The Ichthyogram

March 1999 **Volume 10 Issue 1**

Success on the Fungus Front

Over the past few years several studies have been conducted at the Fisheries Experiment Station (FES) evaluating hydrogen peroxide as a fungicidal treatment for trout eggs. Initially treatment concentrations of 250 and 500 ppm for 15 minutes were evaluated, but were not entirely successful in controlling fungus and egg loss. Follow-up studies tried treatments of 500 ppm for 45 minutes, but due to technical difficulties poor eye-up and hatch resulted. Deformity and survival information collected from these follow-up tests did seem to indicate that treatment durations of 45 minutes were too long. Because of these results, for this current test it was decided to cut the treatment duration to 35 minutes at the same concentration of 500 ppm. Also, a recent article by Gaikowski et al. (Journal of Aquatic Animal Health 10:241-251, 1998) indicated that rainbow trout eggs may be sensitive to hydrogen peroxide during a certain stage of development. They found that hatching success may be improved if hydrogen peroxide treatment was discontinued between 70-140 daily temperature units (DTUs °C).

Based on their findings a decision was made to cut the treatment duration of our test from 35 to 5 minutes during this "critical" period. The five minute treatment was attempted rather than no treatment at all for fear of fungal growth encroaching on the eggs. In February approximately 60,000 rainbow trout eggs of the Fish Lake-DeSmet strain were fertilized at the Egan State Fish Hatchery. The eggs were allowed to water-harden for about an hour and then transported to the FES. Once at the station the eggs were treated for 10 minutes in 100 ppm betadyne, and then divided into nine, 10-L upwelling-type egg jars at 6,300 eggs per jar (three jars per treatment) for the formalin, peroxide, and control treatments.

An additional treatment using paraformaldehyde (formalin that had formed a white precipitate) was evaluated in two clear, 7-L concave-bottomed jars at a density of 3,900 eggs per jar. The paraformaldehyde treatment was conducted because several gallons of formalin had been exposed to freezing temperatures during the winter and small amounts of paraformaldehyde had formed in the bottoms of the containers. The treatment was therefore conducted to see if the nonprecipitate portion of the formalin could be safely used on eggs.

The chemical treatments were started the day following fertilization via peristaltic pumps. Formalin and paraformaldehyde treatments were conducted daily for 15 minutes at 1,667 ppm. The hydrogen peroxide treatment consisted of a 500 ppm concentration of 35 minutes duration for the first six days of the study and from the 12th

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day to eye-up. For the intervening days, (70 - 140 DTU °C at 12.5°C) the duration of treatment was cut to five minutes daily. The control jars were left without chemical treatment.

The control, formalin, and peroxide treatments were inoculated with a fungus that had been previously cultured. For the culture, fungus samples from waste feed out of a raceway were collected and used to start cultures on petri dishes that contained Bacto corn meal agar. These cultures were assumed to contain *Saprolegnia parasitica*, the chief fungus of trout egg loss. When it came time to inoculate the egg jars, $1cm^2$ of the agar culture was removed and placed into small tissue cassettes which were then suspended in the egg jar inflow. The inoculum was replaced one week after the initial inoculation.

Water quality measurements were made once during the test and showed no real differences between the jars. Oxygen averaged about 7.1 mg/L; temperature 12.8 °C; oxygen saturation 78%; nitrogen saturation 111%. A chemical assay was also conducted once during the test to verify hydrogen peroxide treatment concentrations. Ten minutes after the start of the peroxide treatment the concentration was 387 ppm, and after 20 minutes it was 567 ppm.

There were no significant differences between the formalin and peroxide treatments with respect to eye-up, hatch or deformities (Table 1). The control group experienced a significant impact due to fungal growth; 28% of the control eggs were lost, while neither the formalin nor the peroxide treatments exhibited any fungus at all. The paraformaldehyde eggs were not impacted by possible toxicity effects and had similar eye-up and superior percent hatch to the formalin, control, and peroxide treatments. The number of deformities within the peroxide treatment (1.3%) was slightly higher than the control (0.9%) or formalin (1.0%) groups, but the difference was not significant. Deformities within the paraformaldehyde treatment were not measured. The percentage of deformities from the two previous tests conducted at the FES ranged from 2.9-3.3% for hydrogen peroxide treatments.

