The Ichthyogram

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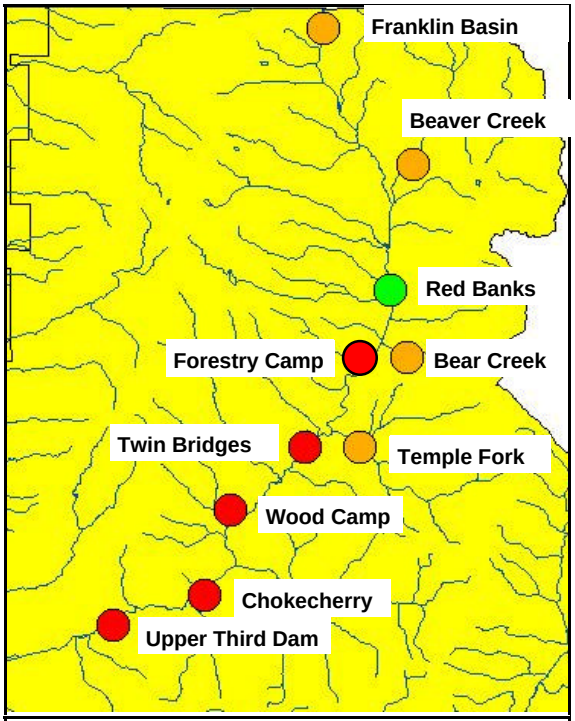
Myxobolus Cerebralis, Other Parasites Found in the Logan River

Fish Pathologists at the Fisheries Experiment Station have announced the discovery of the whirling disease parasite in fish from the Logan River. Cutthroat trout sampled in late August have been found positive at several locations along the river.



Sampling sites on Logan river drainage. Red circles were positive, green circles negative, orange circles not yet completed

Infected fish were found above Third Dam, at Chokecherry Campground, Wood Camp, Lower Twin Bridges and the USU Forestry Camp. Only samples taken at the highest location above Red Banks campground failed to show any sign of the infection. Cutthroat trout were the most widely and heavily infected species.

The investigation was complicated by the discovery of two additional parasites. One similar in appearance to Myxobolus cerebralis, was found in the brain and spinal cord of most fish rather than in cartilage. Little pathology was observed from this parasite. Another parasite was discovered in cysts in bone and cartilage from several of the fish and appeared to be a *Henneguya* species. Finally, confirmation of whirling disease was made by discovery of cartilage lesions containing spores, along with positive results of PCR analysis performed

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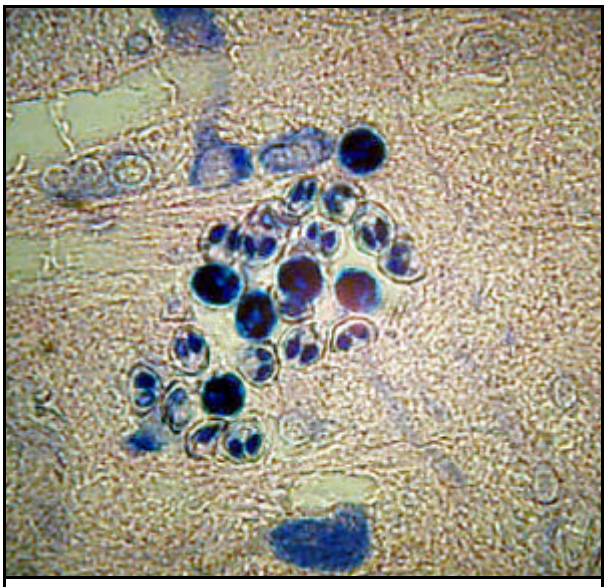
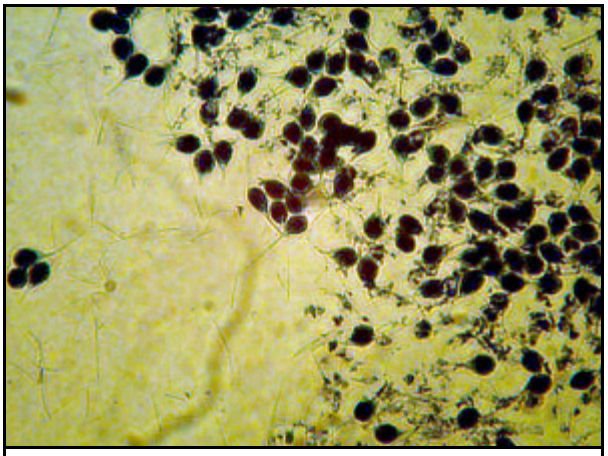
Extensive testing of fish in the Logan River was performed in 1993 after the discovery of the parasite in three private hatcheries, the Little Bear river and lower Blacksmith Fork river in Cache Valley. At that time no infected fish were detected in the Logan. The infection spread to Porcupine and Hyrum reservoirs between 1994 and 1995.



