The Ichthyogram

April 2002 Volume 13 Issue 1

A Review of the Second Season of Mass-Produced Triploid Rainbow Trout

Utah has been moving steadily towards the mass production of triploid rainbow trout. Over the past decade triploidy induction methods such as electrical, magnetic, pressure, and heat shock have been experimented with. Crossing tetraploid females with diploid males, and drugs to induce triploidy were also tried. All of these techniques met with limited success. In the fall of 1999 very good success was achieved by heat shocking eggs at 26-28E C at 20 minutes post fertilization for 20 minutes. For these treatments 97-100% triploids were obtained, but eye-up and hatch were low for the 28E C treatment, 40% and 54% respectively. During the same time a shocking trough was designed, mainly for shocking tiger trout eggs, which allowed us to heat shock large quantities of eggs. Over the course of two visits to the Egan Hatchery in the fall of 1999, over 150,000 tiger trout eggs were heat shocked. We designed the heat shock chamber around one of Egan=s hatchery troughs. See Figure 1 for a more detailed description of the trough design.

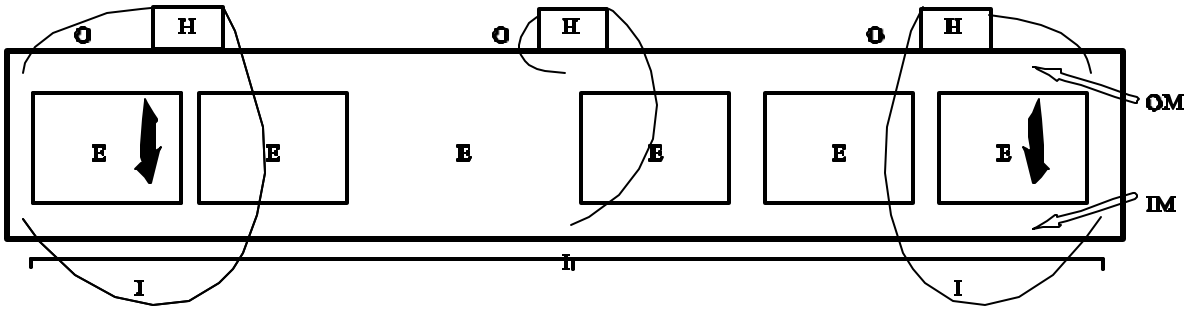

Figure 1. Schematic diagram of Egan=s heat shocking trough. Arrows indicate direction of water flow. H: heat pump; O: pump outlet line; I: pump inlet line; E: egg tray; OM: outlet manifold; IM: inlet manifold.

During that same time frame two year classes of sex-reversed brood stock had been developed. By heat shocking eggs fertilized with the sex-reversed males, all female triploids could be produced. During the fall of 2000, gametes were harvested from these fish and over 1.5 million eggs were fertilized and heat shocked. Eye-up for these egg takes averaged 82%. With this second year of mass-produced triploids, the heat shock techniques were perfected to the point where the next step could be taken. The sex-reversed brood stock were discontinued however, due to the difficulty of producing the brood fish and the difficulty of harvesting milt. The next step consisted of producing mixed-

(Continued on page 2)

inside...

(Continued from page 1)

sex triploids by heat shocking all of the Sand Creek strain of rainbow trout which is a fall spawning strain. It was decided to only do one of the three major strains of rainbows the first year, so that manpower needs could be addressed, and the hatchery staff trained on the technique.

A typical day of heat shocking consisted of one crew, usually 3 people, getting the heat trough set up while the spawn crew got the trailer together and sorted out ripe fish for spawning. In the trough, the inflow and outflow manifolds were hooked up to three heat pumps (Figure 1). The manifolds consisted of perforated **2**" PVC pipe. After the plumbing connections were all made, pre-warmed water was transferred from a six foot circular tank. An array of immersion heaters heated the water in the circular, which generally took 1-2 hours before it reached an adequate temperature to be transferred to the trough.

