Pressure Shock Induction of Triploid Rainbow Trout

November 2007

[1]Ryan Couture
[2]Tim Schamber
[2]Anitra Firmenich
[3]Craig Banner

Oregon Department of Fish and Wildlife (ODFW)

[1]Oregon Hatchery Research Center 2418 East Fall Creek Road, Alsea, OR 97324 (541)487-5510 ryan.b.couture@state.or.us
[2]Roaring River Fish Hatchery 42279 Fish Hatchery Road, Scio, OR 97374 (503)394-2496 roaringriver.hatchery@state.or.us
[3]ODFW Fish Health Lab Oregon State University Fisheries & Wildlife 516 Nash Hall, Corvallis, OR 97331 (541)737-1857 bannerc@onid.orst.edu

Background

The use of sexually sterile fish has many applications in fish culture and fish management. Use of sterile fish in hatchery programs could potentially minimize genetic interactions with native stocks. A need for sexually sterile fish has prompted research in the production of triploid fish. Triploid fish are produced through a number of techniques including chemical, thermal or mechanical methods. Both the thermal and mechanical methods are commonly used in production facilities. In Oregon, thermal (heat) shock is the current standard for production of triploid Rainbow Trout and has been used at a production level since 2002. Although the process is viable, the triploid rate is variable and the survival is often poor. With a goal to produce groups of fish approaching 100% triploid induction rate the use of mechanical (pressure) shock was investigated. Pressure shocking is typically done in a hydrostatic pressure chamber and various projects have reported close to 100% sterilization rate.

In 2007, The Oregon Department of Fish and Wildlife (ODFW) purchased a 2.7-liter capacity portable pressure chamber for inducing triploidy by pressure shocking. This device was tested on rainbow trout eggs at Oak Springs Hatchery and brook trout eggs at Wizard Falls Hatchery. Production number of triploid rainbow trout were being produced at Roaring River Hatchery using propane-fueled on-demand water heaters to heat shock eggs so that hatchery was chosen to compare the pressure and heat shock methods. The rate of triploid induction is determined by ODFW Fish Health Services staff using flow cytometry. The method measures the amount of DNA present in blood cells. Blood cells from a triploid fish will have 50% more DNA than those from a diploid fish due to the presence of the third set of chromosomes. In 2007 nearly 11 million triploid rainbow trout eggs were produced. (2007 Fish Prop annual report)

Methods

A 2.7 liter hydrostatic pressure chamber, manufactured by TRC Hydraulics Inc, New Brunswick, Canada, was used to produce triploid Rainbow trout via mechanical pressure shock. Eggs from the 072 Cape Cod stock of rainbow trout at Roaring River Hatchery were used in the experiment. Eggs were collected from 3 year old fish on November 14, 2007. All of the test groups were generated from one egg take, gametes were pooled (to minimize variation between groups) and a sample of gametes from the pool was used in each of the treatments described in table 1. Control groups were taken from the same pool of gametes. Controls included one standard diploid group and two handling controls for both the “pressure test” and the “duration of shock” parts of the experiment. We placed one control at the start and one at the end of each phase of the experiment to control for time between induction of each group (i.e. groups experienced differing lengths of time between egg take and fertilization). This time varied from 15-90 minutes.

Pooled unfertilized eggs and milt from females and males were transferred separately to the hatch-house. A pooled batch of 15 females (approx. 75,000 eggs, 5,000eggs/female) was combined for the “pressure test” part of the experiment. A pooled batch of 10 females (approx. 50,000 eggs, 5,000eggs/female) was used for the “duration of shock” part of the experiment. The batches were split into groups of 10,000 eggs for each of the test groups and fertilized with the pooled milt. Once the eggs were fertilized, water was added and the time recorded. The eggs were exposed to pressure at 300 Time Temperature Units (TTU’s), approximately 27 minutes after fertilization. The time was

calculated by dividing 300 by the actual temperature of the water in degrees Celsius. The ambient water temperature was 11.11 celcius (52 F). Each group was individually incubated, each in one tray of a vertical flow through incubator.