The results from the above test indicate that we are getting closer in achieving a good hydrogen peroxide treatment, and being able to culture fungus. We did not specifically test to see if a reduction in the duration of peroxide treatments (from 35 to five minutes) during the critical developmental stage was a contributing factor to the success of the treatment, but this should be tested in the future. Overall it appeared that a 500 ppm 35 minute hydrogen peroxide treatment was a successful alternative to the formalin treatment. A treatment cost analysis was made to compare the cost of chemicals used for this particular study. The total cost of formalin used was $26.85 and $7.01 for the hydrogen peroxide. This analysis favors the use of hydrogen peroxide over formalin and the results from the egg data suggest no significant difference in using peroxide or formalin. Further tests on a larger production scale with different water quality parameters may still be necessary.

Ronney Arndt

Table 1. Percent eye-up, hatch, and deformities of rainbow trout eggs treated with formalin, hydrogen peroxide, paraformaldehyde, or left untreated (control).
[1]based on number of eyed eggs [2]based on number of hatched eggs

Treatment	Eye-up %	Eggs with fungus %	Hatch[1] %	Deformities[2] %
control	63	28	63	0.9
formalin	80	0	80	1.0
hydrogen peroxide	82	0	81	1.3
paraformaldehyde	82	0	91	not calculated

Little South Fork Provo River: A Comparison of Population Estimates between 1996 and 1998

The Little South Fork Provo River is a first-order tributary to the Provo River about a kilometer downstream of the confluence with the South Fork Provo River in the Uinta National Forest, and upstream of Woodland, Utah. Land within its watershed is a mixture of pasture and aspen-fir woodland, used primarily for grazing, forest recreation, and timber harvest. In October and November 1997, sampling of fish from the Weber-Provo Canal indicated that spores in head samples from rainbow and cutthroat trout appeared to be *Myxobolus cerebralis*, the causative agent of whirling disease (Wilson 1998. *Ichthyogram* 9 [1/2]:1). This could not be confirmed histologically, but was verified with a polymerase chain reaction assay method.

Results from additional samples in December 1997 were still questionable for multiple locations in the Provo River drainage. Mixtures of spores were observed, some consistent with *M. cerebralis* and some larger; the latter were observed in nervous tissue via histology, but no lesions pathognomonic for *M. cerebralis* were found. A mixed infection was presumed. Lack of deformities and the inability to confirm the disease histologically indicated that whirling disease is a recent introduction to the area and/or infections are light. The Utah Division of Wildlife Resources (DWR) was interested in further sampling to determine if the disease was indeed there and what impact it was having on the native fish population.

Historical population estimates by the DWR for this stream could not be found. However, in 1996 a population estimate was made by Paul Cowley (US Forest Service) and his crew. These data are compared to population estimates made in 1998 by the DWR for two stream segments; one at the same location as Cowley's sample and another downstream.

Methods

On 14 August 1996, a 104 m section of the Little South Fork was electroshocked (Smith-Root Co.; 500 V) by Paul Cowley, Dave Fogle, and Mark Brough. The sample site was at the crossing of the Bench Creek Road (FSR-052; T03S, R07E, Sec32). Stream width averaged 1.85 m, and depth 0.3 m. Water temperature was 10°C (50°F) and conductivity was 180 uS. Population estimates and confidence limits were made from the results of two passes.

On 18 August 1998, the section described above was re-sampled. Water quality at the time was: temperature 13°C, pH 7.5, total hardness 103 mg/l, and total alkalinity 86 mg/l. A 300 foot (91.5 m) section was sampled by two-pass electrofishing. A crew of about 6-7 was involved including Charlie Thompson, Don Wiley, Eric Wagner, Ronney Arndt, and Mark Smith. Average width at this time was 5.0 m.
A second site, downstream of the first, was sampled 19 August 1998 about 1-2 km above the confluence with the Provo River near an A frame cabin on the Bar X Ranch, downstream of a small hand-made rock dam. Mean width was 4.1 m and station length was 91.5 m (300 ft) for the two-pass estimate. Water temperature was 9°C at 11:30 am, pH 8.0, total hardness 120 mg/l, and total alkalinity 86 mg/l.