Spores of unidentifed myxosporean (possibly *Myxobolus neurobius*) in the spinal cord of a cutthroat trout from the Logan river.

The new findings and lack of any symptoms in the infected fish have led biologists to conjecture that the parasite is a recent introduction to the Logan river. It is not known how the infection spread to the river system, but biologists speculate that transport and improper disposal of infected fish parts or movement of contaminated mud on anglers boots may be responsible. The Logan river has been considered at high risk due to its proximity to contaminated watersheds and its popularity with recreationists.

There is no accurate way to determine what impact the parasite will have on the fishery. The Logan river is known for its wild cutthroat trout fishery. Small fish are much more susceptible to whirling disease, but it isn=t known if the parasite has spread to smaller tributaries where newly hatched wild fish are often found. There is no effective treatment for infected fish. Researchers are focusing much effort on finding ways to manage tiny tubifex worms which act as an intermediate host for the parasite.



Spores resembling *Henneguya* sp. from a cartilaginous cyst in a cutthroat trout from the Logan river.

A response team was formed and met on November 23 to oversee further investigations to determine the extent and spread of the parasite. Additional samples were taken from Beaver Creek, the upper Logan river in Franklin Basin, Bear Creek and Temple Fork. Results of these tests are underway. Biologists also plan to sample the lower Logan river within the city of Logan to determine if the infection has spread downstream yet.

Chris Wilson

June Sucker Spawn with the Aid of HCG

The June Sucker (*Chasmistes liorus*) is endemic to Utah Lake. Various factors have led to the decline of this species resulting in the listing of this species as endangered by USFWS. As part of the recovery effort, various year classes have been held at the June sucker culture facility at the Fisheries Experiment Station since 1991. Recently, interest has been renewed in artificial spawning of captive broodstock as a means to increase the number of fish. Unfortunately there is no direct information on optimal dosage regimens, so trial and error approaches have been necessary.

Attempts to induce spawning were reported in a previous article (Ichthyogram, Vol. 9, #3). In that trial, 800 IU/lb of human chorionic gonadotropin (HCG) were administered by an intraperitoneal (IP) injection to five female June suckers showing external signs of ripeness, followed by a repeat injection of 400 IU/lb four days later. The trial was somewhat limited by availability of the compound and uncertainties about the legal use on a threatened species.

There's a sucker born every minute.

Phineas T. Barnum

Despite the limited success, it was felt this approach deserved further investigation. A new trial was designed, utilizing a protocol reported successful for Spotted and White suckers. This regimen called for administration of 1000 IU/kg daily for three to five days.

Five females were selected for the trial. Twenty-four hours after the fourth injection, each of the five females ovulated. One fish died the day after spawning due to repeated handling stress and a bacteremia from *Aeromonas hydrophila*. Ten males were injected on days three and four at 500 IU/kg. Individual paired matings were performed: eggs from each female were fertilized using a male from another year class.

A total of 19,250 eggs were collected from the five females. There were 41.6 slightly adhesive eggs per milliliter. Only one batch of green eggs was counted to avoid handling stress. The stress did not seem to decrease eye-up. It is estimated that four of the females gave about 3,000 eggs each while one gave nearly 7,500 eggs. After rolling eight days at 60^0F in plexiglass eyeing jars the eggs eyed-up. About 80% of the eggs hatched on the fourteenth day. The loss before eye-up was not recorded but is included in the 20% that did not hatch. Fungus was not prevalent. Twelve hundred and eighteen cripples (8%) were picked out on June 14th and 15th 1999. On August 30th 1999 a total of 13532 fish were transferred to Utah State University ponds where they will be used for disease certification.

Dates	Days	Mortality	% of fish
June 16th to June 30th	14	1072	7.6%
July 1st to July 31st	31	126	0.9%
August 1st to August 30th	30	56	0.4%.

Table 1. Mortality among larval June suckers by time period.

Future plans include looking at the effects of HCG on sperm count and sperm motility, cryopreservation of June Sucker gametes, and the use of sperm extenders for the short term preservation of June Sucker milt.

