The Ichthyogram

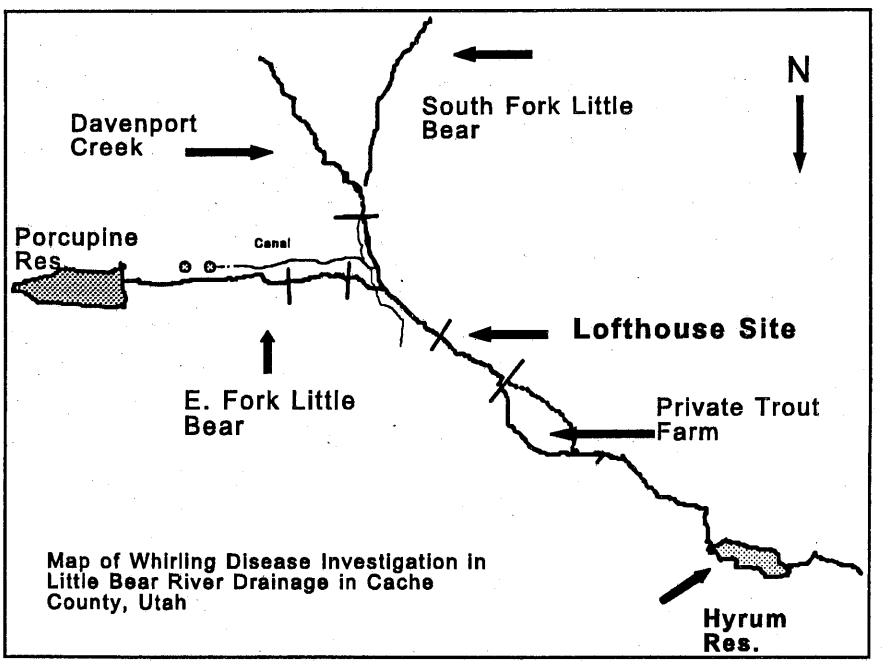
March 1996 Volume 7 Issue 1

Whirling Disease Extends Down Little Bear River Drainage, Affects Kokanee

The geographic range of whirling disease continues to expand in northern Utah. In October and November, biologists sampled the Lofthouse site of the Little Bear River and Hyrum Reservoir. At the Lofthouse site, infected rainbow, cutthroat and brown trout were discovered in October 1995, in contrast to 1994, when no infected fish were detected. At Hyrum Reservoir, infected rainbow trout were detected for the first time ever in November 1995.

The first discovery of the parasite in the drainage was made in 1993, when *Myxobolus cerebralis* was found in fish from a private hatchery and adjacent areas of the Little Bear River several miles upstream. In 1994, the parasite was found further upstream in rainbow trout and kokanee salmon from Porcupine Reservoir, which had consistently tested negative from 1987 to 1994. Presently all year classes of kokanee have shown evidence of infection. Biologists found kokanee which were grossly deformed and infected for the first time in October 1995. The presence of deformites and significant pathology in cranial cartilage contrasts with the observations of

(Continued on page 3)



Map of Whirling Disease Investigation in Little Bear River Drainage in Cache County, Utah

inside...

Triploid Trout Production by Electroporation

Research into the production of sterile rainbow trout has been a priority at the Fisheries Experiment Station (FES). In Utah waters where native cutthroat trout exist and rainbows are stocked, sterile rainbows are needed to avoid hybridization. Methyltestosterone as a tool to produce sterile fish has been withdrawn from our use so an alternate method of sterile fish production is necessary. One method of producing sterile fish is to induce triploidy during the early stages of post fertilization development. Triploidy has been induced in fish by applying several different types of physical shocks to the embryo including thermal, pressure, and electrical.

The use of electrical shock to induce polyploidy has not been extensively researched but it has been shown that electroporation can induce polyploidy in oysters and mussels. Electroporation is commonly used to inject foreign materials into various cell types for a specific purpose. A study by Teskeredžić et al., (Aquaculture 116 (1993): 287-294) did demonstrate that electrical fields could be used successfully to shock coho salmon eggs and produce triploid fish at high rates of survival. Based on the success of producing triploid invertebrates and salmon by means of electrical shock, a series of experiments was designed to determine the efficiency of electroporation in inducing triploidy.