Results and Discussion

In both years, the catch at the upstream site consisted entirely of cutthroat trout and sculpin (presumably mottled sculpin, but not keyed out). At the downstream site in '98, two brown trout were captured (342 mm, 63 mm), as well as an unusually large long-nosed dace (138 mm, 26 g), in

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addition to the cutthroat trout and sculpin. No deformities associated with whirling disease were noted for any fish.

In 1996, cutthroat trout ranged in size from 75 to 275 mm (average = 176.6 mm). Individual weights ranged from 3 to 211 g and averaged 76.5 g. For cutthroat trout over 100 mm, the estimated population for the section was 25 fish (24 fish/100 m). There was also a good distribution of size classes.

In 1998, the 47 cutthroat trout from both passes of the upstream site ranged in size from 22 to 309 mm (mean = 117.9 mm), and individual weights ranged from less than a gram to 290 g (mean = 32.2 g). The estimate for the section was 60 cutthroat trout (66 fish/100 m), which included young-of-the-year. This translated to about 1317 fish/ ha (249 SD) or 42.4 kg/ha (13.2 SD). The histogram of length frequencies (Figure 1) indicated that the number of larger fish was diminished in 1998. This may have been a result of previous sampling and also due to the presence of a campsite where anglers had easier access to the stream than above and below this point. The number of smaller cutthroat trout appear to have increased in response to removing the larger fish.

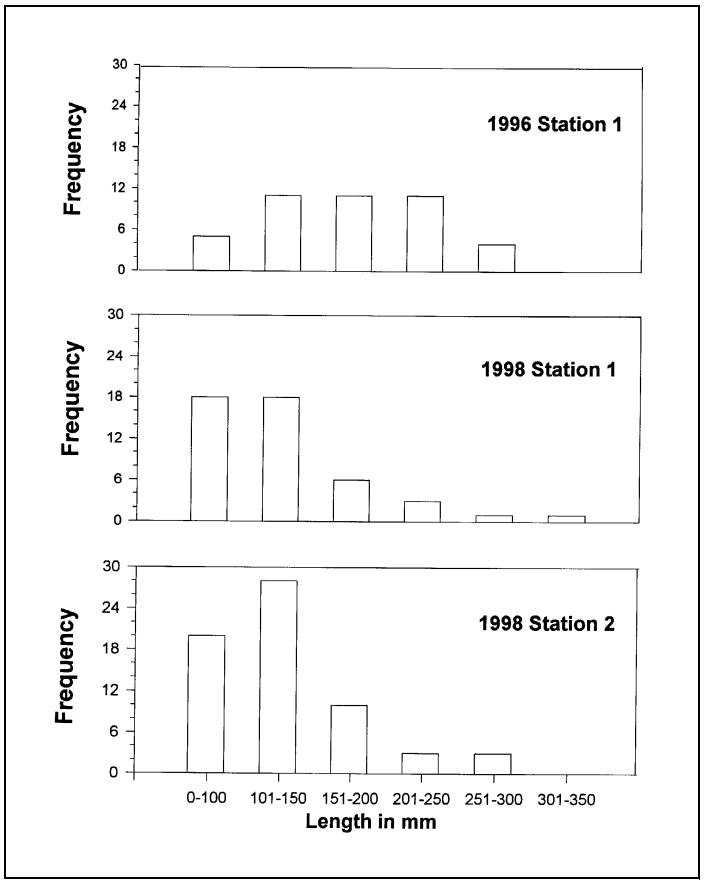


Figure 1. Length-frequency histogram of cutthroat trout from the Little South Fork of the Provo River for an upstream site sampled in 1996 and 1998, and for a downstream site on the Bar X Ranch sampled in 1998 (Station 2).

At the downstream site, the 64 cutthroat trout from both passes ranged in size from 29 to 290 mm (mean = 128 mm) and weighed from 1 to 269 g (mean = 34.0 g). The estimate for the section was 66 cutthroat trout (72 fish/100 m). This translated to 1766 fish/ha (59.4 SD) or 60.1 kg/ha (11.78 SD). The length frequency distribution for this site appeared to indicate a healthy population, with adequate numbers of small fish.