For the "pressure test" experiment, the treatment started at 300 TTU's. Pressure was held for five minutes in the chamber. It took thirty seconds for the chamber to reach final pressure for all groups.

For the "duration of shock" experiment, the treatment started at 300 TTU's. A constant pressure of 9,500 psi was applied to each of the test groups ranging in duration from 3-6 minutes. It took thirty seconds for the chamber to reach final pressure for all groups.

Each of the "Handling Control" groups was handled in the same manner as the respective test groups. They had the same 300 TTU waiting period and placed in the chamber for 5 minutes without pressure applied.

At the end of this project, all healthy surviving fish were included in the hatchery production at Roaring River Hatchery.

Table 1. All Test Groups

TEST GROUPS	NAME	TIME	SAMPLE SIZE	DURATION
#1—Control	Standard Control-Diploid	n/a	10,000	n/a
#2—Pressure Test	Handling Control	300 TTU's	10,000	5 min.
#3—Pressure Test	10,000psi	300 TTU's	10,000	5 min.
#4—Pressure Test	9,500psi	300 TTU's	10,000	5 min.
#5—Pressure Test	9,000psi	300 TTU's	10,000	5 min.
#6—Pressure Test	8,500psi	300 TTU's	10,000	5 min.
#7—Pressure Test	8,000psi	300 TTU's	10,000	5 min.
#8—Pressure Test	Handling Control	300 TTU's	10,000	5 min.
#9—Duration Test	Handling Control	300 TTU's	10,000	5 min.
#10—Duration Test	6 minute duration	300 TTU's	10,000	6 min.
#11—Duration Test	5 minute duration	300 TTU's	10,000	5 min.
#12—Duration Test	4 minute duration	300 TTU's	10,000	4 min.
#13—Duration Test	3 minute duration	300 TTU's	10,000	3 min.
#14—Duration Test	Handling Control	300 TTU's	10,000	5 min.
		TOTAL	**140,000**	

Analysis

Triploid Induction Rate Sampling

Each group was held separately in trays as eggs and baskets as fry at Roaring River Hatchery and cared for by the hatchery crew. When the fry were large enough (June 2008), blood samples were taken and flow cytometric measurement of erythrocyte DNA content was conducted on 120 fish per group to determine the induction rate of each group. The cost of the reagent for this test was funded by OHRC, and the analysis performed in the ODFW Fish Health Lab by ODFW Fish Health Specialist Craig Banner. Sampling was conducted on 120 fry from each of the nine treated test groups for a total

of 1080 samples. The induction rate of each of the nine treated groups is represented in Table 5. The process seemed to be most successful (i.e. 100% induction) in the 10,000psi, 9,500psi and 6 minute duration groups. Five minutes was the standard time for the "pressure test" part of the experiment and will be used as a constant in the future.

Survival Rate Analysis

Survival was recorded at the eyed (Table 2.) and swim-up stage (Table 3.) for each of the test groups and controls. The combined loss for each group can be found in Table 4. The loss shown at each stage is actual loss, not loss in comparison to the diploid or control groups. The average total loss for the diploid and control groups varied between 22-31%, while the total loss for the 10,000 and 9,500psi groups varied from 41-64%. This shows roughly an average increase in loss of approximately 19-33% from the pressure induction process.

Results

Knowledge regarding optimal pressure and duration of shock to minimize egg and fry mortality will be useful for the implementation of pressure shocking at a production level. Ensuring a high degree of triploid induction to minimize impacts on wild stocks is also very important. This will aid in the development of new techniques and technologies to assist in fish culture advances toward conservation and recovery. These goals are consistent with the Native Fish Conservation Policy and the Fish Hatchery Management Policy.

