1985

SALMONID WHIRLING DISEASE

Ken Wolf
U.S. Fish and Wildlife Service

Maria E. Markiw
U.S. Fish and Wildlife Service

SALMONID WHIRLING DISEASE[1]

Ken Wolf and Maria E. Markiw

U.S. Fish and Wildlife Service
National Fisheries Center–Leetown
National Fish Health Research Laboratory
Box 700, Kearneysville, West Virginia 25430

FISH DISEASE LEAFLET 69

UNITED STATES DEPARTMENT OF THE INTERIOR
Fish and Wildlife Service
Division of Fishery Research
Washington, D.C. 20240

1985

[1]Revision of *Fish Disease Leaflet* 47 (1976), "Whirling disease of trout," by G. L. Hoffman.

INTRODUCTION

Salmonid whirling disease—sometimes referred to as "blacktail"—is a chronic, noncontagious parasitic infection of trout and, to a lesser extent, other members of the family Salmonidae. The parasite has specific tropism for cartilage. If the infection is heavy and the fish is young, mortality sometimes results. More commonly, the parasitosis evokes abnormal swimming behavior and the transient melanism of the caudal fin and peduncle that is the blacktail sign (Fig. 1).

When the parasite is given sufficient time for development and the infection is sufficiently intense, the head and axial skeleton become disfigured in some victims (Fig. 2). However, the behavior and appearance of lightly infected fish are commonly normal, or nearly so (Fig. 3).

The parasite belongs to the group of protozoans known as myxosporeans. Although it was first encountered in Europe at the turn of the century, its life cycle was not discovered until the early 1980's. During the intervening years, methods of diagnosis, detection, and identification were developed and improved.

Newly discovered features of the parasite's life cycle plus suggested procedures for detection, identification, and control are discussed here.

Fig. 1. Fingerling rainbow trout showing the blacktail sign of whirling disease. Inability of the fish to control skin pigmentation is a result of neural impairment.

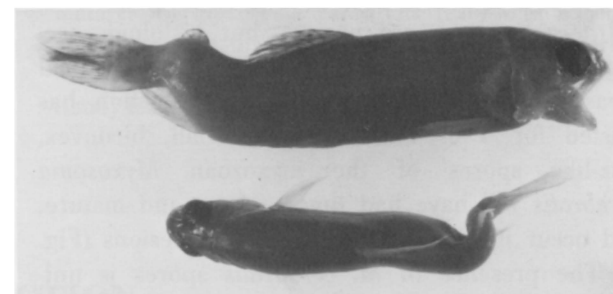

Fig. 2. Extreme distortion of the skeleton caused by whirling disease. These fish were so disabled that they could no longer compete for food, even in a hatchery.

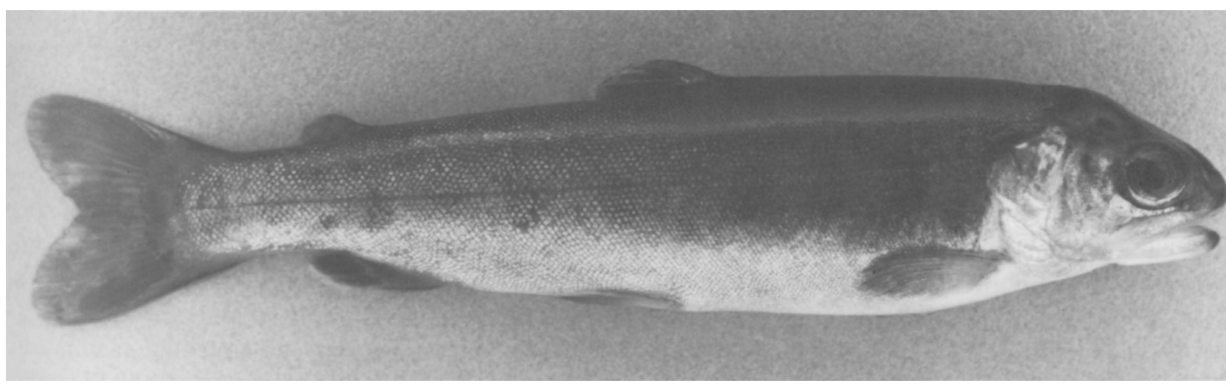

Fig. 3. An otherwise normal-looking fingerling trout showing a slightly sunken dorsal head surface. Outbreaks of whirling disease often involve damage no worse than this. However, such fish usually show whirling behavior.

DIAGNOSIS

Whirling disease can be diagnosed clinically on the basis of changes in fish behavior and appearance. When alarmed or feeding, some infected individuals show an abnormal tail-chasing (whirling) behavior while swimming. The caudal peduncle and tail sometimes become unusually dark or even black (Fig. 1), but that sign is not persistent nor is it specific; moreover, the blacktail sign fades when fish are anesthetized. Deformities of the head or axial skeleton eventually become apparent (Figs. 2, 3). Individually, these signs are less than conclusive, but if all are evident within a population, they collectively constitute a reasonably sound clinical diagnosis.

