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Fish Culture Class Held at FES

Pictured below are successful attendees of the intensive fish culture class recently taught in Logan by the Fisheries Experiment Station staff members and others. The class is held periodically to further educate new State hatchery workers on topics related to coldwater fish production, water quality and fish health topics. Also included in this year's course were new topics, such as warm water aquaculture, humane treatment of animals, lab safety, global positioning systems, aquatic nuisance species, biosecurity and computer presentations. A field trip was made to Kamas hatchery to observe fish production at the new state-of-the-art facility. Spare moments were spent with bouncing balls, eating barbeque and competing in sporting clays at the nearby shooting range.

In addition to the new topics, a number of new instructors took part in teaching the course. They include Gordon Nelson, Don Archer, Adam Kozlowski, Anna Miller, David Thompson, Patrick Goddard, Drs. Robert Schmidt and Mark Miller from USU and Ted Hallows. Former instructors who also taught included Ron Goede, Joe Valentine, Tim Miles, Eric Wagner, Ronney Arndt, Doug Routledge, Roger Mellenthin and Chris Wilson.

A special word of thanks to Kirk Smith for the use of the Cache Valley Hunter Education Center and Cathryn Smith for helping to prepare the materials and getting the troops in plenty of provisions during the meeting.

INSIDE THIS ISSUE:

Pressure Treatment for Triploidy	2
Coldwater Disease Vaccination Trial	4
Sand Filtration Made Feasible	8
Lake Trout Production	10



Use of Pressure Treatment of Eggs to Induce Triploidy in Brook Trout

Sterile brook trout have some appealing attributes. Since they don't reproduce, stunting of growth by overpopulation is not a problem. With a little experience, a fisheries manager can adjust stocking quotas to target a balance between good growth rates and numbers of fish for anglers. This means bigger fish. Another benefit of sterile triploid brook trout is better control of reproduction in areas where native cutthroat trout are a concern. Brook trout are adept at displacing cutthroat trout in areas where cutthroat previously dominated (Dunham et al. 1999; Dunham et al. 2002; Kennedy 2003). Sterile brook trout are a useful tool for managers who wish to appease anglers who prefer brook trout, yet still prevent the cutthroat's extinction.

Pressure treatment as a means of inducing triploidy has been reported for several salmonid species including rainbow trout (Chourrout 1984; Lou and Purdom 1984), steelhead (Guoxiong et al. 1989), coho salmon (Teskeredzic et al. 1993) and chum salmon (Benfey et al. 1988), but not brook trout. In pressure treatment, eggs are put into a metal cylinder and a piston applies hydraulic pressure to the water in which the eggs are held. Data generated by Joe Kozfkay (*in press* Idaho Fish and Game report) indicate pressure treatments of 9500 psig for 5 min at 40 min after fertilization have generated 100% triploid brook trout. The pressure apparatus is expensive and cumbersome, so heat shock has been the method of choice for triploid production. Heat shock has been successful for producing high triploidy rates in Utah's rainbow trout, but for brook trout it has not worked as well.

Production of triploid brook trout has been conducted for several years in British Columbia. According to Tim Yesaki, a fisheries researcher there, high triploidy rates have been achieved with use of heat shock (29.4 C for 7 min duration at 18 minutes after fertilization). Idaho Fish and Game has also been experimenting with sterile brook trout production. In a recent report (Kozfkay 2002), triploid induction rates of 100% were achieved by heat shocks of 27 C (for 20 min, 20 min after fertilization), 28 C (10 min, 10 min after fertilization), and 29.4 C (7 min, 18 min after fertilization). Survival to eyeup averaged 52% at 27 C, 50% at 28 C, and 62% at 29.4 C. Controls (7.5 C) averaged 89% eyeup. Kozfkay repeated the 29.4 C treatment this past year and induction was much lower (82%). Dubé et al (1991) found that the best triploid yield for brook trout was at 28 C for 10 min initiated 15 min after fertilization. Galbreath and Samples (2000) also found that 28 C for 10 min at 10-16 min after fertilization worked most effectively, resulting in 79-99% triploid with a relative survival of 68-71%. This article summarizes an initial evaluation of pressure treatments as a means of inducing triploidy in brook trout.

