized eggs are deposited in a cocoon and young oligochaetes hatch directly from the cocoon 7–26 d later depending on temperature. The newly hatched oligochaetes will become sexually mature within 1–3 mo and after reproduction they undergo several cycles of resorption and regeneration of their sexual organs (Poddubnaya 1984).

Individual *T. tubifex* can remain persistently infected with *M. cerebralis* throughout their lifespan and are capable of shedding actinospores for several periods (Beauchamp et al. 2001, 2002, 2006). Our understanding of the interactions between *M. cerebralis* and its oligochaete host *T. tubifex* are limited and exacerbated due to the difficulty in identification of *T. tubifex* based on (1) external morphology, viz., the chaetae and (2) morphology of the reproductive structures viz., the penis sheath. Chaetae of *T. tubifex* can change shape depending on environmental conditions such as salinity (Chapman & Brinkhurst 1987). Multiple molecular studies (Anlauf 1994, 1997, Anlauf & Neumann 1997, Sturmbauer et al. 1999, Beauchamp et al. 2001, 2002, Stevens et al. 2001, Kerans et al. 2004, Steinbach Elwell et al. 2006, Krueger et al. 2006, Baxa et al. 2008, Rasmussen et al. 2008, Fytilis et al. 2013, Nehring et al. 2014) suggest the existence of cryptic species of *Tubifex* that vary in a variety of physiological characteristics including their susceptibility to *M. cerebralis* infection. The wide distribution of *T. tubifex* and its ability to occupy a variety of habitat types suggest that this species may consist of different ecological races (Poddubnaya 1980, Milbrink 1993) or even cryptic species. Anlauf (1994, 1997) concluded that *T. tubifex* populations from Germany consisted primarily of 3 dominant genotypes and that they differed in their

adult weights, reproduction and relative mortality in response to temperature shifts. Sturmbauer et al. (1999) found that *T. tubifex* species consisted of 5 morphologically indistinguishable lineages (designated I through V) that differed significantly in their resistance to cadmium. In addition, the genetic distances observed between some of these lineages were large enough to suggest the existence of at least 2 cryptic species. Beauchamp et al. (2001) used a similar approach and corroborated the findings of Sturmbauer et al. (1999). *T. tubifex* populations analyzed by Beauchamp et al. (2001) formed several distinct lineages and the genetic distances between some of these lineages were similar to those for well-defined species within *Limnodrilus*, suggesting the existence of cryptic species within the genus *Tubifex*. Nehring et al. (2013) recently demonstrated that a changing demographic in oligochaete populations correlated to changes in levels of infectivity in the upper Colorado river suggesting that genetic differences among populations of *T. tubifex* may influence the severity of WD in trout inhabiting the same region. Granath (2014) recently showed that habitat alteration of *T. tubifex* has an effect in reducing the transmissibility of *M. cerebralis*.

PARASITE DETECTION AND DISEASE INTERVENTION

The 18S rDNA sequences widely used in phylogenetic studies provide means for detection of different life stages of myxozoans. Subsequent development of molecular techniques have focused on using primers from variable regions of the 18S rRNA gene. Assays based on PCR amplification of this gene sequence have been developed for *C. shasta* (Bartholomew et al. 1989, 1995, Bartholomew 1998, Palenzuela & Bartholomew 1999), *M. cerebralis* (Andree et al. 1998, 1999, Kelley et al. 2004), *Kudoa thyrsites* (Hervio et al. 1997), *K. amamiensis* (Yokoyama et al. 2000), and *Tetracapsuloides bryosalmonae* (Kent et al. 1998, Morris et al. 2000, Saulnier & de Kinkelin 1997) and other myxozoans. *In situ* hybridisation procedures have also been developed that combine the specificity and sensitivity of DNA detection techniques with the ability to examine the parasite in its biological context (Antonio et al. 1998, 1999, Morris et al. 1999, 2000), which has been extremely useful in studies of parasite invasion and life cycles and in examining mechanisms of host resistance (Antonio et al. 1999). In addition, a loop-mediated isothermal amplification assay has been developed for the rapid

detection of *M. cerebralis* in both fish and oligochaete hosts (El-Matbouli & Soliman 2005).

WD has been effectively managed in hatchery-reared salmonids by preventing or reducing the exposure of young susceptible fish to the infectious actinospore stages (Hoffman 1990). This has been accomplished by rearing young fish in well water to avoid exposures to the parasite until fish grow and become more resistant, or by modifying pond design to minimize habitat options for the oligochaete host (Wolf et al. 1986). While clinical disease manifestations may be controlled by these measures, fish may still become latently infected and produce myxospores. The stocking of subclinically infected fish is one problem responsible for the spread of WD (Hoffman 1970, Modin 1998). In contrast, the actinospore stages released into the water from infected oligochaetes are short-lived and more susceptible to inactivation (Markiw 1992). Removing or inactivating these waterborne infectious stages is viewed as an essential approach to controlling infection among salmonids when surface waters must be utilized for hatchery rearing.

