The Ichthyogram

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Hybrid Trout Performance and Susceptibility to *Myxobolus cerebralis* at Mill Meadow and Forsyth Reservoirs

Mill Meadow and Forsyth reservoirs in the Fremont river drainage were among the first sites where *Myxobolus cerebralis* was detected in Utah trout in 1991. Significant deformities were noted in rainbow, cutthroat, cutbow hybrids, brook and brown trout in those reservoirs at that time. Those reservoirs were treated with rotenone to eliminate salmonids for a period of time in an attempt to interrupt the parasite's life cycle. With the exception of sentinel fish, those reservoirs have been kept largely salmonid-free since late 1991.

As part of the study looking at the resistance of various trout hybrids to whirling disease, Mill Meadow Res. was stocked with brownbows (rainbow ♀ x brown ♂) and splake (lake ♀ x brook ♂) on June 13, 1996, and rainbows and brake trout (brown ♀ x lake ♂) on July 12. Forsyth Res. was also stocked with brownbows and splake on June 13 and tiger trout (brook ♀ x brown ♂) on July 12. To increase hybrid vigor and hopefully survival, the brownbows, brake and tiger trout had been heat shocked shortly after fertilization to induce triploidy. On October 15 three gill nets per reservoir were set and the following day fish were collected. To determine the growth of the fish and the general health of the fish, necropsies (according to the Health Condition Profile, HCP) were performed on the four trout hybrids as well as the rainbow trout.

Health Condition Profile

In terms of weight and length gains, all five types of trout appeared to grow relatively well (Table 1). At Mill Meadow Res. both the brownbows and the rainbows had higher final weights compared to the splake and brake, but they were also stocked as larger fish. This was also true for Forsyth where the brownbows had the highest final weights but also were stocked at a larger size. At Mill Meadow Res. the specific growth rate was highest for the rainbows, 3.37; followed by the brake trout, brownbows, and the splake (Table 1). At Forsyth Res. the specific growth rate was highest for tiger trout, 2.62; followed by the brownbows; 2.03, and the splake; 1.98. In Mill Meadow Res. both brownbows and splake had higher final weights, lengths, and specific growth rates than their counterparts at Forsyth Res. Overall the growth of the fish in both reservoirs in terms of weight gain, length increase, and specific growth rates appeared to reflect adequate conditions with respect to water quality and food abundance for good fish growth.

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In general, information obtained on the various HCP indices showed the fish to be in good health at Mill Meadow Res. However results from several indices indicated differences between the various hybrids (Table 2). There was a large percentage (95%) of brownbows that had a lenticular opacity (cataract) in one or both eyes. It was unknown whether or not the eyes were functionally blind but they were termed so to follow the convention of the HCP. The pigmentation of these fish suggested at least partial vision. This cataract was absent in the rainbows, splake and brake trout, however with these fish a substantial percentage exhibited a slight but consistent hemorrhaging along the bottom margin of the eye. Because this hemorrhaging was absent in the brownbows it may have been the result of morphological differences influencing damage by the gill nets or possibly by something else other than the physical distress from the netting.

The feeding index scores for the brownbows, rainbows, and splake were all similar, 65%, 55%, and 67% respectively, but the brake had a much higher score, 91%. The feeding index is calculated by subtracting the bile index (a value of 0 to 1) which is a measure of gall bladder fullness and color, from 100. The feeding index therefore measures how recently a fish has eaten with higher scores representing recent feeding. The cataract of the brownbows did not appear to negatively impact the ability of the fish to feed because their score was not substantially different from the others with the exception of the brake trout. Fat levels were highest for the rainbows, 2.7, followed by the brownbows, 2.0, splake, 1.7, and the brake trout, 1.1. Statistical comparisons of condition factor were not made due to morphological differences between the fish, however the rainbows had the highest condition factor, 1.35, followed by the brownbows, 1.16, brake trout, 1.04, and the splake, 0.93.