Two separate trials to induce triploidy have been conducted to date, one in April 1995 using cutthroat trout, and the second, in October, using rainbow trout. In Trial 1 all eggs were shocked at 30 min post fertilization, but in Trial 2 the post fertilization shocking time varied from 15 to 35 min. The fertilization of eggs was staggered at five minutes apart to allow for the experimental shock to take place.

In Trial 1 three main variables, voltage level, number of repeated pulses, and duration of pulse were manipulated during the various treatments. Eggs were exposed to three voltage levels: 250, 375, or 500 V for pulse durations of 99 μsec or 5 msec and for either one pulse or six successive pulses. Three additional treatments were also analyzed: one pulse of 10 msec duration at 250 V and 10 msec pulses at 500 V with either one pulse or six pulses.

In Trial 2 eggs were exposed to a single pulse of either 50 V with a 99 ms shock or 250 V with a 5 ms shock. Within both voltage level and pulse duration regimes treatments were exposed to 1-3 multiple shocks and the post-fertilization shocking time was either 15, 25, or 35 min.

Shocks were administered with an electroporator equipped with an electrode designed to fit a 100 x 15 mm petri dish. After the shock was administered, eggs were rinsed three times with hatchery water, placed back into the flask after which 75 ml an iodophor solution was added and the flask returned to a water bath. Following a 10 min betadyne treatment the eggs were assigned to either a jar or tray incubator unit. Triplicate controls were included for both types of incubation unit. Controls were handled exactly the same as the treated eggs without the application of the electrical shock. Also to overcome possible differences between the two rearing units at least one replicate of each treatment was assigned to an incubator tray. Data collected during both trials included visual 24 h post treatment mortality, percent egg mortality, percent eye-up, and percent hatch. Several days after hatching fry were transferred into troughs which were subdivided to accommodate the different lots of fish. Fish were reared there until appropriate blood was sampled for analysis of triploidy by flow cytometry.

In Trial 1 voltage level, pulse duration, and number of pulses significantly influenced 24 h mortalities (see Table 1). Treatments

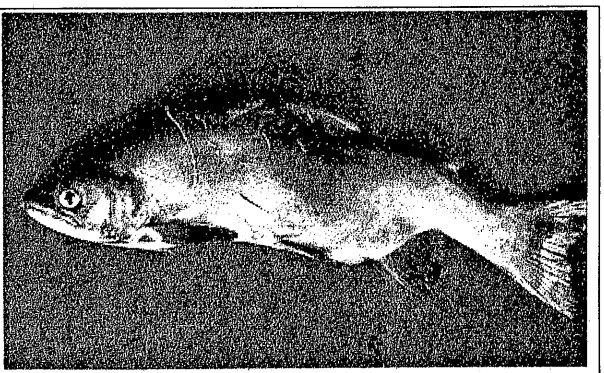
(Continued from page 2)
shocked at 500 V had significantly higher mortalities than those shocked at either 250 or 375 V. Treatments with pulse durations of 99 μs also had significantly lower 24 h mortalities compared to pulse durations of 5 or 10 ms. The number of shocks also appeared to be important, with significantly higher mortalities in treatments receiving 6 shocks compared to those that received 1. Similar trends were also evident with respect to eye- up and percent hatch. Those treatments that underwent the fewest number of pulses for the shortest amount of time and at the lower voltages exhibited the best performance. The percent triploidy induced from Trial 1 is shown in Figure 1. There appeared to be no trend in percent triploidy in relation to the various treatments.

In Trial 2, voltage level (50 vs. 250 V) and pulse duration (5 vs. 99 ms) had no significant impacts on 24 h mortality, eye-up, or hatch. With the exception of one treatment, all treatments undergoing multiple shocks had significantly higher 24 h mortalities compared to those receiving one shock regardless of the post fertilization shocking time (see Table 2). There were some significant differences between treatments with respect to eye-up, but no clear trend was evident. No differences were found between treatments looking at percent hatch. There is some evidence suggesting that eggs used for this trial were either overripe or in some way bad because even the control group only had an average eye-up of 41 %. The percent triploidy induced from Trial 2 is shown in Figure 2. With the exception of treatment J, all treatments that exhibited triploidy were those that underwent a single electrical shock, with the varying post-fertilization shocking .