After the water had reached somewhere close to 27E C it was transferred to the trough and the heat pumps were started. With the pumps running, it was simply a matter of getting the water temperature to the target of 26.5E C, which was accomplished by either adding cool or hot water to the trough. Once the target temperature had been reached, the heat pumps did a great job of maintaining it throughout the day. On busy days in the middle of the spawn cycle, a second trough was set up. For a typical spawn day a crew of three was necessary to keep things running in the hatchery. One person was the designated time keeper and made sure each batch went through the right step at the appropriate time. A minimum of one other person was needed to transfer the eggs through the various stages in the process.

When a batch of eggs was fertilized, the time was noted and the eggs were transferred to the hatchery building where they were rinsed and then placed in line to go into the trough. Because the time from fertilization before they went into the heat bath was 20 minutes, it was not unusual to have 2-3 buckets of eggs awaiting the heat shock. The actual quantity of eggs that came from each fertilization event varied widely and depended on the number of ripe females available. In general a ratio of three females to one male was used for each batch of eggs. After 20 minutes fertilization, the eggs were drained of excess water, and gently poured into aluminum baskets that were approximately 30 cm x 15 cm. The baskets were raised off of the bottom of the trough by attached legs which allowed for water circulation underneath the eggs. Each basket was lined with a mosquito netting liner which facilitated the removal of eggs once the shock was over. Generally 35 oz of eggs (.14,000 eggs) were placed into each basket, which depending on the size of the egg batch, meant dividing single batches among up to three baskets.

As soon as the 20 minute heat shock was over, the mosquito netting liner was bunched up at the top, the Abag@ of eggs was removed, transferred to a large eyeing jar with hatchery water (8-9E C) running through it, and allowed to water harden. Usually, heat shocked eggs from each age class of females were pooled, treated with povidone iodine, and the total number of eggs was determined. Overall the system worked fairly well, although over time, and with the input of those running it, improvements could be made. During the course of the spawning season, staff from the state-wide hatchery system were trained on the system, so there is now a trained pool of people to run the operation in the future.

The process of scaling up the heat shock operation appears to have worked with a few minor glitches. As part of this transition to mass production we were trying to evaluate the overall success by examining percent eye-up, hatch, fry survival, and triploidy induction for each age class of eggs taken during each spawning date over the course of the season

(Table 1). The percent eyed data indicates a drop to 71% from a ten year average of 78%. The percent hatch data varied widely (range of 68-92%, average of 83%), but did not stray from the historical average at the Fisheries Experiment Station for Sand Creek rainbow trout of 84%. The variation in hatch can largely be attributed to several batches being shipped as green eggs the day of spawning. This technique generally results in a reduction in hatch. Blood samples were taken from 30 fish from each lot of fish produced and analyzed by Washington State University for triploidy. The average triploidy induction was over 99%. There was little variation in triploidy induction over the course of the spawn, but the samples from the final egg takes had decreased to 96%; still a respectable result.

Table 1. Summary data from spawning and heat shock of Sand Creek strain of rainbow trout, fall 2001.

Spawn Date	Eye-up (%)[1]	Hatch (%)[1]	Triploid (%)[1] (no. sampled)	Comments
09-04-01	47	80	100 (60)	3 yr old lot was dumped due to poor quality eggs
09-18-01	83	81[2]	100 (88)	2 of 3 lots shipped as green
10-01-01	83	85	100 (90)	
10-15-01	70	82[2]	98 (90)	large fry die-off, possibly en-cephalitis
10-29-01	68	81[2]	100 (90)	large die-off within one week of hatch
11-07-01	73	85	99 (90)	
11-19-01	70	85	96 (32)	lots pooled
Overall Mean	71	83	99	

[1] **Averaged for a given spawning day or days if spawn took two days**
[2] **Based on incomplete data**