At Forsyth Res. the HCP data also indicated the fish were generally in good condition (Table 3). However like at Mill Meadow Res., there appeared to be a higher percentage of brownbows that exhibited cataracts; 90% of brownbows sampled by the HCP had cataracts in one or both eyes and 10% showed hemorrhaging. No cataracts were found among the splake or tiger trout although 80% of the splake and 20% of the tiger trout exhibited hemorrhaging.

The feeding index was high for the splake, 87%, and the tiger trout, 73%, but low for the brownbows, 30% indicating a low level of recent feeding. Fat levels were highest

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Table 1. Growth of rainbow trout and trout hybrids in Mill Meadow and Forsyth Reservoirs

Location	Species	Initial Weight (g)	Final Weight (g)	Initial Length (mm)	Final Length (mm)	Specific Growth Rate*
Mill Meadow	brownbow	13.4	241.9	108	274	2.32
	rainbow	10.5	267.0	97	269	3.37
	splake	9.4	150.1	104	250	2.22
	brake	4.5	64.3	80	184	2.77
Forsyth	brownbow	13.4	168.8	108	243	2.03
	splake	9.4	110.8	104	233	1.98
	tiger	6.3	78.2	85	197	2.62

*** SGR = ((ln weight final - ln weight initial)/number of days) * 100**

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for the brownbows, 2.3, followed by the tiger trout, 2.0, and the splake, 1.5. The brownbows had the highest condition factor, 1.15, followed by the tiger trout, 0.99, and the splake, 0.86.

With the exception of the rainbows at Mill Meadow Res. the brownbows at both reservoirs had the highest fat levels, mean of 2.2, and the highest specific growth rates, mean of 2.18. The presence of the cataracts was unfortunate because the brownbows had done so well otherwise. Because the fat levels and growth rate data indicated they had grown so well one has to wonder whether the cataracts were a recent aberration which had not effected their ability to feed until recently, possibly supported by the Forsyth feeding index data, or that the cataracts played no part in the brownbows ability to feed, possibly supported by the Mill Meadow data. Pat Brown, superintendent of the Loa State Hatchery, has mentioned there is an occurrence of cataracts in brownbows being raised there. Examination of several affected fish showed no evidence of the eye fluke, *Diplostoma* sp, which commonly causes cataract formation in other locations in Utah. Work is underway to characterize and further evaluate the cataracts in these fish.

Ronney Arndt

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Table 2. Select HCP Indices of fish collected at Mill Meadow Reservoir

HCP index	HCP score	brownbow (N = 20)	rainbow (N = 20)	splake (N = 21)	brake (N = 7)
eyes	% blind	95	0	0	0
	% hemorrhagic	0	35	57	58
	% normal	5	65	43	42
feeding index	1-100%	65	55	67	91
fat	0 = no fat 4 = highest score	2.0 b	2.7 a	1.7 bc	1.1 c
condition factor	$(wt/lgth^3) * 10^5$	1.16	1.35	0.93	1.04

Table 3. Select HCP Indices of fish collected at Forsyth Reservoir

HCP index	HCP score	brownbow (N = 10)	splake (N = 10)	tiger (N = 10)
eyes	% blind	90	0	0
	% hemorrhagic	10	80	20
	% normal	0	20	80
feeding index	1-100%	30	87	73
fat	0 = no fat	2.3 a	1.5 b	2.0 ab
	4 = highest score			
condition factor	$(wt/lgth^3) * 10^5$	1.15	0.86	0.99

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Whirling Disease Investigation

The fish subjected to the health condition profile were also examined for the presence of deformities which *might* be associated with infection of *M. cerebralis.* A recent addition to the Health Condition Profile, the **deformity index** was assessed on varying numbers of fish. (See related article from *The Ichthyogram,* Vol. 6 No. 3). A wedge from individual fish or the entire head of small fish was collected and processed by the pepsin-trypsin digest method. The resulting residue was examined microscopically for spores resembling *M. cerebralis.*

At both locations, the brownbow had the highest rate of deformities and incidence of infection, exceeding even rainbow trout. All of the various interspecies hybrids demonstrated the presence of the parasite. This marked the first time that splake, tiger trout and brake trout were found infected in Utah. Brake trout had been tested at sentinel fish at various contaminated locations earlier and always tested negative. However, only 1/7 fish demonstrated the infection, and only one spore was detected in that fish.

