The Ichthyogram

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Utah Begins Triploid Rainbow Trout Production

Research biologists at the Fisheries Experiment Station have announced the apparent success of the first production-scale, heat shocking process for production of all-female, triploid rainbow trout in Utah. Triploids are so named because they possess three sets of chromosomes instead of the normal two, rendering them sterile. Because of the possible impact of sterile rainbow trout males on native cutthroat trout, production of all female triploids is of interest. Triploid males still produce sperm, but the sperm duct is blocked, preventing fertilization. These fish still behave as fertile males, possibly displacing fertile males during courtship. For reasons unknown, female triploids do not produce gametes. Therefore it is desirable to rear all female rainbow trout for areas in which they may compete with spawning cutthroat trout. This may be achieved by altering the hormonal balance in the hatchery feed during the first 60 days. Half of these develop as true males, but the other half are sex-reversed and become phenotypic males, but are genotypic females (i.e., they look and produce sperm like males, but genetically they are female). These are the fish used for spawning to produce all female progeny. These fish are selected from the true-male group by attempting to strip milt from the fish when they reach 2 years of age. If the milt is expressed, it is considered a normal male and discarded. If the milt is not expressed, it is considered a sex-reversed fish (XX **%**) and the gonads are surgically removed for use.

Eric Wagner and George Coombs sort out sex-reversed broodstock prior to spawning at J. Perry Egan hatchery

The surgery was a success, but . . . . **Chris Wilson, assisted by Mark Smith, surgically removes gonads from sterile fish**

Sex reversed broodstock have been produced at the Fisheries Experiment Station and two year classes have been transferred to Egan Hatchery. Work in Fall 2000 was the first attempt at using these for triploid production. Generally these fish are sacrificed for the gonads, creating a yearly need for males. An

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attempt was made to surgically harvest only one gonad and recover the fish for reuse the following year. Although the surgical process was successful and the fish recovered, later complications from suture dehiscence necessitated the euthanasia of the fish. Plans are being formulated to retry the procedure with different suture material in the future.

Gonads are manually pressed through a sieve, releasing sex-reversed milt.

The triploidy process involved heat shocking of eggs at 26-27EC for 20 min at 20 min after fertilization. Based on previous testing, this regime worked well for achieving high percentages of triploids without major egg losses observed at higher temperatures. The heat shocks were conducted in a hatchery trough at the J. Perry Egan State Fish Hatchery using recirculating heater pumps to maintain the temperature. A manifold system of **2** inch PVC with holes drilled in the sides delivered heated water along the length of one side of the trough and recovered it from the other side.

A total of 1.6 million eggs of both the Sand Creek (RTSC) and Ten Sleep (RTTS) strain were treated. Egg survival was relatively high (eyeup average = 82%) so there were many excess eggs that were discarded (See table below). Some combining of lots was done for shipping, resulting in 5 lots that were reared at the state hatcheries in Springville, Loa, Kamas, and at the Fisheries Experiment Station. All but the Kamas lot were progeny from the XX males and should be all female.

Biologist Ronney Arndt administers TLC to heat-shocked trout eggs

The 5 lots were sampled recently by diluting blood from 60 fish per lot in Alsere=s anticoagulant solution. These were kept cold and shipped to Washington State University where Paul Wheeler analyzed the samples using flow cytometry. The lots at the Fisheries Experiment Station, Loa Hatchery and both lots at the Kamas Hatchery were 100% triploid. The Springville Hatchery lot was 98.4% triploid (60 of 61 were triploid). Overall the average triploidy rate was 99.7%.

The results indicate that the first year of production has been a great success. Sterility is beneficial in preventing hybridization with native cutthroat trout. It can also produce bigger fish, since the energy normally put into gonads is put into growth. These differences become more noticeable as the fish matures into the age 3 year class and older, so put-and-take programs would not see much growth benefit. Most of this year's production will be stocked into Strawberry Reservoir. Further production is planned for the future. DWR Sport Fish Managers are considering the sterilization of the majority of rainbow trout production in the state for future stocking.

