The Ichthyogram

July 1999 Volume 10 Issue 2

PROGRESS IN COLORADO RIVER CUTTHROAT RECOVERY

The Colorado River cutthroat trout (*Oncorhyncus clarkii pleuriticus*) is one of two native salmonids located in Utah. The range of this beautiful fish has diminished significantly from pioneer days as a result of many factors, such as poor land and water management, hybridization with other salmonids and overfishing. As such, the species is designated as Asensitive@ by state and federal agencies. A *Conservation Agreement and Strategy* was drafted and signed by state and federal agencies in 1996 to aid in the protection and recovery of this species. The fish has recently been petitioned for listing under the terms of the Endangered Species Act by certain environmental groups, while others groups, such as Utah Trout Unlimited, have not supported such listing at this time, contingent on managing agencies fulfilling their commitments to the Conservation Agreement.

UDWR fish culturist Ron Morrill coaxes eggs from a Colorado River cutthroat trout at Sheep Creek Lake.

To that end, several Utah Division of Wildlife Resource fisheries

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biologists are diligently working toward keeping this fish from being listed. Although, the Northern, Southern and Southeastern regions are involved, some early success has been accomplished by the Northeastern (NER) and Southern region biologists. Work in the Southern region will be covered in a future article.

The NER is considered critical because the Uintah Basin is considered to be one of the last strongholds of the Colorado River cutthroat trout in Utah. Back in the mid 1980's NER discontinued all stocking of cutthroat trout with the hope that in 10+ years they would phase out and the proper endemic subspecies could be reintroduced. Early work using meristics and later electrophoresis was tedious and inaccurate at best, but nevertheless some 110 different waters were placed on a probable list.

With the replacement of meristics and electrophoresis with DNA analysis for subspecies identification, biologists were now able to examine large numbers of streams to not only identify populations of *O. clarki pleuriticus*, but to delineate the percent of hybridization. Of the 42 waters examined thus far by NER fisheries biologists, 25 were found to contain viable populations of *O. clarki pleuriticus* with a relatively low prevalence of hybridization, 15 more samples await processing, and 68 more remain to be examined.

Among the many actions discussed in the Agreement is the creation of wild broodstocks to enhance existing populations and possibly reintroduce the species to a portion of its former range. A genetically pure population of *O. clarki pleuriticus* was identified from the West Fork of the Duchesne River on the south slope of the Uintah mountains in Utah. Fish from that site were pathogen tested and transferred to Sheep Creek Lake, a manmade reservoir on the north slope of the Uinta mountains, under a special management hardship plan. Transferring the fish allowed them to grow to a larger size and produce more eggs in the fertile waters of the reservoir. This water has a controlled inflow via a canal as well as a previously constructed spawning trap. The lake was closed to fishing to protect the fish.

Full pathogen certification was initiated in 1995, and the population was certified pathogen free after the inspection in 1998. Interagency administrative delays prevented a successful egg take in 1998, but the egg take in 1999 has been more successful than expected. Over 150,000 eggs were taken on three spawning dates and transferred to the Fisheries Experiment Station, which is ideally suited to the culture of small groups of cutthroat trout. Thus far, the percentage of green eggs eyed has been close to 90% for the group.

The plans for the fish include holding a portion till 2000 for fin clipping and restocking into Sheep Creek Lake to increase numbers of broodstock there.

Other possible plans include stocking these fish into high mountain lakes on the south slope of the Uintahs for recreational fishing. The reintroduction of these fish under this plan would be contingent on the agreement of state, county and federal partners.

Chris Wilson and Chad Crosby

Do Rainbow Trout Dream of Gravel Raceways?

Over the past several years various studies have been conducted at the Fisheries Experiment Station (FES) to determine the causes of fin erosion and possible improvements in fin condition. Fin erosion can be a common occurrence among fish raised in modern, concrete raceways. It has been theorized that fin erosion is derived from aggression between fish, nutritional imbalances in feeds, rearing densities or environmental factors inherent to a hatchery. Based on a state wide survey conducted by Bosakowski and Wagner (J. World Aquaculture Society 25:308-316, 1994, fish raised in dirt or cobble bottomed raceways were found to have better fins than those raised in concrete raceways. Despite encouraging results (*The Ichthyogram* Vol. 9, issue 3), in follow up tests, gravel floors were difficult to maintain and did not withstand regular cleaning very well.