The results of testing demonstrate that the three year period was not sufficient to eliminate *M. cerebralis* from these reservoirs. The differing exposure times and varying sample sizes of the various species and hybrids limit the conclusions that can be drawn on the relative susceptibilities to the parasite at this time. The data would suggest that brownbows are more prone to develop spores as compared to splake at both reservoirs. The predominance of rainbow trout maternal DNA in the triploid brownbows would possibly predispose them to infection. The recent discovery of *M. cerebralis* in splake in Utah and Colorado contradict some early research findings and have caused some researchers to question whether any species of salmonid is truly immune to infection.

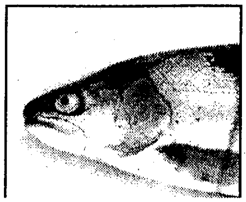

Figure 1. Brownbow showing typical cranial deformity

The majority of the deformities noted were cranial, mandibular and vertebral, which are consistent with lesions commonly associated with whirling disease. (See accompanying figure) However, these lesions are not pathognomonic for the infection and deformities might be expected from otherwise healthy interspecies hybrids. More work is underway to assess the significance of the deformity index in fish infected with *M. cerebralis.*

Given more time, the rainbow trout and other species hybrids may prove to be more or less susceptible to *M. cerebralis* and may demonstrate further deformities. Unfortunately, dam repairs and intensive water use caused the drainage of Forsyth reservoir shortly after samples were taken. Plans are underway to sample Mill Meadow reservoir again in 1997. Chris Wilson

Mill Meadow	% Infection	% Deformity	**Days of Exposure**
Brownbow	18.18% (n=55)	4.52% (n=20)	125
Splake	11.54% (n=26)	0.83% (n=20)	125
Rainbow	13.89% (n=36)	1.67% (n=20)	96
Brake	14.29% (n=7)	1.90% (n=5)	96
Forsyth			
Brownbow	29.31% (n=58)	2.86% (n=10)	125
Splake	20.00% (n=45)	0.00% (n=10)	125
Tiger	17.86% (n=28)	0.71% (n=20)	96

A Comparison of Salinity Tolerance Among Four Cutthroat Trout Strains in 24 hour Tests

As part of a larger study comparing the water quality tolerances of cutthroat trout *Oncorhynchus clarki* strains used in Utah, two saltwater challenge tests were conducted in November 1996. The four strains of cutthroat trout tested were Bear Lake Bonneville (CTBL), Strawberry Reservoir-Electric Lake (part Yellowstone, Bear Lake, and rainbow trout; CTSB), Snake River (CTSN), and southern Bonneville (from Manning Meadow Reservoir; CTBV). Average size of the fish for the tests ranged from 1.3 to 4.5 g.

The salinity was created by dissolving rock salt in the 800 L circular tanks used for the tests. Salinity was measured by specific conductivity and ranged from 23 to 42 mS/cm. Temperature during the tests was 14.5 ± 0.4 C, oxygen was maintained above 6.3 mg/L with compressed air delivered through air stones, and un-ionized ammonia-nitrogen did not exceed 0.008 mg/L.

For the test, there were four cylindrical plastic mesh cages per tank. Ten fish of a particular strain were put into each cage such that each of the four strains was in each tank and exposed to the same water. Two circular tanks were used for each level of salinity. Mortality was determined after 24 h and mean weights determined for each cage and dead fish in each cage. Mortality was analyzed for strain differences by one-way ANOVA for each salinity.

