



PROJECT 4: HATCHERY TROUT EVALUATIONS

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Senior Fisheries Research Biologist**

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Project 4: Hatchery Trout Evaluations

Subproject 2: Sterile Trout Investigations

Subproject 3: Predator Training

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**ANNUAL PERFORMANCE REPORT
SUBPROJECT #2: STERILE TROUT EVALUATIONS**

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ABSTRACT

Increased growth, improved survival, and genetic protection of wild stocks have been suggested as benefits of stocking triploid (i.e., sterile) fish. I examined the relative growth and survival of triploid and diploid rainbow trout *Oncorhynchus mykiss* stocked in four high mountain lakes during 1999. During 2003, 31 test fish were sampled from Crystal, Golden, and Maki lakes. No test fish were sampled from Snowslide Lake. Of these 31 fish, 21 diploids and 10 triploids were identified. Over the three years of sampling, 2001-2003, 107 test fish were sampled. For all lakes and years combined, the catch of diploids far exceeded that of triploids. Overall, 32 triploid and 75 diploid fish were caught (i.e., 2.3 times more diploids were caught). There was no statistical difference in length or weight between the test groups. To gain a better understanding of the performance of triploid fish in high mountain lakes, 15 additional lakes were stocked with mixed-sex diploid, mixed-sex triploid, and all-female triploid rainbow trout. This is in addition to the 16 lakes stocked in 2003. These 31 lakes will be sampled from 2004-2007.

Methods for producing triploid brook trout *Salvelinus fontinalis*, rainbow X Yellowstone cutthroat trout hybrids *O. mykiss* X *O. clarki bouvieri*, lake trout *S. namaycush*, and westslope cutthroat *O. clarki lewisi* were investigated during 2003. Survival to eye-up and to feeding fry for brook trout was 6% and 13% higher for pressure treated eggs than for heat treated eggs. Based on higher survival and 100% induction rates, the 9,500 psi treatment applied at 40 minutes after fertilization (MAF) for 5 minutes was most efficient. Survival and induction rates for pressure treated Henrys Lake hybrid eggs were also higher and more consistent than for heat treated eggs. The highest survival to eye-up ($62 \pm 12\%$) was provided by a 10,000 psi treatment at 46.7 MAF for 5 minutes and exceeded that of controls by 2%. Due to this relatively high survival rate and 100% induction rates, a 10,000 psi pressure treatment at 46.7 MAF for 5 minutes was the most efficient. Overall survival of lake trout from Story Fish Hatchery was higher than from Saratoga or Egan fish hatcheries over the previous two years. Survival to eye-up, hatch, and feeding fry for the pressure-treated production group from Story was 97, 79, and 68%, respectively. All blood samples ($n = 29$) from the Story production group were classified as triploid. These eggs were pressure treated with 9,500 psi at 40 MAF for 5 minutes. Survival for treated and untreated eggs from Saratoga was over 30% lower. Survival and induction rates for heat treated westslope cutthroat trout were low, 15% and 91%, respectively. Additional research is needed to identify an efficient treatment for westslope cutthroat trout.

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INTRODUCTION

Triploid salmonids are functionally sterile, and the common assertion is that sterility provides a fisheries or aquaculture benefit (Benfey 1999). Triploid salmonids produced by temperature or pressure shock may suffer lower fertilization rates, increased mortality, or reduced growth from egg through initiation of feeding (Solar et al. 1984; Happe et al. 1988; Guo et al. 1990; Oliva-Teles and Kaushik 1990; Galbreath et al. 1994; McCarthy et al. 1996). Despite these early rearing disadvantages, triploid performance appears to improve with age. Several investigators reported enhanced hatchery performance in terms of growth and food conversion for age-1 and older triploids (Lincoln and Scott 1984; Bye and Lincoln 1986; Boulanger 1991; Habicht et al. 1994; Sheehan et al. 1999).

Unlike the breadth of previous work reported for triploid salmonids in an aquacultural setting, published literature on the performance of triploid salmonids in natural environments is sparse. Brock et al. (1994) and Simon et al. (1993) reported lower growth and survival for triploid rainbow trout *Oncorhynchus mykiss* compared to diploid controls. In contrast, triploid brook trout *Salvelinus fontinalis* and kokanee *O. nerka* demonstrated the potential for increased longevity in lake habitats (Parkinson and Tsumura 1988; Warrillow et al. 1997). Dillon et al. (2000) reported that stocking of mixed-sex triploid rainbow trout in 16 Idaho streams did not reduce return to creel for anglers compared to mixed-sex diploid fish. Lastly, Cotter et al. (2000) argued that stocking triploid Atlantic salmon *Salmo salar* reduced genetic impacts to wild populations, because fewer triploid fish returned to spawning habitats. These studies provide some background for evaluating the performance of triploid salmonids in natural environments. However, their limited scope, lack of replication, and contradicting results fail to fully address the performance of triploid salmonids stocked to benefit anglers.

The genetic conservation of wild populations is a management priority for the Idaho Department of Fish and Game (IDFG). The IDFG recently established a policy to stock only triploid rainbow trout in systems where reproduction between wild and hatchery fish was possible (IDFG 2001). Implementation of the above-noted policy has resulted in the widespread stocking of sterile rainbow trout in hundreds of Idaho high mountain lakes. In these lakes, temperature and oxygen levels may be low for much of the year, and it has been suggested that sterile fish may not perform well under these conditions (J. Johnston, Washington Department of Fish & Wildlife, personal communication). It is important to determine if stocking of triploid rainbow trout produces satisfactory fisheries in Idaho high mountain lakes. If not, fisheries managers may need to adjust stocking strategies rather than rely on historical stocking levels, as is currently being done.

Induced sterility in other hatchery-produced salmonids including rainbow X Yellowstone cutthroat trout hybrids *O. mykiss* X *O. clarki bouvieri*, westslope cutthroat trout *O. clarki lewisi*, as well as lake trout *Salvelinus namaycush* and brook trout could further reduce impacts of stocking on Idaho's native and wild fish populations. However, sterilization techniques for these species have yet to be developed in IDFG hatcheries. If efficient methods are developed, sterility may provide a method for controlling lake trout density and reduce competition with or predation on cutthroat trout (Kaeding et al. 1996). Another potential benefit of sterility is increased longevity through elimination of normal gonadal development and associated spawning mortality (Ihssen et al. 1990). As mentioned previously, sterility also provides a level of genetic protection for wild stocks. This would be desirable for the management of Henrys Lake, as diploid hybrids have the potential to hybridize with the lake's native stock of Yellowstone cutthroat trout *O. clarki bouvieri* (Rohrer and Thorgaard 1986).

In this progress report, I compare survival and growth of triploid and diploid rainbow trout that were stocked as part of a pilot study in four central Idaho high mountain lakes during 1999. I also document the stocking of an additional 15 high mountain lakes during 2003. These lakes, combined with the 16 lakes stocked during 2001, will allow for a broader scale and more robust evaluation of triploid performance by including larger lakes and increasing the sample size. Furthermore, during this portion of the project an all-female triploid group was stocked along with mixed-sex groups to allow comparison of different options available to fisheries managers. In addition, I summarize efforts to produce triploid brook trout, rainbow X Yellowstone cutthroat trout hybrids, lake trout, and westslope cutthroat trout.

