The Ichthyogram

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NEW TECHNOLOGY VERIFIES DISCOVERY OF WHIRLING DISEASE PARASITE AT ROCKPORT RESERVOIR

Using new technology, the presence of the whirling disease parasite has been detected and verified in Rockport Reservoir in the Weber River drainage. Samples were obtained in June 1997 as part of the ongoing survey and were sent to the Fisheries Experiment Station for analysis. The sample consisted of cranial wedges from twenty-five adult rainbow trout (*Oncorhynchus mykiss*), two subadult rainbows, and twenty-two brown trout (*Salmo trutta*).

The samples were divided into pooled samples of five or less fish and subjected to the pepsin-trypsin digest method for spore detection. One pool of adult rainbows showed the presence of a small number of spores (5/100 fields at 400x), using Ziehl-Nielsen stain. All other rainbow trout and all brown trout samples were negative for spores. The spores were morphometrically consistent with *Myxobolus cerebralis*, the parasite which causes whirling disease. None of the sampled fish displayed any symptoms or deformities commonly associated with the infection.

Traditionally, examination of the head tissue by histopathology is used to confirm the presence of spores as "whirling disease". Due to the low number of spores detected, it was suspected that conventional histopathology techniques would be unsuccessful. As an alternative, pathologists decided to use the polymerase chain reaction technique on the small amount of remaining material. This relatively new technique works by amplifying any existing DNA of the parasite, making it a very sensitive method of detection. The testing was performed by Dr. Robert Ellis at the College of Veterinary Medicine, Dept. of Microbiology at Colorado State University. Using this technique on remaining wedge material, two of the five fish tested positive. The residue of the original examined material which had been exposed to enzyme digestion failed to test positive.

This finding increases the fears of biologists that the parasite has become well established in this river

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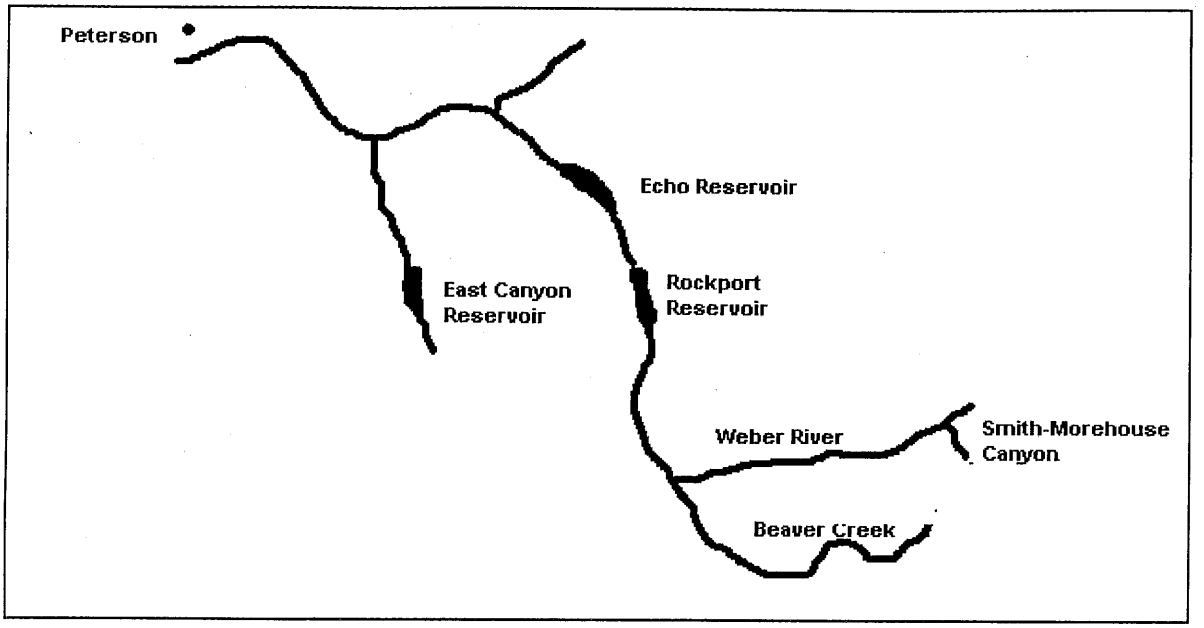