In conclusion it does appear possible to induce triploidy using electroporation. However, given the current results it is apparent that additional work is required. It looks like lower voltages are less damaging to the developing embryos and that multiple shocks and pulses cause high mortality. More traditional methods of shocking to induce triploidy, including heat and pressure, expose the eggs to shocks of anywhere from 1-10 min. Given the low percentage of triploidy from both trials it is possible that we are shocking the eggs for such a small amount of time that only a small percentage of eggs are being exposed to the conditions that may induce triploidy. Future work will try to further narrow the appropriate voltage level, pulse duration, and pulse length to increase the percentage of triploid produced.

(Continued from page 1)
pathologists in Colorado.

Research is underway in 1996 to study possible effects of the infection on kokanee numbers and reproduction. One proposed method would be to monitor redd counts or evaluate deformities in returning spawners. Kokanee are of special concern because of their yearly spawning run, in which large numbers of fish die after spawning, potentially releasing large numbers of spores of the whirling disease parasite into the environment. Chris Wilson



ANOTHER FISH WITH THE COMMON COLD?
This deformed kokanee salmon was sampled from the Little Bear river above Porcupine reservoir in October 1995, individually tested and found infected with *Myxobolus cerebralis.*

Table 1. Percent mortality and survival at various stages of development of cut-throat trout electrically shocked with various voltages, voltage pulse durations and number of pulses.

Treatment Explanation[a] Treatment Code	Percent 24 h mortality[b]	Percent eye up	Percent hatch
250 V 99 Fs 1 pulse, V1A	30 ±35	50±26	40 ±24
250 V 5 ms 1 pulse, V1B	50 ±26	35 ±14	26 ±11
250 V 99 Fs 6 pulses, V1C	38 ±45	33 ±27	26 ±21
250 V 5 ms 6 pulses, V1D	98 ±3	2 ±3	0 ±0
250 V 10 ms 1 pulse, V1E	57 ±33	27 ±15	18 ±11
375 V 99 Fs 1 pulse, V2A	13 ±6	52 ±14	45 ±17
375 V 5 ms 1 pulse, V2B	65±48	25 ±27	19 ±20
375 V 99 Fs 6 pulses, V2C	50 ±10	28 ±9	21 ±6
375 V 5 ms 6 pulses, V2D	98 ±3	0 ±0	0 ±0
500 V 99 Fs 1 pulse, V3A	42 ±28	35 ±21	30 ±20
500 V 5 ms 1 pulse, V3B	90 ±0	10 ±2	8 ±1
500 V 99 Fs 6 pulses, V3C	90 ±13	9 ±9	8 ±8
500 V 5 ms 6 pulses, V3D	100 ±0	0 ±0	0 ±0
500 V 10 ms 1 pulse, V3E	70 ±17	15 ±7	12 ±5
500 V 10 ms 6 pulses, V3F	100 ±0	0 ±0	0 ±0
Control - jar	4 ±6	76 ±11	58 ±14
Control - incubator	6 ±8	73 ±17	67±18
Control - not handled[c]	0	70	60

a = All treatments were shocked at 15 min post fertilization
b = Estimated from visual observations
c = Not replicated. All other treatments run in triplicate

Table 2. Percent mortality and survival at various developmental stages of rainbow trout shocked electrically with various voltages, voltage pulse durations and number of pulses at three post-fertilization times.

Treatment Explanation	Percent 24 h mortality[a]	Percent eye up	Percent hatch[b]
A - 250 V 5 ms 1 pulse shocked at 15 min	78 ±14	9 ±2	96 ±3
B - 250 V 5 ms 1 pulse shocked at 15 and 25 min	91 ±6	4 ±2	75 ±20
C - 250 V 5 ms 1 pulse shocked at 15, 25, and 35 min. Sucrose shocking media	96 ±4	2 ±2	67 ±47
D - 250 V 5 ms 1 pulse shocked at 15, 25, and 35 min. Water shocking media	96 ±2	2 ±1	77 ±21
E - 250 V 5 ms 1 pulse shocked at 25 min	35 ±19	24 ±14	95 ±4
F - 250 V 5 ms 1 pulse shocked at 25 and 35 min	80 ±18	4 ±1	89 ±8
K - 250 V 5 ms 1 pulse shocked at 30 min[c]	37 ±5	16 ±8	93 ±8
G - 250 V 5 ms 1 pulse shocked at 35 min	72 ±16	14 ±4	92 ±7
H - 50 V 99 ms 1 pulse shocked at 15 min	58 ±23	8 ±4	98 ±3
I - 50 V 99 ms 1 pulse shocked at 15 and 25 min	87 ±5	4 ±1	97 ±4
J - 50 V 99 ms 1 pulse shocked at 15, 25, and 35 min	35 ±11	23 ±4	90 ±11
Control: handled as if undergoing one shock	5 ±0	41 ±18	95 ±4

a = Estimated from visual observations
b = Calculated from the percent eye-up, representing 100%
c = treatment replicated as a control from the initial cutthroat trial