The results of whirling disease testing of samples from 1998 indicated that cutthroat trout from the upstream site were negative. At the downstream site just a kilometer or two above the confluence with the S.Fk. Provo, the brown and cutthroat trout both were questionable; i.e., there were spores present, but they may have been another species of Myxobolid such as *M. neurobius* or *M. kisutchi*.

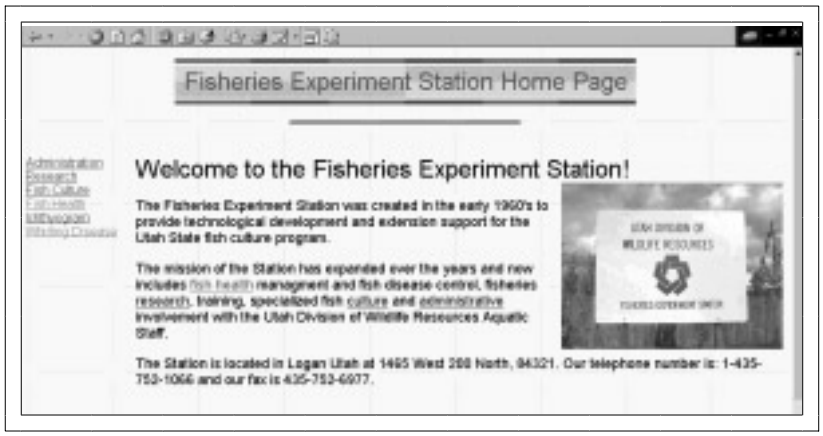
The results thus far do not indicate severe impacts of whirling disease, if indeed it is present. The data presented thus far will hopefully serve as a preliminary look at the impact of whirling disease in the area, providing a baseline for future evaluations.

Eric Wagner

WEB Page for Fisheries Experiment Station Completed

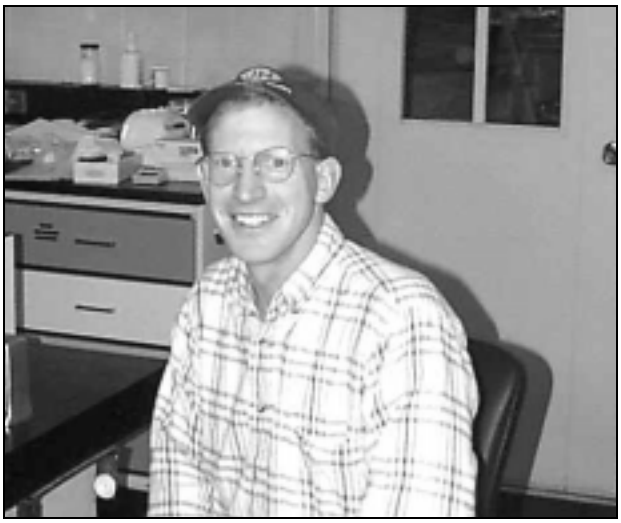
Utah Division of Wildlife Resources recently announced the completion of a long awaited, oft belayed home page for the Fisheries Experiment Station. The page, located at **http://www.nr.state.ut.us/dwr/fes/index.htm** features pages on the four main programs within the station, bio-sketches of the personnel, as well as e-mail links and phone numbers.

In addition, the pages contain a list of recent FES research projects and publications, complete with abstracts, an inventory of fish held at FES, and the list of fish health approved state and federal locations within the state. Information on whirling disease in Utah and do/don'ts for anglers to help prevent further spread of the parasite is listed. Also included is an electronic version of recent editions of the *Ichthyogram* in *Adobe Reader* pdf format.



Plans are underway to update the page on a quarterly basis, or more frequently as needed. Those with questions or suggestions should contact the webmaster at nrdwr.cwilson@state.ut.us.