In the fall of 2003, brook trout eggs from 5-year-old females were fertilized in 3 different lots (replicates), using 5 females and 4 males per lot. The fertilization of the lots was staggered in time to permit pressure treatments of each replicate at the correct time. A 1% saline solution was used as a sperm diluent. Eggs were rinsed after 2-3 min and the eggs water hardened at the hatchery water temperature (8.5 C) until it was time to start the pressure treatment. Pressure treatments of 5 min duration were as follows:

1. 9000 psig at 32 min post-fertilization (p.f.)
2. 9500 psig at 22 min p.f.
3. 9500 psig at 32 min p.f.
4. 9500 psig at 43 min p.f.
5. control (0 psig)

The time required to reach the target pressure varied from 18 to 30 sec. Depressurization required only 1 to 3 sec. Eggs were transferred to net sacks of mosquito netting and transported to the Fisheries Experiment Station (FES), Logan, Utah. The eggs were disinfected with 100 ppm

povidone-iodine for 10 min upon arrival, then transferred to tray incubators. A daily prophylactic treatment with 1667 ppm formalin was used to control fungus. Dead eggs or fry were removed by hand-picking about every other day. When the fish hatched and began to near the swim-up stage, they were transferred to troughs with screen dividers. Blood samples for flow cytometry analysis (Paul Wheeler, Washington State University) were taken from the caudal vein on April 26, 2004.

Results and Discussion

The survival of the eggs to the eyed stage and to hatching was uniformly poor, highly variable, and did not significantly differ among treatments ($p = 0.261$; Table 1). The combination of green egg transport and the warm temperatures at the FES are thought to contribute to the poor survival.

Table 1. Survival and percent triploidy of brook trout pressure treated at two different pressures and various times after fertilization.

Treatment Pressure	Min after fertilization	Survival to eyed egg stage (%)	Survival to hatching (%)	Mean triploidy rate (%)	Sample size
9000	32	28.5 ± 3.2	13.2 ± 9.1	97.9	48
9500	22	16.8 ± 13.4	6.0 ± 6.6	66.7	9
9500	32	22.7 ± 22.0	9.3 ± 19.8	100	28
9500	43	27.9 ± 21.3	9.1 ± 26.1	100	46
0	control	47.4 ± 13.0	27.1 ± 7.7	0	45

Triploidy rates were encouraging in the test, reaching 100% in the 9500 psig treatments conducted at either 32 or 43 min after fertilization. Applying pressure before this time (22 min) did not result in acceptable ploidy rates. Even at lower pressure (9000 psig), the triploidy rates was improved at later times post fertilization. The results indicate a window of opportunity between 32 and 43 min after fertilization for optimal triploidy induction via pressure induction. The triploidy rates achieved in this study and by Joe Kozfkay in Idaho, indicate that pressure treatment is a viable technique for producing sterile brook trout. In Utah, however, we will need to improve overall survival. Future trials should focus on production scale efforts at hatcheries where the temperatures are appropriate for brook trout.

Eric Wagner

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Bacterial Coldwater Disease (BCWD) Autogenous Bacterin Production and Vaccination Trial

Rainbow trout fry syndrome (RTFS), a form of bacterial coldwater disease (BCWD), is a bacterial fish disease caused by *Flavobacterium psychrophilum*. It has been regularly isolated from fish in the Utah hatchery system. The disease has been a recurring problem for hatchery managers in Utah and elsewhere and appears to be getting worse over time. A preliminary evaluation of a *F. psychrophilum* bacterin was conducted by Wilson (1996). The test indicated a reasonable level of success could be expected (20% reduction in mortality) with the Sand Creek strain of rainbow trout (RTSC). Obach and Laurencin (1991) have also successfully reduced mortality of rainbow trout vaccinated with either intraperitoneal injections or bath treatments.