Figure 1. % Triploid of Cutthroat Trout Treated by Electroporation

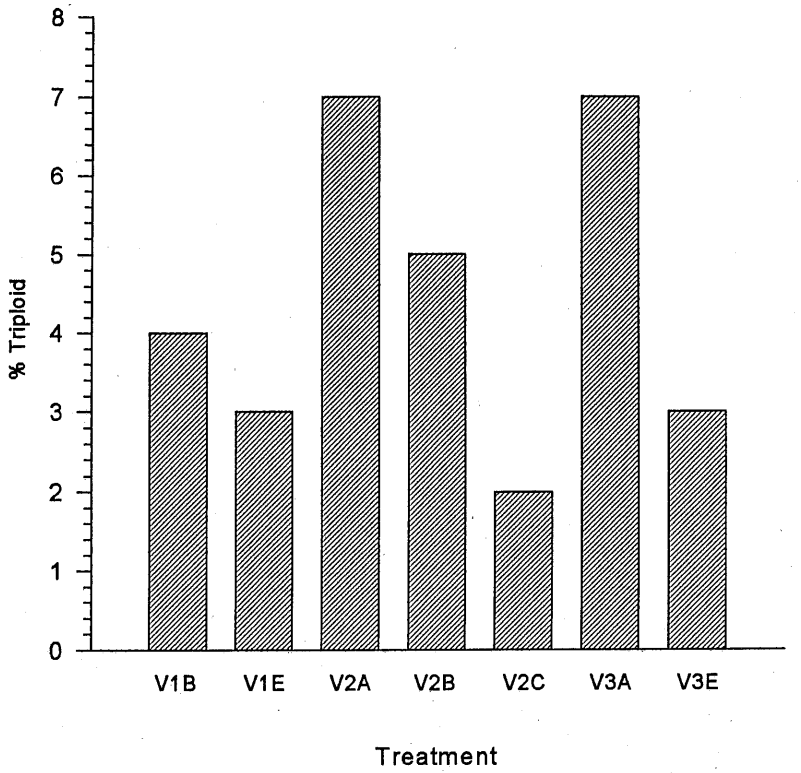
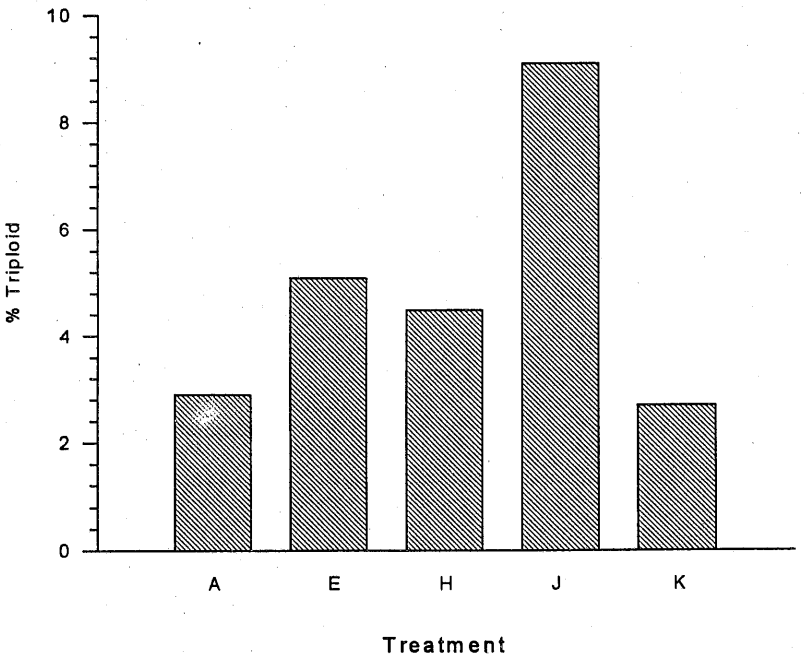