Internal organs are normal, but histologic sections through cartilage show focal to regional areas of lysis and damage. If the infection has existed for 3 or more months, small, biconvex, disk-like spores of the myxozoan *Myxosoma cerebralis* will have had time to form and mature, and occur in or around the cartilage lesions (Fig. 4). The presence of *M. cerebralis* spores is universally considered to be pathognomonic for whirling disease.

Although hematoxylin and eosin are routinely used in histology, those stains do not enhance the appearance of spores of *M. cerebralis*. In contrast, Giemsa, May-Grunwald-Giemsa, and Ziehl-Neelsen stains are decidedly better because the polar capsules react strongly and distinctly; when one of these stains is applied, the spores become prominent and are easily seen.

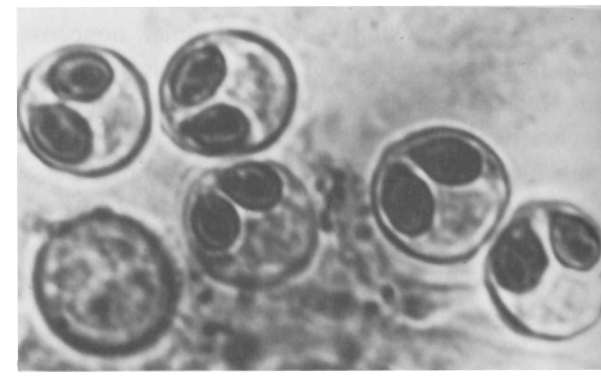

Fig. 4. Stained spores of *Myxosoma cerebralis*. The several spores are 8 to 10 μm in greatest dimension and show two prominent ovate polar capsules within their nearly circular profile.

IDENTIFICATION

As in many myxosporeans, spores of *M. cerebralis* are not particularly distinctive, and attempts at morphologic identification by inexperienced persons commonly result in uncertainty. Referral to a qualified person or laboratory is suggested. The mature spore is biconvex or lenticular and features a nearly circular outline that is 8 to 10 μm in greatest dimension. Two prominent ovate capsules containing coiled filaments of unknown function are at the anterior or polar end of the spore (Fig. 4). However, identification that is based solely on morphology may be erroneous because myxozoans are common parasites, and it is not unusual to find mixed infections of *M. cerebralis* and one or more other myxosporeans. Accordingly, the prudent diagnostician also takes into account the anatomical location of the spores, the geographical location and history of the hatchery, the species of fish involved, and the clinical signs.

The soundest identification is based on size and morphology of the spores, epizootiological data, and serology. In serology, a direct fluorescent antibody technique is used (Markiw and Wolf 1978). Rabbit antiserum against *M. cerebralis* is conjugated with fluorescein isothiocyanate. This preparation reacts with homologous spores and prespores (developmental forms that are smaller than spores and lack polar capsules) and the reactivity can be visualized by fluorescence microscopy, preferably with epiillumination (Fig. 5). Antiserum prepared at the National Fish Health Research Laboratory showed unacceptable cross-reactivity with other genera of myxosporeans when indirect fluorescent antibody tests were used. When the direct test was used, cross-reactivity was found only with another member of the genus *Myxosoma*.

The fluorescent antibody test works best with fresh spores. Spores that have been stored in formalin for a week or more show reduced specific fluorescence, and older specimens show little or none.

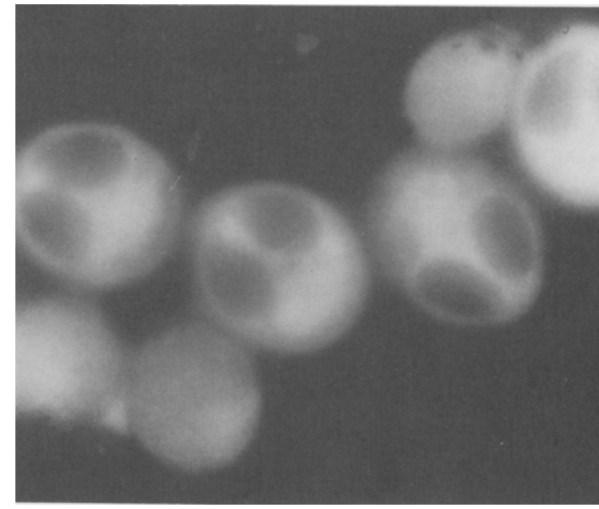

Fig. 5. As seen by fluorescence microscopy, spores and prespore stages (small bodies lacking internal features) of *Myxosoma cerebralis* react with specific antiserum that has been conjugated with fluorescein isothiocyanate. This direct fluorescent antibody test provides serological identification.

DETECTION

Spore detection in full-blown outbreaks of whirling disease—infections that have extended over several months—is relatively easy because mature spores are then abundant. Individual fish sometimes harbor hundreds of thousands of spores. Quantitatively, about two-thirds of all spores are in the head, and more than half of those are in the cartilage of the gill arches (Markiw and Wolf 1974a).

When fish are to be examined for the presence or absence of spores, the sequence of progressively more sensitive procedures that follows is suggested. The search is terminated when spores are found.

