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[Article]

Production of Triploid Lake Trout by Means of Pressure Treatment

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Abstract.—To induce triploidy in lake trout *Salvelinus namaycush*, we treated a production-sized group at Story State Fish Hatchery, Wyoming, with 65,500 kPa at 300 degree-minutes (°C·min) for 5 min and investigated four different pressure treatments (62,053 kPa at 300°C·min for 5 min; 65,500 kPa at 200, 300, or 400°C·min for 5 min) at Saratoga National Fish Hatchery, Wyoming. For the production group, eggs hatched and survived at a higher rate when held in chilled water (10.6°C) than ambient water (11.5°C). For the experimental groups, mean survival of pressure-treated groups to the eyed egg, hatching, and feeding fry stages was at least 15% lower than that of the control group. However, due to high variability, there was no statistical difference in survival among the experimental groups. All fry (n = 178) sampled from the four experimental treatments and the single production group were triploid.

Introductions of lake trout *Salvelinus namaycush* throughout the western United States have led to reduction or elimination of native or recreationally important species (Ruzycki et al. 2003). However, introduced lake trout also support high-yield or trophy fisheries (Martinez and Wiltzius 1995), making them popular with recreational anglers. To maintain harvest rates, fisheries may be supplemented with hatchery-produced fish. Due to their long life span (Donald and Alger 1986) and highly piscivorous feeding behavior (Eby et al. 1995), introduced lake trout populations may be difficult to manage (Johnson and Martinez 2000). The use of sterile lake trout in hatchery-supported fisheries may allow better control of predator population densities and predator–prey ratios, thereby improving management of this species.

Techniques for inducing triploidy (i.e., functional sterility) in salmonids have been widely studied, especially in *Oncorhynchus* spp. and *Salmo* spp. (Ihssen et al. 1990; Benfey 1999). Much of this research was undertaken to improve the performance of salmonids for commercial aquaculture. Triploidy also provides advantages for recreational fisheries management in that normal gonadal maturation is disrupted, and therefore triploid salmonids are unable to reproduce (Thorgaard 1983). This reduces the potential for hybridization between wild and stocked fish and allows hatchery-supported populations to be more easily maintained at desired densities.

Few studies have been conducted on techniques to induce triploidy in char *Salvelinus* spp., and we are aware of no studies conducted to induce triploidy in lake trout. For brook trout *Salvelinus fontinalis*, pressure treatments of 62,053 kPa were most successful (100% triploidy) when applied at 200 degree-minutes after fertilization (°C·min) for 5 min (Benfey et al. 1997). For Arctic char *Salvelinus alpinus*, 65,500-kPa pressure treatments at 225°C·min and 300°C·min yielded 100% triploidy with ''excellent'' survival (Keefe and Benfey 1995). Gillet et al. (2001) produced 100% triploidy in Arctic char by means of a 65,500-kPa treatment at 320°C·min for 5 min, and survival was approximately 90% that of controls. Additionally, thermal treatments have been used to induce triploidy in brook trout (Dube et al. 1991; Galbreath and Samples 2000); however, during two of our pilot studies, survival of thermally treated lake trout eggs

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was less than 10%. Using these published pressure treatments as a starting point, we sought to develop a triploidy induction technique that (1) provided high induction rates in lake trout and (2) provided adequate survival to the eyed egg, hatching, and feeding fry stages relative to that of untreated eggs.

Methods

Large-scale production of triploid lake trout.—We pressure-treated a production-sized group of lake trout eggs (approximately 45,000 eggs) at the Wyoming Game and Fish Department's Story State Fish Hatchery (SFH), Story, on October 8, 2003. Eggs from four females were pooled and combined with the pooled milt from four males. Milt was collected in a plastic bag and was later poured over the eggs. Eggs were rinsed for 2–3 min after freshwater was used to initiate fertilization. Eggs remained in well water in plastic spawning bowls until just before introduction into a hydraulic pressure chamber (model HPC; TRC Hydraulics, Inc., Dieppe, New Brunswick, Canada). The pressure chamber was filled with ambient hatchery water before egg treatment. A shock of 65,500 kPa at 300°C·min was applied for 5 min. The entire process was repeated three times to attain treatment of all eggs. In addition, two females were spawned with two males, and the eggs were left untreated for use in survival rate comparisons. The treated eggs were combined into one upweller incubation jar until eye-up, while the untreated group was eyed in Heath-type incubation trays. Eggs were incubated at 7.5°C. Percent eye-up was determined volumetrically after removing dead eggs with a Jensorter JH fish egg sorter (Jensorter, LLC, Bend, Oregon).

