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The Ichthyogram

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Hydrogen Peroxide: Toxicity to Rainbow Trout Fry and Chemical Stability

The use of hydrogen peroxide (H_2O_2) as a fungicide treatment for salmonid eggs has received heightened interest over the past several years. This is because fungicidal compounds which were previously used extensively by the aquaculture industry are not legal for use on food fish (malachite green) or are declining in popularity due to human safety and environmental concerns (formalin). Unfortunately no new wonder chemical has come along as a replacement to some of these treatments.

However, in a comprehensive study conducted at the LaCrosse Fisheries Research Center (Prog. Fish-Culturist 56: 4) many different chemicals were tested for their efficacy in reducing the infection rate of eggs. Hydrogen peroxide and NaCl along with formalin were found to be the most desirable in their ability to control fungus and their minimal impact on the environment. Formalin is known to be an effective fungicide but there are safety concerns about its use among those whom handle it and concerns about its effect on the environment when it is released in hatchery effluent. Salt (NaCl) is also a relatively effective fungicide but the amounts required to get a desired effect (15,000 ppm) may make its use prohibitive in some cases. Hydrogen peroxide has risen to the forefront as an alternative fungicidal treatment because of its reported effectiveness in controlling fungus and parasites and because it breaks down in the environment into the friendly products of water and oxygen. The FDA has also designated hydrogen peroxide as a drug of low regulatory priority when it is administered at certain concentrations to control fungus on eggs and all life stages of fish.

Initial studies at the Fisheries Experiment Station and other Utah state hatcheries were designed to compare hydrogen peroxide and formalin and their abilities to control egg fungus. The initial results were inconclusive, so further studies are being conducted and planned to determine effective hydrogen peroxide concentrations for the treatment of trout eggs.

Another proposed use for hydrogen peroxide is its application as a treatment of external bacterial infections and parasites. Recently it has been researched

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for its ability to treat trout with bacterial gill disease (*AFS/FHS Newsletter* 23:1) as well as its ability to control the infection rate of sea lice on Atlantic salmon (*Aquaculture International* 2:10).

In order to determine hydrogen peroxide's ability to act as an effective external treatment, information was required on the chemical concentration and duration of treatment that were lethal to the various life history stages of rainbow and cutthroat trout. To address this an experiment was designed where one month post-hatch rainbow trout fry were exposed to five different hydrogen peroxide concentrations ranging from 70-540 ppm and durations of 30, 60 and 120 min. The fry were placed in 75 l aquaria filled to 50 l with a mixture of hatchery water and hydrogen peroxide to obtain the desired concentration. Each tank was provided with supplemental aeration and water temperatures were constant throughout the study (15° C). After the 30, 60 or 120 min treatment, surviving fish were placed into a trough with fresh hatchery water for 96 h and mortalities recorded. After 96 h gill samples were taken from five fish from each replicate and preserved in 10% formalin for subsequent analysis of any histological changes the treatment may have caused to the gills. In addition to these tests, water quality analyses was conducted on the effect of the various concentrations of hydrogen peroxide on pH, hardness, alkalinity and CO2 concentrations. The actual concentration of hydrogen peroxide for each treatment was also assayed using a Hach test kit (ammonium molybdate/sodium thiosulfate titration method).

As might be expected, results from the toxicity trials showed that increases in both duration of treatment and concentration of hydrogen peroxide correspondingly increased the percent fry mortality. Mortalities began to arise in the 170 ppm treatments of both 60 and 120 min. The