The most simple and rapid first step consists of removing and grinding gill arches, and suspending the resulting homogenate in several volumes of water or physiological saline. After the particulate matter has been allowed to settle for a few minutes, several drops of the supernatant are examined microscopically. An objective lens of about 40× is suggested, but experienced persons use 20× or even 10× objectives with confidence. If no spores are found after a search of 5 to 10 min, additional portions of the homogenate should be examined.

If no spores are found during this first simple procedure, one of several more-sensitive methods should be used. The so-called plankton centrifuge method (Prasher et al. 1971; O'Grodnick 1975) is now the most widely used procedure in laboratories where significant numbers of whirling disease examinations are made. (The plankton centrifuge used is Model 903 of G.M. Manufacturing and Instrument Corp., Manuet, New York 10954.) This method works well with either fresh or preserved specimens. It has the advantages of being relatively fast (2–3 h) and of

accommodating the processing of batches of specimens (such as statistical samples) when populations of fish are examined.

When fresh or frozen material is to be processed, heads of fish are removed and held in water at about 50°C for 5 to 10 min to loosen skin, eyes, and other soft tissues for easy removal. The defleshed bony and cartilaginous cranial elements and gill arches are then pooled and reduced by mincing, or by mechanical grinding if much bone is present. The resulting material is suspended in about 10 volumes of water and then reduced by several minutes of processing in a high-speed blender. The homogenized material is then passed through a continuous plankton centrifuge. Centrifuge harvests are collected and suspended in several volumes of water and examined microscopically.

A modification of the plankton centrifuge method resulted in a 10-fold increase in sensitivity (Markiw and Wolf 1980). However, the modification—30 min digestion of the harvest in 0.25% trypsin at pH 7.2 to 7.5—removes residual fish tissue only if the original material is fresh or frozen. Fixation of tissue in formalin denatures protein and prevents enzymatic digestion.

Laboratories that process whirling disease materials are advised to decontaminate equipment after each lot is processed. Half-strength household bleach (about 2.6% sodium or calcium hypochlorite) is suggested because this alkaline solution dissolves spores that might adhere to the instruments and equipment and thus yield a false positive when the next lot is processed.

The most sensitive method of spore detection involves digestion in pepsin and then in trypsin (Markiw and Wolf 1974a,b). The resulting material is then centrifuged on 55% solution of dextrose or sucrose, which retards sedimentation of debris but allows spores to pass through and be concentrated. The method works only with fresh or frozen material—not with tissues that have been fixed in formalin. Elapsed processing time is 6–8 h, but the spores are released efficiently, are concentrated in a small volume, and are virtually free of tissue residues. Accordingly, the method is also used in the preparation of antigens of *M. cerebralis*.

GEOGRAPHIC RANGE

Whirling disease occurs in much of Europe, and all indications are that it originated there. It occurs in the Soviet Union and, apparently by introduction, is now common in the British Isles. It was accidentally introduced into New Zealand and twice into the United States. Halliday (1976) compiled an extensive list of the then-current world distribution of the infection. However, he noted that the cited occurrence in several countries was subject to dispute. The matter could be resolved by making critical examinations in which sentinel populations of susceptible fish and contemporary procedures of spore detection and identification were used.

LIFE CYCLE

The life cycle of whirling disease consists of two phases (Fig. 6): the long-known phase of infection in fish involving the myxosporean *M. cerebralis*, and the newly discovered alternate phase that takes place in *Tubifex tubifex* (Fig. 7), a common oligochaete worm of freshwater environments (Wolf and Markiw 1984). A morphologically distinctive transformation takes place when the organism leaves the fish and begins its change in the worm. The resulting form produced in the worm is an actinosporean provisionally called *Triactinomyxon gyrosalmo*