New Wildlife Technician Joins Whirling Disease Survey Project



Welcome to Robert Montgomery, the newest arrival at the Fisheries Experiment Station. Robert, a native of California, will work on processing/reading samples from the whirling disease survey, as well as wild broodstock pathogen inspections. Robert is a graduate of Utah State University, where he graduated with a B.S. degree in 1998. An avid fly-fisherman, he acts as secretary of the Cache Anglers chapter of Trout Unlimited in Logan.

Robert replaces Art Butts, who entered the graduate school at Utah State University. Art is engaged in a masters project on the population impacts of whirling disease in kokanee salmon at Porcupine Reservoir (and the L.A. Lakers) under Dr. David Beauchamp.

Best wishes to both individuals in their new endeavors!

East Fork Little Bear River: 1998 Population Estimates

In the June 1998 issue of the *Ichthyogram* (9{1/2}:8-10), population estimates for the E.Fk. Little Bear River were presented with respect to whirling disease. The parasite *Myxobolus cerebralis* was discovered in the drainage in 1993, yet its impacts on the salmonid populations are still not well understood. In September 1998, additional sampling was conducted to monitor possible changes.

Two-pass electrofishing was used to capture all fish possible in a 100 m reach. The three reaches sampled in 1997 were sampled again on 10 September 1998. The reach furthest downstream was just above the Liberty Road bridge. The middle reach was in the area that the Division of Wildlife reconstructed to return the stream to a normal meander, and the uppermost reach was between the reconstructed area and Porcupine Dam.

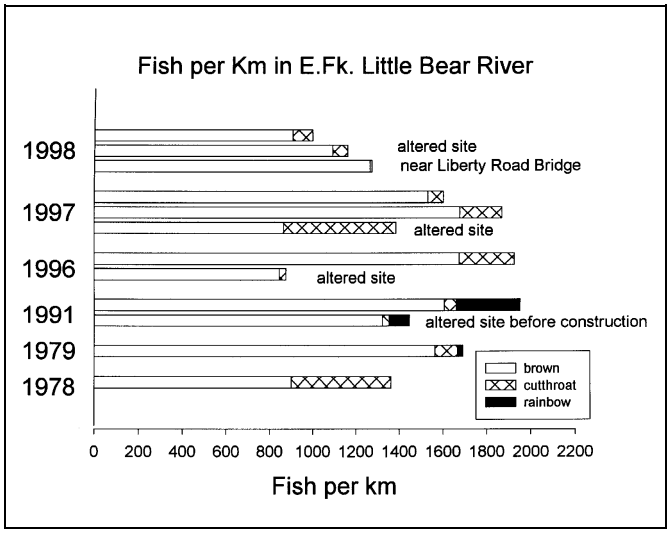


Fig. 1. Estimated number of brown, cutthroat and rainbow trout per km of the East Fork Little River for each of 6 years.

The catch consisted primarily of brown trout, data for which are summarized in Figures 1, 2, and 3. Cutthroat were found in low numbers, primarily in the two upstream reaches. These were probably survivors of the 1997 stocking, averaging 236 mm in total length. No cutthroat or rainbow trout were stocked in 1998. Sloped head deformities were observed in 2 of 17 (11.7%) cutthroat trout; these were shorter than the average (total lengths of 172 and 169 mm). Two additional cutthroat had shortened opercles (259 and 232 mm total length) which may or may not be related to whirling disease. Deformities of the brown trout sampled were limited to obvious hooking injury and one fish with a shortened mandible (<1%).

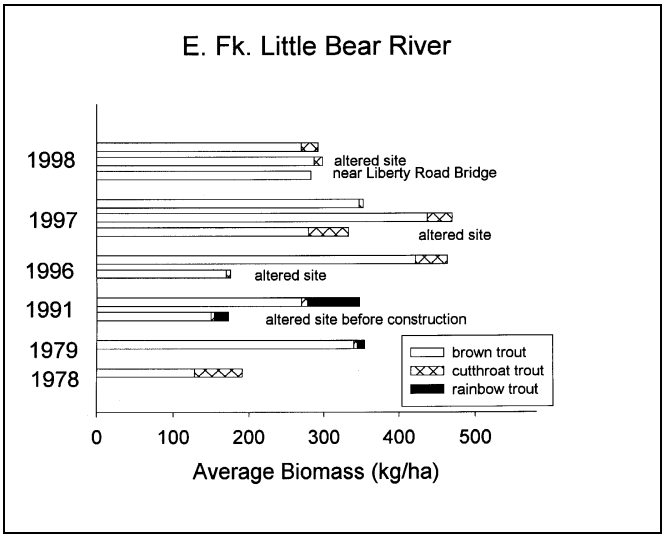


Fig. 2. Average biomass estimates of brown, cutthroat and rainbow trout in the East Fork Little Bear River for each of 6 years.