Bacterin development

A *Flavobacterium psychrophilum* isolate from an epizootic of BCWD at Glenwood (GW; D03-008) was cryopreserved at the Fisheries Experiment Station (FES). The identification of the isolate was confirmed serologically by slide agglutination with specific polyclonal antisera. Also, the isolate was consistent morphologically and biochemically with *F. psychrophilum*. It is generally accepted in the fish vaccine world that a "hot" (high mortality rates) isolate makes the best vaccine. Using the "hot rule" we chose to use the GW isolate for a bacterin, based on the case submission forms provided by the hatchery managers. Because of strain variability among *F. psychrophilum* cultures, it was also wise to use the GW strain in Utah for vaccination development, rather than rely on an out-of-state source. The GW isolate was sent to Novartis AquaHealth, LTD (AquaHealth) who cultured the bacteria in environmentally controlled vats (called fermentors) or flasks using specialized growth media. Then a bacterin was created from the culture by killing the bacteria with ~0.2% formalin, (killing can also be accomplished with heat, chemicals, or pressure). The bacterin was then put through a series of tests to ensure sterility and safety. Once the vaccine culture is formalin killed, it is plated/isolated onto sensitive media and the media observed for a period of time (~3-14 days) to ensure vaccine "sterility." Once the bacterin was deemed sterile, AquaHealth administered the bacterin (probably a 1 minute immersion) to fish (usually 30) and the fish were observed for a period of time (~7-14 days) to ensure vaccine "safety." Once safety and sterility tests were completed by AquaHealth, 14 liters of bacterin were sent to the FES.

Field trials

Based on historical problems with BCWD at GW, Fountain Green (FG) and Loa (LA) state fish hatcheries, field-testing of the bacterin in the Sand Creek strain of rainbow trout was conducted at these 3 hatcheries. At each hatchery, a given lot of rainbow trout was divided into vaccinate (test) and control (as close to normal production as possible) groups. Due to hatchery limitations, only 1 replicate of each treatment was conducted at each hatchery. At FG, 125,700 fish were used for each treatment; at Loa, 53,142 fish were used for the treatment group and 53,496 fish were used for the control group. GW initiated the study with 109,161 fish in the treatment group and 111,628 in the control group.

Vaccination was initiated when test fish were approximately 50 d post hatch, based on previous research by Obach and Laurencin (1991) that indicated immunization prior to this age was not fruitful. A secondary 'booster' bath treatment was given two weeks later at each hatchery. Test fish were taken off feed prior to the bath treatment. Each bath was made by diluting 1 L of vaccine with 9 L of

hatchery water. NaCl was also added to the primary bath (about 2%) to create a hyperosmotic solution, facilitating uptake of the bacterial antigen. Supplemental oxygen was provided in the bath via an airstone during the 1 min bath treatment. Fish were dipnetted (3.6 to 5.1 kg of fish per netful) and left within the dipnet during the bath treatment. Up to 115 kg (253 lbs) of fish were treated with each bath solution. Although 100 kg is recommended as the maximum treated by a single bath, we were near the end of the group and bacterin quantity was a concern, so we decided to continue with the existing bath rather than open a new container. The overage was only on the primary and secondary bath at GW. The time required to treat the fish was about an hour. Controls were not handled, simulating conditions as if no bacterin treatment had been conducted.

Vaccine efficacy was evaluated by comparing mortality, feed conversion, and disease prevalence between vaccinated and control groups. After 3 months, sample prevalence of *F. psychrophilum* was determined at FG for each treatment group by lethally sampling 60 fish per treatment, inoculating brain tissue onto selective media. Due to outbreaks of BCWD at Loa and GW, no final prevalence sample was taken at these hatcheries.

Table 1. Number and weight of fish treated with a *Flavobacterium psychrophilum* bacterin at each of three Utah state hatcheries, the date and fish age at bath treatment.

Results

	Primary bath	Secondary bath
Fountain Green Hatchery		
Date	13 Jan 2004	27 Jan 2004
Age (weeks)	7	9
Total weight (kg)	49	99
Mean weight (g)	0.4	1.0
Salt treatment	Yes	No
Glenwood Hatchery		
Date	12 Jan 2004	26 Jan 2004
Age	7	9
Total weight (kg)	123	204
Mean weight (g)	1.1	1.9
Salt treatment	Yes	No
Loa Hatchery		
Date	2 Dec 2003	16 Dec 2003
Age	7	9
Total weight (kg)	39.9	87.1
Mean weight (g)	0.7	1.6
Salt treatment	Yes	No

The results indicated that the vaccine was ineffective at reducing mortality, relative to controls at both GW and Loa. At GW, the mortality was higher in treated fish (2.6%) than in controls (1.3%; Fig. 1). Higher mortalities prompted a diagnostic examination two weeks after bacterin treatment.

Flavobacterium psychrophilum was isolated from moribund fish. This resulted in treatment of the fish with medicated feed (oxytetracycline at 2 g/100 lb feed), starting 22 days after the bacterin treatment. Control fish were not fed the medicated feed until day 34 after bacterin treatment when mortalities were increasing in that group as well. These fish received only medicated feed on six different days over a 10 d period.