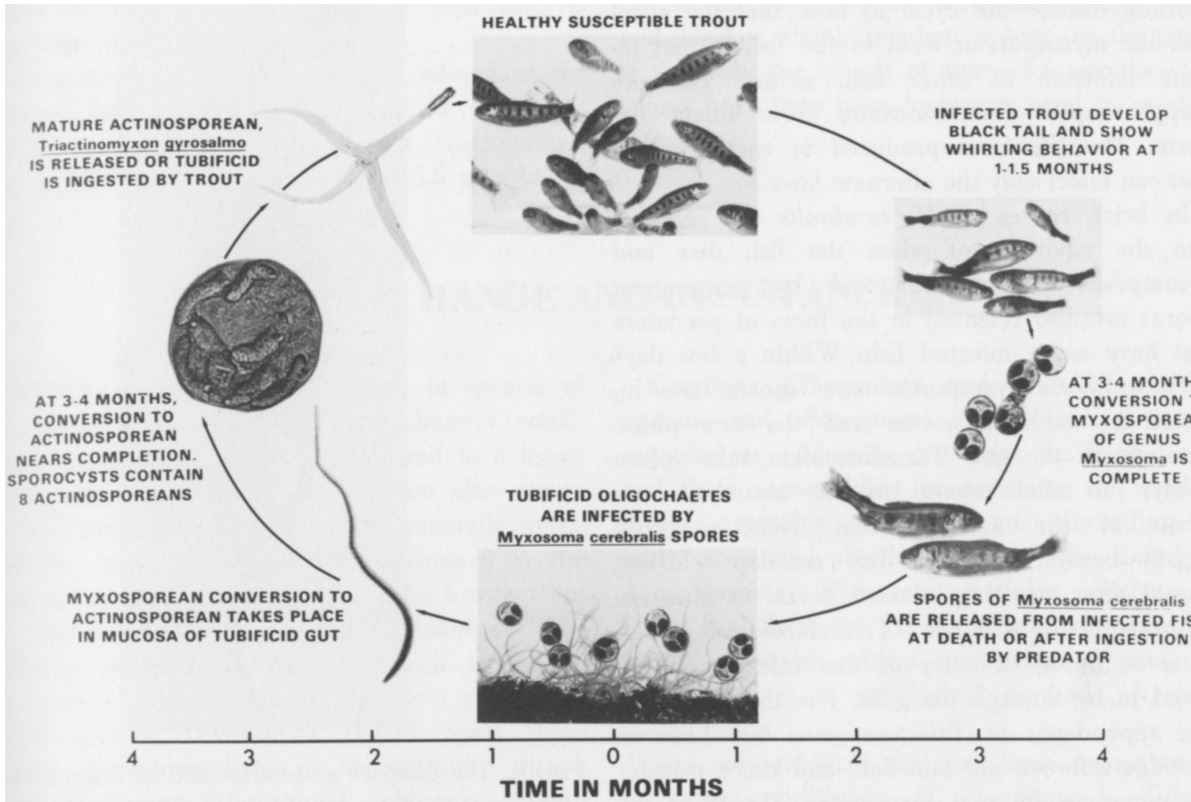

Fig. 6. A diagrammatic representation of the complete life cycle of the whirling disease organism.

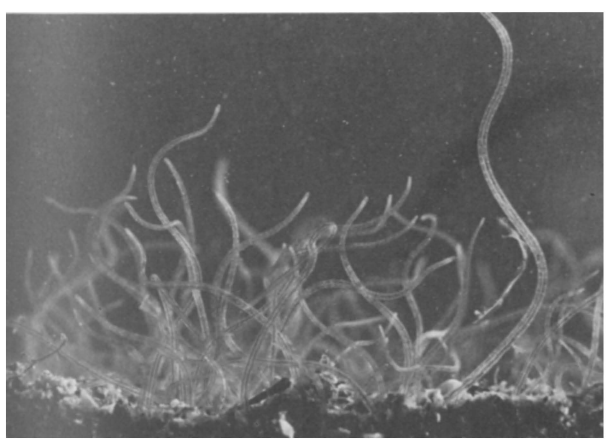

Fig. 7. Tubificid oligochaetes are small reddish worms (2–8 cm long) that are common in organically rich aquatic environments such as earthen trout ponds and streams carrying hatchery effluents.

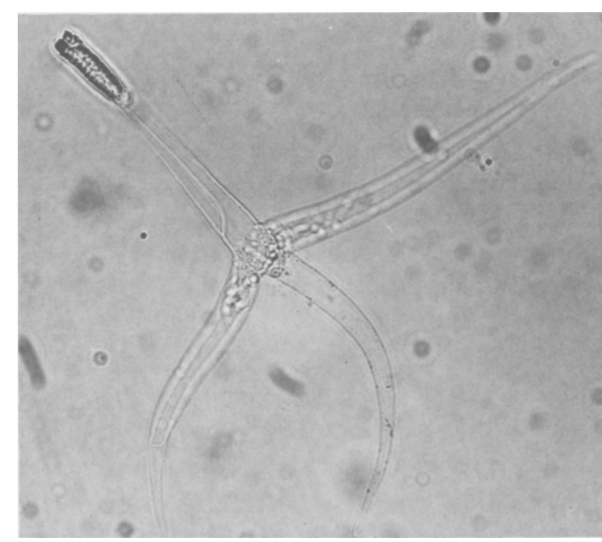

Fig. 8. The organism that initiates whirling disease—a three-parted actinosporean provisionally named *Triactinomyxon gyrosalmo*.

(Fig. 8). It is critical to an understanding of the whirling disease life cycle to note that the small disk-like myxosporean form in the fish cannot initiate infection in other fish; neither can the grapple-shaped *Triactinomyxon* form infect the worm. The life form produced in each kind of host can infect only the alternate host.

In brief, spores of *M. cerebralis* are released into the environment when the fish dies and decomposes or is consumed by scavengers. Spores are also released in the feces of predators that have eaten infected fish. Within a few days at most, the myxosporean-type spores are ingested by tubificid worms and the new phase develops in the gut. Transformation takes place slowly, but after several months abundant new forms of the actinosporean *Triactinomyxon* (Fig. 8) become mature. Fish develop whirling disease after ingesting infected worms or after encountering waterborne *Triactinomyxon*. The route or portal of entry of that infection is believed to be through the gills. The three grapple-like appendages of *Triactinomyxon* are believed to lodge between gill lamellae, and there transfer the numerous internal sporozoites (Fig. 9) to the vascular system of the fish. Once in the fish, spores of *M. cerebralis* mature after about 3 months.