RESEARCH GOAL

1. To enhance hatchery-supported fisheries while reducing genetic risks to indigenous rainbow and cutthroat trout.

OBJECTIVES

1. To increase survival of rainbow trout in high mountain lakes by 25% by stocking all-female triploid fish while maintaining growth rates equal to that of diploid rainbow trout. Assessments will include four high mountain lakes during 2001-2003, an additional 16 lakes during 2004 and 2005, and 15 other lakes in 2006 and 2007.
2. To develop techniques for inducing triploidy in brook trout, rainbow X Yellowstone cutthroat trout hybrids, lake trout, and westslope cutthroat trout at high rates (95-100%) while maintaining adequate survival (not less than 75% of untreated fish).

METHODS

Performance of Sterile Trout in High Mountain Lakes

Pilot Study—1999 Test Groups

McCall regional management personnel purchased normal (diploid) and pressure-treated (triploid) all-female Kamloops strain rainbow trout eggs from Troutlodge, Inc., Sumner, Washington. Eggs were transported to McCall Fish Hatchery (FH) and incubated. Resultant fry were reared in 1 m tanks until they reached 50 mm and then transferred to raceways. Prior to stocking, the diploid and triploid groups were grit marked with green and red fluorescent dye, respectively. Fish were held for two weeks to monitor retention. Equal numbers of diploid and triploid fish were stocked into four lakes near McCall, Idaho with fixed-wing aircraft (Figure 1). On October 15, 1999, 500 diploid and 500 triploid fry were stocked into Maki, Golden, and Snowslide lakes, whereas 250 diploid and 250 triploid fry were stocked into Crystal Lake.

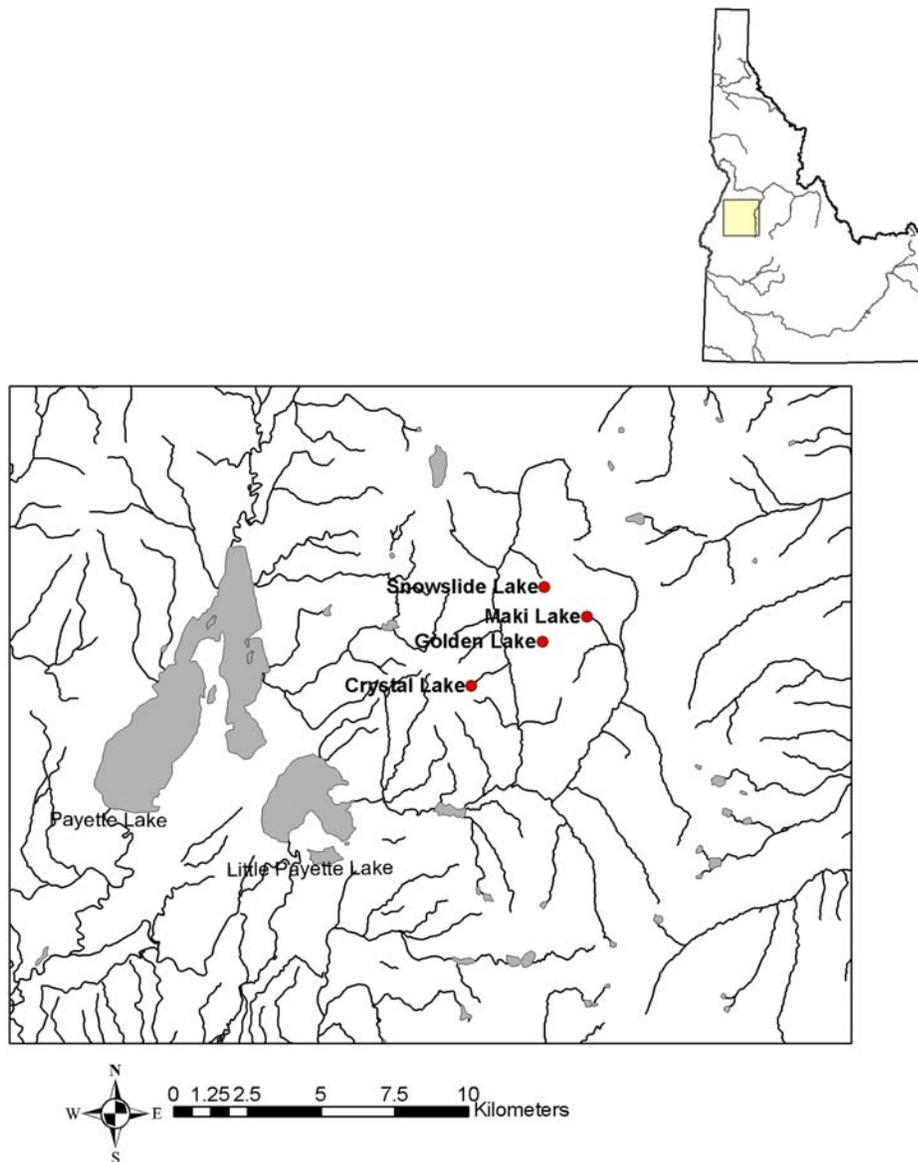


Figure 1. Location of four pilot study lakes stocked with diploid and triploid rainbow trout during 1999.

Lakes were surveyed with floating gillnets and angling from July 28 through July 30, 2002. The experimental gillnets used had 19, 25, 30, 33, 38, and 48 mm bar mesh panels and were 46 m long by 1.5 m deep. Typically, five gillnets were set in the early afternoon and pulled the following morning. While the nets fished, the two- or three-person field crew used spin- and fly-fishing gear to collect additional samples.

Captured fish were identified to species, measured in total length to the nearest millimeter, and weighed to the nearest gram. All rainbow trout were examined for grit mark

presence under a portable fluorescent lantern (Model #UVL-4, UVP, Inc.). Examination for grit dye was conducted in the absence of light within a black plastic garbage bag.

2003 Test Groups

I selected study lakes that had surface areas of approximately four hectares or larger, were on the normal stocking rotation, and that have been stocked historically with rainbow trout. Eggs for the three test groups used in this study were obtained from Troutlodge and IDFG's Hayspur FH. The Troutlodge group was a Kamloops strain that was genetically modified to produce all-female eggs. The Hayspur groups were mixed-sex diploids and mixed-sex triploids. At Hayspur, pooled groups of eggs were split in half. Half of the eggs were shocked at 26°C at 20 MAF for 20 minutes to produce the mixed-sex triploid group, while the other half was placed in rearing containers without being shocked (mixed-sex diploid group). Hayspur eggs remained on station until the eyed stage.

On May 12 and 13, 2003, the three test groups were shipped to Eagle Fish Hatchery as eyed eggs and placed in vertical stack incubation trays. After the button up stage, fry were transferred to 1 m tanks. Fry remained in 1 m tanks through the remainder of the rearing period on 11°C water. From August 11-13, 2003, fry from each group received a separate fin clip. The mixed-sex diploid group was marked by removal of the left pelvic fin, whereas the right pelvic fin was removed from the mixed-sex triploid group, and the adipose fin from the Troutlodge all-female group. Prior to stocking, length and weight were measured from a random sample of fish from each group. Ninety-five blood samples were collected from the treatment groups (Troutlodge-60, Hayspur Triploids-25, Hayspur Diploids-10), stored in Alsever's solution, and shipped to Western Carolina University, where ploidy levels were determined with flow cytometry by Dr. Peter Galbreath.