At Loa, the mortality was 4.6% in the treated group and 1.9% in the control group (Fig. 2). Increasing mortalities at this hatchery also prompted a diagnostic examination. Bacterial isolates from moribund

(Continued on page 6)

(Continued from page 5)

fish developed yellow-pigmented colonies of long, slender, gram-negative rods indicating that a *Flavobacterium* was also present in this case. As a result, vaccinated fish were fed medicated feed (same dose as for GW) for 8 consecutive days starting on day 67 post-vaccination. The control group was also fed medicated feed for 10 days beginning on day 86.

At FG, mortality over the 90 days post-treatment was 1.4% in the control raceway and 1.0% in the treated raceway. Sampling for *F. psychrophilum* at 90 days after treatment at this hatchery indicated that these fish were not specific pathogen carriers. Differences in the performance of the fish between the treatments for each hatchery are shown in Table 2. Separate lot histories were not recorded at FG, so only the mortality data was available for each of the treatments.

Table 2. Comparison of some fish culture rearing and performance variables between vaccinated and control groups at three Utah state hatcheries about 3 months after exposure to a *Flavobacterium psychrophilum* bacterin.

	Glenwood		Loa		Ft. Green
	Control	Treated	Control	Treated	Combined
Max. density index (lbs/ft ³ /in)	0.48	0.51	0.41	0.39	0.35
Flow index (lbs/gal/min/in)	1.03	1.09	1.06	1.07	2.80*
Feed conversion	0.72	0.62	0.88	0.85	2.11
Mean final weight (g) at 3-30-04	12.4	14.3	55.4	58.9	2.6
Temperature (F)	58	58	59	59	52
Mortality (%)	1.3	2.6	1.9	4.6	1.4/1.0

*oxygen injection employed at Fountain Green

The data in Table 2 hint that there may be a growth benefit and better feed conversions from vaccination, though we couldn't statistically test that. Ackerman et al. (2000) noted that growth of rainbow trout was improved in groups vaccinated against *Aeromonas salmonicida* with a microencapsulated bacterin with *Vibrio anguillarum* lipopolysaccharide. In our study, the results in general indicated that the stress of vaccination was more harmful than doing nothing in 2 of 3 hatcheries.

The trials were conducted without any adjuvant and used whole cell preparations. We were interested in trying the most cost-efficient methods first, before attempting more elaborate bacterin preparation techniques. For example, research has shown that adjuvants (non-specific compounds such as heat-killed *Mycobacterium tuberculosis* or bacteria lipopolysaccharides that are added to the specific antigen mix to help stimulate the immune system) can improve immune response (Ackerman et al. 2000; Nikl et al. 1991; Rahman et al. 2000). Adjuvants add to the cost of the vaccine, but may be necessary for an adequate immune response. Also, by breaking the antigen into smaller pieces (subunit vaccines), this may facilitate uptake of the antigen by the white blood cells (macrophages). Rahman (2002) found that the outer membrane fraction of *F. psychrophilum* induced a better immune response than whole cell preparations. Another strategy to try is attacking the bacterium at the source, i.e., the broodstock. Intraperitoneal injections are impractical for large numbers of production fish, but this more efficacious method of immunization is feasible for broodstock. Also different approaches to disinfecting eggs before hatchery transfer might help. Kumagai et al. (1998) found that povidone iodine ranging from 50 to 1000 ppm was ineffective at eliminating *F. psychrophilum* from the surface of salmonid eggs. So, alternative disinfectants like glutaraldehyde need to be evaluated.

Acknowledgments

We appreciated the cooperation of the hatchery personnel in this study, especially Terry Howick, Pat

Ah Heck, another employee at FES...

With the improving news about the Division's budget, we have finally been able to fund the vacant biologist position with the Technical Services team at the Fisheries Experiment Station. And after several months of searching, we were able to find a very qualified biologist to fill the position vacated when **Ernie Dean** retired.

Chris Heck (referred to as Heck, due to the other Chris around here) is a very experienced microbiologist with well over 15 years of experience performing laboratory research. Much of Chris' resume experience comes from our own Utah State University. Recently, he worked at Utah State Biotechnology and Genomics Research center where he was instrumental in the planning and establishment of the fermentation and bioprocessing facility. His Masters degree is from Western State College in Gunnison Colorado where he conducted research on the purification and characterization of isoenzymes involved in plant development and even found time to ski. Prior to his graduate work, Chris completed his studies in Soil Science and Microbiology from the University of Minnesota.