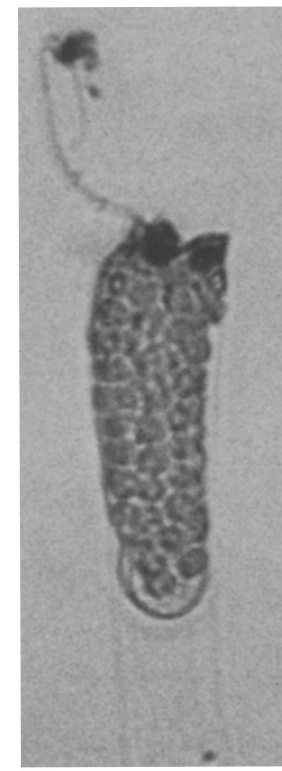

Fig. 9. The epispore or anterior end of *Triactinomyxon* contains three prominent polar capsules (two shown) and 30 to 50 small sporozoites (2–3 μm in diameter).

TRANSMISSION

Whirling disease occurs when fish encounter the infective phase of the protozoan—the waterborne form that is provisionally termed *T. gyrosalmo* or, alternatively, when they ingest the tubificid oligochaete in which the *Triactinomyxon* is produced. Attempts to effect fish-to-fish transmission of whirling disease have been unsuccessful.

INCUBATION

Incubation time is directly related to temperature. Trout fry that are fed infected worms or are exposed to waterborne *T. gyrosalmo* show the blacktail sign after 35 to 45 days at 12°C. Whirling behavior first appears at about the same time or slightly later. Fully mature spores of *M. cerebralis* can first be found in infected fish after 2.6–3.5 months, but spore production continues for weeks and perhaps for months. Incubation time is shortened or length-

ened at temperatures above or below 12°C, to about 50 days at 17°C and 120 days at 7°C (Halliday 1973).

Incubation time in the worm is defined as the interval between first contact with *M. cerebralis* spores and the release of the first *Triactinomyxon*. At 12°C, that interval is about 3.5 months, or about equal to the incubation time required for the development of *M. cerebralis* spores (Wolf and Markiw 1984). Incubation time in the worm is precisely the length of time—3.5 months—that spores have long been known to need to produce infectivity.

HOST RANGE AND RESISTANCE

Whirling disease typically occurs in species of the family Salmonidae. Under husbandry conditions, the usual victims are trout, and to a lesser extent salmon; however, the infection also occurs in wild populations. Investigators generally agree that the rainbow trout (*Salmo gairdneri*) is the most susceptible species and that the brown trout (*S. trutta*) is highly resistant (Halliday 1976; Hoffman and Putz 1969; O'Grodnick 1979). On the basis of tests conducted during 3 years with seven species, O'Grodnick (1979) rated three species as intermediate in susceptibility: brook trout (*Salvelinus fontinalis*), chinook salmon (*Oncorhynchus tshawytscha*), and sockeye salmon (*O. nerka*). The coho salmon (*O. kisutch*) was usually refractory. Although O'Grodnick (1979) found the lake trout (*Salvelinus namaycush*) to be consistently refractory, Hoffman and Putz (1969) reported that exposed lake trout developed clinical signs of whirling disease and produced spores of *M. cerebralis*.

Graylings (*Thymallus*) and whitefishes (*Coregonus* and *Prosopium*), which are generally regarded as salmonids, have not yet been tested and their susceptibility or resistance to whirling disease remains undetermined. According to early accounts, whirling disease was found in nonsalmonids. However, Halliday (1976) believed that these reports might be erroneous. The matter deserves critical reexamination and the application of a serological method of spore identification.

The only tubificid that has been demonstrated to be susceptible to *M. cerebralis* is *Tubifex tubifex*. Members of the genera *Limnodrilus*, *Quistadrilus*, and *Ilyodrilus* were present with *T. tubifex*, but did not yield *Triactinomyxon*. Other genera of oligochaetes that have been tested—*Dero*, *Stylaria*, and *Aeolosoma*—did not produce infectivity for whirling disease (Markiw and Wolf 1983).

IMMUNITY

The immune response of fish to whirling disease has great potential in the development of nondestructive methods of detecting the causal organism. In addition, it provides for possible applications in vaccinating fish to induce immunity. All that can now be said for the immune response, however, is that some evidence has been found that rainbow trout produce antibody against *M. cerebralis* but that little use has been made of this finding. The situation is not clear because, even though antibody is produced, its specificity remains to be demonstrated—or conversely, the tests used need to include adequate precautions to exclude nonspecific reactions. Also, the presence of antibody does not necessarily mean that protection exists.

Relevant literature on the immune response of fish to whirling disease consists of three reports

describing searches for trout antibody against *M. cerebralis*. Griffin and Davis (1978) reviewed reports of two earlier unsuccessful efforts and described their own more productive search. Using an indirect fluorescent antibody technique and serum from rainbow trout known to have whirling disease, they found evidence of antibodies in 16 of 18 samples. However, when serum from specific-pathogen-free fish was tested, 3 of 18 also showed reactivity—presumably a false positive reaction.