Figure 2. % Triploid of Rainbow Trout Treated by Electroporation



Whirling Disease Prevention and Control: A Review

Whirling disease is caused by the myxosporean protozoan *Myxobolus cerebralis*. The disease has been associated with significant declines in rainbow trout *Oncorhynchus mykiss* populations that have been closely monitored on the Madison River in Montana and in Colorado. In the Middle Park, Colorado reach of the Colorado River, Walker and Nehring (1995) observed high mortality of young-of-the-year rainbow trout, reducing age 1+, 2+, and 3+ cohorts to 0.7, 0.5, and 9.7% of the 1994 population, respectively. Subsequent live-cage studies showed that mortality varied among the species tested: brown trout *Salmo trutta*, 2%; Colorado River cutthroat trout *Oncorhynchus clarki pleuriticus* , 10%; Colorado River rainbow trout, 23%; Tasmanian strain rainbow trout 50%. The caged fish had *M. cerebralis* spores in them and exhibited typical signs of the disease, including whirling behavior, skeletal deformities, and black tails. According to an account by H. Novick in the 18 August 1994 "The River Reporter", 95 to 97% of all wild rainbow trout from 1991 to 1993 had been lost in the upper Colorado River. In Montana, rainbow trout densities have dropped 90% in infected reaches of the Madison River (6 April 1995, *Missoulian*).

Clearly, whirling disease can be a significant problem. Its control is difficult given the tenacity of the spore and its longevity in wet muds. The spores can tolerate freezing at -20 C for at least 3 months and the spores are still viable after passage through the guts of predators such as northern pike *Esox lucius*, black-crested night herons *Nycticorax nycticorax*, or mallard ducks *Anas platyrhynchos* (Taylor and Lott 1978; El-Matbouli and Hoffman 1991). There have been reports from Europe of spores remaining viable in dry pond beds for as many as twelve years (Bauer 1962). This article summarizes control methods attempted to date.

For the fish culturist, there are a variety of ways of dealing with the parasite. Since the alternate host for *M. cerebralis* is the oligochaete worm *Tubifex tubifex*, avoiding earthen ponds for culturing fish and keeping concrete systems free of organic waste and sediment are good common sense solutions (Markiw 1992a). Fish exhibit fewer debilitating symptoms of the disease as they grow older, since the cartilage attacked by the trophozoite is largely converted to bone in older fish. Therefore, stocking dirt ponds with larger fish is an option for managers not stocking fish in the wild; Hoffman (1990) recommends using fish that are at least 6 cm long. Rasmussen (1965) reported success in Danish trout farms by rearing rainbow trout to 5 cm in concrete tanks before stocking into infected dirt ponds. Disinfection of in-coming water is possible with ultraviolet radiation. Hoffman (1974) found that 2537 Angstrom units of UV light at dosages of 35,000, 43,000, and 112,000 microwatt sec/cm^2 were effective in controlling infection of rainbow trout fry. Filtration of water through a 25 μm commercial filter cartridge did not reduce or eliminate the disease (Hoffman 1974). However, Hoffman et al. (1962) noted that sand-charcoal filters had been used successfully in France.

Disinfection of hatcheries and ponds is feasible with chemicals (Table 1). Calcium cyanide was effective in disinfecting ponds, whereas quicklime was less effective (Bauer 1962). Calcium cyanamide (488 g/m^2) used for disinfection of dirt ponds and chlorine gas (300 ppm) for disinfection of incoming spring water were effective in preventing the recurrence of whirling disease the following year in a Pennsylvanian trout

(Continued on page 8)

(Continued from page 7)
hatchery (Hoffman and Dunbar 1961). Tests with quicklime in simulated tests by Hoffman and Hoffman (1972) were effective in preventing infection of rainbow trout. Treatment of simulated earthen ponds with either 4550 g/m^2 hydrated lime (CaOH) or 1200 ppm chlorine on the wet mud did not destroy all the spores (Hoffman and O'Grodnick 1977). However, when the spores were not protected by the mud, 10 ppm chlorine was sufficient to kill the spores (Hoffman and O'Grodnick 1977). Chlorine at 200 ppm gave variable results (Hoffman and Putz 1969). Chlorine at 400 ppm killed 36-90% of spores (Hoffman and Hoffman 1972). Prophylactic treatment with chlorine can be effective. Chlorine was effective in reducing infection by 73% in one group and 63% in another group of trout treated weekly with 0.5 ppm for 2 h over a 4 mo period. Presumably, the triactinomyxon stage of *M. cerebralis* and tubificid hosts were killed by the chlorine, whereas the treatment was not toxic to the fish (Markiw 1992a). The use of chlorine may be hampered by the U.S. Food and Drug Administration (FDA), the Environmental Protection Agency and state water quality regulations, as it is not an approved compound for discharging from hatcheries.