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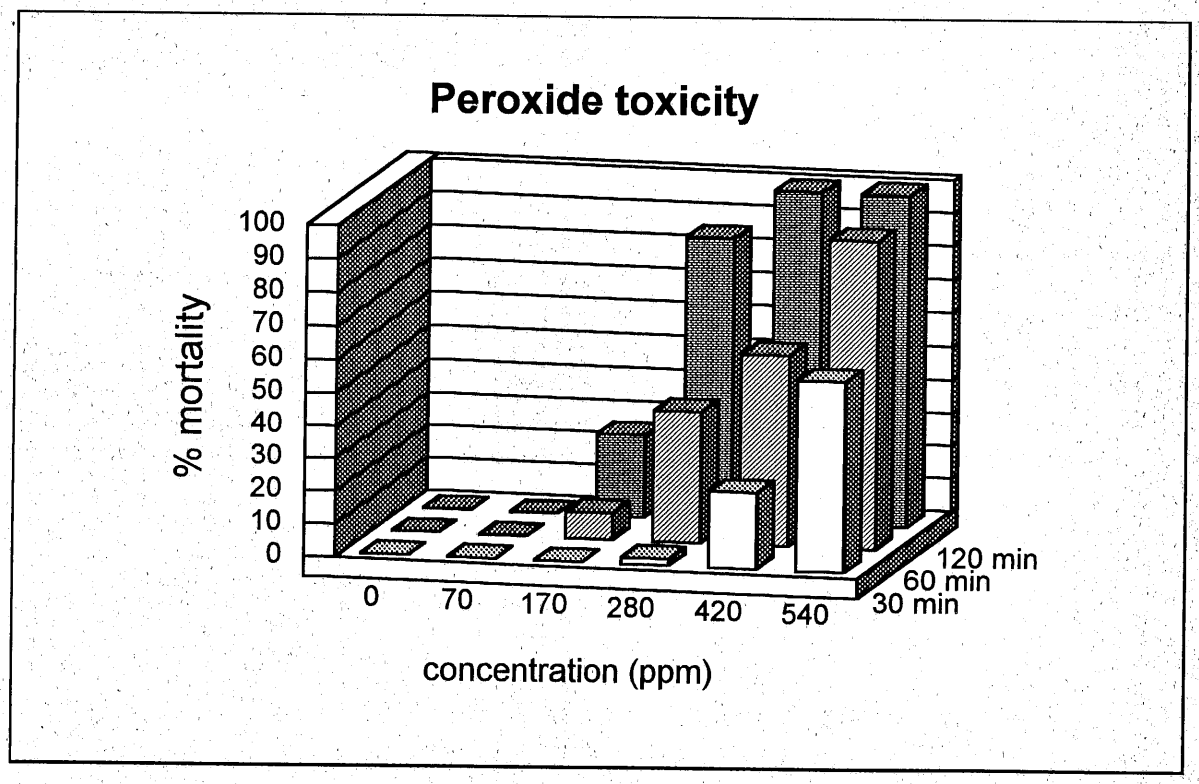

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420 and 540 ppm treatments of 120 min resulted in 100% mortality (treatment concentrations listed are the actual assayed concentrations). It was obvious that fish exposed to a given concentration survived the treatment better when exposed to it for a shorter length of time. For example the 280 ppm-30 min treatment had a mean mortality of 2% compared to 85% mortality for the 280 ppm-120 minute treatment. Data from 96 h mortalities exhibited no clear trend however mortalities were centered around the 170 and 280 ppm-120 min treatments which had mortalities of 45 and 13% respectively. In general it appeared that for all treatments the majority of the mortality occurred during the actual treatment with the exception of the 170 ppm-120 min group which suffered the greatest mortality during the 96 h period.

Information collected from the water quality analysis showed little impact from the addition of hydrogen peroxide on pH, hardness, alkalinity, or CO2 concentrations. Something interesting did result from our analysis of the various concentrations used for the treatments. Assays of all five concentrations gave results that were consistently 60% of the calculated concentration. Even analysis of our hydrogen peroxide supply (35%) showed it to be 60% of its actual concentration. The concentration values listed above are those obtained from the assay and are not the original calculated values. Assuming the Hach test kit assay is accurate, our stock hydrogen peroxide must have broken down over time prior to the tests because assays of hydrogen peroxide concentrations at the initiation of toxicity tests and 24 h later did not differ, indicating stability over that time period. These results underscore the need to assay the stock solution prior to treatment.

Future work at the Fisheries Experiment Station will be directed towards testing the toxicity of hydrogen peroxide at different life stages as well as the toxic effects to different species. It would also be interesting to help quantify the actual efficacy of hydrogen peroxide to reduce or eliminate bacterial infections and parasites and to further determine the effects of treatment duration, hydrogen peroxide concentration and other factors such as organic load and water quality differences on treatment efficacy.

Ronnie Arndt

Aquaculture Training at the FES

The two week intensive cold-water aquaculture training will be presented at the Fisheries Experiment Station from September 18 - 29, 1995. This training has been presented to all UDWR aquaculture personnel to date and is offered whenever enough new people are present for a class. In addition to fish culture techniques, other topics such as hatchery records, fish disease diagnostics, therapeutic agents and safe use of hazardous substances will be covered.