The critical point in immunological studies thus far reported is that the myxosporean phase used as the source of parasite antigen is not the stage that is infectious for fish. The new knowledge that the stage termed *T. gyrosalmo* is the infectious stage for fish suggests that that form should be tested as antigen in serological procedures. Moreover, that stage should be the more logical antigen for use in immunization, because it is the form that the fish first encounters and probably has some antigenic components not present in the myxosporean form (*M. cerebralis*).

CONTROL

The broad term control includes the component aspects of avoidance, therapy, chemoprophylaxis, accommodation, decontamination, eradication, and prevention.

Avoidance requires a starting brood stock that is free of whirling disease plus a water supply that is free of the initiator *T. gyrosalmo* or its invertebrate host *Tubifex tubifex*. As an added precaution, fish stocks from geographic regions where whirling disease is enzootic should be avoided. Although all indications are that eggs from infected brood stock do not harbor spores of *M. cerebralis*, discretion suggests that such eggs should not be taken to hatcheries that are free of whirling disease.

Therapy, or an effective treatment of existing whirling disease—i.e., reduction of mortality or clinical signs or both—remains to be developed. Moreover, unlike some other parasites that can be eliminated with drug treatment, *M. cerebralis* is likely to persist in a population in spite of therapy. In that respect, whirling disease is like certain bacteremias such as furunculosis and renibacterial kidney disease. Therapy can effectively reduce losses, but inevitably results in an attendant development of pathogen carriers among the survivors. Although it is not considered therapy, continuous administration of drugs—chemoprophylaxis—has been tried for control, but it too has been found wanting. In no instance was infection prevented, but the most effective products did achieve a measure of reduction in the number of spores produced. Taylor et al. (1973), who fed six drugs to young trout throughout a 1-year test period, found that the greatest benefit was among fish that had been fed furazolidone; they had fewer spores than did control fish and those fed antiprotozoal drugs. However, food containing furazolidone was not palatable, and growth of fish so treated was only half that in the nontreated controls. O'Grodnick and Gustafson (1974, 1975), who conducted continuous feeding tests with 20 different drugs, concluded that, although they exhibited toxicity to trout, the best drugs—furoxone, benomyl, and fumagillin—all somewhat inhibited spore development. Whirling disease has not yet been prevented with drugs, and prospects for effective and practical chemoprophylaxis are poor.

Accommodation is the approach used to minimize mortality and clinical signs in facilities where the principal water supply carries infectivity that cannot be removed or where low levels of infection are acceptable. Accommodation is practiced in Europe, where trout are raised for the table and the stocking of fishing waters is not a major consideration. Economics also contributes to the practice of accommodation. Although the technology is available to decontaminate water supplies that bear infectivity, the

methods require capital investment for equipment and continuing cost of operation and maintenance. Accommodation is based on the fact that fish develop resistance to whirling disease with increasing size or age. In practice, eggs are incubated and the resulting fry and fingerlings are reared in pathogen-free water as long as possible. The objective is to rear fish to fingerling size or larger before crowding necessitates moving them to production facilities and contaminated water. Fingerlings reared in pathogen-free water for several months or more may become infected, but the effects of whirling disease are greatly reduced; the fish often behave normally and appear to be perfectly healthy.

Common methods of food preparation—cooking, canning, and pickling—inactivate spores of *M. cerebralis* in infected fish. Under properly controlled conditions that ensure 66°C for 40 min, hot smoking also kills spores (Wolf and Markiw 1982). Accordingly, such products pose no threat to the spread of the disease.

Where whirling disease cannot be avoided and where the potential for spread of the infection is not a consideration, the propagation of resistant species of salmonids is another approach to accommodation. Brown trout and coho salmon are notably resistant to the disease; however, these species can become infected and not show signs of the condition.

Most of the literature dealing with decontamination, eradication, and prevention was published before the infective stage, *T. gyrosalmo*, and its invertebrate host, *Tubifex tubifex*, were known. In addition, conclusions drawn from such early work were based on methods of spore detection that are much less sensitive than those available today. In the best method of assessment now used, sample populations of susceptible young "sentinel" fish are used to determine the results of decontamination or eradication procedures.

In theory, tubificid worms can be killed by thorough drying or by chemical treatment of their aquatic soil habitat. In practice, however, only partial kills have been effected. Climatic factors and worm biology contributed to the incomplete success. As examples, precipitation occurs at most locations where trout are raised and, although earthen holding facilities might be drained, the soil realistically cannot be thoroughly dried. Some worms usually survive as a result of their burrowing ability and reproduction by the formation of resistant cocoons, and the invariable presence of burrows of vertebrates and invertebrates in aquatic habitats. Chemicals such as calcium hydroxide or calcium cyanamide have been recommended for and used in decontamination or eradication.

It is worth noting, however, that such recommendations were made before the role of tubificids was recognized. In practice, whirling disease has not been eradicated by chemical treatment of earthen holding facilities. It has not been possible to maintain effective concentrations of chemical compounds long enough. Distribution of chemicals to uniformly adequate depth is probably a contributing factor. Success has been achieved when the water supply was free of infectivity and when earth was replaced with concrete in the construction of holding facilities.