After the initial sorting to remove dead eggs, surviving eyed eggs were transported to the Idaho Department of Fish and Game's Grace Fish Hatchery, Grace. The pressure-treated and untreated groups were split in half, placed in Heath-type incubation trays, and stored in adjacent vertical flow-through incubation stacks. One stack was supplied with chilled water (10.6°C), whereas the other was supplied with ambient-temperature water (11.5°C). For each temperature treatment, the pressure-treated eggs were split equally into eight Heath-type incubation trays, whereas untreated eggs were split equally into two trays. Chilled water was provided by a 8,018-kJ (7,600 British thermal units) in-line chiller (Pro-Cool model; Area, Inc., Homestead, Florida). A StowAway temperature logger (Onset Computer Corp., Bourne, Massachusetts) was placed in the top tray of each stack to monitor temperature until fry were transferred to indoor raceways. Dead eggs and fry were removed by hand and counted on a daily basis.

Experimental pressure treatment.—We conducted experiments in cooperation with the U.S. Fish and Wildlife Service at Saratoga National Fish Hatchery (NFH), Saratoga, Wyoming, on October 28, 2003. Four 8-year-old females and four males were stripped and spawned in the same manner as described in the previous section. Approximately equal numbers of fertilized eggs were split five ways (four treatment groups and one control). We tested two different pressure levels and three post-fertilization times: (1) 62,053 kPa (9,000 pounds per square inch gauge [psig]) at 300°Cmin, (2) 65,500 kPa (9,500 psig) at 200°C·min, (3) 65,500 kPa at 300°C·min, (4) 65,500 kPa at 400°C·min, and (5) a control group at ambient temperature (9.3°C), which was handled in the same manner as pressure-treated eggs. Treatment durations were standardized at 5 min (Lincoln 1989). To prevent time conflicts, the fertilization time for the third treatment was delayed by approximately 10 min. After removal from the pressure chamber, eggs were placed in Heath incubation trays, which were placed in vertical flow-through stacks in random order. The process was replicated three times with different groups of eggs. Eggs and fry were handled and enumerated in the same manner as described in the previous section except that at hatch, fry were transferred to 15 individual rearing containers. Each circular fiberglass container (2.5 L) and was supplied with freshwater at a rate of 0.5–1.0 L/min. Fry remained in the containers until blood was collected for ploidy testing.

Ploidy testing.—At 75 d posthatch, a random sample of 30 fry was collected from the production-sized group at Story SFH. Additionally, samples of 15 fry from each of the 12 pressure-treated groups and 5 fry from each of the three control groups were anesthetized in a lethal dose of tricaine methanesulfonate (MS-222). Blood samples were collected by severing the caudal peduncle and placing the posterior end of each fry in a 1.5-mL microcentrifuge tube filled with Alsever's anticoagulant solution (Alsever and Ainslie 1941). The DNA content for samples from each treatment–replicate combination was used as an index of ploidy and was determined with flow cytometry at Washington State University (Thorgaard et al. 1982).

Statistical analysis.—A replicated comparison of the effect of chilled and ambient water on survival of production eggs was not possible due to

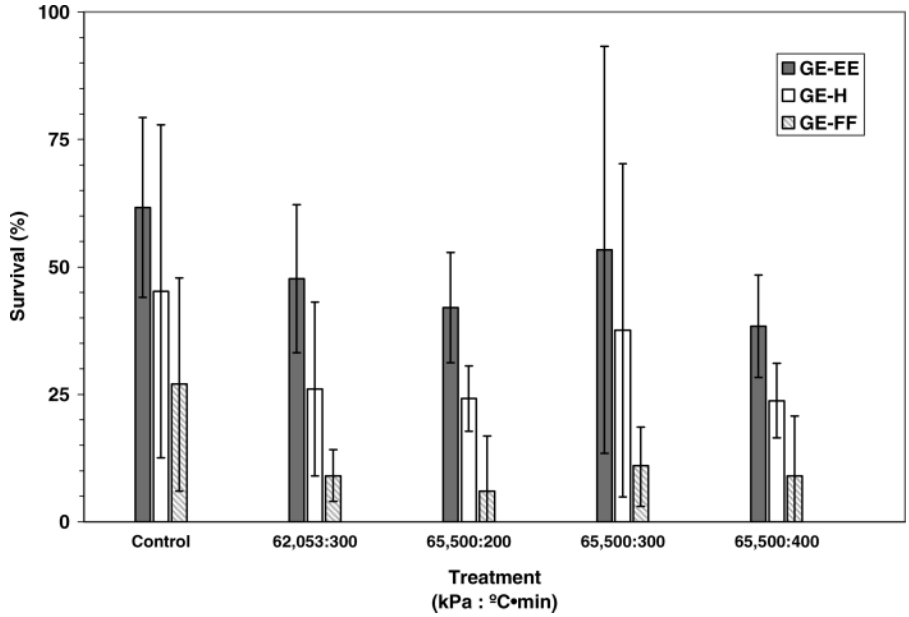

FIGURE 1.—Survival of green lake trout eggs (GE) to the eyed egg (EE), hatching (H), and feeding fry (FF) stages in experimental treatments evaluating the effects of two gauge pressures (62,053 or 65,500 kPa for 5 min) and three different postfertilization application times (degree-minutes [°C·min]). Error bars represent 95% confidence intervals.

pseudoreplication (Hurlbert 1984). Nonetheless, for comparative purposes, we calculated mean survival values and 95% confidence intervals (CIs) for the eight trays within the chilled and ambient stacks (Brown and Austen 1996). For the experiments, survival data were arcsine transformed prior to analysis. A one-way analysis of variance (ANOVA) was used to compare survival rates among experimental treatments (SPSS 1993). The significance level (α) was set at 0.05. Tukey's least-significant-difference test was used for subsequent mean comparisons.