Lot Number [1]	Total green eggs	Total eyed eggs	eyeup (%)	notes
001018RTSCEG05	146,344	114,260	78.1	crossed with 2 y-old XX%; 4&5 y-
001108RTSCEG05	73,511	48,708	66.2	last take of RTSC; 4&5 y-old eggs
001108RTSCEG03	117,838	106,596	90.5	3 yr old x normal %
001120RTTSEG03	161,454	125,112	77.5	First take of RTTS
001120RTTSEG04	126,293	84,680	67.0	2y XX%; eggs dumped
001120RTTSEG05	297,860	179,452	75.5	2 y XX%
001129RTTSEG03	283,383	254,127	90.0	2 y XX%; eggs dumped
001129RTTSEG04	201,102	205,320	97.4	2 y XX%; 195 oz dumped
001129RTTSEG05	212,784	191,268	94.1	2 y XX%
total:	**1,620,569**	**average:**	**81.8**	

[1]The first three RTSC lots were combined and sent to Kamas Hatchery (Lot 001108RTTPEGSC). The lot of RTTS fertilized 11-20-00 (5-year-old females) was sent to Kamas Hatchery as Lot 001120RTTPEG05; the other eggs from that date were dumped (excess of quota) as well as the 3-year-old & lot and 195 oz from the 4-y-old & lot of 11-29-00. The 4-y-old lot of 11-29-00 was split: 207 oz went to Loa Hatchery as lot 001129RTTPEG04. The remaining 306 oz were combined with the 5 y-old eggs which were sent to Springville Hatchery as lot 001129RTTPEG05.

Overall, sterile rainbow trout production has a bright future in Utah. Future research at Egan Hatchery will examine the potential for triploidy of brook, brown, and lake trout for specific management needs. by *Eric Wagner*

New Faces at the FES

Ryan Hillyard has joined the Research team at the FES, replacing Robert Montgomery who is pursuing additional classwork. Ryan is a graduate of Utah State University, receiving a B.Sc. in December 2000 in Fisheries and Wildlife Biology. Ryan worked for Idaho Fish and Game for two summers, gaining experience with hydroacoustics, GPS, limnology, and tagging. He helped with the triploid survival and performance evaluation as well. He is active with the Logan chapter of Ducks Unlimited. He is a Cache Valley native and he and his wife currently reside in Smithfield.

Also new to FES is **Dr. Linda Chittum**, who occupies the position of Fish Health Specialist. Linda is a recruit from the Colorado Division of Wildlife, where she worked at the Brush Fish Health Laboratory for the past 5 years. Linda obtained her D.V.M. degree from Colorado State University. Linda will be focusing her efforts on fish health inspections and wild fish pathogen surveys. Linda is an avid waterfowl hunter and angler. She has applied for certification as a fish pathologist and fish health inspector with the American Fisheries Society.

Using AquaMats[7] to Enhance Growth and Improve Fin Condition for Rainbow Trout

AquaMats[7](Meridian Applied Technology Systems, Calverton, Maryland) are a type of artificial seaweed with a high surface area that are designed to encourage colonization and growth of algae, zooplankton and other aquatic organisms. In the past they have been used in aquaculture to provide structure in ponds and as a substrate for the growth of aquatic plants and animals. They have also been used in coastal areas as artificial reefs. In an aquatic environment, once the AquaMats[7] are colonized, they may provide a secondary source of nutrition for fish. These invertebrate prey items including crustaceans such as amphipods, ostracods, copepods, and aquatic insects may contain nutrients which might enhance fin condition. The spatial arrangement of mats in a raceway may also be beneficial in reducing fin erosion. Dorsal fin damage can be attributed to aggression between fish and aggression may be the result of dominant fish fighting for and protecting feeding sites within a raceway. The preference of fish for habitats that contain structures has been documented, however whether the presence of structure would break up a raceway environment to the point that aggression, and therefore fin erosion reduced, has not been determined. The purposes of the following two studies were to test beneficial aspects of aquatic growth on the AquaMats[7] to cultured rainbow trout, and positive effects of the physical structure of the mats on reducing aggression between fish. Positive results from this study may be manifested in enhanced fish growth and possibly improved fin condition. For both tests, short (46 cm) and long (61 cm) mats were used. For the first test the mats were placed into the test raceways in an alternating short to long pattern with the short ones being approximately 8 cm off the bottom and the long ones 15 cm off the bottom. The shorter mats did not break the water surface, while the longer ones overhung the water surface in a downstream direction by 20-30 cm. To test to see if the mats were contributing supplemental nutrition to the fish, two different treatments were included. Three raceways served as controls, three contained mats which were removed twice weekly and cleaned, and three contained mats that were not cleaned. Rainbow trout were stocked at a density of 8,100 fish per raceway when they had reached 1.7 g/fish (270 fish/lb).