Although chemicals that are selectively lethal for tubificids are not now known, such compounds could, if applied regularly and at adequate concentrations, result in eradication. However, possible effects on other aquatic biota would have to be considered.

Decontamination of waterborne infectivity has been effected; the best results have been obtained by combining filtration to remove or reduce suspended material with ultraviolet irradiation (Hoffman 1974, 1975).

ANNOTATED BIBLIOGRAPHY

Griffin, B. R., and E. M. Davis. 1978. *Myxosoma cerebralis*: detection of circulating antibodies in infected rainbow trout (*Salmo gairdneri*). J. Fish Res. Board Can. 35(9):1186–1190.

Indirect fluorescent antibody technique was used to detect circulating antibodies against *Myxosoma cerebralis*. Antibodies were found in 14 of 18 infected fish. Of 18 sera from specific-pathogen-free or control fish, 15 showed no evidence of antibodies. Correlation between the presence of spores and antibodies was significant ($P \leq 0.01$), but less than absolute.

Halliday, M. M. 1973. Studies on *Myxosoma cerebralis*, a parasite of salmonids. II. The development and pathology of *Myxosoma cerebralis* in experimentally infected rainbow trout (*Salmo gairdneri*) fry reared at different water temperatures. Nord. Vet. Med. 25:349–358.

Experiments with fish held at 7, 12, or 17°C showed that maturation of *Myxosoma cerebralis* was temperature dependent. The highest temperature accelerated the process and the lowest one slowed it.

Halliday, M. M. 1976. The biology of *Myxosoma cerebralis*: the causative organism of whirling disease of salmonids. J. Fish Biol. 9(4):339–357.

Somewhat outdated, but nevertheless the most comprehensive review of the subject. More than 100 references are cited.

Hoffman, G. L. 1974. Disinfection of contaminated water by ultraviolet irradiation, with emphasis on whirling disease (*Myxosoma cerebralis*) and its effect on fish. Trans. Am. Fish. Soc. 103(3):541–550.

Whirling disease was avoided under experimental conditions in which waterborne infectivity was passed through a 25-μm filter and an ultraviolet irradiation unit that provided at least 35,000 microwatt seconds per square centimeter at a wavelength of 2,537 angstroms.

Hoffman, G. L. 1975. Whirling disease (*Myxosoma cerebralis*) control with ultraviolet irradiation and effect on fish. J. Wildl. Dis. 11(4):505–507.

Combined 25 μm filtration plus ultraviolet irradiation at 18,000 or 27,650 microwatt seconds reduced but did not eliminate infectivity. A dosage of at least 35,000 microwatt seconds is suggested.

Hoffman, G. L., and R. E. Putz. 1969. Host susceptibility and the effect of aging, freezing, heat, and chemicals on spores of *Myxosoma cerebralis*. Prog. Fish-Cult. 31(1):35–37.

Coho and chinook salmon and lake trout tested showed clinical whirling disease and produced *Myxosoma cerebralis* spores. The work confirmed an earlier finding that spores had to be "aged" to yield infectivity. Frozen spores survived only 18 days. Heat and drastic chemical treatment gave presumptive evidence of spore death.

Markiw, M. E., and K. Wolf. 1974a. *Myxosoma cerebralis*: isolation and concentration from fish skeletal elements—sequential enzymatic digestions and purification by differential centrifugation. J. Fish. Res. Board Can. 31(1):15–20.

Defleshed skeletal elements are reduced in size, and subjected to pepsin and then trypsin digestion the resulting material is centrifuged through a sugar solution that retains debris but allows spores to sediment. The method is sensitive and provides purified spores for use as antigen. The method does not work with fixed specimens. The report concludes with a list of stepwise procedures to be used for efficient detection and diagnosis.

Markiw, M. E., and K. Wolf. 1974b. *Myxosoma cerebralis*: comparative sensitivity of spore detection methods. J. Fish. Res. Board Can. 31(10):1597–1600.

A sequence of four methods was applied. The most sensitive method was that of pepsin then trypsin digestion plus differential centrifugation. One group of fish with a population incidence of 18% yielded about 150 spores per fish—a light infection—whereas more heavily infected populations yielded as many as 10,000 spores per fish.

Markiw, M. E., and K. Wolf. 1978. *Myxosoma cerebralis*: fluorescent antibody techniques for antigen recognition. J. Fish. Res. Board Can. 35(6):828–832.

Rabbit antiserum was prepared against homogenates of prespores and spores of *Myxosoma cerebralis*. When it was conjugated with fluorescein isothiocyanate and used in indirect fluorescent antibody tests on other myxosporeans, an unacceptable level of cross-reactivity occurred. In direct tests, cross-reactivity occurred only with an organism of the genus *Myxosoma*. The antiserum provides a serological method of identification. (In later research, Wolf and Markiw [1984] showed that the preparation gave a homologous reaction with *Triactinomyxon gyrosalmo*, and thus supported the claim that an actinosporean was the alternate life stage of *M. cerebralis*.)