Chris's breadth of experience in microbiological and molecular biological lab techniques along with his practical experience with a plethora of laboratory equipment will be a valuable asset in the growing fish health program at FES. Outside of his studies, Chris has a passion for back-country skiing, rock climbing, and road biking. In addition to his own hobbies, Chris is forced by his wonderful wife, Joyce, to enjoy the wonderful world of gardening (and weeding), and we have plans on making him a crazed fisherman and hunter. Chris can be reached at (435) 752-1066 extension 13.

(Continued from page 6)

Brown, and Dean Meacham. Thanks to Hugh Mitchell at AquaHealth for making the bacterin.
Eric Wagner and David Thompson

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Making Sand Filtration Economically Feasible

For the past three years we have been investigating the potential of sand filtration as a means to filter out triactinomyxons (TAMs), the water-borne, fish-infectors of whirling disease. The initial test indicated that when using the appropriate-sized sand (particles of $>180\ \mu\text{m}$), 99% of infection could be prevented (Ichthyogram, Oct. 2002, Vol. 13, Issue. 3). Because faulty back flushing was seen as the weak link in our first test, the follow-up test concentrated on improving the back flush procedure. Sand filters must be periodically back flushed to remove trapped debris. So, for our second attempt (Ichthyogram, July 2003, Vol. 14, Issue 2), an experiment was conducted to improve back flush procedures: 1) *extended backflush*, in which the duration of backflushing was increased to theoretically flush out more triactinomyxons, 2) *diverted return flow*, in which water that was headed for the tanks with fish, immediately after backflushing, was diverted for 5 min to "re-seat" the sand in the filter, and 3) *slow sand filtration*, in which water was passed through a larger filter at a slower rate and no backflushing was conducted. The results from this test revealed the only fish 100% clean of *Myxobolus cerebralis* were within the diverted back flush treatment. z

At the conclusion of that past study we felt confident in our filter design and back flush protocol, so our next direction of research was to make a system that could be economically installed and maintained at a state hatchery. To accomplish this, a reliable source of appropriate-sized sand was required. The sand used in the previous two trials was laboriously sifted to remove the smaller size fractions. For the second experiment this required almost two weeks of labor using a mechanical sifter to acquire the necessary quantities. For production-level filters, this technique would not be sufficient. At the urgings of Roger Mellenthin, culturist extraordinaire here at the FES, we decided to take a closer look at some of the commercially available sand blasts sands which were cheap and easily obtainable. We narrowed the choices down to four different sizes that might be successful: # 7030 had an effective size (ES) of $100\ \mu\text{m}$, #4010 ES = $250\ \mu\text{m}$, #4060 ES = $300\ \mu\text{m}$, and a ruby garnet sand, which had an ES of $180\ \mu\text{m}$. The product number relates to the sands size composition and each had a unique effective size, which relates to the quantity of smaller sand grains. Effective size is defined by that size fraction at which only 10% of smaller particles remain, and it is important because smaller size fractions reduce filter flow.

Preliminary testing with all four sands, and combinations thereof indicated the sand #7030 and the garnet sand would not work due to the restrictions in flow they caused due to their smaller ES. As a result it was decided to test both #4010 and #4060 in the same sand filter/recirculation system used in previous tests. For this test sand depth was increased to 10 inches, an increase from four inches used during the first test and an increase from seven inches used for the second. Beginning on Dec. 19, 2003, rainbow trout (Sand Creek strain) were stocked into the experimental systems and the next day TAMs were added to the systems above the sand filters. TAMs were added to the systems three times per week for all but the final two weeks of the sixteen-week test. For each exposure 13,000 TAMs were added to the head box that fed water to the three filters comprising a treatment.

At the conclusion of the test, all fish were euthanized, lengths and weights were measured. Also a deformity index, designed to quantify cranial and skeletal deformities, was conducted. The presence or absence of deformities such as vertebral, mandibular, cranial, or opercular deformities was noted and quantified for each treatment. Fish without deformities were categorized as normal. Such deformities are typical clinical signs of whirling disease. The fish heads were removed and then processed using the pepsin-trypsin digest method. A portion of the homogenate was sent to an independent laboratory (Pisces-Molecular LLC) for assaying of *M. cerebralis* via PCR, however those results are still pending.