The histogram of brown trout length frequencies (Figure 3) continues to indicate that juvenile brown trout numbers remain low. The cause may be predation, whirling disease, or some other unknown factor. However, at the population level, the number of brown trout per km and biomass estimates did not differ significantly from 1997 data (*t*-test, $p > 0.05$). Total kg/ha (cutthroat and brown trout data combined) did not differ either, but total fish per km was higher in 1997 ($p = 0.043$). This is likely due to the harvest

Recent Training Sessions Hosted by FES

As part of their mission of technology transfer and training, personnel from the Fisheries Experiment Station recently completed several different educational sessions held in the last few months.

Jim Flanagan, George Coombs and Joey Comp examine a laboratory specimen at the egg taking workshop

In December, state hatchery workers attended the second installment of an egg taking and handling workshop. The session was a followup to the introductory course held earlier in 1998. The course was conducted by Eric Wagner and Ronney Arndt, with assistance from Ron Goede, Joe Valentine, Tim Miles and Chris Wilson.

The Health Condition Profile was taught in March 10-11 by Ron Goede. The course was attended by 26 students, among them new DWR biologists and hatchery workers, biologists from the U.S. Forest Service and graduate students from Utah State University. Ron reports the HCP will be undergoing some changes in the coming month, including insertion of the newest Deformity and Lesion Indices, creation of a computerized "digital" atlas, and reprogramming of the popular "AUSUM" computer program (created in DOS) to a more versatile Windows spreadsheet format.

A Fish Disease/ INAD workshop was held for state hatchery superintendants and others at a special sesssion prior to the Bonneville chapter AFS meeting in Moab on March 15. Tim Miles and Chris Wilson covered some of the more common hatchery disease problems, reviewed diagnostic protocols and unveiled a planned involvement in a New Animal Drug investigation for the use of Chloramine-T for bacterial gill disease.

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of cutthroat trout from the system and their low abundance in 1998.

This winter Porcupine Reservoir was drained for installation of a valve and repairs to the dam. This resulted in high flows during reservoir drainage (during brown trout spawning) and low flows during repair and refilling this spring. The impacts of this on the stream and the salmonids below are unknown at this time. The impact of an environmental disturbance like this in concert with the presence of *Myxobolus cerebralis* is unknown, and the impact would be difficult to attribute to either cause or the combination of the two. Further sampling should provide additional data.

Eric Wagner

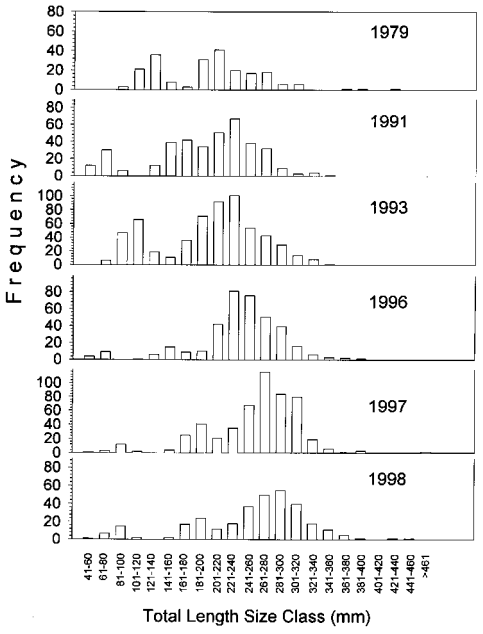

Fig. 3. Histogram of brown trout total lengths (mm) electrofished from the East Fork Little Bear River for 6 different years, all sites pooled.

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

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