An area of fin erosion research that has not received much attention is what inherent qualities there are to a dirt or cobble raceway that may lead to good fin quality. Is it the physical structure of the substrate, the appearance of the substrate or possibly supplemental prey items living in the gravel that contribute to good fins? Gilham and Baker (J. Endocrinology 105:99-105, 1985) found when rainbow trout were raised for several weeks in either black or white tanks and then exposed to various stressors, that fish held in the black tanks had an enhanced stress response.

The purposes of this study were two-fold. First, to evaluate a gravel substrate for raceways that would withstand regular cleaning. And secondly, to determine if it is the actual presence of gravel in the raceway, or simply the appearance of gravel. That is, to determine whether or not a two dimensional gravel pattern as a raceway substrate would have the beneficial impact on fin quality that the actual presence of gravel had.

Sand Creek strain rainbow trout were stocked into nine separate concrete raceways at 2,600 fish per raceway. Three raceways contained a two dimensional gravel pattern as a substrate (2D), three contained a three dimensional gravel substrate (3D), and three were left untreated as controls. Water was supplied to the study by a well which had the following general characteristics: temperature = 13° C, oxygen = 9.3 mg/L, hardness = 274 mg/L. Supplemental oxygen was added through low head oxygenators. The fish were hand fed a floating commercial trout formulation (Silver Cup). All nine raceways were inventoried monthly for weight gain, and fin measurements were made at approximately the same time and used to calculate relative fin index values (fin length/total length * 100). Necropsies according to the Health Condition Profile (HCP) were performed twice during the study as well.

The backing material used for the 2D and 3D treatments were sheets of a prismatic plastic material normally used as coverings for fluorescent light fixtures. The 2D panels were spray painted with a pattern to mimic cobble to an average coverage of 75% with a variety of Anatural@ colors (i.e. black, brown, white, grey, tan). Once painted, they were coated with a thin layer of a 3:1 epoxy laminating resin (Fiberglass Coatings, Inc., St. Petersburg, FL.). The 3D panels were first sanded, then coated with a layer of the epoxy after which a single layer of gravel was placed on the resin.

Throughout the study no significant differences were found in growth between the treatments. By the end of the study, control and 2D fish averaged 32 g/fish and

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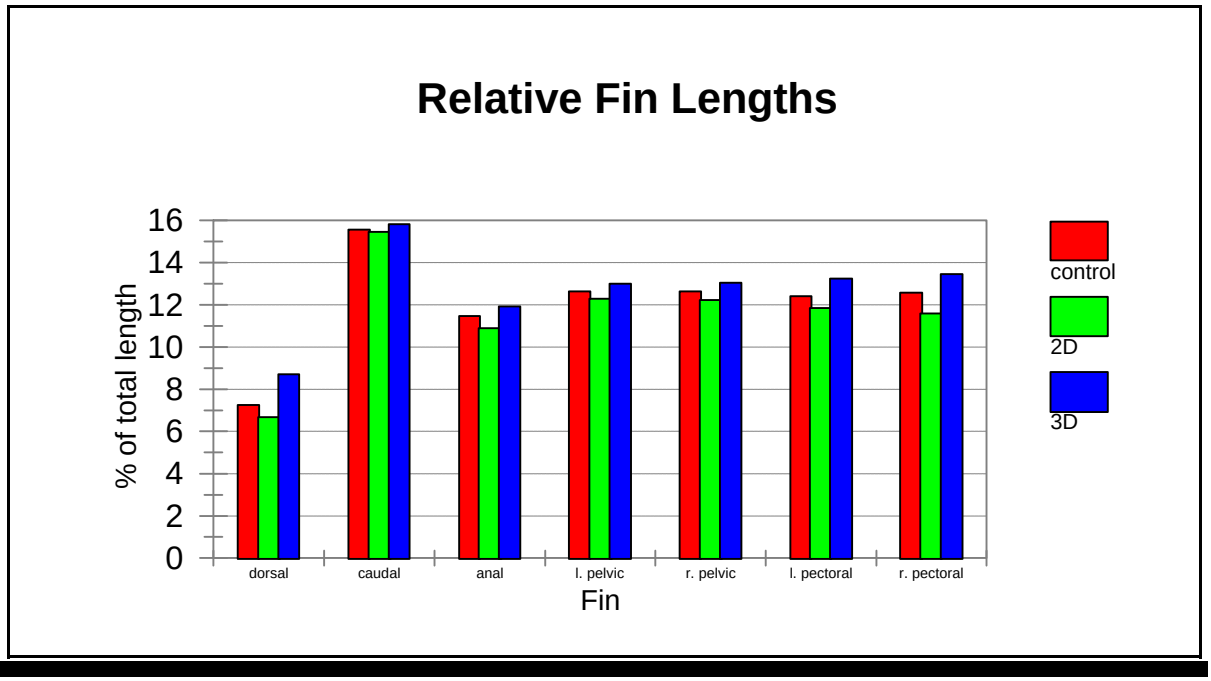
the 3D fish were 33 g/fish. No significant differences were found in specific growth rate or feed conversion ratio between the three groups of fish. Specific growth rates were 1.67 for control and 2D fish and 1.70 for 3D fish. Feed conversions were 0.76 for control and 3D fish and 0.78 for 2D fish. Relative fin index data revealed several consistent differences between groups and consistent trends throughout the study. By day 47 of the study dorsal, left pectoral, and right pectoral fins were all significantly better for the control and 3D fish compared to the 2D fish. Left and right ventral fins were better for the 3D fish compared to the 2D fish, and the control fish scored intermediately. On day 74, dorsal, right ventral, and both pelvic fins were significantly better for the 3D fish compared to the 2D fish. The fins of the control fish were not different from either group. By day 109 of the study, dorsal, anal, and both pectoral fins were significantly better for the control and 3D treatment fish compared to the 2D fish. Scores from the final fin measurements taken, day 137, indicated a similar trend. Dorsal, anal, both ventral, and right pectoral fins were significantly better for the control and 3D treatment fish compared to the 2D fish. Caudal and left pectoral fins were significantly better for the 3D fish compared to the 2D fish, and the control fish scored intermediately.