For back flushing, the diverted back flush protocol derived from the second test was used for both sand treatments. For the first seven weeks, the filters were back flushed once weekly, and for the remaining seven weeks the filters were flushed twice weekly. As part of the back flush procedure, all water flushed from the individual filters was collected and drained through a $100\ \mu\text{m}$ sieve to determine the quantity of sand lost during the back flush. This was done to get a handle on how quickly sand was lost from a filter during routine operations so that that replacement sand could be periodically added. This same procedure was conducted on water collected during the period after the back flush when water was diverted from the fish tanks for a five-minute period.

As in the previous tests, the fish survived reasonably well in the recirculation systems as evidenced by fish growth and mortality (Table 1). Infection quantified by PTD showed no infection within the two treatments and the negative controls. However the spore load among the positive controls, over 90,000 spores per head, was reasonably high.

The sand filters performed well as in the previous tests. The average flow rate during normal operations was just over two L/min ($8 \text{ m}^3/\text{m}^2/\text{hr}$) for the two sand treatments. To avoid losing sand in the discharge because of its smaller ES, the #4010 sand had to be back flushed at a lower rate (6 l/min; $22 \text{ m}^3/\text{m}^2/\text{hr}$) than the #4060 treatment (8 l/min; $29 \text{ m}^3/\text{m}^2/\text{hr}$). Despite the lower flow rate, the #4010 treatment lost more sand, on a cumulative basis, during the test, 195 mL, than the #4060 treatment, which lost only 3 mL of sand. Approximately six weeks into the test, small amounts of sand began to discharge from the sand filters of two of the three #4010 filters. During the remaining ten-week extent of the test, those two filters discharged a cumulative average of 61 mL of sand.

Even with these few problems it appears we have mostly debugged the sand filter technology as it applies to whirling disease and the removal of TAMs from contaminated hatchery water supplies. Pending the PCR results, it appears either sand size was effective at filtering out the *M.c. actinospores* (TAMs). The only concern at this point might be the sand loss associated with using the #4010 sand. Because the #4010 sand has a smaller ES, it may be better at filtering out smaller particles than the #4060 sand, although our infection results seem to indicate both sand sizes performed equally.

We are currently testing the #4010 sand in a 3" diameter commercial sand filter at flows of 40-50 L/min to look at sand loss during forward flow, and during and after the back flush procedure. If those results are not encouraging, we will move on to the larger #4060 sand. In either case we feel we have an adequate filter design, back flush protocol, and choice of sands to make sand filtration a reality at hatcheries with *Myxobolus cerebralis*-contaminated water supplies.

Table 1. Final fish weights, percent mortality, percent deformities, and *Myxobolus cerebralis* spore load per fish head for fish reared in systems supplied with TAM-contaminated water filtered through different grades of sand-blast sand.

Treatment	Final Weight (g/fish)	Mortality (%)	Deformity (%)	M.c. spores per fish head
#4060	5.6	1.7	3.3	0
#4010	5.5	1.7	1.7	0
Negative control	5.6	0	0	0
Positive control	5.3	0	8.3	91,233

Table 2. Filter performance during forward and back flush flow during sixteen-week period. Mean cumulative sand loss during the back flush procedure, the diversion interval, and during normal operation.

Treatment	Mean forward flow (L/min)	Mean back flush flow (L/min)	Cumulative sand loss during back flush (mL)	Cumulative sand loss during diversion (mL)	Cumulative sand loss during forward flow (mL)
#4060	2.3	8.0	21.5	2.8	0
#4010	2.2	6.3	21.8	195.0	61.3
- control	2.1	–	–	–	–
+ control	2.3	–	–	–	–

Ronney Arndt

Lake Trout Triploid Production, Fall 2003

Production of sterile lake trout for Bear Lake, UT-ID, was attempted again this last fall. The results from heat shocks the last two years have been disappointing, so Joe Kozfskay (IDFG) and Eric Wagner (UTDWR) coordinated to pursue pressure treatment as an alternative method of inducing triploidy (sterility), based on success with Arctic char (*Salvelinus alpinus*). Pressure treatment of 9500 psi for 5 min at 300 degree C-min has given nearly 100% triploid Arctic char (Eric Johnson, Rochester, WA, pers. comm.). A similar treatment has been used successfully to produce sterile brook trout (*Salvelinus fontinalis*; Benfey et al. 1997).