Roger Mellenthin

Effect of Povidone Iodine on TAM Viability

When fish eggs are collected from wild broodstocks and other sources outside the hatchery, the potential for bringing a pathogen into the hatchery is a serious concern to hatchery managers. As a precaution, povidone iodine (1-ethyenyl-2-pyrrolidinone homopolymer and 1-vinyl-2-pyrrolidinone polymer iodine complex) is used routinely for disinfection of fish eggs in water that potentially carry pathogens. Betadine**7** or PVP-Iodine is a commercial product that is 10% povidone-iodine (1% active iodine). The concentration of this stock solution typically used to disinfect salmonid eggs is 1% or 100 ppm of active iodine (Ross and Smith 1972; Amend 1974). While this concentration has been shown to be effective for controlling the majority of external bacteria and viruses in *in-vitro* tests (Amend and Pietsch 1972; Ross and Smith 1972; Chapman and Rogers 1992; Goldes and Mead 1995; Kumagai et al. 1998), the effect on *Myxobolus cerebralis* has not been tested. Vital staining has been useful for many applications, including the testing the viability of *M. cerebralis* (Markiw 1992).

A series of tests were conducted on triactinomyxons (TAMs), the infective stage of the salmonid parasite *Myxobolus cerebralis,* to determine the concentration of iodine needed to kill them. For each concentration, three to six replicate tests were conducted. For each test, 2 mL of the test iodine solution were mixed with 2 mL of TAM stock solution in a test tube and left at room temperature (15-20 C) for 10, 30 or 60 min. The pH of the mix was 6.5. A minute or two before the time was up, the mixed solution was poured into a 10 um mesh filter to start filtering. At the allotted time, the filter retentate was rinsed with 20 mL of hatchery well water. This process took several minutes, after which 100 uL aliquots of retentate were transferred to 3-4 microscope slides. The slides were subsequently stained with 50-75 uL each of propidium iodide (52 mg/L) and fluorescein diacetate (100 uL of 5 mg/mL stock solution diluted with 8.3 mL hatchery well water). Control slides were made from the TAM stock solution and stained as noted above. The concentration of the iodine stock solution was verified with a commercial colorimetric test. The same stock solution was used for all tests.

After incubation in a refrigerator at 4-7EC for at least 45 min, the TAMs were observed by epifluoresence microscopy. The TAMs were classified as either red (dead), green (viable), or red and green (possibly viable).

The results of the iodine tests are presented in Table 1. Povidone-iodine concentrations of 50% or 5,000 ppm of active iodine for an hour were required to kill greater than 99% of the TAMs. This is 50 times the concentration typically recommended for treatment of fish eggs for bacterial and viral disinfection (McFadden 1969). These results should be confirmed by attempting to infect fish with treated TAMs.

Clearly, higher concentrations of iodine are needed to adequately disinfect incoming water for *M. cerebralis*. The question becomes AHow high can we safely go?@ Amend (1974) tested the toxicity of Betadine and Wescodyne**7** (1.6% active iodine in the form of 9.1% polyethoxy polypropoxy polyethoxy ethanol-iodine complex, 8.74% nonylphenoxypolyethanoxyethanol iodine complex and 82% inert ingredients) to rainbow trout eggs. Amend (1974) found that toxicity was dependent upon pH and the stage of development of the eggs. At pH 6.9, the LC_{50} for active iodine was 1480 ppm in a 15 min treatment or 1050 ppm in a 60 min treatment of eyed eggs. If the solution was buffered, the LC_{50} of eyed eggs was increased to greater than 2000 ppm at either pH 7.0 or 8.0.

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Povidone-iodine concentration % (ppm active iodine)	Duration (min)	Dead (%)	Viable (%)	Possibly viable (%)	range of TAM numbers per replicate (n)
0.0 (0.0)		1.5	91.2	7.3	10-100 (24)
1.0 (100)	10	64.3	16.5	19.2	82-100 (3)
2.5 (250)	10	67.2	25.4	7.4	76-104 (4)
5.0 (500)	10	66.3	11.7	22.0	100-100 (3)
50.0 (5,000)	10	76.5	9.3	14.2	100-160 (3)
50.0 (5,000)	30	90.7	8.8	0.4	70-102 (3)
50.0 (5,000)	60	99.3	0.5	0.2	91-117 (6)

Table 1. Mean percentage of non-viable, viable, and possibly viable triactinomyxons after exposure to various concentrations of povidone-iodine. Iodine concentrations are given as a percentage of the commercial povidone-iodine stock solution (10% povidone-iodine) and in active iodine concentration in ppm (given in parentheses). Control values were pooled.