Looking at the end result, the largest ever production of triploid rainbows in Utah was successful. Over the course of the Sand Creek spawning season almost three million eggs were processed. The eye-up was reduced, but most literature does indicate that heat shocking to induce triploidy may result in this decrease. Concerns were raised about the water quality of the shock trough, in particular the dissolved oxygen. One reading of 6 mg/L was obtained during the fall, which is safely above the critical dissolved oxygen level of 2 mg/L for the first 10 days post fertilization. Some hatchery managers have also reported a decrease in fry survival and an increase in size variation among a given lot, while others have reported better growth and lower feed conversions. As the summer progresses some of this information will be collected and evaluated to see what the complete results are. To address issues of egg survival and fish performance, a series of experiments is being planned over the next two years during which each of the three strains of rainbow trout will be evaluated in their diploid and triploid versions side by side in a hatchery. As the fish reach catchable size, they will be subjected to various stressors and their post stocking survival will be evaluated. Some literature has indicated that triploids do not handle stress or marginal water quality as well as diploids. Also, to improve survival we may experiment with administering the iodophor treatment to each batch of eggs immediately after it is removed from the water bath.

(Continued on page 6)

Identification of *Henneguya salmonicola* in Mountain Whitefish in the Logan River, Utah

Mountain whitefish *Prosopium williamsoni* from the Logan River near Logan, Utah were necropsied after a significant fish kill resulting from sediment release from First Dam. The fish were collected November 15, 2001. White cysts (plasmodia) were found in the muscle tissue of 5 of 16 fish (31%). Cysts per infected fish ranged from 1 to 12, with no tendency for left or right sides to have more or less on a side. The cysts were generally spherical, ranging in size from 2-7 mm in diameter.

The milky fluid within the cysts contained myxospores typical of *Henneguya*, i.e., featuring two convex valves with caudal processes (hereafter referred to as tails) enclosing two polar capsules and a sporoplasm. Measurements were made at 600 and1000x using a calibrated ocular micrometer on myxospores from 5 different cysts, examining 12-15 myxospores per cyst. There was little difference among the cysts, so data were pooled (*n* = 63) and presented in Table 1.

Table 1. Comparison of measurements of *Henneguya salmonicola* from the Logan River with published data. All measurements are in microns.

	***Prosopium williamsoni* in Logan R., Utah** **mean (range)**	***Oncorhynchus kisutch* in Stickeen R., Alaska (Ward 1919)** **mean (range)**	***Oncorhynchus kisutch* and *O. gorbuscha*, Puget Sound and south-east Alaska (Fish 1939)** **fresh mean / fixed mean**
Total length: mean (range)	43.1 (32-52)	(42.7-57.0)	44.7/42.0[b]
Spore length	10.8 (8.9-12.9)	12.4 (12.0-14.2) (8.4-8.6)[a] (8.6-9.5)[a]	10.4/9.3
Spore width	9.7 (7.9-11.9)	7.9 (7.1-8.4)	8.9/7.3
Spore depth	7.5 (6-9)	4.8	
tail length (longer tail)	32.2 (22-43)	34.5 (30.8-38.2) (34.2-36.8)[a]	34.3/32.7
tail length (shorter tail)	30.4 (22-43)		28.1/25.5
left polar capsule length	5.0 (4.0-5.9)	(3.7-4.6)	4.6/4.4
left polar capsule width	2.4 (2.0-3.5)	(1.6-2.8)	2.7/2.3
right polar capsule length	5.0 (4.0-5.9)	(3.7-4.6)	4.2/3.7
right polar capsule width	2.4 (1.5-3.5)	(1.6-2.8)	2.7/2.3
polar filament length	43.7 (19-65)		
number of polar filament coils	4-6		5-6

[a]additional spores were measured and the range is presented for these.
[b]The first of the two numbers is for living myxospores (*n* = 200), the second is for fixed and stained myxospores (*n* = 200), total length derived from summing means for spore length and tail length.

(Continued on page 5)