Various drugs have also been tested, with limited success (Table 2). The antibiotic Fumagillin (dicyclohexylamine) fed to rainbow trout (medicated pellets contained 0.1% Fumagillin) reduced clinical infections of whirling disease; 73-100% of non-medicated fish had severe infections whereas only 10-20% of medicated fish harbored spores (El-Matbouli and Hoffman 1991). In drug efficacy tests with rainbow x cutthroat trout hybrids fed medicated feed, Taylor et al. (1973) found that furazolidone inhibited spore formation. However, the drug affected feed palatability and growth in this group was half that of controls. Also, some fish on furazolidone still had trophozooites and granulomas. Russian literature (Bauer 1962) suggested that osarsol added to feed was effective in controlling the disease. Acetarsone (Stovarsol) suppressed the disease, but did not eliminate it (Hoffman et al. 1962). Similarly, Markiw (1992a) noted that furoxone, benomyl, proguanil and clamoxyquin reduced losses and infection of youg salmonids, but none prevented or totally eliminated the disease. Even if these drugs were effective, they are not registered for use in fish. Registration of the drug through the FDA generally requires many years of testing and millions of dollars.

For those trying to manage whirling disease in natural waters, the options are fewer. For programs relying upon stocked fish, stocking larger fish (> 6 cm) should be evaluated. Fish should not be transferred from positive sites. The disease is not considered egg-transmissible, so eggs taken from fish in WD+ waters may be used if no other disease-free sources are available. This may benefit expansion programs for sensitive species such as cutthroat trout. Stocking of infected fish into infected areas is not recommended. This practice may exacerbate problems by increasing the dose of triactinomyxons. This hypothesis needs to be tested in the wild, but Markiw (1992b) demonstrated that rainbow trout exposed to low numbers (1 or 10) of triactinomyxon did not develop spores. Higher doses of triactinomyxon resulted in proportionately more spores being recovered from infected fish, presumably overwhelming the immune system.

In Utah, removal of trout from the upper Fremont River drainage to break the life cycle of the parasite is being tried, but the experiment is still in progress. In Michigan, chlorination and rotenone treatment of the Tobacco River did not eliminate the parasite (CRWC 1988). However, levels of the infection have been low, and have not amplified over the last eight years in that section of the river (J. Hnath, Michigan Dept. Natural Resources, pers. comm.).

Table 1. A list of chemicals causing distortion and probable death of *Myxobolus cerebralis* spores.

Chemical	Concentration	Citation
Calcium hydroxide	0.5 and 2.0%	Hoffman and Putz (1969)
Calcium oxide (quicklime)	0.25, 0.5, and 1.0% 380 g/m^2 (3360 lb/acre)	Hoffman and Hoffman (1972)
Hydrogen Peroxide	30%	Lom (1964)
Potassium hydroxide	0.25, 0.5, and 1.0%	Hoffman and Hoffman (1972) Uspenskaya (1957)
Sodium hydroxide	1.0%	Uspenskaya (1957)
Available chlorine as sodium hypochlorite	1,600 ppm	Hoffman and Putz (1969)
Alkyl dimethylbenzylammonium chloride (Roccal)	200 and 800 ppm	Hoffman and Putz (1969)
Calcium cyanide	4,000 kg/ha	Bauer (1962)
Urea	saturated solution	Lom (1964)

Table 2. A list of drugs tested for use against *Myxobolus cerebralis*.

Drug	Concentration	Results (% reduction of incidence) Lot 1	Lot 2	Citation
Acetarsone (Stovarsol)	10-1000 mg/kg fish/d (3 d/wk for 6 mo)	suppression		Hoffman et al. 1962
Amprolium	13-18 mg/kg B.Wt.	17	0	Taylor et al. 1973
	24-44 mg/kg B.Wt.	50	0	Taylor et al. 1973
Fumagillin DCH	1 g/kg feed fed at 1%B.W.	73		El-Matbouli and Hoffman 1991
Furazolidone	152-194 mg/kg B.Wt.	100	39	Taylor et al. 1973
Merck 930	8-15 mg/kg B.Wt.	0	---	Taylor et al. 1973
	33-64 mg/kg B.Wt.	0	0	Taylor et al. 1973
Nicarbazin	6-14 mg/kg B.Wt.	17	0	Taylor et al. 1973
	30-60 mg/kg B.Wt.	22	0	Taylor et al. 1973
Oxytetracycline	68-152 mg/kg B.Wt.	39	---	Taylor et al. 1973
Sulfamerazine	15-36 mg/kg B.Wt.	0	0	Taylor et al. 1973