Results

For large-scale production efforts at Story SFH, overall survival for treated and untreated eggs was high. For eggs treated with 65,500 kPa at 300°C·min, survival from green eggs to eyed eggs was 97%. No mortality to eye-up was seen in untreated eggs. After treated eggs were split into two groups, the mean survival of treated eggs was higher when hatched on chilled water than ambient water (i.e., 95% CIs did not overlap). Treated eggs held in chilled water exhibited 80% ± 1% (mean ± 95% CI) survival to hatch and 67% ± 2% survival to the feeding fry stage, whereas treated eggs held in ambient water had 77% ± 1% survival to hatch and 60% ± 3% survival to the feeding fry stage. These differences represent a 4% increase in survival to the hatching stage and a 12% increase in survival to the feeding fry stage by the use of chilled water. Mean survival to the hatching and feeding fry stages was 98% and 96%, respectively, for untreated eggs held in chilled water. Untreated eggs held in ambient water exhibited 96% survival to hatch and 91% survival to the feeding fry stage.

For the experimental treatments, the highest mean survival to eye-up (53%), hatch (38%), and the feeding fry stage (11%) was provided by a pressure treatment of 65,500 kPa at 300°C·min (Figure 1). However, due to the high degree of variability within treatments, 95% CIs were wide (26–87% of the means for the eyed egg and hatching stages) and overlapped for all treatments. There was no statistical difference in survival to eye-up or hatch among the pressure treatments tested (one-way ANOVA, $P > 0.166$).

Ploidy was analyzed for a total of 209 fish from the experimental and production groups. All pressure-treated fish were found to be triploid. For the production group from Story SFH, 29 samples were triploid (one sample was unreadable). For the 12 pressure-treated experimental groups, 149 out of the 165 samples were found to be triploid (16

samples were unreadable). For the control group, 13 of the 15 samples were classified as diploid (two samples were unreadable).

Discussion

In this study, pressure treatments of 62,053 and 65,500 kPa proved to be effective at producing large numbers of triploid lake trout. All pressure-treated fish from both brood sources were triploid. The minimum pressure level needed to attain 100% triploidy was not found and may be lower than the lowest level we tested (62,053 kPa). Pressure levels used in this study were consistent with those that successfully induced triploidy in other *Salvelinus* species (Benfey et al. 1997; Gillet et al. 2001).

Survival of treated and untreated eggs from the Story SFH production group was increased by the use of a lower hatching temperature. Due to space and monetary constraints, we were unable to replicate the survival rate comparison for eggs hatched on ambient (11.5°C) versus chilled water (10.6°C). However, for all 10 paired trays (eight treated, two untreated), survival rates were higher for eggs hatched on chilled water than on ambient water. The chilled water used in our study was still warmer than the temperature normally used for rearing lake trout. Ostergaard (1987) reported that survival of lake trout to the posthatch stage was threefold higher for fish reared on chilled water (5.3°C) than for those reared on ambient water (9.3°C). Dwyer (1987), however, reported no statistical difference in survival among groups of eggs reared at 1.8, 6.4, and 9.8°C.

We were unable to find a statistically significant difference in survival among experimental treatments tested at Saratoga NFH. This may be due in part to the variable and high rates of mortality seen in the experimental groups. Although dead eggs were removed daily, fungus from dead eggs was difficult to control in some Heath trays and may have confounded treatment effects by spreading to and killing live eggs. Since survival to the hatching stage was six- to sevenfold higher for the production group from Story SFH and fungus or disease outbreaks would be less likely at that facility, additional testing should be conducted there to determine the minimum pressure threshold that provides near 100% induction while maximizing survival.

Based on these results and without additional testing, it is apparent that large numbers of triploid lake trout may be produced with adequate survival and high triploidy induction rates even in hatcheries with less-than-ideal hatching temperatures. Assuming that the loading and unloading of the pressure chamber require 1 min each, approximately 25,000 eggs could be treated at about 7-min intervals. At this rate, several hundred thousand eggs could be treated daily with one unit, and only slight modifications to normal spawning procedures would be needed. This could allow replacement of diploid lake trout stocking with triploid stocking in areas where reproduction by stocked lake trout or spawning of wild and hatchery-produced lake trout is undesirable. However, no information is available on the performance of triploid lake trout in recreational fisheries. In order to assure that triploid lake trout provide similar fisheries, a detailed examination of poststocking performance is needed.

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