High incubation temperatures may also have been a factor in poor survival the last two years. For example, Ostergaard (1987) found that lake trout reared on chilled water (3.7 to 7.7 C) had higher survival to hatching (44-72%) than controls (37-44%) at 8.3 to 10.6 C in one brood year. Similar results were also noted the next year in which survival to hatching was 68.4% for chilled water treatment compared to 50% for controls. However, Dwyer (1987) found that there was no difference in the survival rate of lake trout hatched and reared at three different water temperatures (10.0, 6.7, 1.7 C). Combs (1965) noted temperatures of 5.8 to 10.0 C had the lowest egg mortality for kokanee, with constant temperatures of 1.7 C and 3.0 C inducing 80% and 37.3% mortality, respectively. Baird et al. (2002) found that brook trout eggs reared at 9.4 C had lower survival (40-57%) than at 5.1 C (60-73%). March (1995) found that hatching success of Arctic char was significantly higher at 3 C than 6 C in 15 of 18 family groups.

The sterile lake trout development work was pursued on three different fronts:

- 1) Story Hatchery, Story, Wyoming,
- 2) Saratoga National Fish Hatchery, Saratoga, WY, and
- 3) Egan State Fish Hatchery, Bicknell, UT.

Due to the political pressure for stocking lake trout, sterile or not, and the desire to adequately protect endemic species, there was a need to try multiple strategies in case one method did not produce sterile fish. Egan Hatchery provided lake trout eggs that were to be fed 17- α methyl-testosterone (MT) feed upon reaching the swim-up stage. Pressure treatment of lake trout eggs occurred at Story State Fish Hatchery, WY and at the federal hatchery in Saratoga, WY. Blood from pressure treated fish was analyzed by flow cytometry by Paul Wheeler, Washington State University, to determine the ploidy status.

Egan Hatchery lake trout

About 58,900 eggs from Egan Hatchery were fertilized on 10 Dec 03 and transported to the Fish Lake facility and incubated using 4-5 C water from Twin Creeks. Pat Brown, of Loa Hatchery, graciously agreed to care for the eggs while they were there. At eyeup, the eggs were transferred to the Fisheries Experiment Station, Logan, UT (FES). At the FES, the fish were to be treated with MT to induce sterility, but we did not receive approval of an Investigational New Animal Drug permit (INAD) for MT in time to initiate the special feeding. Due to hatchery closures, the demand for the space and water for rainbow trout, and the lower values of diploid lake trout, rainbow trout culture became a higher priority and the lake trout were discontinued.

Story State Hatchery

At Story, a production lot of eggs (48,676) was treated on 7 October 2003 with 9500 psig for 5 min at 40 min after fertilization. A commercial pressure unit owned by Idaho Fish and Game was used for the pressure treatment. A small number (7,100) of untreated control eggs were also taken at the same time. The eggs were reared until eyeup at Story, and then transported to the Grace State Fish Hatchery, Grace, Idaho. At Grace, half the eggs were reared on a chiller system (51 F or 10.6 C),

distributed among 10 egg incubation trays. The other half was incubated at ambient hatchery water temperatures (54 F or about 12.2 C), similarly distributed among 10 trays.

Eyeup of the pressure treated group was 97% and the control was 100% (Table 1). Survival from green egg to hatching of the chilled water group averaged 80.1% for the pressure treated group and 98.0% for controls. On ambient temperature, survival to hatching was only slightly less; 78.7% for treated eggs and 96.3% for controls. Survival of the pressure treated group relative to controls indicated that pressure treatment results in a 10-20% drop in survival, similar to heat shocked eggs. The triploidy rate for the pressure treated group was 100% ($n = 29$).