(Continued from page 8)

For management of naturally reproducing salmonid populations in positive waters, selection of resistant species or strains is one of the few options currently available. Salmonids vary in susceptibility, with the following list ranking the most common in order of decreasing susceptibility: rainbow trout, sockeye salmon *Oncorhynchus nerka*, golden trout *O. aguabonita*, cutthroat trout *O. clarki*, brook trout *Salvelinus fontinalis*, steelhead *O. mykiss*, chinook salmon *O. tshawytscha*, Atlantic salmon *Salmo salar*, brown trout, coho salmon *O. kisutch*, lake trout *Salvelinus namaycush*, and splake (lake x brook trout hybrid)(O'Grodnick 1979; Markiw 1992a). Walker and Nehring (1995) reported that the kokanee in Colorado appear to be more resistant to whirling disease than previous literature indicates.

Cutthroat trout are more resistant to the disease than rainbow trout. Walker and Nehring (1995) noted that Snake River finespotted cutthroat trout in a state hatchery that shared a similar lot history with similar-sized rainbow trout were negative (n = 20) whereas rainbow trout had infection rates of 65-70%. In a single trial with greenback cutthroat trout *O. clarki stomias*, M. Markiw noted that rainbow trout yielded 15.6 times more spores than the cutthroat trout (Walker and Nehring 1995). With these susceptibility differences in mind, cutthroat trout may be better candidates for stocking or wild-fish management in infected waters, especially in the West.

The best management is to avoid contaminating negative waters, containing the infection through enforcement of disease regulations, public education, and disinfection. Thorough drying of contaminated mud can kill spores (Hoffman and O'Grodnick 1977). Heat has been effective in causing the distortion and probable death of spores. Hoffman and Putz (1969) examined spores after heating in 0.85% saline to 60, 80, and 100 C. These temperatures were effective in killing spores whereas temperatures of 40 C or room temperature were not. Later tests by Hoffman and Markiw (1977) indicated that heating spores for 10 min at 90 C was effective in killing the spores as determined by methylene blue staining (killed spores take the stain, live spores do not). Heating at lower temperatures progressively reduced the percentage killed (80 C, 98%; 70 C, 60 %; 60 C, 34%; 50 C, 24%) in five trials. Heating for longer periods (up to 100 min) at 70 C increased the percentage of spores that were stainable, but still did not reach 100% (Hoffman and Markiw 1977). Smoking fish at 66 C for 40 min was effective in killing spores (Wolf and Markiw 1982).

Future research into control of the disease is needed. Immunological studies have indicated that rainbow trout produce antibodies against *M. cerebralis*, but protection against infection has not been demonstrated (Griffin and Davis 1978; Markiw 1992a). Enhancement of the immune response may be one avenue of research. Hybrids of resistant salmonid species are being evaluated in Utah for use in infected reservoirs. Resistance of various strains of rainbow trout need to be determined. A greater understanding of the environmental determinants influencing the severity of the disease may lend greater insight into control measures that minimize mortality. Until future research provides additional approaches to controlling whirling disease, the data summarized above should be helpful in the control and eradication efforts.

Eric Wagner

(Continued on page 11)

(Continued from page 10)

References

Bauer, O. N. 1962. The ecology of parasites of freshwater fish. Bulletin of the State Scientific Research Institute of Lake and River Fisheries 49:3-189. (Translated from Russian for the National Science Foundation, Washington, DC by the Israel Program for Scientific Translations, Jerusalem).

CRWC(Colorado River Wildlife Council) 1988. Whirling disease management in North America. Colorado River Wildlife Council Emergency Conference Proceedings.

El-Matbouli, M., and R. W. Hoffman. 1991. Effects of freezing, aging, and passage through the alimentary canal of predatory animals on the viability of *Myxobolus cerebralis* spores. Journal of Aquatic Animal Health 3:260-262.

El-Matbouli, M., and R. W. Hoffman. 1991. Prevention of experimentally induced whirling disease in rainbow trout *Oncorhynchus mykiss* by Fumagillin. Diseases of Aquatic Organisms 10:109-113.

