427

WHIRLING DISEASE OF TROUTS CAUSED BY *Myxosoma cerebralis* IN THE UNITED STATES

SPECIAL SCIENTIFIC REPORT–FISHERIES No. 427

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FISH AND WILDLIFE SERVICE

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Glenn L. Hoffman, Clarence E. Dunbar,[1/] and Arthur Bradford[2/]

ABSTRACT

This disease has recently appeared in North America for the first time. It appeared in a Pennsylvania trout hatchery and may have spread from there to the Lamar National Fish Hatchery which is on the same watershed, and from Lamar to the Kensington State Hatchery in Connecticut in transferred fish. The parasite develops in the cartilage, primarily of the head of very small trout. Symptoms of blacktail, whirling, spinal curvature and misshapen heads follow skeletal damage.

It was not possible to infect rainbow trout fry experimentally with the spores although the incidence was very high in one of the hatcheries. The development of the parasite was studied in infected fish brought from the Benner Spring Hatchery to Leetown. In histological sections the parasite can be seen as a small multinucleate trophosoite at 3 months after infection. The first spores can be seen at 4 months and persist for at least 3 years. Spores can be found in wet mounts prepared from head cartilage.

A program for control has been started, and the incidence appears to be declining in the Benner Spring Hatchery. The spring water reservoir was chlorinated, the ponds cleaned and potassium cyanamide applied. Acetarsone (Stovarsol) was fed to one lot of small trout at high concentrations with no noticed side effects, but control of whirling disease was not determined.

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This disease, apparently of central European origin, has more recently appeared in Russia, Italy, and the United States. Trout usually become infected during the first few weeks of feeding, mortalities ensue and most of the surviviors exhibit disease symptoms for 3 or more years. The spores gain entrance to the fish, presumably through accidental ingestion, and the sporoplasm of the spore emerges and migrates to the cartilage, mainly that of the head. The very small sporoplasm, now called a trophosoite, grows and its nuclei divide repeatedly to form a much larger organism which finally produces the spores. During the growth of the parasite much host cartilage is eroded and the skeleton weakened, resulting in the symptoms -- whirling, black tail, gaped jaws, misshapen heads and trunks. The whirling is caused by damage to the cartilaginous capsule of the organ of equilibrium; the blacktail is caused by damage to the skeleton in the region of the nerves which control the posterior pigment cells.

1/ Eastern Fish Disease Laboratory, Leetown (P.O. Kearneysville), West Virginia.
2/ Benner Spring Research Station, Pennsylvania Fish Commission, Bellefonte, Pennsylvania.

To the best of our knowledge no other group of parasites has representatives which develop in cartilage or bone of the host, but among the Myxosporidia there are 6 other species which have been so recorded. They are: Myxobolus aegelefini from the head skeleton of Gadus, Pleuronectes, Molva, Merluccius (marine) (Kabata, 1957); Myxobolus dentium from the base of the teeth of Esox masquinongy (Fantham et al. 1939); Henneguya brachyura from the fin ray of Notropis (Ward, 1919); Henneguya sp. in the cartilage of gill of Pomoxis (Davis, 1923); and Myxosoma sp. in the head cartilage of Lepomis macrochirus (Hoffman, 1961, unpub. research). To date the pathogenicity of cartilage parasites has been studied only in M. cerebralis infections.

During the past 3 years, we have studied the symptoms, the cause, and have attempted to infect fish experimentally to study methods of control and treatment. Our results are here incorporated in a review of the subject.

HISTORICAL AND GEOGRAPHICAL

The parasite and disease were first described as Lentospora cerebralis by Hofer (1903) in Germany. His associate, Marianne Plehn (1904, 1924) described the disease, the parasite, and the histopathology in greater detail. Schäperclaus (1931, 1954) also described the disease in detail and outlined a method of control. Heuschman (1940, 1949), Tack (1951), and Luling (1952) in Germany have also studied whirling disease and its control. Vanco (1952) records it from France, Kocylowski (1953) from Poland, Scolari (1954) from Itally, Dyk (1954) and Volf (1957) from Czechoslovakia, Uspenskaya (1955, 1957) in trout and salmon from Russia, Schäperclaus (1959, pers. comm.) from Denmark, and Bogdanova (1960) in salmon from S.E. Russia. Hoffman and Dunbar (1961) briefly reported on it from the United States.

The chronology of the reports indicates that the disease originated in Central Eurpoe. In support of this hypothesis is the fact that the brown trout, a native of Europe, may become infected but is resistant to the disease, whereas the imported rainbow trout becomes seriously diseased.

OCCURRENCE IN THE UNITED STATES

Whirling disease appeared in brook trout at the Benner Spring Fish Research Station, Bellefonte, Pennsylvania, in 1956; one pond of fish was affected. European trout were circumstantially implicated in the appearance of whirling disease at Benner Spring; sample purchases of frozen imported table fish may have been fed accidentally, or their viscera discarded in streams. The epizootic became severe in the hatchery in 1957 and 1958 at which time a tentative diagnosis was made by Dr. S. F. Snieszko. This was verified histologically by Dr. E. M. Wood, Fish Pathologist, Seattle, Washington. In the spring of 1960 an attempt was made to eradicate the parasite by using calcium cyanamide (1/10 lb. per sq. ft.) in the dirt ponds as recommended by Dr. Schäperclaus of Germany, and chlorine gas (max. 300 ppm) in the spring water source and small reservoir. A few trout were killed in the small reservoir, but it was not certainly known if this was the source of infection for the hatchery. There was no recurrence of the disease in 1961 fingerlings in the hatching building. We believe the disease is under control and can be eliminated completely in a year or so. Whirling disease did reappear later in some of the dirt ponds, however. As long as the spring water supply remains uninfested there probably will be no recurrence of the disease in the hatching building. We hope the spores presumably remaining in and around the ponds will eventually be eliminated.

The National Fish Hatchery, Lamar, Pa., is on the same watershed as Benner Spring and in 1960 Mr. Fred Howard discovered the disease there. The disease was verified by the Eastern Fish Disease Laboratory. There were only a few fish affected, and we believe that only one series of ponds was contaminated. The Lamar Hatchery plans to treat the ponds, when drained, with calcium cyanamide. All diseased fish were incinerated.