Two days of this course will be devoted to assessment of the Health/Condition Profile (HCP). This portion of the course will be open to other UDWR personnel who have not received the HCP training before. Seating for this portion will be limited, so contact Ron Goede at 752-1066 for more information and reservations.

Utah Brood Stock History: FishLake-DeSmet Rainbow Trout

Utah's Fish Lake-DeSmet rainbow trout (RTFD) resulted from a mix of fish from the two sources, Fish Lake in Sevier County, Utah, and DeSmet Lake, Wyoming. The DeSmet Lake eggs were received at the Fisheries Experiment Station on June 23, 1969 and transferred to Mantua Hatchery on Sept. 24, 1970. The eggs from Fish Lake were derived from wild stocks spawning up Twin Creeks on Aug 22, 1969. The two groups were kept separate initially, but the screen between them collapsed and the two have been wedded together since.

The source of the fish stocked into Fish Lake is unknown. Utah received rainbow trout eggs in 1883 and 1893 from the McCloud River, California. In 1894 and 1895, 43,880 eggs were received from Neosho, Missouri. The disposition of these fish is unknown, but were likely distributed around Utah and Salt Lake counties. The Murray Hatchery (now long gone) received its first eggs in 1899-1900 from the U.S. Fish Cultural Station, Portland, Oregon. In the subsequent years, many public waters were stocked, but it is not known if or when Fish Lake was among those. Ron Goede, Fisheries Experiment Station director, mentioned that Fish Lake was stocked with DeSmet rainbow trout in the years just prior to collecting eggs. Therefore it is highly probable that the Fish Lake rainbow trout were DeSmet Lake origin fish.

The source of the rainbow trout that were originally stocked into DeSmet Lake, WY is unknown, but a Fisheries Research Report by J.W. Mueller and L.C. Rockett (Monogr. 3, WY Game and Fish, Cheyenne, 1980). DeSmet Lake was first stocked in 1896 with cutthroat, lake, kokanee, and brook trout. Rainbow from several sources were stocked later, mostly as fry. After 1953, larger fish were stocked to improve survival and two strains appeared: a fall spawning and a spring spawning strain. The last year that fall spawning rainbow trout were planted was 1957. Only rainbow trout from DeSmet eggs have been planted since 1958, except 5,000 Kamloops rainbow trout planted in April, 1962. Eggs from the spring spawning strain were collected from 1958 to 1984, and some of these eggs were those that initiated the Utah RTFD stock. Wyoming Fish and Game biologists noted that the DeSmet rainbows were not returning to the creel very well, so the strain was discontinued. Eagle Lake rainbow were fin-clipped and stocked into DeSmet Lake; in subsequent years the DeSmet rainbows were culled from the fish traps on the spawning tributaries and the fin-clipped fish kept. By 1991, the original DeSmet strain was gone.

Although it is no longer a broodstock in Wyoming, the strain lives on in Montana where it is called the Harrison Lake strain. DeSmet fish were stocked into this lake in 1976 and probably mixed with rainbow trout already in Harrison Lake (Thurston Dodson, pers. comm.). This lake is a source of wild eggs currently for MT. Dodson also notes that the strain is harder to catch than some of the other strains used in Montana, but is a good strain for stocking into lakes.

The RTFD are spring spawners, currently spawning at Egan Hatchery between mid-Jan and mid-May. This is a shift of about a month from the spawning period of the

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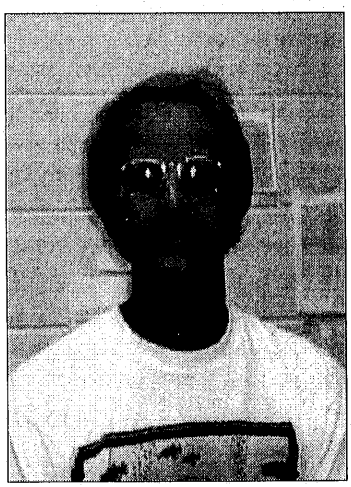
first egg takes in 1973-1974. Tests were conducted at the Fisheries Experiment Station from 1973 to 1976, comparing the performance of several strains of rainbow trout. The Fish-Lake DeSmet fish had higher feed conversion (1.5) than Sand Creek, Shepherd of the Hills, Ten Sleep, Beity, New Zealand, and Albino strains. It also had the highest variability (CV) in length and weight of catchables. The percentage of cripples (2.5%) and the hatching success (43%) were also lowest for RTFD. Although early growth was inferior to the other strains, by the time fish were catchables, mean length was greatest for the RTFD. In contrast, Harold Kinkaid (1986, *Fish Culture in Fisheries Mgmt.*) noted that RTFD had the highest percent eye-up (94%) and the best feed conversion of all the Utah strains listed.