Griffin, B. R., and E. M. Davis. 1978. *Myxosoma cerebralis*: Detection of circulating antibodies in infected rainbow trout (*Salmo gairdneri*). Journal of the Fisheries Research Board of Canada 35:1186-1190.

Hoffman, G. L. 1974. Disinfection of contaminated water by ultraviolet irradiation, with emphasis on whirling disease (*Myxosoma cerebralis*) and its effect on fish. Transactions of the American Fisheries Society 103:541-550.

Hoffman, G. L. 1990. *Myxobolus cerebralis*, a worldwide cause of salmonid whirling disease. Journal of Aquatic Animal Health 2:30-37.

Hoffman, G. L., and C. E. Dunbar. 1961. Studies on *Myxosoma cerebralis* (Hofer) Plehn (Protozoa: Myxosporidea) the cause of whirling disease of trout. Annual meeting of the American Society of Parasitologists, Abstract 53, August 27-31, Lafayette, IN. Journal of Parasitology 47 (4, section II): 29.

Hoffman, G. L., C. E. Dunbar, and A. Bradford. 1962. Whirling disease of trouts caused by *Myxosoma cerebralis* in the United States. Special Scientific Report No. 427, U.S. Fish and Wildlife Service, Washington, DC.

Hoffman, G. L. Sr., and G. L. Hoffman, Jr. 1972. Studies on the control of whirling disease (*Myxosoma cerebralis*). Journal of Wildlife Diseases 8:49-53.

Hoffman, G. L., and M. E. Markiw. 1977. Control of whirling disease (*Myxosoma cerebralis*): use of methylene blue staining as a possible indicator of effect of heat on spores. Journal of Fish Biology 10:181-183.

Hoffman, G.L., and J. J. O'Grodnick. 1977. Control of whirling disease (*Myxosoma cerebralis*): effects of drying, and disinfection with hydrated lime or chlorine. Journal of Fish Biology 10:175-179.

Hoffman, G. L., and R. E. Putz. 1969. Host susceptibility and the effect of aging, freezing, heat, and chemicals on spores of *Myxosoma cerebralis*. Progressive Fish-Culturist 31:35-37.

Lom, J. 1964. Notes on the extrusion and some other features of myxosporidian spores. Acta Protozoologica 2:321-327.

Markiw, M. E. 1992a. Salmonid whirling disease. Fish and Wildlife Service Leaflet 17. Washington, D.C.

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

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

Rasmussen, C. J. 1965. Control of whirling disease in Danish trout farms. European Inland Fisheries Advisory Commission Technical Paper No. 2: 14-15.

Taylor, R. E .L., S. J. Coli, and D. R. Junell. 1973. Attempts to control whirling disease by continuous drug feeding. Journal of Wildlife Diseases 9:302-305.

Taylor, R. L., and M. Lott. 1978. Transmission of salmonid whirling disease by birds fed trout infected with *Myxosoma cerebralis*. Journal of Protozoology 25:105-106.

Uspenskaya, A. V. 1957. The ecology and spreading of the pathogen of trout whirling disease- *Myxosoma cerebralis* (Hofer, 1903, Plehn, 1905) in the fish ponds of the Soviet Union. Bulletin of the All-Union Scientific Research Institute Fresh-water Fisheries 42: 47-55 .

Walker, P. G., and R. B. Nehring. 1995. An investigation to determine the cause(s) of the disappearance of young wild rainbow trout in the Upper Colorado River, in Middle Park, Colorado. Colorado Division of Wildlife, Brush, Colorado.

Wolf, K., and M. E. Markiw. 1982. *Myxosoma cerebralis*: inactivation of spores by hot smoking of infected trout. Canadian Journal of Fisheries and Aquatic Sciences 39:926-928.

The Ichthyogram is a quarterly publication of the Fisheries Experiment Station, Utah Division of Wildlife Resources, Logan Utah 84321.

Editor: Chris Wilson
Contributors: Eric Wagner
Ronnie Arndt
Chris Wilson

This newsletter is printed on recycled paper .

Send comments or change of address to:
EDITOR, The Ichthyogram
1465 West 200 North
Logan, UT 84321

STATE OF UTAH
NATURAL RESOURCES
Wildlife Resources

The Ichthyogram

Fisheries Experiment Station
1465 West 200 North
Logan, UT 84321