drainage. The parasite was first discovered in the drainage in 1993 at a private pond in Smith-Morehouse Canyon which had received fish from a recently infected private hatchery. All parties were cooperative in working with Utah Division of Wildlife Resources biologists to promptly remove the fish and treat the pond with calcium oxide. The pond reportedly was self-contained and did not drain into the Weber River. In 1996, brown trout (5/10) from a stretch of the lower river near the town of Peterson showed the presence of spores consistent with *M. cerebralis*, but the infection was very slight and hasn't been confirmed with additional samples to date. Samples from Rockport and Echo reservoirs in 1996 had failed to show the parasite. Fisheries managers and pathologists are currently obtaining additional samples from various location in the drainage to determine the extent of the parasite.
Chris Wilson

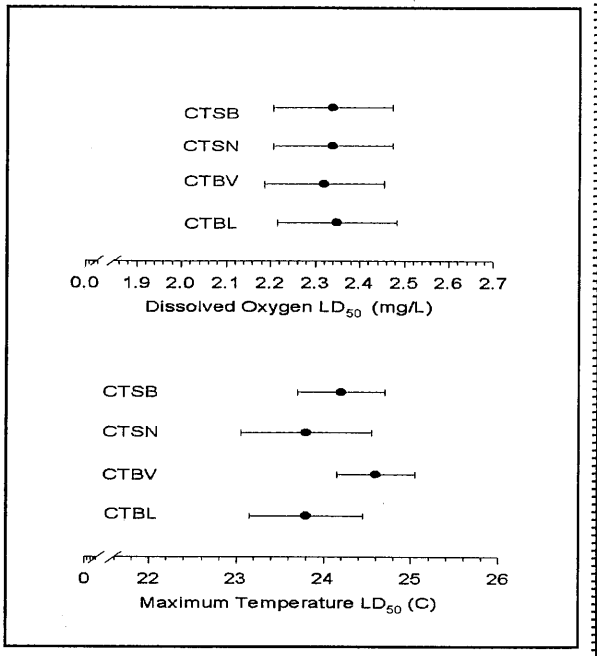
GOEDE RECEIVES AWARD FROM TROUT UNLIMITED

Ron Goede, director of the Fisheries Experiment Station, received the Professional Conservation Award at the Annual Convention of Trout Unlimited in August 1997 at Knoxville, Tennessee. The Award was made primarily for Ron's tireless work over the years in drawing attention to protection of the health of wild fish. In particular, his work in alerting the fish health community to the potential dangers to trout from whirling disease was noted.

Although the Award was originally presented in Ron's absence at the meeting in August, a special presentation was made at the Wildlife Board Meeting on September 24 in Salt Lake City. Paul Dremann, vice-president for conservation issues of the Utah Council of Trout Unlimited presented the award.

Comparative Resistance of Four Strains of Cutthroat Trout to Extremes of Temperature and Hypoxia

Cutthroat trout (*Oncorhynchus clarki*) of four strains were exposed to high temperature, high salinity, or low dissolved oxygen in several separate tests to determine any inherent differences that may make them better suited for certain environments. The salinity tests were summarized in *Ichthyogram* 7(4). The four strains tested were Bear Lake Bonneville (CTBL), southern Bonneville originating from Manning Meadow Reservoir, Monroe Mountains, Utah (CTBV), Strawberry Reservoir strain from from Electric Lake, Utah (CTSB), and the Snake River strain (CTSN) originating from wild stock in Wyoming via the Jackson National Fish Hatchery.