Test fish were stocked from August 14-22, 2003 (Figure 2). Test fish were loaded into plastic stocking bags containing approximately 4 liters of water at a density of 250 fish per bag. Bags were filled with pure oxygen, sealed, and stored in chilled water during transport. Lakes were stocked from fixed-wing aircraft or with all-terrain vehicles.



Figure 2. Locations of 15 mountain lakes in Idaho stocked during 2003 and used to compare the relative performance of Hayspur mixed-sex diploid, Hayspur mixed-sex triploid, and Troutlodge all-female rainbow trout.

Production of Sterile Trout

Brook Trout

I conducted thermal and pressure shock experiments to induce triploidy in brook trout at Henrys Lake FH on October 30, 2003. Fertilized eggs from each replicate were produced by combining the gametes of two females with two males. Eggs were fertilized in a non-iodized saline solution (concentration of 7.4 g/L) to increase sperm viability and improve fertilization

rates. Eggs were placed in temporary containers until introduction into the heat bath or pressure chamber. Approximately equal numbers of fertilized eggs (measured by displacement method) were split four ways (three treatments and one control). The heat treatment was 29.4°C at 18 MAF for 7 minutes. The two pressure treatments used 9,500 psi and five minute duration at 200 and 300 Celsius minutes after fertilization (CMAF). After thermal or pressure treatment, eggs were transferred to incubation trays and placed in vertical flow-through stacks. The process was repeated with two other females and two other males for each of the second and third replicates.

The hydraulic pressure chamber, Model HPC™, used during this experiment was built by TRC Hydraulics Inc., Dieppe, New Brunswick, Canada. The 2.7 liter chamber was filled with ambient hatchery water before egg treatment. Fertilized eggs were placed in a perforated aluminum cylinder for loading and unloading and transferred to incubation trays after the shock was applied. The heat shocking unit consisted of an individual 51 L insulated cooler. Coolers were fitted with inlet and outlet hoses and attached to one recirculating heat pump (PolyScience Inc., Model 210). Temperature of the hot water bath was set and monitored with a VWR traceable monitoring thermometer ($\pm 0.1^\circ\text{C}$). After treatment, all eggs were incubated at Henrys Lake FH. Eggs were enumerated at the eyed stage, and subsamples ($n = 200$) were transported to Eagle FH for further rearing and survival enumeration. When fish reached approximately 30 mm, 25 blood samples were collected from each treatment by replicate group, stored in Alsever's solution, and shipped to North Carolina State University, where ploidy levels were determined with flow cytometry by Dr. Jeff Hinshaw.

Henrys Lake Hybrid Trout

I conducted a heat and pressure shock experiment to induce triploidy in rainbow x Yellowstone cutthroat trout hybrids at Henrys Lake Fish Hatchery on March 25, 2003. Three males were used for each of the four replicates, while three females were used for the first replicate, and five females were used for the second, third, and fourth replicates. After introduction of the milt and light stirring, eggs were rinsed with freshwater to initiate fertilization. Approximately equal numbers of fertilized eggs (measured by displacement method) were split four ways (three treatments and one control). One pressure treatment, two heat treatments, and one control were compared. The pressure treatment consisted of a shock of 10,000 psi applied at 40 MAF for five minutes. The two heat treatments were 29.4°C at 18 MAF for 7 minutes and 28°C at 15 MAF for 20 minutes. All eggs were incubated, hatched, and reared at Henrys Lake Fish Hatchery. Eggs were enumerated at the eyed and hatch stages to determine survival. Twenty blood samples were collected from each treatment by replicate group, stored in Alsever's solution, and shipped to Washington State University (WSU), where ploidy levels were determined with flow cytometry by Paul Wheeler. A small experimental chamber (0.5 L capacity) was borrowed from WSU and was used to apply pressure shocks during this experiment. The same heat shock equipment, as described in the previous section, was used.

Lake Trout

I pressure treated a production group of lake trout eggs at Story State FH, Story, Wyoming on October 8, 2003. Four females were spawned with four males at three separate times for approximately 45,000 eggs. Eggs were treated using the TRC unit, and a shock of 9,500 psi at 300 CMAF for five minutes was applied. In addition, two females were spawned with two males and not treated, and acted as a control for survival rate comparisons. The

treated eggs were combined into one upweller until eye-up, while the control group was eyed in an incubation tray. Eggs were shipped to Grace FH at the eyed stage, where half were hatched in chilled water and the other half were reared on ambient water. Onset Optic StowAway® temperature loggers were placed in the top tray of the chilled and ambient stacks to monitor temperature until fry were transferred from incubation trays to raceways. Survival and induction rates were estimated in the same manner as described in the brook trout section.

Experiments to induce triploidy in lake trout were conducted in cooperation with the Utah Division of Wildlife Resources-Fisheries Experimental Station, Logan, Utah and the U.S. Fish & Wildlife Service at Saratoga National FH, Saratoga, Wyoming on October 28, 2003. Four eight-year-old females were spawned with four males for each of the three replicates. Eggs were rinsed with fresh water to initiate fertilization. Eggs remained in spawning bowls until just before introduction into the pressure chamber. Approximately equal numbers of fertilized eggs were split five ways (four treatments and one control). Experimental treatment pressures of 9,000 or 9,500 psi were applied at 200, 300, or 400 CMAF for five minutes. After removal from the chamber, eggs were placed in incubation trays and placed in vertical flow through stacks in random order. The process was repeated three times with different groups of eggs (replicates). The TRC pressure chamber was used to apply the shock. At the eyed stage, eggs were transported to Grace FH for rearing. Survival and induction rates were estimated in the same manner as described in the brook trout section.

In addition to the experiments at Saratoga National FH, a pressure shock of 9,500 psi at 250 CMAF for five minutes was applied to the fertilized eggs from four females and four males for production purposes. This process was repeated four times to yield a production group of approximately 104,000 eggs. Eggs were treated in the same fashion as in the experiments, except that eggs were eyed in upwellers and then after being transported to Grace FH, half of the production group was hatched on chilled water and the other was hatched on ambient water. Thermographs were placed in the top tray of the chilled and ambient stacks to monitor temperature until fry were ponded. Survival and induction rates were estimated in the same manner as described in the brook trout section, except that samples were sent to Paul Wheeler at Washington State University for ploidy analysis. Thirty samples were collected from each of the two production groups, and 15 samples were collected from each treatment by replicate group for the Saratoga experimental lots.

Westslope Cutthroat Trout

I conducted heat shock experiments to induce triploidy in westslope cutthroat trout at Hayspur FH on March 3, 2003. Due to the low number of eggs per female, seven females were fertilized with milt from three males for each of the three replicates. Approximately equal numbers of fertilized eggs were split five ways (four treatments and one control). Survival and induction rates were compared across the four heat treatments and one control. Heat shock treatments were applied at 26-29.4°C at 10, 18, or 20 MAF and for durations of 7, 10, or 20 minutes. Eggs were shocked in experimental coolers, and survival and induction rates were estimated in the same manner as described in the brook trout section. Twenty blood samples were collected from each treatment by replicate group, stored in Alsever's solution, and shipped to Western Carolina University, where ploidy levels were determined with flow cytometry by Dr. Peter Galbreath.