In December 1961, Mr. Lyle Pettijohn diagnosed Myxosoma cerebralis disease in fingerling rainbow trout from the Kensington State Fish Hatchery, Kensington, Connecticut. The hatchery personnel had noticed symptoms,

apparently of M. cerebralis disease, about a year earlier. The source of infection may have been in one lot of trout transferred from Lamar to Kensington in 1959.

To our knowledge whirling disease caused by M. cerebralis has not been verified anywhere else in North America. Dr. R. Bangham (pers. comm.) recalls identifying M. cerebralis at a northern Wisconsin hatchery about 1945 but no specimens are available for our verification. We have seen similar whirling at three other hatcheries but could find no M. cerebralis and assume that some physiological disturbance or other disease may also cause this symptom. No other characteristic symptoms were present in these fish.

If the spreading of this disease is not halted shortly, it can be expected to show up in trout and salmon hatcheries where infected fish are transferred and particularly those which have earthen ponds, trout in spring water reservoirs or stream water supply.

SYMPTOMS AND THE COURSE OF THE DISEASE

The symptoms are thoroughly discussed by Plehn (1904), Schäperclaus (1954) and Uspenskaya (1957) but will be reviewed here because of the lack of a previous English discussion. The symptoms we have seen are identical with those reported in Europe.

Trout may become infected up to one year of age (Schäperclaus, 1954) but usually become infected during the first few weeks of life--the earlier the infection, the more severe the disease because of the greater amount of cartilage present in younger fish. As we have pointed out, we were not able to prove that trout become infected by ingesting the spores, but this should be assumed until disproven. The disease takes the following course:

Period of "incubation". After exposure to the spores a lapse of 40-60 days ensues before the symptoms of whirling disease are evident (Schäperclaus, 1954). Our experimental fish in which we were never able to demonstrate the parasite exhibited symptoms in 12 to 16 days. We mention it here because there may be other conditions which simulate M. cerebralis whirling disease. We do not know for certain whether the disease causes mortalities during the "incubation" period. The parasites are so small during this period that histological verification is probably impossible or extremely difficult and mortalities might be attributed to other factors.

Initial symptoms. The most obvious symptoms, tail-chasing, whirling and black tail (fig. 1), become evident at about 40 to 60 days and may last about 1 year. The trophozoites have invaded and eroded the cartilage of the developing skeleton. Rather large "lesions" containing the parasites and debris can be seen in histological preparations. The cartilaginous capsule around the auditory-equilibrium organ behind the eye is usually invaded. Perhaps toxins released by the parasite (Plehn, 1904) or simply weakening of the capsule destroys the equilibrium of the fish to such an extent that each time it is disturbed or tries to feed it goes into a frantic tail-chasing whirl. This tail-chasing type of whirling differs from the horizontal spiraling of the fish along its long axis which is characteristic of a virus disease, infectious pancreatic necrosis (Snieszko and Wolf, 1958) and possibly hexamitiasis (octomitiasis) (Davis, 1953). Small fish, up to 3 months of age (about 2 inches long) may become so exhausted that they fall to the bottom and remain on their sides until they regain their strength. It is during this period that mortalities are likely to occur. Other debilitating factors such as other parasites, bacterial or viral diseases or malnutrition will probably increase mortalities. During the early part of this period the whirling symptom is at its worst, but it tends to subside gradually until it is only rarely seen in fish one year post-infection.

Very often the cartilage of the vertebral column, posterior to the 26th vertebra, is simultaneously affected (Plehn, 1904; Schäperclaus, 1954). The sympathetic nerves which control the caudal pigment cells have their origin at about the 26th vertebra. Apparently the weakened skeleton at this point causes pressure on the caudal nerves and pigment cell control is lost, hence the black tail. The black tail tends to dissappear earlier than the whirling symptoms. One lot of about 4,000 rainbows at Kensington became infected when brought to the hatchery at

Figure 1:--Photograph of black-tail rainbow trout caused by Myxosoma cerebralis - about 3 months post-infection. Photograph by S. F. Snieszko.

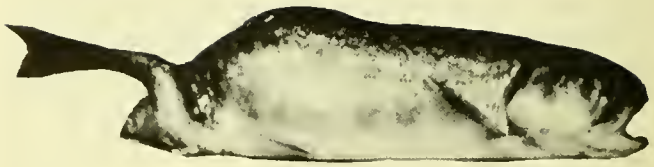


Figure 2:--Photograph of eastern brook trout with spinal curvature and black tail due to Myxosoma cerebralis infection. Photograph by S. F. Snieszko.

6 months of age; an estimated 100 percent of these developed whirling symptoms, but no black tail.

Survivors' symptoms (figs. 2 and 3). Those infected fish which were not killed by the parasite during the early stages of the disease tend to recover although they may be misshapen, particularly in the head. The black tail and whirling usually disappear but the spinal curvature and misshapen head may reflect permanent damage. The two most common and obvious head symptoms are the sunken areas behind the eyes and the permanently open or twisted lower jaw. All of these symptoms are caused by the loss of cartilage during bone formation when the skeleton is weakened and support is lost. During the latter part of the first year much of the damaged area is filled in with an epitheloid granuloma type of tissue which tends to proliferate in many instances and cause secondary damage. Plehn (1904) p. 163 has an excellent illustration of such a tissue proliferating from a vertebra and causing pressure on a sympathetic nerve. Presumably these "recovered" fish can live for a long period of time so long as they are not crippled too badly to feed. In spite of apparent recovery, however, the spores remain in the tissues of the fish for at least 3 years.

Figure 3:--Photograph of yearling rainbow trout with spinal curvature due to Myxosoma cerebralis infection.

TRANSMISSION AND HOST SPECIFICITY

It has long been assumed that, if ingested, the spores taken directly from infected trout will infect other trout (Plehn, 1904; Schäperclaus, 1931, 1954). As previously discussed, we were not able to do this experimentally at Leetown, and to our knowledge no one else has. Therefore, all knowledge concerning transmission and life cycle has come from studies on material collected during epizootics.