Temperature tests were conducted either as 96 h or critical thermal maximum (CTM) tests using juvenile fish acclimated to either 13.6°C or 18.0°C. Two end points were used in the CTM tests: temperature at first loss of equilibrium (CTM_{eq}) and the onset on spasms (CTM_s). Mean CTM_{eq} and CTM_s were significantly higher for fish acclimated at 18.0°C (29.5, 29.8°C respectively) than those acclimated at 13.6°C (28.1,28.6°C). There were no significant differences in CTM_{eq} among strains acclimated to 18.0°C, but CTM_s was significantly higher ($P = 0.03$) for CTBL (30.0) than for CTSN (29.6) or CTBV (29.7°C). For tests with fish acclimated at 13.0°C, there were no significant differences among the four strains in CTM_{eq} or CTM_s.

In 96-h tests, differences among strains varied among the nine tests. Overall, it appeared that the CTBV had slightly better survival at warmer temperatures than the other strains tested. In this study, cutthroat trout had upper incipient lethal temperature (UILT) limits ranging from 23.8 to 24.6 °C. Other researchers have indicated that rainbow trout tolerate slightly higher values (25 to 26 °C) at similar acclimation temperatures. Size differences in thermal tolerance were not significant in this study, similar to results from other researchers with brook trout, Arctic char, and yellow perch.

Hypoxia tolerance was determined in 9 tests by either slowly decreasing the dissolved oxygen (DO) over time (6-7 h) or by putting fish into a constant level of DO and recording mortality after 24 h. Resistance time (elapsed time from stocking until loss of equilibrium) was recorded when a fish died and median values were analyzed. The LD_{50} was 2.34 mg O_2/L for all strains combined and LD_{50}s did not differ among strains. Similarly, there were no significant differences among strains in resistance time or 24 h mortalities.

Overall, the results indicated that the southern Bonneville form of cutthroat trout has slightly better temperature and salinity tolerance than the other strains tested, possibly making it a better native fish for waters with high salinity or summertime temperatures.

Eric Wagner

Effect of Crystal Violet Stain and Hypo-osmotic and Hyper-osmotic Solutions on Recovery of Triactinomyxons, the Infective Stage of *Myxobolus cerebralis*

Previous tests conducted with triactinomyxons (TAMs) to determine fall velocities were frustrated by an inability to recover the TAMs added to a buret. Tests to determine if TAMs would settle out on a density gradient (Percoll) were also frustrated in part by poor recovery. This experiment was undertaken to explore the effects of crystal violet stain and other solutions (de-ionized water, tap water, 60% Percoll) on the structure and observability of TAMs under a microscope over a 24 hour period. Treatments tested were: 1) stain only 2) stain + de-ionized (DI) water, 3) stain + tap water, 4) DI water only, 5) tap water only, and 6) 60% Percoll solution. Percoll is a commercially available liquid that separates into a variety of densities upon centrifugation and is used routinely for cell separation.

On 7-17-97, TAM stock solution (about 3 TAMs/μL preserved in 10-20% formalin) was mixed gently and 240μL placed into each of twelve previously labeled test tubes, two tubes per treatment. Crystal violet stain, DI water and tap water, and 60% Percoll solution were added to the TAM stock solution as shown in Table 1.

The test tubes were kept at room temperature throughout the duration of the experiment. Two slides were made for each test tube for each of three time periods: baseline (t=0), 6-8 hr, and 23-24 hr. The amount of liquid put on each slide was adjusted so that an equal number of TAMs would be expected on each slide; e.g., 34μL/slide for treatment 1, while the remaining treatments had 180μL/slide. Sampling was begun directly following filling of the test tubes. The test tube was mixed with a vortexer and then the sample was carefully pipetted from the middle of the solution onto microscope slides and the elapsed time from mixing of the test solutions was recorded. Total counts were made of the observed TAMs on each slide by light microscopy at 100x. Following the initial count (t =.0), the first twenty four slides (two from each test tube, four from each treatment) were placed in a humid container to inhibit drying and allow for later counting. Slides counted after the first twenty four were cover slipped and discarded after viewing.