Fin scores tabulated during the two HCPs revealed no significant differences. For the first HCP, the control and 2D fish scored 0.3, and the 3D fish scored 0.2. For the second HCP, scores were 0.4 for the control fish, 0.5 for the 2D fish, and 0.2 for the 3D group. The HCP system ranks fins from 0-2, with 0 = no erosion and 2 = active erosion. All other indices measured according to the HCP were within normal ranges for rainbow trout and none of them were significantly different between treatments with the exception of the hematocrit and leucocrit scores measured during the first HCP. Hematocrit percentages for the control and 3D treatments were 36%, and 39% for the 2D treatment. Leucocrit scores were also significantly different for the control and 3D fish, 0.9%, compared to the 2D fish, 0.6%.

Even with regular cleaning, the artificial substrates among the 3D treatments did host a consistent quantity of algal growth that was not found among the control or 2D treatments. Cursory substrate scrapings from the raceways consisted of 60-80% of filamentous algae and diatoms with occasional chironomid larvae and nematodes common to all three treatments.



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However, the 3D treatment had a greater quantity of chironomids, copepods, and various snails.

This study evaluated the impact of two and three dimensional raceway substrates on the fin condition and degree of fin erosion of rainbow trout. Also of interest was the ability to construct a raceway substrate which contained cobble and that stood up to the rigors of daily hatchery life. Fish health, growth, or survival were not influenced by substrate type. The 3D fish had marginally better final weights, specific growth rates and feed conversion ratios, but these differences were not significant. The darker color of both the 2D and 3D raceways did not improve growth or survival.

The only differences between treatments that came out solely during the first HCP were the higher hematocrit and lower leucocrit levels found for the 2D treatments compared to the control and 3D groups. Although values for both hematocrit and leucocrit for all three groups of fish fall within the range of what is considered normal, the higher hematocrit and lower leucocrit levels for the 2D fish may be indicative of a slightly higher level of stress among that treatments.

Fin condition was significantly influenced by treatment type. Those fins that were influenced by treatment type were generally better for the 3D and control fish compared to the 2D fish. The only time the 3D fish had a significantly better fin measurement than the controls was for the day 137 comparison where the dorsal fin was better for the 3D fish compared to the control or 2D fish. In general, there were no real differences between the 3D and control fish, but there were consistent differences between the 3D and 2D treatments.