In 1973, the Mantua Hatchery RTFD broodstock numbered 988 females and 462 males. That year 19,500 eyed eggs were shipped to Egan Hatchery. Between 7 Dec 1973 and 28 Mar 1974, 11 egg takes from the Mantua stock resulted in 1,493,345 eggs which were shipped 'green' to Egan Hatchery to supplement the future brood stock initiated the year before. In the 1977-78 brood season, an estimated 4.4 million eggs were produced at Egan by 3,048 5-yr-old and 1,004 4-yr-old females (average of 1086 eggs per female). At DeSmet Lake, the average number of eggs per female from 1958 to 1979 ranged from 560 to 1,994, and averaged 1,340 over the period. Blaine Hilton, Egan Hatchery superintendent, currently is getting an average of about 1450 to 1500 eggs/female. He noted that this egg production is slightly better than most of the other rainbow trout strains reared at Egan.

Fresh Faces at FES

The new office manager at FES is **Debie Kohler.** Debie took the position formerly held by Lisa Lacaden, who recently resigned to get married. Debie is a native of Idaho Falls and has lived in the Logan area for 10 years. She is a former student at Utah State University and studied physics and philosophy. She has one daughter, Sasha, who is 8 years old. She enjoys water and snow skiing as well as writing short stories. Welcome to the crew, Debie!

Congratulations to **Roger Mellenthine**, who has worked as a fish culture technician on the June sucker project. He will be leaving Logan and the FES for his new full-time position as a wildlife technician at the Midway hatchery. The June suckers are going to miss you, Roger!



The newest biologist at FES is **Ronnie Arndt**, who is working in the research division with Eric Wagner. Ronnie is a Utah native and obtained his B.S. degree in fisheries management from Utah State University. He obtained his masters degree at the University of Washington in 1994, focusing his studies on aquaculture and nutrition. Prior to returning to Utah, he spent a year at Fish Technology Center in Bozeman, MT, working as a fish nutriitionist.

Ronnie and his wife Martha currently have one daughter Caitlin. He is working on research with polyploidy, efficacy of therapeutants and fish nutrition. Welcome to FES, Ronnie!

Hatchery Performance of Cutthroat Trout Fed a Commercial Salmon or Trout Diet

There has been some discussion among Utah culturists concerning the merits of feeding a salmon diet to cutthroat trout, *Oncorhynchus clarki.* At the Glenwood State Hatchery, cutthroat trout fed salmon diets have not performed as well as those on trout diets. At the Mantua State Hatchery, cutthroat trout juveniles and brood stock are routinely fed salmon diets. The warmer temperatures at Glenwood (58 F or 14.5 C) than at Mantua (48 F or 9 C) may be a factor.

Ron Roubidoux, the hatchery superintendent at Mantua, initiated an experiment in 1970 with cutthroat trout from Strawberry Reservoir (mostly Yellowstone cutthroat trout , *O. c.* bouvieri). He split the fish into two raceways when they were 5.4 fish/lb (84 g each). The fish were fed either a salmon or trout diet until they were spawned two years later. Final mean weight was slightly higher in fish fed the salmon diet (567 g or 0.80 f/lb) than the trout diet (540 g or 0.84 f/lb). The egg production was also quite different between the diets. Only 20 females (2.6%) fed the trout diet spawned as 2 y-olds compared to 40 females (5.0%) fed the salmon diet. In addition, the eggs from trout-feed females were smaller (602 eggs/oz) than eggs from fish on salmon feed (557 eggs/oz). Eggs from females fed trout feed were pale yellow in color compared to the deep orange color of eggs from fish on salmon feed. Total egg production was the same for both groups (1.25 oz per ♀).