For the second test three raceways were fitted with AquaMats[7] which were placed into the raceways parallel to their length. Two rows of mats were placed down the length of the raceway in an alternating short to long pattern such that one row began with a short mat followed by a long one with the next row following an opposite pattern. Midway along the length of each row one mat was hung off of the raceway wall at the water surface so that the entire surface area of the mats extended over the surface of the water. The mats were not cleaned for the duration of the 106 day test. For this test rainbow trout were stocked at a density of 5,100 fish per raceway once the fish had reached 2.1 g/fish (216 fish/lb).

The results from the first test revealed no significant differences, with respect to growth, between the treatments. By the end of the study all fish averaged 35 g/fish. No significant differences were found in specific growth rate or feed conversion ration between test fish. The hypothesis that fish may be getting additional food items off of the mats was not substantiated; in fact the FCR was higher, although not significantly so, for the non-cleaned group indicating they were not converting feed as efficiently. The surface of the mats in the non-cleaned raceways did host a consistent quantity of algal growth that was not found among the control or cleaned treatments. Cursory substrate scrapings consisted of almost exclusively filamentous algae with an occasional but rare chironomid larvae.

Cumulative mortalities (%) were significantly higher for the non-cleaned and control groups (1.4%) compared to the cleaned group (1.1%). Two months into the study there was a small outbreak of what was cursorily diagnosed as columnaris. The contamined raceways, which were randomly distributed among treatments, were treated with a 100 ppm hydrogen peroxide solution for 15 minutes for two days . During the infection period mortalities were not significantly higher for any one treatment, so the cumulative mortality for the non-cleaned fish must be attributed to some other reason.

Relative fin index calculations made from the fin measurements revealed several significant differences at the end of the study. The control fish had significantly better anal and ventral fins than the non-cleaned while the cleaned fish were not significantly different from either group. The poorer anal and ventral fin scores of the non-cleaned fish compared to the control fish may be explained by fish behavior in raceways with mats. For both treatments containing the mats the fish were segregated into the areas between mats and schooled in a circular direction within this compartment. This may have confined the fish more so than if they had the entire raceway to utilize.

During the course of the second test, and by its conclusion, no significant differences were found, with respect to growth, between the treatments. By the end of the study both control fish and treatment fish averaged 32 g/fish. No significant differences were found in specific growth rate or feed conversion ratio between the two groups of fish. As in the first test, the hypothesis that fish may be getting additional food items off of the mats was not substantiated. Cumulative mortality was slightly higher for the treatment fish (2.5%) compared to the controls (1.8%), but this difference was not significant.

There did appear to be some transient effects of mat use on fin condition. Relative fin index calculations made from the fin measurements revealed several significant differences throughout the course of the study. After one month, both pectoral fins were significantly longer for fish reared in raceways with mats compared to controls. After two months, all fins were significantly longer for the treatment fish. However by the end of the study, only the left pectoral fin was significantly longer for the treatment fish. It is possible the relative ratio of mat area to raceway volume may have been influential. At the end of August, the quantity, and therefore the area of mats in the raceways was doubled. Subsequent fin measurements made at the end of September revealed a consistent trend of better fin condition among fish from raceways with mats compared to controls. By the end of October, this trend had all but disappeared. It is possible that the relationship between the mat area to raceway volume, and the fish density was conducive to reduced fin erosion, but that as fish grew, and density increased, the positive affect of mats on fin condition decreased.