Trout fry can be infected by exposing them to water containing the silt, and presumably the spores, of ponds from a hatchery epizootic (Schäperclaus, 1931). One assumption is that the spores are ingested accidentally, but the possibility of an invertebrate transport host has not been ruled out. Another assumption is that spores are freed from infected fish when they decompose or are crushed. Uspenskaya (1957) has found isolated spores in various organs of the fish and suggests that they may be carried away from the site of infection by blood or lymph and be deposited in other organs. If this includes the intestine, they could be shed while the fish is still alive. However, it has not been determined whether fish can be infected by this method. Apparently the freed spores accumulate in the ponds (particularly earthen ponds) and the severity of the epizootic depends on the number so accumulated. Small trout up to four months are most severely affected. The disease can be controlled therefore, but perhaps not eliminated, by keeping the trout in spore-free water until they are four months or more of age. Older fish may become infected, but are usually not seriously affected because ossification of the skeleton prevents massive infection. Such fish, however, may act as "carriers".

On the basis of epidemilogical evidence the spores are apparently very resistant to drying, freezing, and survive a long period of time (Plehn, 1904, 1924; Schäperclaus, 1931, 1954). We have kept 2 vials of spores for 3 years, one at room temperature, the other at about 6° C. At the end of 22 months all spores appeared normal, but at 3 years the sporoplasm has completely disappeared from 85 percent and did not appear normal in the remaining 15 percent. We believe they were 100 percent non-viable.

It is likely that the spores can be carried from pond to pond or hatchery to hatchery on boots and other equipment. Schäperclaus (1931) found myxosporidean spores in the feces of kingfishers at an affected hatchery and believes that the disease can be spread in this manner.

Most myxosporidean species show varying degrees of specificity for certain species or closely related species of fish. They are also usually specific for a certain organ or tissue. Myxosoma cerebralis is no exception--it has been found in rainbow trout (Salmo gairdneri); eastern brook trout (Salvelinus fontinalis); brown trout (Salmo trutta) and recently in salmon (Salmo salar) (Uspenskaya, 1957), grayling (Volf, 1957), and in Salvelinus leucomaenis, S. malma, Oncorhynchus keta and O. masu (Bogdanova, 1960). Rainbow trout are most seriously affected by the disease, brook trout somewhat less severely, and brown trout show no symptoms at all but may act as "carriers". Likewise, any symptom-free but infected rainbow or brook trout may be serious "carriers". The initial infection in a fish is always in cartilage, but loss of cartilage and proliferation of tissue may leave the spores outside of the skeleton in little cyst-like structures.

LIFE CYCLE

The complete life cycle of Myxosoma cerebralis (Lentospora c.) has never been determined experimentally (fig. 4). We know of no one who has demonstrated the experimental life cycle of any Myxosoma or related Myxobolus or Henneguya species. These are all histozoic parasites. Plehn (1904, 1924), Schäperclaus (1954), and Uspenkaya (1957) assume that the spores are ingested, the sporoplasm leaves the spore, penetrates the intestinal mucosa and migrates to the cartilage. However, this has never been verified experimentally. Kudo (1930), p. 313, reviews experimental infections and cites two workers who successfully infected fish with three coelozoic myxosporideans--Myxidium, Chloromyxum, and Leptotheca; but none of the histozoic Myxosoma, Myxobolus, or Henneguya are mentioned.

The various tissue stages from hatchery epizootics have been described by Plehn (1904)

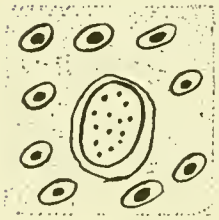
INFECTED SALMONID CONTAINS SPORES
4 MO TO AT LEAST 3 YRS AFTER
BEING INFECTED

SIDE FRONT

SPORE RELEASED UPON DEATH
OF FISH

SPORES MAY BE FOUND IN LESIONS
IN BONE AND GRANULOMAS FROM ABOUT
4 MO AFTER INFECTION TILL AT LEAST
3 YRS

SMALL FISH PROBABLY BECOMES
INFECTED BY INGESTING SPORES
—NOT PROVEN EXPERIMENTALLY



MULTINUCLEATE SPOROPLASM, NOW
CALLED TROPHOZOITE, MAY BE FOUND
IN CARTILAGE FROM 40 DAYS TO 3 MO
AFTER INFECTION

POLAR FILAMENTS EXTRUDE IN INTESTINE.
SPOROPLASM EMERGES, PROBABLY INVADES
INTESTINAL WALL AND MIGRATES TO HEAD
CARTILAGE

Figure 4:--Life cycle of Myxosoma cerebralis. (Note that experimental infection of fish has never been achieved).

and Schäperclaus (1931). In neither instance, however, was the material from experimental infections, i.e., the fish were not exposed to infection under controlled conditions. Schäperclaus (1931) held newly feeding fry in infested ponds for 11 days and then observed them in aquaria supplied with uninfested water. Symptoms of black-tail disease (tail-chasing whirl and black tail) developed at 35-46 days. The fish recovered in about 2 months but the infections were not verified histologically (Schäperclaus, 1961, pers. comm.). It is not known for certain exactly how they acquired the parasites.

The sporoplasm becomes the multinucleate amoeboid trophozoite that can be seen in the histological sections of cartilage from about 40 days post infection (Schäperclaus, 1954) to 3-4 months. The trophozoite grows and the nuclei divide and differentiate to produce units known as pansporoblasts, containing 12 nuclei each which eventually produces 2 spores at about 4-6 months. Spores are probably released after the death and disintegration of the fish. However, Uspenskaya (1957) has found spores in various organs other than skeleton and believes that some make their way to the outside through the intestine during the 4-9 month phase of the disease. Spores have been found in fish up to 3 years of age (Uspenskaya, 1957). It is assumed that newly feeding trout fry become infected by ingesting spores which have been released from the skeleton and associated lesions of older fish that have died or been crushed. However, no one has reported on establishing the disease experimentally by feeding fresh spores to fry or by holding the fry in water to which fresh spores have been added.