Saratoga Hatchery

Pressure treatments at the Saratoga National Fish Hatchery were conducted on 28 October 2003. Both a production group and treatments of a controlled experiment were pressure treated using the commercial pressure device. The experiment tested two³ different pressure levels and three times post fertilization will be compared without a control:

- 1) 90009500 10000 psi for 5 min at 32 min post fertilization (p.f.)
- 2) 9500 psi for 5 min at 22 min p.f.
- 3) 9500 psi for 5 min at 32 min p.f.
- 4) 9500 psi for 5 min at 43 min p.f.
- 5) control at ambient temperature (9.3 C), handled the same as treatment eggs

A total of three replicates were conducted, using pooled eggs from four 9-year-old females and pooled sperm from four males for each replicate. Replicate lots were staggered to accommodate the time needed to empty the pressure vessel and get the next batch in. A portion of the replicate pool was delayed a few minutes before fertilization to avoid a time conflict with Treatment 3; one batch served for treatments 1, 2, 3, and 5 and the delayed batch was for treatment 3. About 5,000 to 6,500 eggs were used for each treatment within a replicate. The eggs were incubated in Heath trays at Saratoga Hatchery until eyeup, whereupon the eggs were transported to the Grace Hatchery in separate cloth netting bags in a water cooler the same day.

An additional production lot of 83,100 eggs was also pressure treated at Saratoga in four different batches, using 9500 psig for 5 min at 27 to 28.5 min after fertilization. The eggs were incubated in an egg jar. Upon reaching eyeup, these eggs were transported and reared at the Grace Hatchery, with half being reared at ambient temperature (12.2 C) and half on the chilled water (10.6 C). The production lot eyeup was 30.0% and survival to hatch was 15.4%. The triploidy rate was 100% in this group also ($n = 27$).

The survival to eyeup among the replicates of the pressure treated groups ranged from 33.9 to 64.8%, whereas controls ranged from 53.9 to 68.1% eyeup (Table 1). The control survival to eyeup was significantly higher (one-way ANOVA, $P < 0.05$) than the 22 and 43 min pressure treatment at 9500 psig, but did not significantly differ from the other treatments. Survival from green egg to hatching among replicates of the treated eggs ranged from 21.2 to 49.5%, and controls ranged from 31.4 to 57.6%. These data also indicate significantly reduced survival of pressure treated groups relative to untreated controls for all treatment levels except 9500 psi at 32 min after fertilization. The percent triploidy was 100% in all the pressure treatments and controls were 100% diploid (Table 2).

The success of the Story production lot, both in terms of survival and triploidy rates, has led to the decision to use that source and the pressure recipe used there for future triploid lake trout needs for Bear Lake, UT-ID.

Eric Wagner

(Continued on page 12)

(Continued from page 11)

Table 1. Survival of lake trout eggs exposed to various levels of pressure at various times after fertilization and incubated at two different water temperatures. Within the experimental group at Saratoga Hatchery, means that are not significantly different from each other within the column have a common letter following the mean.

	Pressure (psig)	Min after fertilization	Survival to eyed egg (%)	Survival ¹ to Hatch (%)
Story Hatchery, 10.6 C Pressure-treated	9500	40	97	80.1
Control	0		100	98.0
Story Hatchery, 12.2 C Pressure-treated	9500	40	97	77.2
Control	0		100	96.3
Saratoga Hatchery, 10.6 C	9500	27-28	30.0	14.7
Saratoga Hatchery, 12.2 C	9500	27-28	30.0	13.4
Saratoga Hatchery Experimental Group, 12.2 C	9000	32	47.6 ab	26.0 a
	9500	22	42.0 a	24.2 a
	9500	32	53.3 ab	37.6 ab
	9500	43	38.3 a	23.8 a
	0		61.6 b	45.2 b

¹survival from green egg to hatching

Table 2. Percent triploidy rate of lake trout exposed to various levels of pressure at various times after fertilization.

	Pressure (psig)	Min after fertilization	Triploidy rate (%)	Sample Size
Story Hatchery production lot	9500	40	100	29
Saratoga Hatchery production lot	9500	27 to 28	100	27
Saratoga Hatchery Experiment				
	0	32	0	13
	9000	32	100	41
	9500	22	100	41
	9500	32	100	27
	9500	43	100	40

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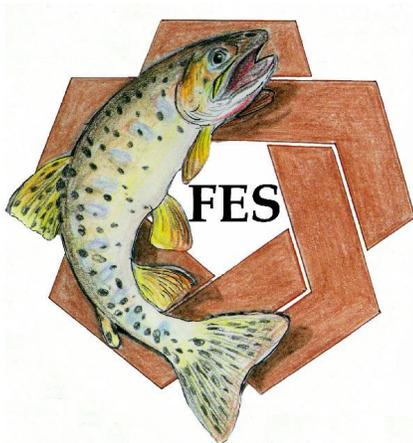
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