There are a number of *Henneguya* species that have been reported in salmonid muscle tissue. These include *H. salmonicola* (Ward 1919), *H. kolesnikovi* in *Coregonus* (Gurley 1893), *H. zschokkei* in *Prosopium coulteri* and *Leucociscus leucociscus* (Gurley 1893), and *H. tegidiensis* in *Coregonus clupeoides* (Nicholas and Jones 1959). Outside of Salmonidae, other muscle infecting species include *H. malapteruri* in *Malapterurus electricus* (Ali 1999), *H. travassosi* in *Astyanax fasciatus* and *Leporinus copelandi* (Jakowska and Nigrelli 1953), *H. schizurus* in *Esox lucius* eye muscle (Gurley 1894), and *H. monurus* in *Aphredoderus sayanus* (Gurley 1893). In some Myxozoa summaries (Shulman 1966; Lom and Dykova 1992), H. *kolesnikovi, H. zschokkei, H. tegidiensis* and *H. salmonicola* are considered to be synonymous. However, the original description of *H. zschokkei* by Gurley (1893) describes the tails as ›6 to 8 times longer than the body, attenuating posteriorly, curved and undulating.‹ This would translate to a tail length of 62-83 um for the body size observed in this study, which is much longer than the 32 um tail length actually measured. Also, the tails were not curved and undulating. The illustration of *H. zschokkei,* by Gurley (1894; Fig. 1) indicated that this myxospore is quite different from *H. salmonicola* described by Ward (1919; Fig. 2). For *H. kolesnikovi* and *H. monurus*, no myxospore measurements were ever given; only cyst dimensions were provided for the former (10-30 x 7-20 mm; Gurley 1893). *H. monurus* also had only one tail. The measurements reported for *H. tegidiensis* (Nicholas and Jones 1959) indicated that it closely matches those for *H. salmonicola* and those recorded in this study. The primary differences are the divergent tails and the lack of sutural folds along the margin of the spore body (see Fig 3). The divergent tail may be a function of formalin fixation and the folds are not always clear, so it is possible the two species are the same. Molecular-based research is need to better define potential species differences and similarities.

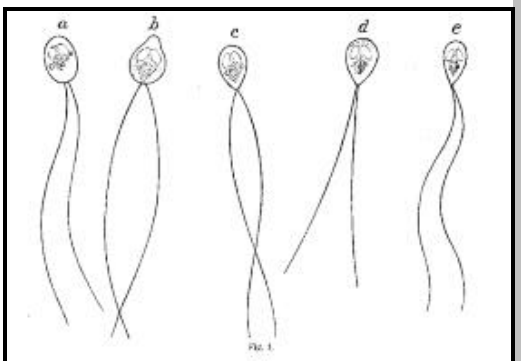

Figure 1. *Henneguya zchokkei* illustration from Gurley (1894).

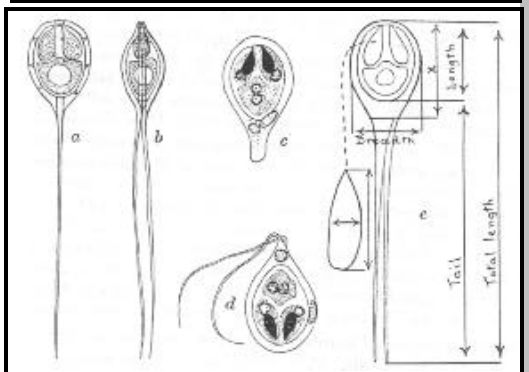

Figure 2. *Henneguya salmonicola* illustration from Ward (1919).

H. malapteruri differs from the myxospores observed by featuring larger spore bodies (14-18 um) and longer polar capsules (5-7.3 um; Ali 1999). *H. travassosi* differs by a shorter total length (26.3-28 um) and narrower width (3.8-4.8 um).

Comparison of the measurements of this study with those described for other *Henneguya* species with a tropism for muscle tissue indicated that *Henneguya salmonicola* Ward 1919 was the species observed.

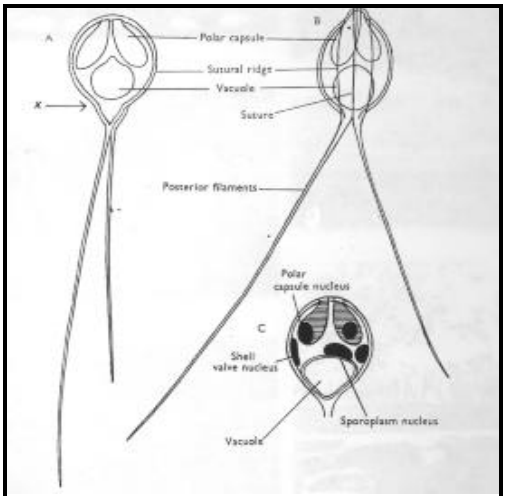

Figure 3. *Henneguya tegidiensis* illustration from Nicholas and Jones (1959).