In an attempt to reproduce the life cycle and to test disinfectants, we set up 47 experiments in 1959. Spores were fed to 47 lots of 6-24 rainbow and brook trout each. The fish ranged from 2 weeks to 4 months of age. Individual lots of the spores were treated with one of the following: sodium hypochlorite, sodium hydroxide, formalin, phenol, calcium oxide, zephiran chloride (Roccal^{R}), calcium cyanamide, drying, heating, freezing, and others (94 fish) served as controls. The aquarium water was not changed until absolutely necessary in order to retain the spores. Compressed air was supplied and the aquaria cooled by immersion in running 54° F. spring water. Of the entire group of fish, 17, including two controls in uncontaminated, but otherwise similar aquaria, developed whirling symptoms at 12 to 19 days post exposure. Only one developed black tail. Whirling (tail chasing type) alone cannot be used as proof of M. cerebralis infection because we have seen whirling at two hatcheries where no spores could be found. Presumably other conditions, perhaps certain types of malnutrition, can cause whirling. Therefore, we attempted to verify our studies by identifying the developing stages of the parasite in histological sections, but we were not able to demonstrate any parasites.

In 1960 seven lots of 10-75 (total 290) newly feeding rainbow fry were placed in aquaria at 54° F. Suspensions containing many spores were prepared by homogenizing infected yearling trout heads with the Waring blendor or macerating with mortar and pestle. This material was added to the aquaria just after the fish had begun to feed and the water was not changed in order to retain the spores. Another lot was also fed such a suspension for 6 days. All fish were kept in the "contaminated" water for 5 to 14 days and then transferred to running spring water. Whirling was seen in a very few at about 2 weeks but no infections have been verified and no symptoms persisted. From these experiments we assume that a transport host, pond envoronment, or different water condition is necessary for transmission of the disease. Another possibility for our negative results is that we may have rendered the spores non-viable during handling. We were not able, however, to ascertain any damaging factors and the spores appeared normal microscopically.

We have likewise been unable to infect very young bluegills with the spores of a different Myxosoma sp. that occurs in bluegill cartilage.

In an attempt to determine whether fry can become infected prior to feeding, 4 lbs. of rainbow sac-fry were kept at the affected Benner Spring Hatchery for 10 days and then brought to Leetown, before feeding, for rearing in uncon-

taminated water. They were observed for 5 months; except for one which developed whirling symptoms, growth and behavior were normal. The affected fish was sectioned at 3 months of age and the developing stages of M. cerebralis were readily recognized in the cartilage.

DEVELOPMENT STAGES IN TROUT

Prior to 3 months (fig. 5)

Presumably the sporoplasm has made its way through the intestinal wall and migrated via blood or lymph channels to the cartilage, mainly of the head. This seldom affects fish over 12 months of age (Plehn, 1904, 1924; Schäperclaus, 1931, 1954). Our attempts to infect fish experimentally failed, so we could not demonstrate this stage. Schäperclaus (1954) p. 379 (our fig. 5) has an excellent photomicrograph of the developing trophozoite which has "eroded" a cavity for itself in the cartilage. As near as we can determine this is from a 40-day-old fry, so the parasite must be 40 days or less of age.

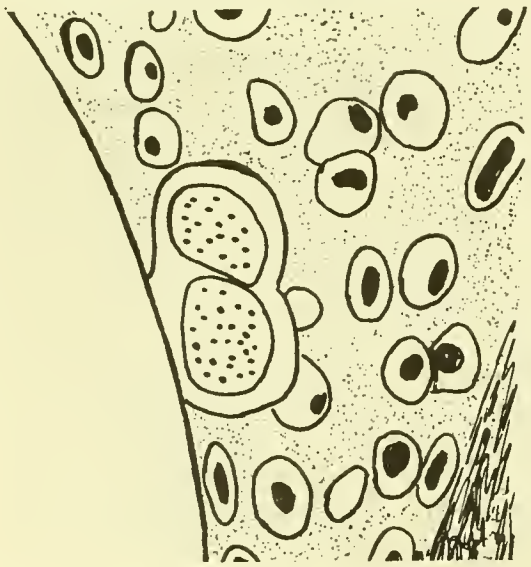


Figure 5:--Trophozoite of Myxosoma cerebralis in cartilage at about 40 days post-infection (drawn from photo of Schäperclaus, 1954).

Three months (figs. 6, 7, and 8)

Our only specimen of known age is this one. It was from one of many sac-fry brought from Benner Spring, Pa., during the epizootic, to Leetown for rearing in water free of M. cerebralis. Since it must have become infected at Benner Spring, we know the approximate age of the parasite. At this stage the parasites are multinucleate ameboid trophozoites and in cross sections range from 5 x 5 to 30 x 8μ in diameter; the smaller ones are possibly cross sections of elongate trophozoites. There are at least 18 nuclei about 1-1/2 - 2μ in diameter in each trophozoite. These exist in lesions measuring about 300 x 100μ in the cartilage. Also in the lesions are freed cartilage cells and remnants of disintegrating cells and cartilage matrix. Apparently in normal osteogenesis in the trout studied, the cartilage of the skeleton in certain places is eroded from within by vascular action at the same time that bone is being laid down on the outside of the skeletal structures. Blood vessels penetrate the cartilage, and pockets of it are eroded, presumably by enzymes released from the capillaries. These pockets of cartilage erosion (fig. 9) resemble M. cerebralis lesions, but contain blood vessels, disintegrating cartilage cells and cartilage matrix and sometimes multinucleate host cells but no parasites. The multinucleate cells resemble phagocytic giant cells, but Ruth[3/] suggested that they might be tissue cells that have failed to divide.

Four months (figs. 10, 11, and 12)

The multinucleate trophozoite has grown considerably but is still in the cartilage. Some of the nuclei have divided repeatedly to form groups of nuclei (or cells?) which are now known as pansporoblasts. These distinct units produce the spores, usually two each. Kudo (1960) believes that myxosporideans are distinctly multicellular at this stage and, therefore, much different from other protozoa. Kudo (1930) and Noble (1944) have reviewed pansporoblast formation in other Myxosporidia. At this age some of the pansporoblasts have already produced spores. The trophosoite (entire parasite) has probably reached its maximum size at this stage; those measured were up to 1 mm in greatest diameter although we do not know for certain whether two or more trophozoites may be in such close association that their boundaries are not discernible.

3/ Ruth, Delbert. Anatomy Department, John Hopkins Medical School, Baltimore, Maryland, pers. comm., 1961.

Figure 6:--Photomicrograph of Myxosoma cerebralis in cartilage at 3 months post-infection (x 100). See fig. 7 for labelling.