It may be possible to explain these results by looking at the growth of algae and associated aquatic invertebrates that were found in the algal growth growing on the raceway substrates. Compared to the 2D raceways, the 3D raceways had a qualitatively higher amount of chironomid larvae, copepods, and snails. It is possible that these potential prey items provided the 3D fish with a supplemental food source; a food source that may have provided additional minerals or micronutrient in quantities to positively impact fin condition. Lellis and Barrows (Aquaculture 156:229-240, 1997) demonstrated that steelhead trout fed a krill-based diet exhibited improved fin condition compared to fish fed a fish meal-based diet. They theorized that the krill-based diet, which contained naturally higher levels of copper, in some way improved the process of collagen formation in fin rays than the fish meal-based diet, which contained higher levels of iron, calcium and phosphorus. This line of reasoning may be substantiated by the results from a previous study conducted last year at the FES. For this study fish were reared in concrete raceways and raceways coated with a resin to smooth raceway surfaces and thereby reduce abrasions and fin erosion (The Ichthyogram Vol. 9 issue 4). The results from that study revealed better fin condition among the control fish in the concrete raceways compared to the fish in the coated raceways. For that study and for this current one it is possible that the resin coated raceway walls and substrates were so smooth the did not allow colonization of algae and aquatic invertebrates which may have served as supplemental food items and possibly improved fin condition. This may be a weak theory, but it is one that requires further investigation.

In conclusion, the combination of materials used for the 3D treatment proved to be very durable and were not excessively difficult to clean. Very few cobble pieces fell off during the study despite being scrubbed several times a week. Although the gravel substrate design for this study is probably not practical for a production scale hatchery, subsequent design modifications and improvements may make it a realistic alternative in the near future to all concrete raceways.

Ronney Arndt

New Faces at the FES

Clint Brunson, a native of Aurora, Utah, has been working as a seasonal with the research program since late May. He will be continuing his senior year in the Fish and Wildlife program at Utah State University this fall. His hobbies include sports, and outdoor activities like fly fishing and bow hunting.

Amy Howa began working with the research program in July. She will be on board for a year, working on a whirling disease project federally funded through the National Partnership on Management of Wild and Native Cold Water Fisheries. Amy hails from Salt Lake City and is a recent graduate from the Biology Department at Utah State. The project will focus on the effects of water quality variables (pH, dissolved oxygen, hardness/alkalinity, and salinity) on the viability of the triactinomyxon, the infective stage of *Myxobolus cerebralis,* the causative agent of whirling disease. Her interests include long-distance running and outdoor activities.

TAMs Get Creamed in Separator

Triactinomyxons (TAMs) are the infective stage of the myxozoan parasite *Myxobolus cerebralis*, the causative agent of whirling disease. To better understand the dynamics of the disease in wild streams, it is desirable to quantify the number of TAMs in given stream and compare it to other infected streams. This tool could help identify ›hot spots‹ and environmental variables that contribute to the decline of the salmonid fishes present.

In a continuing effort to develop a technology to efficiently separate and concentrate TAMs from water, a test was conducted to evaluate the feasibility of using cream separator technology. The separator works by spinning rapidly, and the centrifugal force pushes the solid material in the water towards the wall of the container. The solids-free water then moves up the column and trickles out the top. The test was conducted with the invaluable help of Chris Heck of the Biotechnology Center at Utah State University, using a separator there at the Center.

For the test, about 18,400 TAMs were added to 20 L of water. This was pumped through the cream separator at normal speed which was 0.83 L/min. A mylar sheet within the separator column collected the solids material which was rinsed into a vial for later examination. The water that passed through the top of the separator was filtered through a 20 um mesh via a perstaltic pump; this retentate was examined also. The total number of TAMs were counted on five slides of 100 ul each from both samples. There were an average of 0.6 TAMs per slide in the sample off the mylar sheet, corresponding to an estimated recovery of 702 TAMs or 3.8% of the original number added. A piece of TAM was observed in the sample, indicating physical destruction of some of the TAMs. In the 20 um mesh retentate, an estimated 1,320 TAMs (7.2%) were recovered. About 28% of these were damaged, with the processes ripped off or mangled.