The information collected from the second test indicated that the use of AquaMats[7] was partially beneficial to good fin condition in the culture of rainbow. The results from the first test indicated that the baffle-type placement served to congregate the fish in the area between mats thereby increasing density and possibly fin erosion. That would explain the fin measurement results from the test. In the second test the placement of mats was adjusted to allow for free movement by the fish while at the same providing physical structure in an attempt to by break up the homogeneity of the environment and reduce territorial aggression. In both tests the was no evidence the mats provided secondary nutrients to the point that growth was improved. It is possible the density of mats in the given raceway volume was not high enough for an effect to have been seen.

Ronney Arndt

Triactinomyxon Polar Filament Discharge: Effects of Mucus, Magnetic Fields, and MS-222

Polar filaments are slender threads within teardrop shaped structures known as polar capsules (see Figure 1). Polar capsules are a diagnostic characteristic of members of the phylum Myxozoa and are also present at the tip of the *Myxobolus cerebralis* triactinomyxon (tam). Polar filaments evert from the capsule, attaching to the host, thus providing the means by which sporozooites within the tam can invade the host tissue. Attempts to prematurely fire the polar filament may be useful for preventing infection of the fish host. The mechanism of polar filament discharge remains a mystery. The natural triggers for discharge have yet to be determined.

Figure 1

Mucus would seem to be a logical candidate for a discharge agent, but results appear to vary among species. Yokoyama et al. (1995) was able to discharge filaments of raabeia-type actinospores of *Myxobolus cultus* using mucus from a variety of fish species, including goldfish *Carassius auratus*, common carp *Cyprinus carpio*, loach *Misgurnus anguillicaudatus*, rainbow trout, catfish *Parasiluris asotus*, and japanese eel *Anguilla japonica*. These authors also noted that mucin from bovine submaxillary gland was similarly active. Uspenskaya (1995) was also able to discharge filaments of *Zschokkella nova* using mucus of goldfish. Xiao and Desser (2000) observed polar filament extrusion and ameboid movement of the sporoplasms of actinospores after 1-2 min in a mix of lake water and mucus. The extrusion of aurantiactinomyxon, neoactinomyxum, echinactinomyxon, and raabeia forms was induced by mucus from a variety of species including brown bullhead *Ameiurus nebulosus*, yellow perch *Perca flavescens*, pumpkinseed *Lepomis gibbosus*, creek chub *Semotilus atromaculatus*, golden shiner *Notemigonus crysoleucas*, common shiner *Luxilus cornutus*, white sucker *Catostomus commersoni*, and fathead minnow *Pimephales promelas*. The percentage of extruded polar filaments varied with species and actinospore, but no actinospore was species specific. However, Xiao and Desser (2000) noted that some triactinomyxon forms were discharged by mucus from a narrower range of species. For example, for the 'triactinomyxon C' form (Xiao and Desser 1998), discharge was induced in >90% of the actinospores by common shiner, fathead minnow, and golden shiner mucus, but was 12% or less in the others, including the other cyprinid (creek chub). Triactinomyxon 'F' had an even narrower range, discharging 77% in mucus of common shiner, 12% in golden shiner, and 8% or less in the remaining species.

Mucus-induced discharge of the *Myxobolus cerebralis* triactinomyxon has been more elusive. El-Matbouli et al. (1999) failed to extrude polar filaments of *M. cerebralis* triactinomyxons using rainbow trout mucus. We have conducted similar tests with negative results. We conducted a follow-up experiment to determine if mucus in combination with mechanical contact would trigger polar filament extrusion of triactinomyxons of *M. cerebralis*. Mucus scraped with a glass slide from a rainbow trout was wiped onto a glass rod. The rod was stirred for 1 min within a 1 mL suspension of tams in well water. The mucus on the rod was wiped onto a microscope slide for examination and 200 µL of the tam solution remaining in the test tube was added to the slide as well. Of 116 tams observed at 400X, no extruded polar filaments were seen.