Impact of changing temperature

Temperature is a dominant factor influencing the development of the parasite in the worm and the fish. El-Matbouli et al. (1999a,b) examined the effect of water temperature on development, release and survival of parasite stages in *T. tubifex* and found that the highest level of actinospore production occurred at 10 and 15°C; at higher or lower temperatures spore release was minimal. This may explain why researchers detect the most severe cases of WD in rainbow trout in the 10–15°C temperature range. Baldwin et al. (2000) using a sentinel cage method observed a direct correlation between both prevalence and severity of infection of *M. cerebralis* in rainbow trout and temperature. The highest prevalence of infection and the most severe lesions were found in rainbow trout in an enzootic area with water temperatures between 10 and 12°C. Thus, if yolk-sac trout larvae are emerging from the gravel bottom at these temperatures, rainbow trout reproduction could be severely affected. Additional studies conducted by the Montana Department of Fish, Wildlife and Parks (MDFWP) also show a correlation between water temperature and infection rates in sentinel fish. A seasonal periodicity in actinospore release may perhaps explain the peaks in infection rates of sentinel fish seen by MDFWP and the correlation between

water temperature and severity of *M. cerebralis* infections in rainbow trout (Baldwin et al. 2000, Gilbert & Granath 2001). When temperatures cycle seasonally, as occurs in most natural waters, there may be cyclical shedding of actinospores from a single worm (Thompson et al. 1999, Nehring & Thompson 2003). El-Matbouli et al. (1999b) studied infected oligochaetes at higher water temperatures (>25°C) and have shown that these may overcome infection. Recovered oligochaetes, however, can be reinfected if new spores are available at water temperatures below 20°C. In streams and rivers where WD has caused major trout declines, most likely there is a high release of actinospores in spring and early summer when water temperatures approach 15–16°C. Unfortunately, this is the same temperature period when newly hatched rainbow trout are at their most susceptible age for infection and subsequent development of WD (El-Matbouli et al. 1999a,b). However, in colder waters, *M. cerebralis* actinospores can remain viable and infective for much longer periods.

Chemical treatment

The inactivation of myxospores by either chemical or physical treatments, or potentially by novel biological means, are promising approaches to minimize the spread and potential long-term impact of WD on artificially propagated and naturally reproducing salmonid populations (Beauchamp et al. 2006). Hedrick et al. (2008) found that myxospores both in mud and in suspension are resistant to chlorine. Binding of chlorine to organic matter during treatment probably reduces the active concentrations that reach the myxospores, resulting in a compromised effectiveness of the disinfection process. Myxospores firmly embedded and surrounded by soil and mud would probably be little affected by the disinfectant applied. Quaternary ammonium compounds, particularly the third generation or 'dual quats', are widely used as industrial disinfectants. They serve as cationic surface-active agents that adsorb to, penetrate, and subsequently disrupt cell membranes. Hedrick et al. (2008) suggest that myxospores treated with ADBAC (a commonly used quaternary ammonium compound) at concentrations below 1500 mg l⁻¹ are not fully inactivated. The data of Hedrick et al. (2008) support earlier hypotheses that myxospores are very resistant stages able to withstand environmental extremes, thus significantly hampering control or eradication of the parasite. The apparent disparity between resistance and susceptibility may

result from the co-evolution/selective pressure as mentioned earlier. This might include the ability to tolerate temperature and chemical conditions encountered when passing through key vectors such as predatory birds and fish (El-Matbouli & Hoffmann 1991), but not conditions that are less likely to be routinely encountered in salmonid habitats, such as water temperatures above 20°C, freezing and drying.

Ultraviolet (UV) irradiation

Hoffman & Byrne (1974), Hoffman (1975) and Hedrick et al. (2000) have shown UV radiation to be effective in preventing WD. Hoffman & Byrne (1974) showed that complete protection occurred at calculated UV doses of 35 mJ cm⁻², while calculated UV doses of 27.7 mJ cm⁻² did not provide complete protection (Hoffman 1975). Hedrick et al. (2007) showed complete protection against WD at UV doses as low as 40 mJ cm⁻². Cumulative doses of actinospores need to be considered in order to understand the difference between laboratory and natural exposures. Water treatments with UV under natural exposure conditions have shown that levels of actinospores present at any one point in time will be significantly less than 1000 actinospores fish⁻¹, a concentration found to be completely inactivated in the laboratory challenges of rainbow trout by Hedrick et al. (2007). Therefore, a UV dose continuously maintained for hatchery water supplies of 40 mJ cm⁻² or greater should be highly effective in prevention of WD. Most recently, Hedrick et al. (2012) showed that treatments with UV irradiation at doses from 10–80 mJ cm⁻² either prevented (20–80 mJ cm⁻²) or significantly lowered (10 mJ cm⁻²) actinospore load in experimentally exposed juvenile rainbow trout. Prior treatments of juvenile rainbow trout with UV-treated actinospores (10 and 20 mJ cm⁻²) resulted in a reduced prevalence of infection (Hedrick et al. 2012). Hedrick et al. (2000, 2007, 2012) provide supporting evidence that the early stages of *M. cerebralis* that attach to and then migrate through the host epidermis rely more on chemical and mechanical mechanisms than upon active gene transcription, a process susceptible to one of the primary damaging effects of UV irradiation at the nucleic acid level (Trombert et al. 2007). Thus, the decline and disintegration of UV-treated parasites described in Hedrick et al. (2012) is consistent with the hypothesis that attachment and penetration steps are not significantly impaired by UV irradiation but that subsequent development is compromised. Parasites damaged by UV irradiation