RESULTS

Performance of Sterile Trout in High Mountain Lakes

Pilot Study—1999 Test Group

During 2003, a total of 31 test fish were captured from Crystal, Golden, and Maki lakes. Of these 31 fish, 21 were diploids and 10 were triploids. Most of the diploids (12) and all of the triploids (10) were caught from Golden Lake. No triploids were caught from either Crystal or Maki lakes, whereas two and seven diploids were caught, respectively. During 2003, gill nets were fished for 71-79 h per lake and an additional 0.5-3 h of hook and line sampling was conducted (Table 1). Catch per unit effort for gill nets ranged from a low of 0.03 fish per hour in Crystal Lake to a high of 0.23 fish per hour in Golden Lake. For hook and line sampling, CPUE ranged from a low of zero fish per hour in Crystal Lake to a high of 1.0 fish per hour in Golden Lake. Due to the very small sample sizes of triploids in all lakes and the limited amount of angling effort expended, it is not possible to compare the relative efficiency of the two gears at capturing diploid and triploid fish in 2003.

Table 1. Catch, effort, and catch per unit effort (CPUE) for diploid and triploid rainbow trout surveys conducted on three high mountain lakes in Idaho during 2003. Test fish were stocked as fry during October 1999.

Lake Name	Date	Triploid						Diploid					
		Gill Net			Hook and Line			Gill Net			Hook and Line		
		Catch	Effort (h)	CPUE	Catch	Effort (h)	CPUE	Catch	Effort (h)	CPUE	Catch	Effort (h)	CPUE
Crystal	7/30	0	79	0.00	0	1	0.00	2	79	0.03	0	1	0.00
Golden	7/29	9	78	0.12	1	3	0.33	9	78	0.12	3	3	1.00
Maki	7/28	0	71	0.00	0	2	0.00	6	71	0.08	1	2	0.67
Totals		9	228	0.04	1	5	0.20	17	228	0.07	4	5	0.80

Over the three year sampling period from 2001 to 2003, a total of 107 test fish were captured. Annual catch was fairly consistent with 34 fish captured in 2001, 42 in 2002, and 31 in 2003 (Figure 3). However, lake to lake variation in catch was less consistent. Only 11 fish were caught from Crystal Lake, whereas 55 and 41 fish were caught from Golden and Maki lakes, respectively. For all lakes and years combined, the catch of diploid fish far exceeded that of triploid fish. From the total of 107 test fish, the catch consisted of 32 triploid and 75 diploid fish, thus the catch of triploids was 43% of the number of diploids caught. Within lakes, this discrepancy was the largest in Crystal Lake where the catch of triploids was 22% that of the diploid catch, followed by Maki Lake at 41% and Golden Lake at 49%.

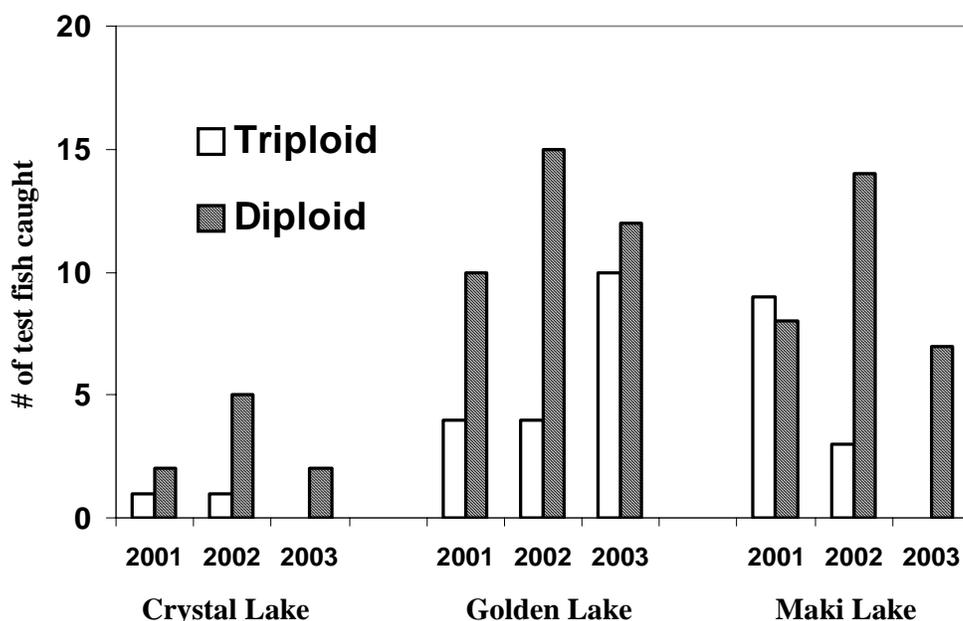


Figure 3. Yearly catch of diploid and triploid rainbow trout in three pilot study lakes near McCall, Idaho.

Comparison of growth between test groups could only be made for Golden Lake, as few fish and no triploids were caught from Maki and Crystal lakes during 2003. In Golden Lake, mean total length of the diploid group ($\bar{x} = 352 \pm 9$ mm; $n = 12$; Figure 4) was not statistically different from the triploid group ($\bar{x} = 342 \pm 11$ mm; $n = 10$), based on overlapping confidence intervals. Compared to mean length values from 2001, triploid fish grew slightly faster than diploids over the two years between 2001 and 2003 sampling events. The triploid group increased by 114 mm, while the diploids increased by 104 mm.

Also in Golden Lake, there was no statistical difference in weight between the diploid group ($\bar{x} = 464 \pm 46$ mm; $n = 12$; Figure 4) and triploid group ($\bar{x} = 429 \pm 44$ mm; $n = 10$) based on overlapping confidence intervals. Between the 2001 and 2003 sampling periods, the diploid and triploid group increased in mean weight at approximately the same rate (302 g and 296 g, respectively).