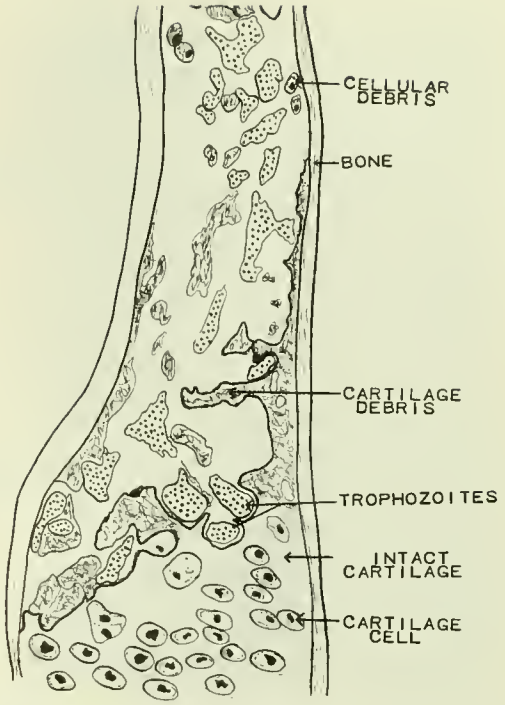


Figure 7:--Drawing of Myxosoma cerebralis in cartilage at 3 months post-infection. (Made from same slide as fig. 6) Drawn with aid of microprojection.

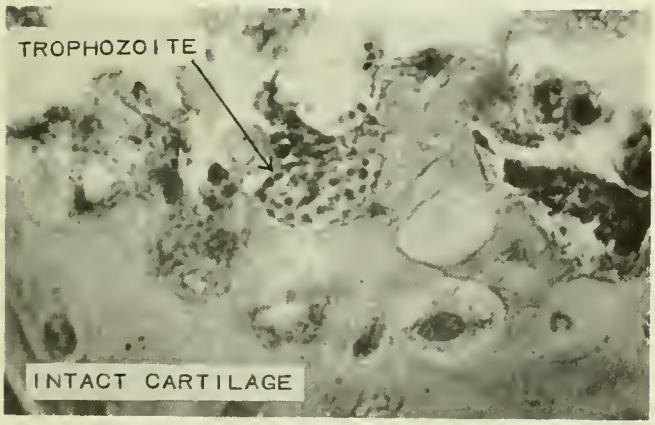


Figure 8:--Photomicrograph of Myxosoma cerebralis in cartilage at 3 months post-infection (x 430).

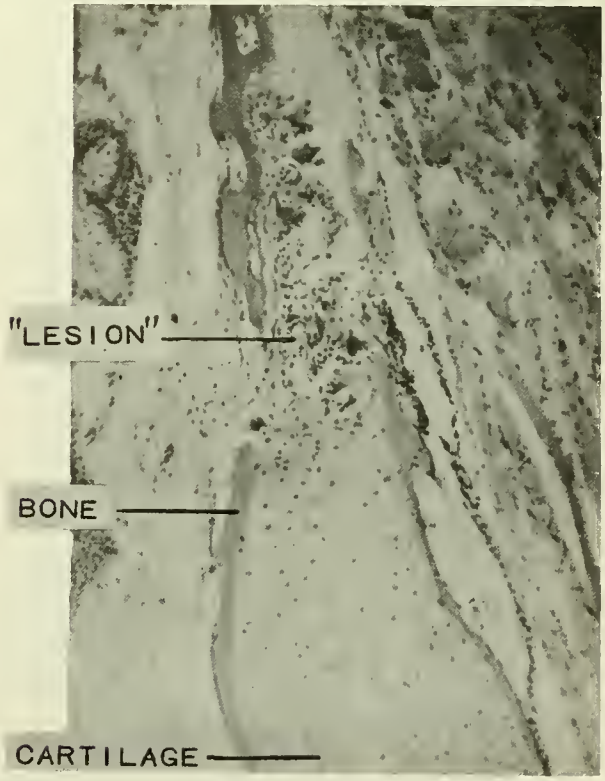


Figure 9:--Photomicrograph of normal cartilage resorption in trout about 3 months of age. These "lesions" grossly resemble Myxosoma cerebralis infections.

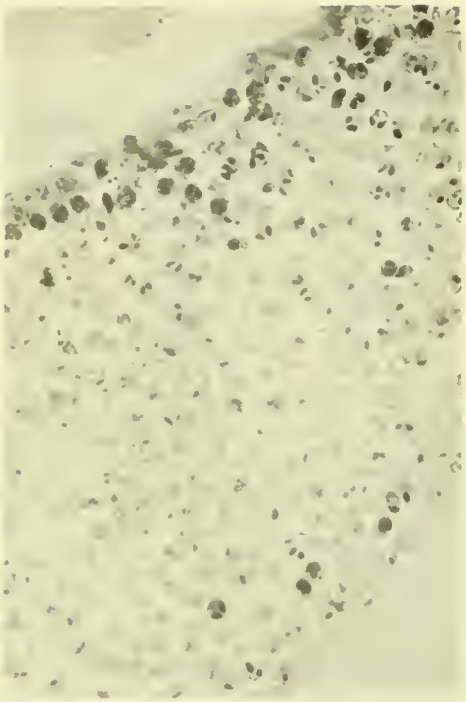


Figure 10:--Photomicrograph of Myxosoma cerebralis in cartilage at 4 months post-infection. Note that two spores develop in each pansporoblast. Stained with Giemsa's to show spores (x 430).

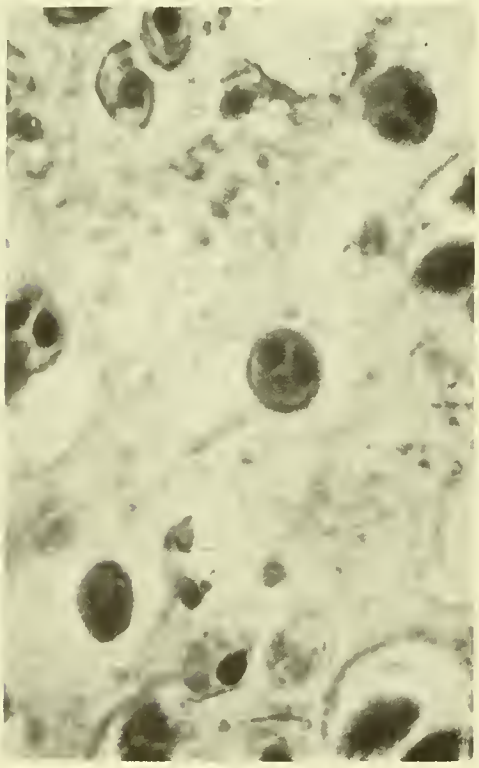


Figure 11:--Photomicrograph of Myxosoma cerebralis in cartilage at 4 months post-infection. Stained with Giemsa's to show spores (x 970).