This project was very useful as a preliminary study to understand what range of pressure treatments and range of exposure times produce high levels of triploid induction. The data reinforces existing literature regarding pressure shock induction of triploidy in Rainbow Trout and provides direction for a more focused and intensive investigation for the following year (2008-'09). For the following spawning season we plan to investigate the increase of the maximum pressure group from 10,000psi to 10,500psi. Five minutes was the standard time for the "pressure test" part of the experiment and will be used as a constant in the future. We would also like to investigate extending the time between fertilization and shock. We would compare the relative induction rates and mortality between groups in Time Temperature Units (TTU's) of 300 and 375TTU's. There is evidence from a study done by Alaska Department of Fish and Wildlife that this increase in TTU's (i.e. increase in time between fertilization and pressure shock induction) may provide a more consistent induction rate of 100% as well as a decrease in associated mortality. The work in Alaska has shown 100% survival at 375 TTU's in comparison to the diploid/control groups. To gain confidence in our findings, we plan to have three replications of each test group for the following years study.

Table 2. Egg Loss

TEST GROUP	LIVE EGGS	EGG LOSS	PERCENT EGG LOSS
#1—Control	8,410	2,102	19.996%
#2—Handling Control	9,339	2,227	19.25%
#3—10,000psi	8,151	3,676	31.08%
#4—9,500psi	5,675	7,221	55.99%
#5—9,000psi	7,689	5,336	40.97%
#6—8,500psi	8,631	6,320	42.27%
#7—8,000psi	7,151	4,692	39.62%
#8—Handling Control	9,700	3,803	28.16%
#9—Handling Control	8,122	3,248	28.57%
#10—6 minute duration	7,015	4,534	39.26%
#11—5 minute duration	6,488	2,689	29.3%
#12—4 minute duration	6,694	4,563	40.53%
#13—3 minute duration	5,178	6,334	55.02%`
#14— Handling Control	8,069	2,638	24.64%

Table 3. Fry Loss

TEST GROUP	FRY LOSS	PERCENT FRY LOSS
#1—Control	285	3.89%
#2—Handling Control	352	3.77%
#3—10,000psi	1120	13.74%
#4—9,500psi	988	17.41%
#5—9,000psi	971	12.64%
#6—8,500psi	1574	18.24%
#7—8,000psi	1352	18.91%
#8—Handling Control	522	5.38%
#9—Handling Control	344	4.24%
#10—6 minute duration	598	8.52%
#11—5 minute duration	661	10.19%
#12—4 minute duration	1125	16.81%
#13—3 minute duration	918	17.73%
#14— Handling Control	232	2.88%

Table 4. Combined Loss (Egg and Fry)

TEST GROUP	PERCENT EGG LOSS	PERCENT FRY LOSS	TOTAL LOSS
#1—Control	19.996%	3.89%	22.70%
#2—Handling Control	19.25%	3.77%	22.29%
#3—10,000psi	31.08%	13.74%	40.55%
#4—9,500psi	55.99%	17.41%	63.66%
#5—9,000psi	40.97%	12.64%	48.42%
#6—8,500psi	42.27%	18.24%	52.79%
#7—8,000psi	39.62%	18.91%	51.03%
#8—Handling Control	28.16%	5.38%	32.03%
#9—Handling Control	28.57%	4.24%	31.59%
#10—6 minute duration	39.26%	8.52%	44.43%
#11—5 minute duration	29.3%	10.19%	36.50%
#12—4 minute duration	40.53%	16.81%	50.52%
#13—3 minute duration	55.02%`	17.73%	62.99%
#14— Handling Control	24.64%	2.88%	26.80%

Table 5. Triploidy Induction Rates

TEST GROUP	PECENT TRIPLOIDY INDUCTION
#1—Control	n/a
#2—Handling Control	n/a
#3—10,000psi	(100%) 118/118
#4—9,500psi	(100%) 120/120
#5—9,000psi	(98.3%) 118/120
#6—8,500psi	(94.2%) 113/120
#7—8,000psi	(74.6%) 88/118
#8—Handling Control	n/a
#9—Handling Control	n/a
#10—6 minute duration	(100%) 120/120
#11—5 minute duration	(97.5%) 117/120
#12—4 minute duration	(99.2%) 119/120
#13—3 minute duration	(95%) 114/120
#14— Handling Control	n/a