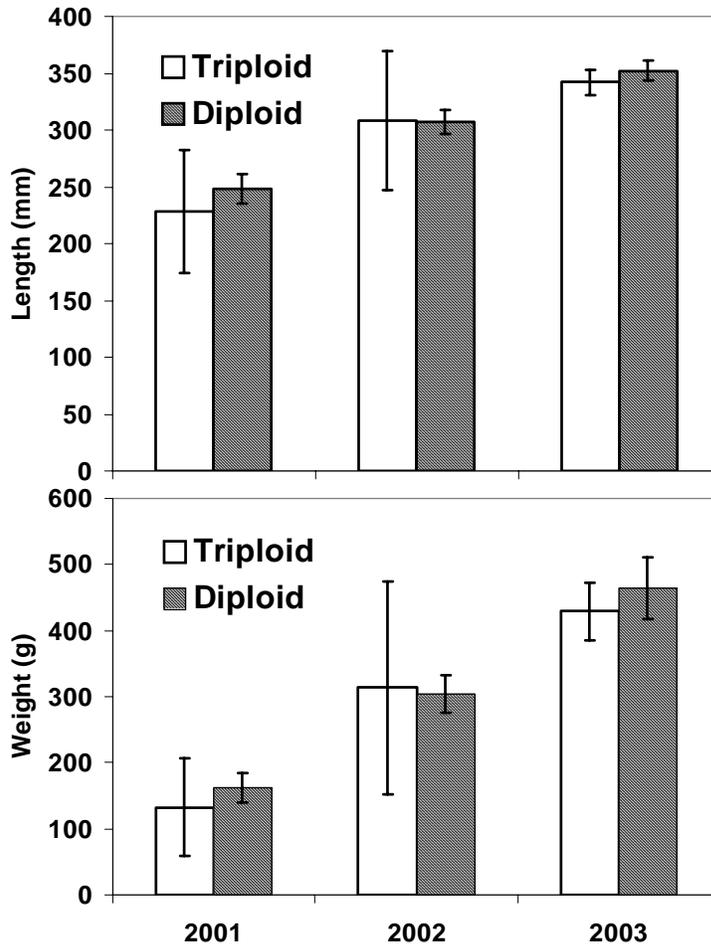


Figure 4. Mean length and weight of triploid and diploid rainbow trout that were stocked during October 1999 and sampled in June 2002. Error bars indicate upper and lower 95% confidence limits.

2003 Test Group

Fifteen high mountain lakes were stocked with test fish from August 14-22, 2003. Twelve of the lakes were stocked from fixed wing aircraft, whereas the remaining three, Blackwell, Big, and Rough lakes, were stocked from all-terrain vehicles (Table 2). Thirteen of the lakes were stocked with equal numbers of test fish: 500 Hayspur triploids, 500 Hayspur diploids, and 500 Troutlodge all-females. The two exceptions were Oreamnos and Blue lakes, where fewer Hayspur triploids were stocked. Due to loss of oxygen in one of the stocking bags during transport, only 440 Hayspur triploids were stocked in Oreamnos Lake. In addition, due to a shortage of available fry at the end of the stocking period, only 400 Hayspur triploids were stocked in Blue Lake. Both lakes received 500 fish from each of the other two test groups. At the time of stocking, there was no statistical difference in the length of the mixed-sex Hayspur diploids (62 ± 1 mm) and mixed-sex Hayspur triploid (62 ± 1 mm) groups, but the Troutlodge all-female group was slightly longer (65 ± 1 mm). There was no statistical difference in weight

between the mixed-sex Hayspur diploid (1.8 ± 0.1 g), mixed-sex Hayspur triploid (1.8 ± 0.1 g), and the Troutlodge all-female group (2.0 ± 0.1 g).

Table 2. Description of 15 study waters in Idaho stocked with Hayspur mixed-sex diploid, Hayspur mixed-sex triploid, and Troutlodge all-female rainbow trout during 2003.

Lake Name	IDFG Catalog #	Date Stocked	Hayspur Triploid	Hayspur Diploid	TL AF Triploid	UTM - E	UTM - N	Zone	Size (ha)	Elevation (m)
Blackwell	09-0000-0366	8/14/03	500	500	500	578944	4979995	11	5.3	2101
Heart-Reg 4	10-0000-0164	8/18/03	500	500	500	658388	4822996	11	4.2	2542
Kane Canyon	15-0000-0208	8/18/03	500	500	500	728605	4851742	11	4.8	2813
Perkons	10-0000-0170	8/18/03	500	500	500	663171	4846357	11	4.1	2653
Heart -Reg 3B	10-0000-0292	8/19/03	500	500	500	660767	4866896	11	5.2	2617
Leggit	10-0000-0262	8/19/03	500	500	500	657652	4848254	11	7.7	2600
Lynx Creek #1	10-0000-0264	8/19/03	500	500	500	653883	4856031	11	5.3	2569
Big	15-0000-0183	8/20/03	500	500	500	269934	4845567	12	5.8	2958
Rough	15-0000-0186	8/20/03	500	500	500	270957	4844656	12	3.3	2925
Edna #2	09-0000-0241	8/21/03	500	500	500	661147	4870395	11	4.3	2563
Oreamnos	09-0000-0169	8/21/03	440	500	500	654770	4879486	11	4.1	2486
Ingeborg	10-0000-0306	8/21/03	500	500	500	657127	4868027	11	9.5	2723
WF Buckhorn #1	07-0000-0484	8/22/03	500	500	500	590225	4972271	11	12.9	2123
Buckhorn #3	07-0000-0494	8/22/03	500	500	500	590225	4972271	11	12.9	2123
Blue	07-0000-0370	8/22/03	400	500	500	601076	4998164	11	5.0	2222

Production of Sterile Trout

Brook Trout

Survival to eye-up and to feeding fry for Henrys Lake brook trout was higher for eggs treated with pressure than for those treated with heat. Survival to eye-up (59%) was equal for the two pressure treatments tested and exceeded survival rates provided by heat by 6% (Table 3). Mean survival to eye-up for controls (72%) was 13% higher than survival for pressure treated eggs. After 200 eyed eggs were isolated from each treatment-replicate combination, the highest survival (82%) to the time fry initiated feeding was provided by a pressure shock of 9,500 psi at 40 MAF for 5 minutes.

Table 3. Survival and triploid induction rates of brook trout from Henrys Lake during 2003. Experiments directly compared the efficiency of heat and pressure treatments. Life stage is abbreviated as EE for eyed eggs and FF for feeding fry.

Shock (°C or PSI)	MAF	Duration	Rep	Survival to EE (%)	Mean Survival to EE (%)	Survival to FF (%)	Mean Survival to FF (%)	Induction Rate (%)	Mean Induction Rate (%)
29.4	18	7	1	37	53	44	66	96	82
			2	64		74		50	
			3	59		79		100	
9,500	26.7	5	1	51	59	62	70	100	100
			2	72		69		100	
			3	53		80		100	
9,500	40	5	1	47	59	66	82	100	100
			2	74		88		100	
			3	57		94		100	
Control			1	57	72	73	80	0	0
			2	80		81		0	
			3	81		86		0	

Analysis of blood samples indicated that pressure treatment was more efficient than heat for inducing triploidy in brook trout. All 150 blood samples from the six pressure treatment by replicate groups were determined to be triploid. Mean induction rate for the heat treatment tested was 82%. A single replicate (#2) within this heat treatment tested at 50%. Based on higher survival from eyed egg to feeding fry and 100% induction rates, the 9,500 psi treatment applied at 40 MAF for 5 minutes was the most optimal for inducing triploidy in brook trout eggs at Henrys Lake.

Henrys Lake Hybrid Trout

Survival to eye-up and induction rates for pressure treatments of Henrys Lake hybrids were higher and more consistent than those for heat treatments. Survival of controls to eye-up averaged $60 \pm 21\%$ (Table 4). Survival of the two heat treatments averaged below 30%. The highest survival to eye-up was provided by the 10,000 psi treatment at 46.7 MAF for 5 minutes. Survival for this treatment ($62 \pm 12\%$) exceeded that of controls by 2%.

Induction rate results were available for only 10 of the 20 treatment by replicate combinations due to spoilage of some samples during shipping. For the 29.4°C at 18 MAF for 5 min duration treatment, induction rates were low and averaged 10% (Table 4). For the other three treatments, induction rates were 100% for all replicates tested. Based on survival rates that exceeded controls and 100% induction rates, the 10,000 psi pressure treatment at 46.7 MAF for 5 minutes was the most optimal for inducing triploidy in hybrid eggs.