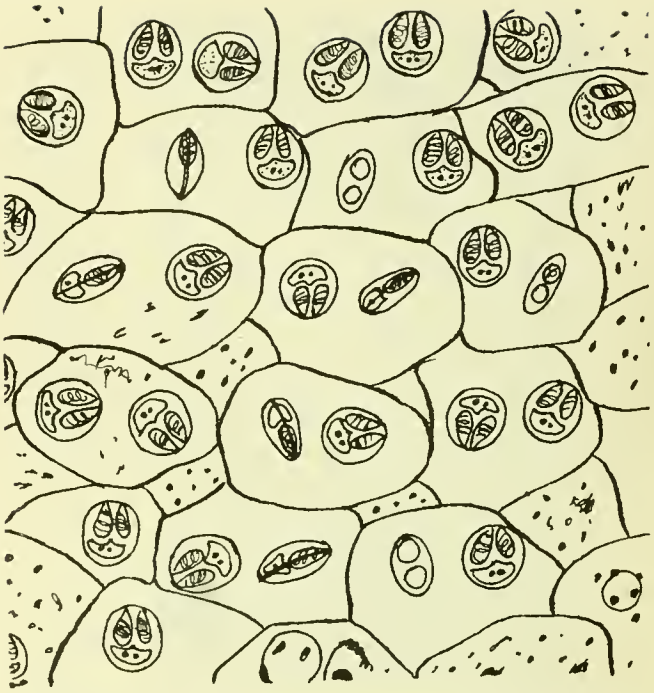


Figure 12:--Free-hand drawing of Myxosoma cerebralis pansporoblasts containing two spores each. Four months post-infection.

Eight months and older (figs. 13 and 14)

At this time all of the pansporoblasts have produced their spores, and these can be seen in the "lesion". Apparently the rest of the parasite disintegrates. The spores appear to remain in the lesion site permanently--Uspenskaya (1957) found them in 3-year-old fish. We have found many spores in 2-year-old fish and presume that they may remain much longer. It has been assumed that the spores remain in the site of the lesion, but she cites evidence that some of them are transported, presumably by blood, to other organs including the liver and lumen of the intestine. We believe that this is not the usual means of spore dissemination.

HISTOPATHOLOGY

There is no evidence of histopathology during the incubation period of 40 to 60 days. However, after this, as the trophozoite grows, cartilage is eroded, skeletal support is weakened, and simultaneously there is a proliferation of epithelioid-type granuloma about the parasite and its spores.

1. Forty days. Slight erosion of cartilage. Schäperclaus (1954) p. 379 (our fig. 5).

2. Sixty-five days. Still trophozoite only; no tissue proliferation. Schäperclaus (1931) p. 542, 554.

3. Three months (figs. 6, 7, and 8). Cartilage still being eroded by trophozoites in a lesion-like cavity in cartilage; cellular and cartilage debris present in lesion, bone being formed at periphery of skeleton. No tissue proliferation or inflammation.

4. Four months (figs. 10, 11, and 12). Some spores now present, but still no proliferation.

5. Eight - 12 months (fig. 13). Spores present. Trophozoites no longer present. Epithelioid proliferation of fish tissue now surrounds the mass of spores. This cyst-like structure is sometimes referred to as a granuloma which may cause pressure on vital organs (Plehn, 1904).

DIAGNOSIS

Before development of the spores (about four months) the disease can be tentatively diagnosed on the basis of the symptoms--tail-chasing, whirling, and black tail. Verification can be made only by finding the ameboid stages in the cartilage of the head in histological section. Very early stages are more difficult to find but at three months they are often easily found and consist of trophozoites 5 to 30μ in size with many nuclei about 1-1/2 to 2μ in diameter.

From 4 months post infection to at least 2 years, the spores can be found easily in wet mounts or histological section. Wet material may be prepared by dissecting out the auditory capsule and crushing it (Plehn, 1904) or splitting the head lengthwise and scraping the posterior part of the cranium with a scalpel to free the spores. A more reliable method for fish, up to at least 2 years of age, is to cut up the head with scissors and macerate in a Waring blendor in about 50 ml of water--the spores can usually be found in a random drop.

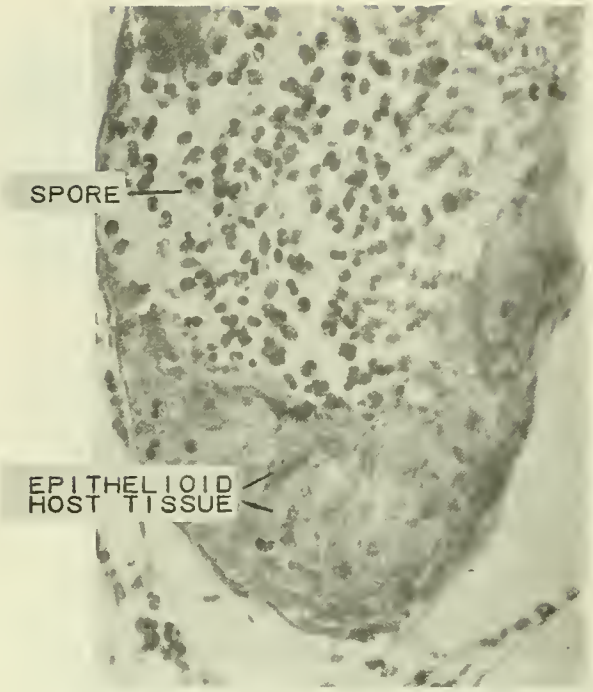


Figure 13:--Photomicrograph of Myxosoma cerebralis spores in an epithelioid granuloma at 12 months post-infection. Stained with Giemsa's to show spores (x 430).