When eggs were water hardened in iodine, 25 ppm of iodine was safe, but 100 ppm resulted in significant mortality. Leary and Pederson (1988) noted reduced survival in rainbow trout eggs water hardened in 1.24% Betadine buffered to pH 8.0. If eggs were allowed to water harden for 30 min, Amend (1974) reported no significant impacts on egg mortality, hatchability, or abnormalities at concentrations of 25, 100, or 200 ppm iodine. McFadden (1969) noted that up to 2.5% povidone iodine for 10 min was not toxic to eyed rainbow trout eggs, but concentrations of 3, 4, and 5% (300-500 ppm iodine) resulted in eggs surviving less than 24 h (no pH given). Alderman (1984) noted that concentrations of iodine from 75 to 200 ppm at pH values of 6.5, 6.75, or 7.5 for 10 min were safe for eyed Atlantic salmon *Salmo salar* eggs. For eyed rainbow trout eggs, Alderman (1984) tested concentrations of 50 to 4,000 ppm iodine for 10 min at pH levels from 3 to 8 and found that the LD_{25} was about 800 ppm at pH 6.0 and in excess of 3000 ppm at pH 7.0. For freshly fertilized rainbow trout eggs, Alderman (1984) found that mortality was also highly variable among females; 800 ppm iodine (either 10 min post-fertilization or after 30 min of water hardening) resulted in nearly complete mortality for eggs from some females and less than 10% for others.

Based upon the above research, achieving 5,000 ppm iodine without killing the eggs might be possible for eyed eggs at pH 8, but unrealistic for freshly fertilized eggs. Alternative chemicals for disinfection may be necessary. Glutaraldehyde was superior to iodine, chloramine-T, and sodium hypochlorite in treatment of plaice (*Pleuronectes platessa)* eggs (Salvesen and Vadstein 1995); concentrations of 400-800 mg/L for 5-10 min was recommended for Atlantic halibut (*Hippoglossus hippoglossus)* eggs, whereas a shorter contact time (2.5 min) was recommended for Turbot (*Scophthalmus maximus)* (Salvesen et al. 1997). For treatment of largemouth bass (*Micropterus salmoides)* eggs, acriflavine (500-700 ppm for 15 min) was the disinfectant recommended by Wright and Snow (1975) over five other disinfectants.

Given the above data, exploration of alternative disinfectants may be necessary for prophylaxis against organisms such as *M. cerebralis* actinospores that may be carried with

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New Faces at the FES

Amy Howa has resigned from her job working with the whirling disease research grant. The large amounts of time at the microscope did not suit her, so she is moving on. In her place we are fortunate to have **Mark Smith**. He is a familiar face at the FES, having worked as a seasonal in the research program with whirling disease and also with the culturists as a technician caring for the June suckers. He is a recent graduate of the fisheries program at Utah State University. He has "hit the ground running" with the viability research and should generate some interesting data which he will present at the next whirling disease symposium.

We also wish to welcome **Quinn Cannon**, who has replaced Mark in the part-time culture technician position. He is starting his sophomore year at Utah State. He volunteered at the FES during his freshman year and collected some useful information on the firing of nematocysts of Cnidarians to compare with the mechanisms of polar filament extrusion of Myxobolids. He worked for the summer with the US Forest Service, working with native cutthroat trout surveys under the direction of Paul Cowley. He is a hard working individual and will now be gaining some additional experience in fish culture to further develop his career.

Health Condition Profile Software Getting a Facelift

AUSUM, the software program associated with necropsy based Health Condition Profile is getting an update. Roger Mellenthin is working with Ron Goede to re-create the program in *Quattro Pro* for Windows, version 8. The original program was created in the DOS based version of the Lotus spreadsheet, which is not Y-2-K compliant. This update should overcome that obstacle and allow for easier printing of summary reports. In addition, the overall deformity, skin lesion and fin deformity indices will be included for the first time. This current effort is considered a prelude to eventual conversion of the program for use with Microsoft *Access* or *Excel*, to accompany a statewide conversion to those programs.

The program is expected to be posted on the state network for access by state hatchery workers and fisheries biologists by the end of the year. Out of state users who wish to upgrade can contact Ron Goede at nrdwr.rgoede@state.ut.us.

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eggs into the hatchery. In the meantime, consideration should always be given to the source of water used and where water is dumped when bringing in eggs from the wild, taking care not to contaminate water supplies. When using iodophors, a fish culturist would also be wise to heed the recommendations of Chapman and Rogers (1992): use at least a 4:1 iodophor to egg volume ratio, make new solutions when treating multiple batches, and circulate the iodophor well during treatment.

Eric Wagner

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