Salmon diets have a higher percentage of protein (48%) than trout feed (40%). Also, a greater portion of the protein component in salmon diets is comprised of fish meal than in trout feed. Crude fat is also slightly higher in salmon feed (11%) than trout feed (10%). Vitamins A, D and E concentrations are higher in salmon feed (10,000, 500, and 250 IU/kg, respectively) than trout feed (8,000, 450, and 200 IU/kg). The additional protein and vitamin supplements increase the salmon diet costs by about $0.04/lb ($0.09/kg). Is it worth it? A controlled experiment at the Fisheries Experiment Station set to find that out for cutthroat trout reared at 55 F (13 C).

Cutthroat trout of the Bear Lake Bonneville strain (*Oncorhynchus clarki* utah) were reared for 241 d in concrete raceways, feeding either a commercial salmon or trout diet. There were three replicates for each diet. Fish were fed five times a week, eight times a day until day 30, six times a day until day 136, and 4 times a day thereafter. Fish were fed at or near satiation by observing waste feed and subsequently reducing amounts slightly. Adjustments were made such that each raceway received the same percentage of body weight of feed. The percent of body weight fed changed as the fish grew, ranging from 4.2 to 1.6%.

Final mean weight was significantly greater for cutthroat trout fed the commercial salmon diet than in fish fed the trout diet ($P = 0.035$; Table 1). Feed conversion was better for fish on the salmon diet (0.80) than the trout diet (0.91; $P = 0.028$). However, mean length, condition factor, and health and condition profile parameters were not significantly different between the two diets, with the exception of leucocrit and hematocrit which differed only for one of three samples taken during the course of the experiment. Relative fin length measurements of all rayed fins provided a similar result, with fins that were progressively worse over time, but not differing between feed

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treatments.

Results indicated that cutthroat trout fed the salmon diet and reared at 13°C grew at a slightly faster rate than fish fed the trout diet, but the difference may not warrant the extra cost for the salmon diet. Further tests at different temperatures and sizes (i.e., fry, broodstock) are needed to refine the feeding techniques for cutthroat trout.
Eric Wagner

Table 1. Feed conversion, mortality, mean weight (N=620 to 636), and mean length (N=30) for cutthroat trout after 241 days of feeding with either a commercial trout or salmon diet. An asterisk indicates a significant difference between diets ($P < 0.05$).

Parameter	trout diet	salmon diet
Feed conversion	0.91	0.80*
Mortality (%)	2.5	2.66
Mean weight (g)	41.88	48.51*
Mean length (mm)	165.2	174.6

AN UPDATE ON WHIRLING DISEASE IN UTAH

Contrary to some reports in the tabloid press, Utah's whirling disease (WD) program is continuing on track. Recent sentinel fish testing in the Fremont river drainage in southern Utah has yielded some encouraging results. This drainage was rotenoned annually for 3 years in an attempt to interuupt the life cycle of the parasite by eliminating the fish host.

In 1994, fish were placed in sentinel cages in the lower Fremont River near Bicknell, the Spring Creek area near Loa and Forsyth reservoir. All of the sites had previously tested positive for *Myxobolus cerebralis*, the parasite causing WD. Rainbow trout tested after 4+ months at all sites have failed to develop spores of the parasite. Brake trout were tested at the latter two sites and also failed to produce spores.

In the adjacent Otter Creek drainage, results have also been encouraging. The portion of the drainage between Koosharem Reservoir and Otter Creek Reservoir was also rotenoned for 3 years after the discovery of the disease in private hatcheries. Rainbow trout from Otter Creek reservoir tested in 1995 again failed to demonstrate infection with WD. This reservoir has been considered at high risk due to possible downstream movement of the infective spores.

In Cache Valley of northern Utah, the encouraging trend continues. Infected fish have not been found beyond the original site on the Blacksmith Fork river. Repeated testing on the Little Bear river in 1994 has failed to demonstrate any more infected fish. On Porcupine reservoir, rainbow trout and kokanee were again found positive in 1995, but at barely detectable levels. Wild brown and cutthroat trout continued to test negative.

The results, while encouraging, are considered as preliminary. Pathologists caution that the program cannot be considered a success or failure at this point. Further testing of greater number of sentinel fish over a longer period of time will be necessary. At this time, different species (lake, cutthroat, rainbow, brake) are being subjected to a comparative infectivity study in Forsyth reservoir. Biologists in the southern region are planning to restock some of the previously contaminated streams with the more resistant brown trout.

Chris Wilson

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