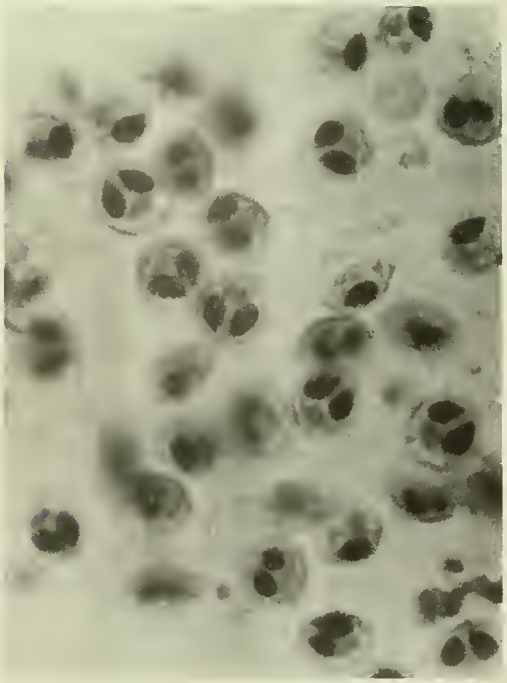


Figure 14:--Photomicrograph of spores of Myxosoma cerebralis from rainbow trout at 12-15 months post-infection. Stained with Giemsa's (x 970). Photo by Dr. E. M. Wood.

In histological section the early stages (1-1/2 to 3 month post infection) can be recognized by their many small nuclei in the lesions in the head cartilage (figs. 5, 7, and 8). Lesion-like structures in normal bone formation (fig. 9) resemble whirling disease lesions. Sometimes these contain large multinucleate host cells (giant cells) which may be confused with parasites.

In histological section of the head the spores stain blue with Giemsa's (figs. 10, 11, 13, and 14), blue with carbol toluidin blue (Schäperclaus, 1931) and red with aniline water-saffranin (Plehn, 1904).

CONTROL AND TREATMENT

A modified version of Schäperclaus' (1954) and Tack's (1951) recommendations for control follows:

1. Destroy all fish from ponds containing fish known to be infected. Incineration or deep burial is recommended.

2. Water supply. That which supplies the hatching house fry and early fingerlings (up to 8 months at least) should be spore-free spring or well water. There should be no fish in the water system before it reaches the hatching house. Stream water may be used only if the stream contains no fish. No satisfactory filtering device for whirling disease has been described for hatcheries in the United States, but we have heard that sand-charcoal filters have been used successfully in France.

3. Disinfection of hatching house. Clean thoroughly (this is more important than chemical disinfection). We have found that overnight soaking with the following will dissolve the spores or cause them to extrude their polar filaments and probably render them uninfective: sodium hypochlorite (commercial or home laundry bleaches) as per instructions on container; zephiran chloride (Roccal^{R}) 800 ppm; sodium hydroxide 1/2 percent, calcium oxide (quicklime) 1/2 percent. It is imperative to have spore-free facilities for fry because the younger the fish the more seriously they are affected by whirling disease.

4. Disinfection of concrete ponds. Drain ponds and immediately apply calcium cyanamide at about 0.04 lb./sq. ft. (1780 lb./acre). Allow to stand 3-4 weeks, clean thoroughly, wet down and repeat treatment. Some of the chemicals listed in 3 above would probably be satisfactory for small ponds. It would take about 10 times as much chemical if used in water-filled ponds. Quicklime and hydroxides are strongly caustic chemical agents and personnel using them should wear protective clothing and use goggles and respirators.

5. Disinfection of earthen ponds. This is the most difficult aspect because organic matter usually interferes with disinfectants. Drain the ponds and immediately apply calcium cyanamide as in 4 above. Allow to stand a month or more and clean out silt as thoroughly as possible. Haul out to a farm field for plowing under or bury deeply. Fill pond with water, drain and immediately apply calcium cyanamide as before. Flush out and refill ponds 2 weeks or more later and stock with fish; Tack (1951) allowed 6 weeks before adding fingerlings.

Schäperclaus (1954) recommends quicklime as the chemical of second choice to be used at the same rate as calcium cyanamide. Snow and Jones (1959) have used quicklime successfully in treating bluegill ponds for fish diseases. We believe that the hot reaction of quicklime which produces a high transient pH is effective in killing spores--a pond so treated is safe to use 10 days after treatment with no flushing necessary. Sodium hydroxide is probably more effective than lime but may be more expensive.

6. Restocking hatchery. Eggs or fry should be obtained from a known uncontaminated source. Fry should be kept in hatching house as long as possible (3-8 months) because it is usually easier to control the disease here. It is advisable to maintain 2 series of ponds until it is certain that the disease is eradicated.

The first series of ponds should be concrete raceways supplied with spore-free water. Use great care not to contaminate with spores or infected fish. Keep fish here only 3 months.

It takes 4 months for the spores to develop, therefore, these ponds could not be contaminated by fish as long as the hatchery house, ponds, and water supply are spore-free. Pick off mortalities twice daily; incinerate or bury deeply.

The second series of ponds may be concrete or earthen. For best results the fish should be 8-12 months or more of age when stocked here. The older the fish when infected, the less serious the disease and it is doubtful that 13-month-old fish can be infected--certainly not heavily.

Following this routine it is expected that some disease will show up in the last series of ponds. If whirlers and mortalities are incinerated, however, one can expect the disease to disappear in 2-3 years. It may be necessary to treat earthen ponds annually for 2-3 years.

7. No fish from an affected hatchery should be transferred to an unaffected hatchery. However, the disease probably cannot be transmitted by eggs (Schäperclaus, 1931). It must be kept in mind that infected brown trout and lightly infected rainbow and brook trout may serve as carriers although they show no symptoms.

8. Fish from an affected hatchery should not be stocked in fishing waters unless there is no other hatchery on that watershed and no natural trout reproduction or stocked fingerlings. It is better to destroy the infected fish than to take a chance on spreading the disease which has apparently spread from Central Europe to Russia, Italy, and now North America in the last decade. Disposal or transport by fishermen of spore-bearing fish is an ever-present threat to spread of the disease.