The results indicated that a high percentage of TAMs were being destroyed in the process of passing through the separator, making this technology a poor choice for TAM filtration and concentration. The small percentage of TAMs passing through the system indicated that a single pass would not be sufficient for treating incoming water supplies either. However, it is conceivable that a few of the separators in series may be an effective means of treating hatchery water supplies, perhaps in conjunction with other technologies such as UV radiation.

Eric Wagner

Drug Use and Triploid Production

Techniques for producing sterile rainbow trout have been in development for several years at the Fisheries Experiment Station (FES). The sterile trout are needed for stocking waters with cutthroat trout where hybridization is undesirable, such as Strawberry Reservoir. Sterility can be obtained by shocking eggs during the early stages of post fertilization development which induces triploidy (3X chromosomes). Some of the early attempts involved pressure, electrical, and magnetic shocks to induce triploidy. These studies met with varying degrees of success, however few treatments resulted in greater than 50% triploids produced from a given lot of eggs. Efforts for the last year have been concentrated on heat shock based on the success reported by other state and federal research programs.

With this study, heat shock was used in combination with chemical shock. A class of drugs called methylxanthines, which includes caffeine, theophylline, and theobromine were of interest because they are thought to interrupt the second polar body formation by suppressing the spindle apparatus. If the polar body is not extruded by the egg shortly after fertilization, then the egg retains a set of chromosomes and triploidy results. MS-222, a common fish anesthetic, was also of interest because being an anesthetic, it had the potential to interrupt the cell cycle during the crucial time required to induce triploidy.

Eggs and milt were collected from rainbow trout of the Fish Lake-DeSmet strain at the Egan hatchery. For fertilization a 1% salt solution was used as a diluent and eggs were washed one minute after fertilization. At 20 minutes post fertilization, small batches of eggs (≈2,500 eggs) were subjected to the various shock treatments. All shocks occurred in plastic coolers that were fitted with circulating heat pumps which maintained the temperature at 28° ± 0.1° C. The coolers were filled with 40 L of hatchery water which had been brought up to temperature. For the shock, eggs were placed into a mosquito netting basket which was lowered into a perforated aluminum basket that sat about 1 cm off the bottom of the cooler. This design allowed for quick removal of eggs and for good water circulation around the eggs. The shock duration was 20 minutes after which the eggs were immediately removed from the coolers and placed in Heath-type incubators. The treatments consisted of a heat treatment without chemicals, caffeine at concentrations of 1 or 10 mM, theobromine at concentrations of 1 or 10 mM, and an MS-222 treatment at a concentration of 25 mg/L. All of the treatments were heated and maintained close to 28° C. A control was also included and it simply followed the treatment protocol but with the eggs going into a cooler containing ambient hatchery water at 8.3° C.

Once the eggs had eyed, they were bumped and sorted and then were transported to the FES and placed in an incubator until they hatched. After hatch, the fry were placed in small flow-through troughs where they were grown out to a size of 4-6 cm. Blood was collected from the fish at this point and analyzed for triploidy via flow cytometry.

Eye-up was low for all treatments and ranged from a low of 4% for the theobromine 1mM treatment to 25% for the theobromine 10 mM treatment. The controls averaged 72%. There were no survivors to the eyed stage among the caffeine 10 mM treatment. The percent hatch

Treatment	% Eye-up	% Hatch	% Triploid	N
control	72	95	0	60
heat	8	50	94	28
MS-222	20	62	98	42
caffeine 1mM	17	47	83	40
caffeine 10mM	0	-	-	-
theobromine 1mM	4	44	100	33
theobromine 10mM	25	61	97	61

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was also low for all groups, except the controls (95%), and ranged from 44% for the theobromine 1 mM treatment to 62% for the MS-222 group.

Excluding the control group, there were no significant differences among the treatments with respect to percent eye-up, hatch and percent triploidy. During grow-out there were no discernable differences between the control and treatment groups with respect to mortalities, appearance, or growth. The percent triploidy produced were consistently high for all treatments with the low being 83% for the caffeine 1mM treatment and the high being 100% for the theobromine 1mM group. No significant differences between treatments in the percent triploidy produced suggested that heat treatment alone in the absence of the test chemicals/drugs would be adequate to achieve a high level of triploidy. This trial reiterates that heat shock can be an effective method of triploid induction, but the technique must be improved to increase the eye-up and hatch to make it a realistic method of producing sterile trout on a large production scale.

Ronney Arndt

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