Table 4. Survival and triploid induction rates of Henrys Lake hybrids during 2003. Experiments directly compared the efficiency of heat and pressure treatments.

Pressure (psi) or Temperature (°C)	MAF	Duration (min)	Replicate	Survival to Eye-Up (%)	Mean Survival (%)	Standard Error	Induction Rate (%)	Sample Size	Mean Induction Rate (%)
10,000	40	5	1	75	47	18	—	—	100
			2	36			100	12	
			3	77			—	—	
			4	0			—	—	
10,000	46:40	5	1	90	62	12	100	14	100
			2	31			100	17	
			3	71			100	15	
			4	56			—	—	
29.4	18	7	1	2	21	15	—	—	10
			2	67			0	20	
			3	5			—	—	
			4	11			20	15	
28	15	20	1	0	29	11	—	—	100
			2	30			—	—	
			3	56			100	12	
			4	29			100	19	
Ambient	15	20	1	93	60	21	—	—	0
			2	57			0	18	
			3	1			—	—	
			4	87			0	19	

Lake Trout

Overall survival of lake trout from Story FH was higher than experienced at Saratoga or Egan FH over the previous two years. Survival to eye-up for the treated production group from Story was 97% (Table 5). No mortality to eye-up was seen in controls. Survival to hatch and feeding fry for the treated groups were 79 and 68%, respectively. Survival to hatch for the control group was 95%. The use of a chiller reduced the ambient water temperature from 11.5 to 10.6°C. Lower water temperature improved the mean survival to hatch rate by 5% for the treated group and 2% for the control group. All blood samples (n = 29) from the Story production group were classified as triploid.

Table 5. Survival and triploid induction rates of lake trout from Saratoga National and Story State fish hatcheries, Wyoming during 2003 pressure treatment experiments. Life stage is abbreviated as EE for eyed eggs and FF for feeding fry. The bottom two lines of this table contain information for the production group produced at each lake trout brood facility.

Pressure Level : MAF : Duration	Replicate	Survival to EE (%)	Mean Survival to EE (%)	Survival to FF (%)	Mean Survival to FF (%)	# of blood samples	Induction Rate (%)
Control	1	63	62	31	27	3	0
	2	54		17		5	0
	3	68		32		5	0
9,000 : 32 : 5	1	41	48	11	9	15	100
	2	50		7		15	100
	3	52		8		11	100
9,500 : 22 : 5	1	47	42	11	6	14	100
	2	39		4		13	100
	3	40		3		14	100
9,500 : 32 : 5	1	35	53	14	11	14	100
	2	65		8		13	100
	3	60		10		0	NA
9,500 : 43 : 5	1	34	38	14	9	14	100
	2	42		5		13	100
	3	39		7		13	100
9,500 : 32 : 5	Saratoga	30	—	5	—	27	100
9,500 : 40 : 5	Story	97	—	68	—	29	100

Overall egg survival to eye-up and hatch for experimental treatments at Saratoga was much lower than for Story. The survival of untreated eggs to eye-up was 62%, over 30% lower than that from Story. Mean survival to eye-up of experimental treatments varied from 38 to 53%, whereas survival to feeding fry varied from 6 to 11%. The highest survival to eye-up and feeding fry stage was provided by shocking eggs with 9,500 psi at 300 CMAF (32 MAF) for 5 minutes. Contrary to what would be expected, survival for the 9,000 psi treatment applied at the same CMAF was lower than the 9,500 psi treatment. A total of 149 blood samples were collected from treated fish, and all were classified as triploid.

The production group from Saratoga performed similarly to the experimental groups in that overall survival was low. Survival to eye-up for fish treated with 9,500 psi at 300 CMAF (32 MAF) for 5 minutes duration was 30%. No control group was available for survival comparison. The use of chilled water improved mean survival rates but only slightly. Mean survival to hatch was 6.1% for eggs hatched on chilled water and 4.3% for eggs hatched on ambient temperature water.

Westslope Cutthroat Trout

Survival rates to eye-up for heat treated westslope cutthroat trout were low. Survival of the untreated controls averaged $19 \pm 8\%$ (Table 6). For the four heat shock treatments tested, survival did not exceed 15% to eye-up. The highest treatment survival of $13 \pm 6\%$ was provided by the 26°C at 20 MAF for 20 minutes duration.

Mean induction rates for the four heat treatments ranged from 73-91% (Table 6). Individual replicates within the 26, 27, and 28°C treatments possessed triploid induction rates of 100%. However, induction rates for other replicates within these treatments were as low as 25-79%. The highest mean induction rate of 91% was provided by the 28°C treatment at 10 MAF for 10 minute duration.

Table 6. Survival and triploid induction rates of westslope cutthroat trout during 2003 Experiments directly compared the efficiency of four heat treatments. The eyed egg stage is abbreviated as EE.

Temperature (°C)	MAF	Duration (min)	Replicate	Survival to EE (%)	Mean Survival to EE (%)	Standard Error	Induction Rate (%)	Mean Induction Rate (%)	Standard Error
Control	20	20	1	35	19	8	5	3	2
			2	10			5		
			3	13			0		
26	20	20	1	24	13	6	95	73	24
			2	6			100		
			3	8			25		
27	20	20	1	16	11	3	90	80	15
			2	7			100		
			3	9			50		
28	10	10	1	14	8	3	95	91	6
			2	5			79		
			3	5			100		
29.4	18	7	1	12	9	2	80	85	3
			2	6			86		
			3	8			90		

DISCUSSION

Performance of Sterile Trout in High Mountain Lakes

In 2003, gill net and angling surveys from the pilot study lakes showed that the catch of diploid rainbow trout exceeded that of triploids by over two times. Combined with the catch statistics from 2001 and 2002 (Figure 5; Kozfkay and Megargle 2001; Kozfkay 2002), the total catch of diploids was 2.3 times higher than for triploids over the three years of sampling. Even though these results are preliminary and were drawn from only three lakes within a small geographical area, the drastic performance difference is concerning, especially since hundreds of high mountain lakes have been stocked exclusively with triploid rainbow trout since 2001, using stocking densities that were based on past experience with diploid fish. Similarly poor performance was noted for coho salmon *O. kisutch* in Johnson Lake, Alaska (Rutz and Baer 1996). Relative catch frequencies for diploid and triploids were 56% and 44% in 1994 and 75% and 25% in 1995, respectively. A similar performance pattern was noted for rainbow trout in six Alaskan lakes (Brock et al. 1994). At age-0, the catch of all-female triploid trout in baited fyke nets was 34% less than the number of mixed-sex diploid trout caught. At age-2+, the

percentage increased to 39%. In contrast, triploid brook trout outperformed diploid brook trout in an Adirondack lake due to reduced emigration of triploid females (Warrillow et al. 1997). A greater percentage of diploid brook trout (32%) emigrated from the lake than triploids (17%). The authors speculated that emigrants suffered higher mortality than lake residents, which reduced the availability of diploids to lake anglers.

Due to the small number of fish caught from each test group within lakes, length and weight measurements were highly variable and yielded wide confidence intervals, especially for triploids. The small sample sizes prevented any statistically valid comparisons regarding differences in length or weight between groups. The inclusion of 16 lakes in 2001 and 15 lakes in 2003 in this study will enable a detailed evaluation of longevity and growth differences between triploid and diploid fish across a broader geographical area.