There were significant differences in mortality among the strains, depending upon the salinity (Table 1). Snake River cutthroat trout experienced the highest mortality in both tests with significant mortality occurring at 29-32 mS/cm (about 19 ppt). Differences among the Utah strains did not occur until higher levels were reached (about 36-40 mS/cm or 22-24 ppt). The southern Bonneville strain from Manning Meadow had significantly better survival at the higher salt concentrations than any of the other strains. At about 36 mS/cm, the CTBL had significantly higher mortality than the CTSB. However, at 38 mS/cm, the mortality was similar between the two strains.

The higher survival of the CTBV suggested that there are some ecologically significant differences between the CTBV and CTBL strains despite both being considered *O. clarki utah*. The CTBL has evolved in a large oligotrophic lake, whereas the stock in Manning Meadow Reservoir was derived from stream-adapted fish from southern Utah. Clearly, these stocks should be maintained as separate strains.

The salinity levels causing mortality should not be considered upper lethal limits for fish in the wild since the exposure in this study was only for 24 h. Longer exposures would be expected to induce additional mortality and at lower concentrations. However, the tests do suggest that there are significant strain differences, with CTSN exhibiting the lowest salinity tolerance and CTBV the highest. Eric Wagner

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UPCOMING EVENTS

- **Annual Whirling Disease Symposium: Expanding the Data Base: 1996 Research Progress Reports.** March 6 - 8, 1997. Eccles Conference Center, Utah State University, Logan, Utah. For more information contact the Whirling Disease Foundation at 406-585-0860. (whirling@mcn.net).
- **Pathogens and Diseases of Fish in Aquatic Ecosystems: Implications in Fisheries Management.** June 3 - 4, 1997, Sheraton Airport Hotel, Portland, Oregon. For more information, contact Ray Brunson at 360-753-9046.

Table 1. Comparison of mortality among four cutthroat trout strains in 24-h saltwater challenge tests.

Test 1				
Specific conductivity mS/cm	CTBL	CTBV	CTSN	CTYL
0.4	0	0	0	0
22.9-29.3	0	0	0.10	0
28.8-32.4	0_a	0_a	0.40_a	0.05_a
33.1-33.4	0_a	0.05_a	0.66_b	0_a
Test 2				
0.4	0	0	0	0
35.9-36.2	0.60_c	0_a	0.95_d	0.30_b
38.1-38.1	0.80_{ab}	0.45_a	1.00_b	0.84_{ab}
40.8-41.9	1.00_b	0.75_a	1.00_b	1.00_b

Crosses Between Tetraploid and Diploid Rainbow Trout: A Preliminary Look

Triploid fish are generally considered sterile, a useful property for some fisheries management programs and aquaculture. For example, sterile tilapia do not stunt, growing much larger than counterparts in breeding swarms. Cutthroat trout may also reproduce with rainbow trout, threatening the native gene pool of cutthroat trout. Sterile fish production for Strawberry Reservoir, UT by use of 17-α methyl testosterone has been discontinued due to regulation of the compound by the U.S. Food and Drug Administration and concerns about safe handling of the hormone. Loss of the hormone has prompted a search for other sterilization methods to meet the needs of anglers and the trophic niche filled by rainbow trout.

Production of triploid trout has gained popularity in recent years. Ploidy is created by shocking the eggs at a critical time after fertilization, causing retention of the second polar body of the egg or suppression of an early mitotic division. Various shocks have been tried, including chemical, high or low temperature, or pressure shocks. Shock treatments have not consistently produced 100% triploidy or sterility, rendering these techniques undesirable for native species management. At the Fisheries Experiment Station, the research team is still exploring other shocks, including electrical shocks and magnetic fields.