The results indicated that TAM numbers were reduced over time in treatments with the crystal violet stain, especially those treatments in which there was just stain or DI water (Table 2). The effect of the stain was attenuated in the tap water treatment. The stain did improve the detection of TAMs as Barry Nehring and staff(1997) have observed; significantly more TAMs were observed in baseline treatments with stain than in those without it (Table 2). By the end of the first day (2nd time period) and also after 24 hr, the tap water treatment had significantly lower TAM numbers than the tap water + stain treatment. This may indicate that the stain may actually have been helpful in maintaining TAM integrity over time in that medium or that the TAMs were easier to see and hence more were counted. In DI water, the opposite was true; TAM numbers were significantly lower in the DI water+stain treatment than DI water alone at the end of the first day and after 24 hr. The Percoll treatment resulted in similar numbers to that or the unstained tap and DI water treatments. Over time however, the higher osmolality of the Percoll solution resulted in noticeable shrinkage of the TAMs. Conversely, DI water resulted in swelling of the TAMs as they absorbed water.

Regression analyses between TAM number and actual time for each treatment were significant for treatment 1 only (r^2 = 0.96). When data for each treatment were analyzed by ANOVA for differences among the three time periods, significant differences from baseline values were observed for treatments with stain as well as the tap-water only treatment.

So what happened to the TAMs in the first two treatments? Re-examination of the baseline slides indicated that the TAMs were

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still present, so the TAMs did not rupture with some osmotic imbalance. It was thought that perhaps more TAMs were taken from the test tubes for the baseline samples and thus less left to be sampled from the tube at a later time. If this were true, total TAM numbers over the three time periods would be the similar. This hypothesis was tested by chi-square analysis. The number of TAMs counted on the four slides for each treatment was totaled over time. This total was compared to the expected TAM numbers over time (1032 TAMs based on random distribution in the solution) using chi-square analysis separately for each treatment. There were significant differences between expected and observed TAM counts for treatments with stain and for the tap-water-only treatment ($P \leq 0.001$). The tap water+stain treatment actually had significantly more total TAMs (1368) than expected, whereas all other significant differences were the result of less TAMs ($\leq$816) than expected. Since total TAMs were less than expected and TAMs were still visible on the slides after 24 hr, something must have occurred in the test tubes that made them very fragile and disintegrated upon vortexing the test tube.

In summary, tests indicated that DI water with crystal violet stain, or stain alone resulted in a significant reduction of TAMs over 24 hr. TAM detection was significantly enhanced with the stain, so stain is recommended just before microscopic examination. Use of tap water rather than de-ionized water for any dilutions is also recommended. In follow up tests to evaluate the fall velocity of TAMs, water samples were stained after the sample was drawn off. This resulted in almost complete recovery of the total number of TAMs added to the tap water in the buret.

Eric Wagner / Mark Smith

Table 1. Composition by volume (µL) of solutions tested for survival of triactinomyxons.

	Treatment					
	stain	stain+DI water	stain+tap water	DI water	tap water	60% Percoll
TAM stock solution	240	240	240	240	240	240
Crystal violet stain	45	45	45			
DI water		1215		1260		
Tap water			1215		1260	
60% Percoll						1260
Total volume	285	1500	1500	1500	1500	1500

Table 2. Mean number of triactinomyxons per slide (TAMs, $N = 4$) for each of six treatments over three time periods. Treatments are: 1, TAM+stain; 2, TAM+stain+de-ionized water; 3, TAM+stain+tap water; 4, TAM+de-ionized water; 5, TAM+tap water; and 6, TAM+60% Percoll. Means that are not significantly different between treatments within a time period are followed by a common letter.

	Treatment					
Time	1	2	3	4	5	6
base-line	152.5 ±51.0a	118.7 ±43.6ab	167.5 ±40.5a	98.2 ±13.4b	76.7 ± 10.5b	77.2 ±2.7b
6-8 hr	35.7 ± 18.3a	55.2 ±29.7ab	99.0 ±14.5c	95.5 ±30.5c	54.0 ±10.2ab	71.0 ±7.6bc
23-24 hr	15.7 ± 3.2a	3.5 ± 3.7a	75.5 ± 9.9c	95.7 ±26.8c	52.2 ±4.2b	69.2 ±9.4bc

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