(Continued on page 6)

(Continued from page 5)

This discovery represents the first report of *Henneguya salmonicola* in Utah. It also is the first report of this myxosporidian in mountain whitefish.

H. salmonicola has been a problem in the salmon industry of the Northwest and British Columbia, negatively impacting the quality and salability of fillets. This is due to the unsightly cysts in the flesh and the proteases released by the cysts (Patashnik and Groninger 1964). Research has indicated that salmon are infected in freshwater before seawater migration (Boyce et al. 1985). The distribution of the parasite is not well defined, but *H. salmonicola* has been reported from Alaska, British Columbia, the Kamchatka pennisula, and Washington (Ward 1919; Fish 1939; Boyce et al. 1985). It has been reported from a wide variety of salmonids including coho, chum, chinook, sockeye and pink salmon, and steelhead. It appears that mountain whitefish may now be added to the list. Further sampling of sympatric salmonids in the Logan River is needed to see if these species (brown and cutthroat trout) are also susceptible hosts.

Eric Wagner

References

Ali 1999. *Henneguya ghaffari* sp. n. (Myxozoa: Myxosporea), infecting the Nile perch *Lates niloticus* (Teleostei: Centropomidae). Diseases of Aquatic Organisms 38:225-230.

Boyce, N.P., Z. Kabata, and L. Margolis. 1985. Investigation of the distribution, detection, and biology of *Henneguya salmonicola* (Protozoa, Myxozoa), a parasite of the flesh of Pacific salmon. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1405.

Fish, F.F. 1939. Observations on *Henneguya salmonicola* Ward, a myxosporidian parasitic in Pacific salmon. Journal of Parasitology 25:169-172.

Gurley, R.R. 1893. On the classification of the Myxosporidia, a group of protozoan parasites infesting fishes. Bulletin of the U.S. Fisheries Commission 11:407-420.

Gurley, R.R. 1894. The Myxosporidia, or psorosperms of fishes, and the epidemics produced by them. Bulletin of the U.S. Fisheries Commission 26:65-304.

Jakowska, S.and R.F. Nigrelli. 1953. The pathology of myxosporidiosis in the electric eel, *Electrophorus electricus* (Linnaeus), caused by *Henneguya visceralis* and *H electrica* spp. nov. Zoologica 38:183-191.

Lom, J., and I. Dyková. 1992. Protozoan parasites of fishes. Elsevier, Amsterdam.

Nicholas, W.L., and J.W. Jones. 1959. *Henneguya tegidiensis* sp. nov. (Myxosporidia), from the freshwater fish *Coregonus clupeoides pennantii* (the gwyniad). Parasitology 49:1-5.

Patashnik, M., and H.S. Groninger, Jr. 1964. Observations on the milky condition in some Pacific Coast fishes. Journal of the Fisheries Research Board of Canada 21:335-346.

Shul=man, S.S. 1966. Myxosporidia of the U.S.S.R. Nauka Publishers, Moscow-Leningrad. Translation from Russian by S. Sharma for the U.S. Dept. Interior and Nat. Sci. Found. 1988.

Ward, H.B. 1919. Notes on North American Myxosporidia. Journal of Parasitology 6:49-64.

(Continued from page 3)

There were some negative aspects associated with switching to all triploid production. An increase in manpower and a slight decrease in egg production make the eggs more expensive to produce. However, the benefits allow for a wide array of management decisions. The driving goal of this project has been to provide a sterile rainbow trout for increasing management options at such places as Strawberry Reservoir. Those options are now a reality. The Egan Hatchery, with help from other hatchery staff, has demonstrated they can produce a heat-shocked rainbow that is 99% triploid. These fish allow for the continued successful management of native cutthroat populations, while at the same time, managing for state-wide rainbow trout fishing opportunities.