Markiw, M. E., and K. Wolf. 1980. *Myxosoma cerebralis*: trypsinization of plankton centrifuge harvests increases optical clarity and spore concentration. Can. J. Fish. Aquat. Sci. 37(12):2225–2227.

Typical harvests of spores from plankton centrifuge processing were digested with 0.25% trypsin for 30 min at pH 7.2–7.5. More than 20% additional spores were released, resulting in a 10-fold increase in concentration. Reduction of tissue residues aided visualization. The method does not work with specimens that have been fixed.

Markiw, M. E., and K. Wolf. 1983. *Myxosoma cerebralis*: (Myxozoa: Myxosporea) etiologic agent of salmonid whirling disease requires tubificid worm (Annelida: Oligochaeta) in its life cycle. J. Protozool. 30(3):561–564.

The so-called "aging" process during which spores develop or become infectious was demonstrated not to occur endogenously. Instead, a tubificid oligochaete of the genus *Tubifex* was essential to production of infectivity. Trout developed whirling disease after having been fed tubificids from a fish hatchery where the disease was known to occur or tubificids that had been kept with *M. cerebralis* spores for about 4 months. Tubificids of the genera *Dero, Stylaria*, and *Aeolosoma* did not develop whirling disease infectivity.

O'Grodnick, J. J. 1975. Whirling disease (*Myxosoma cerebralis*) spore concentration using the continuous plankton centrifuge. J. Wildl. Dis. 11(1):54–57.

The plankton centrifuge method of spore release and concentration is described. (See also Prasher et al. 1971.)

O'Grodnick, J. J. 1979. Susceptibility of various salmonids to whirling disease (*Myxosoma cerebralis*). Trans. Am. Fish. Soc. 108(2):187–190.

During a period of 3 years, seven species were tested. The rainbow trout was found to be most susceptible, and brown trout and coho salmon the most resistant. Sockeye and chinook salmon and brook trout were judged to be intermediate in response. In contrast with findings of Hoffman and Putz (1969), lake trout were completely refractory.

O'Grodnick, J., and C. C. Gustafson. 1974. A study of the transmission, life history and control of whirling disease of trout. Pa. Fish Comm., Fed. Aid Fish Restor., Prog. Rep. F-35-R-6. 31 pp. Mimeographed.

The work summarized showed egg transmission to be unlikely; also that the infection was not prevented by chronic feeding of nine medicinal compounds. The sequence of histological changes in infected cartilage is il-

lustrated xerographically. Concluding sections on disinfection with calcium hydroxide and ultraviolet radiation indicated that infectivity was reduced but not eradicated.

O'Grodnick, J., and C. C. Gustafson. 1975. A study of the transmission, life history and control of whirling disease of trout. Pa. Fish Comm., Fed. Aid Fish Restor. Prog. Rep. F-35-R-7. 34 pp. Mimeographed.

Twelve drugs were tested in chronic feeding trials. The most effective ones reduced but did not prevent spore development. Sequential development of the parasite was followed by histology and illustrated xerographically. Soilborne infectivity was reduced by the application of calcium hydroxide. Waterborne infectivity was eliminated by filtration combined with ultraviolet irradiation.

Prasher, J. B., W. M. Tidd, and R. A. Tubb. 1971. Techniques for extracting and quantitatively studying the spore stage of the protozoan parasite *Myxosoma cerebralis*. Prog. Fish-Cult. 33(4):193-196.

First report of the use of a plankton centrifuge for concentrating *Myxosoma cerebralis* spores.

Taylor, R. E. L., S. J. Coli, and D. R. Junell. 1973. Control of whirling disease by continuous drug feeding. J. Wildl. Dis. 9(4):302-305.

Six drugs were fed continuously for 1 year to young susceptible trout that were held in water containing whirling disease infectivity. Spore development occurred in all lots but was least in fish fed furazolidone. That compound was unpalatable, however, and retarded fish growth by 50%.

Wolf, K., and M. E. Markiw. 1982. *Myxosoma cerebralis*: inactivation of spores by hot smoking of infected trout. Can. J. Fish. Aquat. Sci. 39(6):926-928.

Carcasses of 2-year-old infected rainbow and brook trout were held in 8% brine for 16 h and then smoked at 66°C for 40 min. The complete process inactivated spores of *Myxosoma cerebralis*, but brining alone did not.

Wolf, K., and M. E. Markiw. 1984. Biology contravenes taxonomy in the Myxozoa: New discoveries show alternation of invertebrate and vertebrate hosts. Science 225(4669):1449-1452.

Describes the discovery of the life cycle of *Myxosoma cerebralis*. After host fish death or ingestion by a predator, spores enter the environment and infect tubificid oligochaetes. A new phase begins and ends several months later with maturation of new forms known as actinosporeans. The transformed organism, provisionally named *Triactinomyxon gyrosalmo*, infects trout and initiates the alternate phase of whirling disease.

Note: Use of trade names does not imply U.S. Government endorsement of commercial products.

☆ U.S. GOVERNMENT PRINTING OFFICE: 1985—576-063/25219 REGION NO. 8