We hypothesized that perhaps a magnetic field could induce polar filament extrusion, so we tested the effect of a refrigerator magnet with or without mucus. The magnet strength was 0.45 Gauss as measured by a gaussmeter. Mucus scraped from the side of a rainbow trout (250 mm) anesthetized with tricaine methanesulfonate (MS-222) was transferred to each of 3 slides. Tams from a stock solution in hatchery well water were

added (100 uL) on top of the mucus. These slides were exposed to the magnetic field for 1 min. Control slides of tam stock only were not exposed to the magnetic field. Three additional slides of mucus and tams were not exposed to the magnetic field. Three additional slides were exposed to the magnetic field for 1 min without any mucus present. Results of this test indicated that magnetic field effects alone (9.0 ± 2.0% discharge) did not differ significantly from controls (4.3 ± 2.3%). However, mucus induced significantly higher (ANOVA, p <0.001) discharge rates either with (39.3 ± 3.0) or without the magnetic field present (31.3 ± 16.3).

The effect of mucus in this test was notably different from the previous experience, so we attempted to find out what factor was different between this and the previous trial. Use of MS-222 anesthetic in the second trial was suspected and further testing indicated that discharge was affected by the anesthetic. A 9.09 mg/mL (10 uL of MS-222 stock of 100 mg/mL diluted with 100 uL of tam stock solution) MS-222 solution induced 68.0 ± 5.7% discharge of at least 1 polar filament. This concentration of anesthetic is much higher than that typically used for anesthesia of fish, so we conducted two series of tests to examine the dose response of polar filament discharge to MS-222.

In the first series of tests, concentrations of MS-222 ranging from 0.091 to 8,182 mg/L were tested. For each concentration, MS-222 and tam stock solution was mixed on each of 3 slides and from 38 to 395 tams were observed on each slide. If at least 1 of the 3 polar capsules had discharged it was considered 'fired'. The relationship between dose and discharge was highly variable (Figure 2).

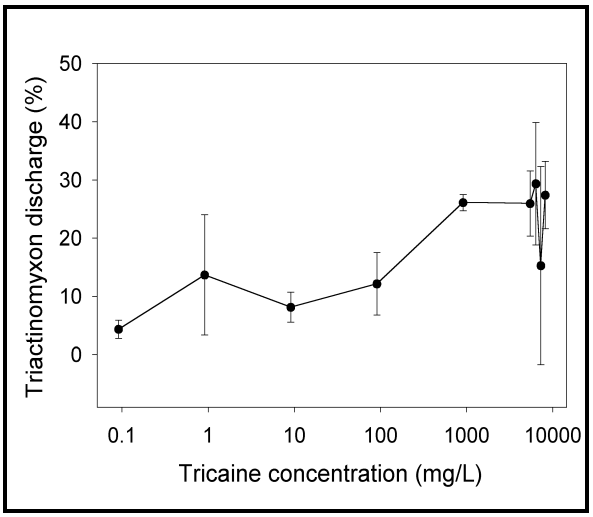

Figure 2. Polar filament discharge vs. MS-222 dose

Results indicated that MS-222 caused significantly higher rates of discharge than observed in controls, but only at concentrations at 909 mg/L and above. At 90.9 mg/L of MS-222 discharge was about 12.2%, but did not significantly differ from controls (1.9%). More tests were conducted in the range between 50 and 300 mg/L MS-222. Three slides of 100 tams each were examined, testing concentrations of 50, 100, 200, and 300 mg/L MS-222. This range covers the typical doses used for anesthetizing fish. The percent discharge in this range (18-33%) did not differ significantly from controls (23.7%). However, the results show some effect on discharge, though the concentrations were high enough that the discharge effect of mucus we observed in previous tests does not appear to be related to MS-222 per se. Further study is needed to examine the synergistic effect of mucus and MS-222.

by *Eric Wagner*

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Initial Look at the Effect of Sonication on *Myxobolus cerebralis* Triactinomyxons

A number of different methods for killing the infective stage of the *Myxobolus cerebralis* parasite have been tested recently here at the Fisheries Experiment Station. Effective control strategies included freezing, temperatures above 75 C for 5 min, drying for an hour, and chlorine at 260 ppm or greater for >1 min. These techniques can be useful for disinfection of equipment, but for treating hatchery water supplies these methods are impractical. Research has indicated that ultraviolet light (UV) is capable of disinfecting water supplies, but high cost and hydraulic head loss with these systems may limit their implementation. Alternative treatment strategies should be explored.