Production of Sterile Trout

Previous applications of heat treatment to brook trout eggs at Henrys Lake provided high induction rates (Kozfkay 2002), but survival to eye-up was slightly lower than desired, approximately 70% that of untreated eggs. It was, therefore, desired to increase survival rates while maintaining high induction rates. Research has indicated that pressure treatment of salmonids eggs may provide slightly higher survival rates than for heat treated eggs (Palti et al. 1997; Thorgaard 1983). Additionally, the use of pressure is thought to apply a more uniform treatment across larger batches of eggs and, therefore, would be more optimal in a production setting (Lincoln 1989). Results from 2003 brook trout experiments indicated that the use of pressure treatment increased survival in comparison to heat treated eggs (82% as opposed to 74% relative to untreated eggs). Pressure also provided higher and more consistent induction rates (100%; n = 150). In contrast to the 2002 experiment, induction rates provided by heat shock were lower in 2003 (50-100%), indicating that induction rates for heat treated eggs may be influenced by several factors, such as variable treatment temperature, batch size, or gamete quality. Since 2003 experiments were completed by splitting the same groups of eggs, it appeared that triploid induction rates for pressure treated eggs were not influenced by one or more of these factors.

During 2002 experiments, survival and induction rates for pressure treated Henrys Lake hybrid eggs were higher than for heat treatments tested in previous years. However, no direct comparison was made on the effect of these two techniques within the same year or on the same groups of eggs. Additionally, eggs for the 2002 experiment were drawn from production lots and, therefore, selection of the optimal pressure treatment may have been partially influenced by differing egg quality or other factors. The 2003 experiment was designed to directly assess the relative performance of the two techniques for producing triploid Henrys Lake hybrids and to remove bias associated with differing egg quality or other factors. In 2003, the use of pressure treatment provided higher survival and induction rates than heat treatment. Furthermore, the survival of pressure treated eggs exceeded that of untreated eggs, which has been known to occur in interspecific hybrid crosses (Seeb et al. 1988; Scheerer and Thorgaard 1983; Scheerer et al. 1987). Based on similar results from the last two years of experiments, I conclude that pressure treatment of 10,000 psi pressure treatment at 47 MAF for 5 minutes is a better alternative for inducing triploidy in Henrys Lake hybrids than heat treatment.

Experiments for inducing triploidy in lake trout during previous years, 2001 and 2002, yielded poor survival rates. The majority of the mortality was focused at the time of hatching and was at least partially caused by abnormal development and blue sac disease (Dwight Aplanalp,

IDFG, personal communication; Ostergaard 1987). Although the few survivors tested were identified as triploid, survival of lake trout eggs and fry were well below rates acceptable for hatchery management or to meet stocking requests for Bear Lake. It was assumed that heat did not provide a viable alternative for producing sterile lake trout. Therefore, experimentation in 2003 focused on pressure treatment to increase survival rates. Experimentation at Saratoga NFH with the use of pressure treatment failed to increase survival rates sufficiently to meet management goals. Some improvement in survival was noted; however, survival of untreated eggs to initial feeding was still less than 30%, and survival of all treated groups was 11% or less.

A nearly identical pressure treatment technique (9,500 psi at 40 MAF for 5 minutes) was applied to a production group of lake trout eggs at Story State Fish Hatchery, Wyoming. Survival of these eggs to the feeding fry stage (63%) was seven fold greater than eggs treated at Saratoga, and all fry tested were triploid. These results would allow stocking requests for Bear Lake to be met in future years. Based on these observations, the poor survival experienced with Saratoga eggs was not caused by heat or pressure treatment nor by additional handling mortality, but by some other factor. A possible explanation was water temperature. Water temperatures at Grace FH where lake trout eggs are hatched are 11.5°C, which is considered warmer than optimal for rearing lake trout. The Saratoga broodstock was held and eggs were eyed at water temperatures of 9.5°C. Story water temperatures are much lower, 3-5°C. It was possible that the combination of warmer than optimal water temperatures for incubating and hatching eggs at Saratoga and Grace FH, respectively, created conditions unfavorable for normal development or disease outbreak (Ostergaard 1987). In contrast, when eggs were incubated at more favorable temperatures at Story, survival increased substantially, in spite of high hatching temperatures at Grace FH. It may be possible to increase hatching rates at Grace by using chilled water. Conversely, since Story FH currently has an excess of lake trout eggs, it would be more cost efficient to simply request additional eggs.

In the first year of testing, 2003, at Hayspur FH, no good technique for inducing triploidy in westslope cutthroat trout with heat treatment was developed. Survival was highest for a treatment of 26°C at 20 MAF for 20 minutes duration, but induction rates were low, 73%. Conversely, the treatment (28°C : 20 MAF : 20 minutes duration) with the highest induction rate, 91%, had poor survival, 8%. These results may have been negatively influenced by the age of brood fish (2+) and small egg sizes, as the survival of untreated eggs was less than 20%. Additional testing must be conducted to determine whether survival may be improved with older brood fish with larger eggs and whether induction rates may be improved with the use of pressure treatment.

RECOMMENDATIONS

1. Determine if the poor survival rates observed for triploid fish observed in pilot study lakes exist across a wider geographical area by examining the 31 lakes stocked in 2001 and 2003.
2. Transfer and integrate pressure shocking techniques for inducing triploidy in Henrys Lake brook trout and rainbow X Yellowstone cutthroat trout, as well as lake trout, to resident fish hatchery personnel.
3. Evaluate pressure treatment as a method for improving survival and induction rates in other species, such as rainbow trout, westslope cutthroat trout, and kokanee.

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**ANNUAL PERFORMANCE REPORT
SUBPROJECT #3: PREDATOR TRAINING**

State of: Idaho

Grant No.: F-73-R-26, Fishery Research

Project No.: 4

Title: Hatchery Trout Evaluations

Subproject #3: Predator Training

Contract Period: July 1, 2002 to June 30, 2003

ABSTRACT

The ability of juvenile salmonids to learn to recognize predators and initiate avoidance behaviors in aquaria has been well established, but field evaluations are sparse. In this second pilot study, I began research designed to test whether the survival and eventual return to creel rate of fingerling rainbow trout *Oncorhynchus mykiss* could be increased by exposing them to piscine predators prior to release. Adult rainbow trout were introduced into production raceways at Nampa and Hagerman fish hatcheries. The predators used for training did not select prey that were statistically shorter than average, unlike in the preceding study. In a subset of the study waters ($n = 4$), I assessed short-term, post-release mortality caused by predation. For these four study waters combined, 242 predators were caught and examined. These predators contained a total of 327 prey fish. Fingerling rainbow trout were the primary prey item encountered (88%). Of the 242 predators examined, 138, or 57%, had consumed at least one recently-stocked test fish. Nearly all fingerlings were found in the stomachs of one predatory species, largemouth bass *Micropterus salmoides*. Overall, 284 fingerling rainbow trout were recovered from stomachs which yielded an average of 2.1 fingerlings per predator (excluding predators with empty stomachs), or 0.85 fingerlings per predator (including predators with empty stomachs).