Suppression of the disease, but not elimination, with Acetarsone (Stovarsol) at 10 mg per kilogram of fish daily on 3 consecutive days with weekly intervals between each course was reported by Scolari (1954). He recommends that it be used for 6 months for (partial) prophylaxis. Apparently the drug has no serious side effects on the fish. We fed it to several thousand rainbow trout for eleven months at Benner Spring at concentrations as high as 100 times that recommended by Scolari (1954). Although these Acetarsone experiments have not been completed as of this writing, the preliminary results are not promising. We thus believe it should be tested further before recommending it for prophylaxis of whirling disease.

SUMMARY

Whirling disease (black-tail), caused by the myxosporidean, Myxosoma cerebralis, is reported from the United States. It has made its appearence at 2 trout hatcheries located on the same watershed in Pensylvania and at another one in Connecticut. Young rainbow and Eastern brook trout were severly affected. European brown trout were not severely diseased, but probably served as "carriers".

Many attempts to transmit the disease in the laboratory failed.

The developmental stages of the parasite, histopathology, diagnosis, and control are discussed. The severity of the disease in the hatcheries has been reduced, but not eliminated, by removing fish from the water source and treating the ponds with calcium cyanamide.

Preliminary experiments with Acetarsone (Stovarsol), although inconclusive, indicate that the drug is not vary toxic to trout.

LITERATURE CITED

Bogdanova, E. A.
1960. Natural habitat of the myxosporidean (Myxosoma cerebralis - whirling disease) at Sakhalin (S. E. Russia). Rep. USSR Academy of Science, Vol. 134, no. 6, pp. 1501-1503.

Davis, H. S.
1923. Studies on the sporulation and development of the cysts in a new species of Myxosporidia, Lentospora ovalis. Journal of Morphology, Vol. 37, no. 3, pp. 425-542.

1953. Culture and diseases of game fishes. University of California Press, 332 pp.

Dyk, V.
1954. Nemoci y. nasich ryb. (Diseases in our fish). Praha, Cezechoslovakia.

Fanthan, H. B., A. Porter, and L. R. Richardson
1939. Some Myxosporidia found in certain freshwater fishes in Quebec Province, Canada. Parasitology, Vol. 31, no. 1, pp. 1-77.

Heuschmann, O.
1940. Über die Ausbreitung der Drehkrankheitung in Suddentschland. Allgem. Fischereizeitung Vol. 65, no. 3, pp. 17-19.

1949. Die Drehkrankheiten der Salmoniden. Allgem. Fischereizeitung Vol. 74, no. 12, pp. 216-217.

Hofer, B.
1903. Über die Drehkrankheit der Regenbogenforelle. Allgem. Fischereizeitung Vol. 28, pp. 7-8.

Hoffman, G. L., and C. E. Dunbar,
1961. Studies on Myxosoma cerebralis (Hofer) Plehn (Protozoa: Myxosporidia) the cause of whirling disease of trout. Journal of Parasitology, Vol. 47, no. 4, Sec. 2, 29 (abstract).

Kabata, Z.
1957. Note on a new host of Myxobolus aeglefini. Journal of Parasitology, Vol. 47, no. 1 and 2, pp. 165-168.

Kocylowski, B.
1953. Choroby ryb i ich knalkanie. Gespodarka rybna 5, No. 1, pp. 24-26.

Kudo, R. R.
1930. Myxosporidia. (In Problems and methods of research in protozoology. Ed. Hegner, R. W. and V. M. Andrews. McMillan Co., New York, pp. 303-324).

1960. Symposium on taxonomy in protozoology. Talk given at annual meeting of American Society of Protozoologists at Stillwater, Oklahoma, September, 1960.

Luling, K. H.
1952. Die Erreger der Knotchen-Baulen und Drehkrankheit unserer Fische. Ostereichs Fischerei, Vol. 5, no. 8, pp. 171-173.

Noble, E. R.
1944. Life cycles in the Myxosporidia. Quarterly Review Biology, Vol. 19, no. 3, pp. 213-235.

Plehn, M.
1904. Über die Drehkrankheit der Salmoniden. [Lentospora cerebralis (Hofer) Plehn.] Archiv für Protistenkunde. Bd., Vol. 5, pp. 145-166.

1924. Praktikum der Fischkrankheiten. Schweizerbart'sche, Stuttgart. 479 pp.

Schäperclaus, W.
1931. XXI. Die Drehkrankheit in der Forellenzucht und ihre Bekämpfung. Zeitschrift für Fischerei. Bd. Vol. 29, pp. 521-567.

1954. Fischkrankheiten. Akademie-Verlag, Berlin. 708 pp.

Scolari, C.
1954. Sull'impiego dello Stovarsolo nella profilassi del "capostorno" O "lentosporiasi" delle trote d'allevamento. Clin. Veterin., Vol. 77, no. 2, pp. 50-53.

Snow, J. R., and R. O. Jones.
1959. Some effects of lime application to warm-water hatchery ponds. Given at 13th Annual Meeting S.E. Association of Game and Fish Commissioners, Duplicated, 15 pp.

Tack, E.
1951. Bekampfung der Drehkrankheit mit Kalkstickstoff. Fischwirt. Nr. 5, pp. 123-130.

Uspenskaya, A. V.
1955. Biology, distribution and economic importance of Myxosoma cerebralis, the causative agent of the twist disease of trout. Lectures of the Academy of Science, USSR. Vol. 105, no. 5, pp. 1132-1135.

1957. The ecology and spreading of the pathogen of Myxosoma cerebralis (Hofer, 1903; Plehn, 1905) of trout in the fish ponds of the Soviet Union. (In Parasites and Diseases of Fish, ed. by G. K. Petrusheveski. Vol. 42, Bulletin, Institute of Freshwater Fisheries. English Translation--Office of Technical Services, U.S. Department of Commerce, Washington, pp. 47-55).

Snieszko, S. F. and K. Wolf.
1958. Infectious pancreatic necrosis of salmonid fishes. U.S. Fish and Wildlife Service, Fishery Leaflet 453.

Vanco, R.
1952. Contribution a l'etude de la pathologie des alevins de truite. Paris, 92 pp.

Volf, F.
1957. The first cases of the whirling disease among our salmonid fish in our hatcheries. Sbornik Československé Akad. Zemědělských věd. Živočišná Výroba. Ročnik 2(XXX), pp. 425-428.

Ward, H.
1919. Notes on North American Myxosporidia. Journal of Parasitology, Vol. 6, no. 2, pp. 49-64.

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