may also be more susceptible to the action of immune cells, i.e. macrophages which have been shown to be associated with elimination of residual developmental stages in the subcutis of rainbow trout at 4–6 d p.e. (El-Matbouli et al. 1995).

The modification and presentation of antigens able to stimulate a strong immune response is one hypothesis for the often striking protection provided by the short-term survival of UV-treated parasites in vaccinated hosts (Wales & Kusel 1992). Protection was most pronounced in fish receiving parasites treated with lower doses of UV irradiation where more protracted replication of UV-treated parasites likely served to better stimulate immunity. The immune response to antigens shared between actinospores and myxospore stages of *M. cerebralis* (Markiw 1989) may further enhance host protection induced by UV-treated parasites by allowing recognition and destruction of developmental stages of the parasite during invasion and development. UV-treatment of *M. cerebralis* provides a putative vaccine that is effective by significantly reducing or eliminating the severity of chronic WD in rainbow trout (Hedrick et al. 2012). Vaccine development for *M. cerebralis* will in part be limited by the current inability to propagate *M. cerebralis* life stages outside of its 2 hosts. Alternatively, the high sensitivity of *T. tubifex* to UV-B irradiation (290–320 nm) can be exploited as an intervention strategy against WD (Soni & Joshi 1997). Soni & Joshi (1997) demonstrated that even small increments in UV-B irradiation resulted in high *T. tubifex* mortality, suggesting that a change in UV-B flux has direct impact on *Tubifex* biomass production.

CONCLUSION

WD among wild trout populations due to *M. cerebralis* is a pressing issue considering its severe negative economic and ecological impact in the wild and also in aquaculture where WD has had its greatest impact. Husbandry methods such as hatching eggs in spore-free water and rearing fry up to the 'ossification' stage in tanks or raceways — some of the prime methods employed to prevent infection of rainbow trout fry — have proved successful in Europe (Ghitino 1970). Considerable new knowledge of the parasite biology, the 2 hosts and the environmental factors influencing their interaction are still growing. New diagnostic methods provide both greater protection against the inadvertent spread of the parasite and serve as important tools to investigate funda-

mental biological and ecological mechanisms of host–parasite interactions. These studies are essential and will ultimately yield knowledge required to enforce control of WD among wild trout. Until that time, we need to further our understanding of the parasite biology and host invasion patterns while concomitantly relying on prudent and practical prophylactic measures conducted by resource managers to minimize the spread of the disease in aquaculture and in wild trout.

FUTURE PERSPECTIVES

The identification of the metallothionein B gene (*metB*) as a candidate for involvement in WD resistance needs to be substantiated. It is unlikely, however, that a single gene is responsible per se in conferring WD resistance. A mechanistic understanding of how salmonid genes differentially express during parasite development in susceptible or resistant salmonid hosts needs to be established. Thus, a chronological study of WD in the TL (susceptible) and GR (resistant) strains of rainbow trout using digital gene expression (DGE) analyses needs to be conducted in order to evaluate the salmonid transcriptome response in WD. Using next-generation RNA-sequencing, gene ontology (GO) annotation and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database analyses, the physiological and pathophysiological relevance of the DGE analyses in WD susceptibility or resistance would be validated and further substantiated by quantitative real-time PCR (qPCR) experiments. Functional genomics and chemical biology tools such as RNA interference technologies to study gene function would offer insights into specific signaling mechanisms for e.g. signaling pathways involving *metB*. Recent studies have highlighted the functional conservation between teleosts and mammals with respect to proteins that belong to the family of suppressors of cytokine signaling (SOCS) which control inflammatory responses mediated via the JAK/STAT signaling pathway (Maehr et al. 2014, Skjesol et al. 2014). Atlantic salmon SOCS1 is a potent inhibitor of interferon-mediated JAK/STAT signaling in teleosts (Skjesol et al. 2014). As the response of the GR and TL rainbow trout strains to WD is linked to the interferon system (Baerwald et al. 2008), it would be of pertinent interest to investigate the role of STAT3, an upstream regulator of metallothionein genes, as a candidate target in WD resistance (Baerwald 2013). Besides, the above transcriptomic approach could also be employed to explore

how differences in WD susceptibility could relate to the presence or absence of particular gene polymorphisms, thus enhancing our understanding of host–parasite interaction mechanisms in WD.

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