Another method of producing triploids reported by French researcher D. Chourrout is to cross tetraploid brood stock (4N) with diploids (2N) to produce sterile triploid offspring. Chourrout reported that success of this technique varied from 0 to 100%, with a mode of about 40%. In 1992 and 1993, tetraploid rainbow trout were created by pressure treatment and reared to maturity at the Fisheries Experiment Station (FES) and at Egan State Hatchery, Bicknell, Utah. Diploid rainbow trout of the same strain were also reared at each hatchery. In the fall of 1995, the first 4N x 2N crosses were attempted and the results are summarized in this article. Egg survival data for the 1996 crosses are also presented.

In the fall of 1995, several crosses were made between 4N and 2N fish using standard dry fertilization techniques. On 11 October 1995, the eggs from three females were pooled and split into two groups: one fertilized by four 4N males and the other by three diploid males (control). On 25 October 1995, eggs from one 4N female were fertilized with sperm pooled from three diploid males. Half this pool was also used to fertilize diploid females as a control. Additional eggs from this same 4N female were fertilized by a pool of two 4N males. Another cross between 4N males (pool of three) and 2N females (pool of 3) was made on the same date. On 16 November 1995, an additional cross was made at the FES between a 4N male and a 2N female (3-year-old). On 27 November 1995, a single 4N female at the Egan Hatchery was fertilized with pooled sperm from two 2N males. At the FES, the eggs were incubated in trays at 12.5 C, and at Egan Hatchery, 9 C. The fish were reared on a standard trout diet until the blood wa analyzed by flow cytometry for triploidy.

The results of the crosses were inconclusive. At the Fisheries Experiment Station, the survival of the crosses and controls was extremely poor, indicating some problem with the quality of the eggs rather than the cross attempted. The few fish surviving were analyzed for ploidy status and the results indicated a very small percentage were triploid (Table 1). However at Egan, the cross between the 4N ♀ and 2N ♂ resulted in 100% triploidy (n = 65).

For crosses made in the fall of 1996 at the FES, similar poor results were noted. Percent eye-up ranged from 7.3 to less than 1% in the 4N x 2N crosses in 1996, and controls ranged from 15 to 61.5% eye-up. Ploidy determinations have not been made for these groups yet.

Temperature and hardness are the primary differences between hatcheries: 8 to 9 C and 90 mg/L respectively at Egan, 13 to 17 C and 222 mg/L at the Fisheries Experiment Station). According to the U.S. Fish and Wildlife Service hatchery handbook, the water temperature at the Fisheries Experiment Station is at the limit suggested for rainbow trout (54 F) and may be responsible for the poor survival. The role temperature plays in the development of triploidy needs to be examined further. Perhaps at lower temperatures, there is more time for proper development of triploid cells.
Eric Wagner

Table 1. Percent survival to the eyed stage and hatching (percentage of eyed eggs) and triploidy of progeny from crosses between tetraploid (4N) and diploid (2N) rainbow trout. *ND=not determined yet **=Produced at Egan Hatchery (8-9 C)

Fertilization Date	Cross description	Number of eggs fertilized	Percent eye-up	Percent Hatch	Percent Triploid (N)
11 Oct. 1995	4N♂ x 2N♀	3740	1.8	95.4	2.1 (48)
11 Oct. 1995	2N♂ x 2N♀	2231	34.9	99.6	0.0 (12)
25 Oct. 1995	4N♂ x 4N♀	711	0.0		
25 Oct. 1995	2N♂ x 4N♀	2287	0.0		
25 Oct. 1995	2N♂ x 2N♀	1062	1.5	100	10 (10)
25 Oct. 1995	4N♂ x 2N♀	4602	0.2	100	20 (05)
16 Nov. 1995	4N♂ x 2N♀	5307	0.0		
27 Nov. 1995	2N♂ x 4N♀		82.6	98.3	100 (65)**
02 Oct. 1996	4N♂ x 2N♀	4886	5.12	5.10	ND*
21 Oct. 1996	4N♂ x 2N♀	3144	1.78	1.65	ND
21 Oct. 1996	4N♂ x 2N♀	4666	0.09	0.04	ND
01 Nov. 1996	4N♂ x 2N♀	8171	7.32	6.95	ND

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