Ronney Arndt

New Home for June suckers

The June suckers at the Fisheries Experiment Station (FES) were moved to the new facility in January. The new and previous facilities are now on warmer water, 64-66^0 F compared to the previous temperature of 60^0 F. Nine lots have been transferred to date. One lot is being used in a feed study, which will run into May (see photo 1). There are five treatments with three replicates each. The feeds that are being compared include: Bio Kyowa (previous diet), Bozeman Fish Technology Center Razorback Formula (present diet), Silver Cup trout diet, Bio Oregon Bio Vita diet and Bio Oregon Bio Flake diet.

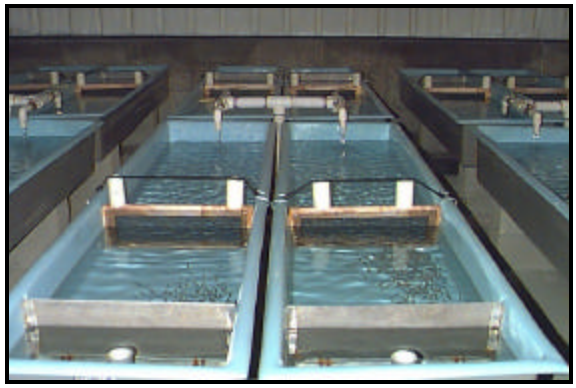

Photo 1. Feed study troughs in the new facility

Preparations for the 2002 fish lots from the Provo River and brood stock at the FES have begun. Tanks and equipment are being installed and the final plans are being made for the upcoming studies. The first will be an induced spawning study using human chorionic gonadotropin (HCG). The next will be a second feed study using fish received in late spring of 2002. The culturing of brine shrimp and rotifers began in March to refine techniques before the feed study begins (see photo 2).

Eriek Hansen

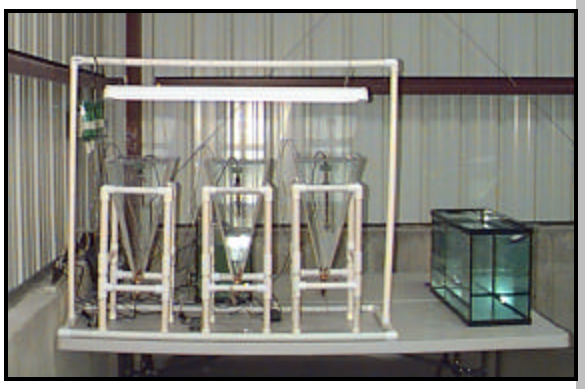

Photo 2. Brine shrimp hatchers in the new facility

Newest Faces at FES

We're proud to announce the latest additions to the staff at FES.

Wildlife biologist II **Mary George** earned her B.S. degree in biology and chemistry from Southern Alabama university. She comes most recently from New Mexico and has previously worked at the Aquatic Animal Health Lab in Colorado. She brings a broad experience in laboratory techniques to the fish health laboratory.

Michael Colvin is working as a wildlife technician in the fish disease lab. He is a native of New York and earned his B.S. degree in aquaculture at Unity College in Maine. He has worked previously for Idaho Fish & Game, evaluating performance of hatchery trout.

New biologist Mary George (center) keeps a wary eye on wildlife technicians Mike Colvin (l) and David Latremouille (r)

David Latremouille is a native of Halifax, Nova Scotia and earned degrees in biology and fisheries at both New Brunswick and New York. He is working as a wildlife technician in the research program for Eric Wagner. Welcome to all these newcomers!

The Ichthyogram is a quarterly publication of the Fisheries Experiment Station, Utah Division of Wildlife Resources, Logan Utah 84321.
Editor:
Chris Wilson (nrdwr.cwilson@state.ut.us)
Contributors for this issue:
Ronney Arndt (nrdwr.rarndt@state.ut.us)
Eric Wagner (nrdwr.ewagner@state.ut.us)
Eriek Hansen (nrdwr.ehansen@state.ut.us)
This newsletter is distributed electronically at http://www.udwrfes.org/ichthyog.htm .

Send comments or change of address to:
EDITOR, The Ichthyogram
1465 West 200 North, Logan, UT 84321

The Ichthyogram

Fisheries Experiment Station
1465 West 200 North
Logan, UT 84321
http://www.udwrfes.org/