One alternative is the use of ultrasound. It is used routinely in laboratories around the world to clean laboratory equipment. Other applications of ultrasound include welding, visualization of soft tissue structures, and disruption of tissues. Applications rely on a variety of different frequencies and intensities to achieve the desired result. A series of tests were conducted here using an ultrasonic cleaner (Branson 2200 R3), but manipulation of frequency and intensity was not possible with the unit. Time was the only variable that could be tested.

For each test, triactinomyxons (tams) harvested fresh from worm cultures were added to well water in the sonication chamber. Control slides were made prior to this by putting 25 to 50 ì L of tam stock solution on each of three slides and staining with fluorescein diacetate and propidium iodide (50 ì L each). The sonicator was turned on for the designated time period (5 to 13 min). The water from the sonication chamber was filtered through a 10 ì m mesh filter and the retentate was rinsed into a 50 mL centrifuge tube. On each of 3 slides, 100 ì L of the sonicated solution was mixed with the vital stains. After at least 45 min incubation in a refrigerator, the slides were examined under epifluorescence at 100 x. Tams were classified as either live (green stained spore body), dead (red), possibly viable (mix of red and green), empty (no spore body present), or broken (only recognizable pieces of tams found). Estimates of the number of tams added were derived from the control slides and the number recovered after sonication were estimated from the treatment slides. Control viability varied among the treatment dates (p = 0.02), so the data were analyzed separately for each time period, comparing arc-sine transformed percentages between treatment and control with a *t*-test.

There were significant reductions in the percentage of viable tams from treatment groups relative to controls up to 11 min. At the higher durations, there were more tams added initially to the sonication chamber because most of these were destroyed in the sonicator. This provided enough recovery that tams could be examined. On a percentage basis, the percent viable in the 11 min and 13 min treatments did not significantly differ from controls, despite the fact that 99% of the tams had been destroyed. Those that had been killed were not recovered (i.e., stained red and observed) and were presumed destroyed by sonication. One effect of sonication was an increase in temperature. After 10 min sonication, the temperature had climbed 3EC, and after 13 min, 5EC.

Minutes of sonication	Viable (%)	Non-viable (%)	Possibly viable (%)	Empty (%)	Recovery (%)
control	81.6	3.7	7.2	7.1	
5	11.6	24.1	39.7	24.6	
7	2.8	26.5	39.9	30.9	
9	0.0	44.5	22.2	33.3	
10	58.3	12.5	29.2	0.0	1.9
11	72.2	0.0	27.8	0.0	1.2
13	44.4	0.0	55.6	0.0	0.6

Table 1. Percent (mean, *n* = 3) of viable, non-viable, empty (no spore body present), and possibly viable triactinomyxons of *Myxobolus cerebralis* after sonication for 5 to 13 min, based on vital staining. Data from controls was pooled for this table (*n* =18). Percent recovery is also presented for the last 3 time treatments.

At the highest duration (13 min), recovery of tams was only 0.6%. There was a drop in recovery over time among the latter 3 treatment times. Unfortunately, no data were collected concerning recovery percentages for the previous data points. Also to be determined is the percentage of tams possibly lost on or through the 10 ì m filter. Despite these deficiencies, the data suggest that sonication can cause significant reductions in tam numbers, but may require long treatment times. Further work is needed in which frequency and intensity of the sound wave can be manipulated to maximize the efficacy of tam destruction. Possible combinations of the sonication technology with other methods such as filtration may hold promise as well.

By *Eric Wagner*

Ron Goede Continuing to Recover

At press time, Ron Goede, former director of the Fisheries Experiment Station, was scheduled for release from LDS Hospital in Salt Lake City to a transitional care unit at Logan Regional Hospital on June 2. Goede was seriously injured in a one car accident in April and sustained chest injuries along with multiple fractures.

His recovery to this point has been quite remarkable, but he will still have to undergo orthopedic surgery to his left arm in the weeks to come. He expresses his thanks to well wishers who have sent cards and gifts. Best of all, he hasn't lost his sense of humor!

He does have access to e-mail and would enjoy hearing from his friends at rgoede@sisna.com.

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