Fingerling consumption by predators did not differ between test groups. Due to partial digestion, only 99 of the 284 ingested rainbow trout fingerlings, or 35%, could be identified to test group. For the four reservoirs combined, 48 fingerlings were identified from the predator-trained group, whereas 51 fingerlings were identified from the control group. Based on overlapping confidence intervals, there was no statistical difference between the mean number of fingerlings ingested per reservoir for the control group, 12.8 (± 18) fingerlings/reservoir, and for the predator-trained group, 12 (± 19) fingerlings/reservoir. Overall survival of the test groups through the first fall after stocking was low. No test fish were caught from Crane Falls, Little Wood, Succor Creek, Condie, Glendale, or Dierkes reservoirs. From the other four study waters, a total of 24 test fish were caught. By group, the catch consisted of 11 fish from the control group and 13 fish from the predator-trained group.

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INTRODUCTION

A fish's ability to recognize predators is determined primarily by genetics and prior experience (Huntingford 1993). Prey species that evolved in predator-rich environments are able to recognize predators quickly and elicit predator avoidance strategies without prior exposure to predators (Johnson et al. 1993). Prey species that evolved in predator-poor environments seem to lack this innate ability but may learn to recognize predators after one or a series of attacks on conspecifics (Patten 1977). Learning is thought to occur through social communication, which is transferred by visual, olfactory, or other cues (Suboski et al. 1990).

By eliminating piscine and avian predation along with other causes of natural mortality, production fish hatcheries are able to supply large numbers of salmonids to habitats that would support few or no fisheries. However, by removing early life-history survival constraints, the behavior of stocked trout is altered from that of their wild counterparts (Berejikian et al. 1996; Dickson and MacCrimmon 1982). Hatchery trout are often more aggressive (Mesa 1991; Fenderson et al. 1968) and show less ability to recognize and react to predators (Berejikian 1995; Healey and Reinhardt 1995). These altered behavioral characteristics may explain, in part, why the survival rate of cultured trout is lower than that of wild trout or trout produced directly from wild parents (Fraser 1981; Miller 1951; Miller 1953).

Several researchers have trained naive prey to recognize predators and elicit avoidance behaviors. The survival rate of predator-conditioned coho salmon *Oncorhynchus kisutch* fry was 25% greater than the survival rate of naive fry when exposed to torrent sculpin *Cottus rhotheus* in artificial stream channels (Patten 1977). Similarly, juvenile coho salmon exposed to predation events from behind clear partitions were over twice as likely to avoid unrestrained lingcod *Ophiodon elongatus* than untrained salmon (Olla and Davis 1989). Thompson (1966) used an electrified fish model to train juvenile Chinook salmon *O. tshawytscha*, and after stocking found two and a half times more untrained fish than trained fish in the stomachs of piscine predators. Brown et al. (1997) demonstrated that naive fathead minnow *Pimephales promelas* learned to chemically recognize a predator, northern pike *Esox lucius*, in less than four days, but visual recognition did not occur until several days later. Rainbow trout do not possess the same alarm pheromones as cyprinids, but appear able to recognize the scent of injured conspecifics and predators (Brown and Smith 1998).

Although the majority of the literature suggests a benefit to training naive prey, at least two researchers have concluded that predator training had no benefit. The use of an electrified loon *Gavia immer* model failed to increase the post-release survival of brook trout *Salvelinus fontinalis* (Fraser 1974). He observed that conditioned fish moved 0.5 m laterally when the model approached and speculated that this behavior had no survival benefit. Berejikian et al. (1999) were able to train Chinook and coho salmon to recognize potential predators in aquaria, but did not observe a post release survival improvement. They speculated that trained and untrained fish formed mixed-group schools after stocking and that predator recognition and avoidance behaviors were passed from trained fish to untrained fish through social communication.

No studies have been designed to improve predator avoidance of rainbow trout on a production scale. However, these studies would be desirable, as the survival of fingerling rainbow trout in Idaho and elsewhere is often low. For instance, creel surveys conducted in Cascade Reservoir from November 1990 through 1992 indicated that less than 1% of fingerlings stocked returned to the creel (Dillon and Alexander 1995). Similarly, the return to creel rate of

fingerlings in Magic Reservoir is often very low. From 1992-1995, return to creel rates ranged from 0.1% to 5.8% (Teuscher et al. 1998). Given such low return rates and the results of previous studies, increased post-release survival associated with predator training could dramatically increase the efficiency of the resident hatchery trout program in systems where predation limits survival.

RESEARCH GOAL

1. To increase the post-release survival and return to creel rates of rainbow trout stocked as fingerlings.

OBJECTIVES

1. To undertake a pilot study that within one year assesses survival advantages of predator training at the hatchery production scale.
2. In the next four years, evaluate whether post-stock survival of predator-trained fingerlings can exceed that of untrained fingerlings by 25% or more.

METHODS

Fish used for this experiment were triploid Hayspur-strain rainbow trout (T9). Test fish were reared in two pairs of production raceways at Nampa Fish Hatchery and one pair of production raceways at Hagerman Fish Hatchery on Tucker Springs water. For each pair of raceways, one raceway was designated as a control, while the other was designated for experimental purposes. In the control raceways, fingerlings were reared conventionally, as in most Idaho Department of Fish and Game (IDFG) resident fish hatcheries. In the experimental raceways, rearing techniques were the same except that large rainbow trout were introduced as predators (Kozfkay 2002) for approximately 15 d immediately before stocking.

Prior to the introduction of predators, fingerlings from each raceway were crowded and held for fin clipping. From April 8 through May 9, 2003, control fingerlings were marked by excision of the left ventral fin. Predator-trained fingerlings were marked by excision of the right ventral fin. During marking, no sorting occurred except that deformed individuals were removed from the raceways and euthanized.

Rainbow trout that would be used as predators were selected from catchable-size groups (200-250 mm) and held an additional 3-4 months to allow for sufficient increases in size (350 mm). These fish were reared at the hatchery where training occurred to reduce the possibility of introducing disease. After the fingerling test groups recovered from marking stress in 5-6 d, large rainbow trout were introduced as predators into each of the experimental raceways at a ratio of approximately one predator for every 5,000 fingerlings. Before introduction into an experimental raceway, each predator was measured to the nearest mm and weighed to the nearest gram. Predators were also individually marked with jaw tags to allow monitoring of growth during the training period. On April 18, 2003, seven predators ($\bar{X} = 490 \pm 42$ mm, 1506 ± 482 g) were introduced into raceway 4 at Hagerman Fish Hatchery (FH). At

Nampa FH, eight predators ($\bar{x} = 396 \pm 20$ mm, 719 ± 109 g) were introduced into raceway 10 on April 28, 2003, and five predators ($\bar{x} = 366 \pm 49$ mm, 580 ± 196 g) were introduced into raceway 11 on May 19, 2003. Predators were not restrained in any manner and had full access to all portions of the experimental raceways.

After the 15 d training period and immediately prior to stocking, I collected a random sample of 100 fingerlings from each pair of experimental and control raceways. To compare relative size of the fingerlings from each group, length was measured to the nearest mm and weight was measured to the nearest gram. Also at that time, all predators were recaptured and measured. In addition, I examined the stomach contents from all predators from each of the three experimental raceways. Stomach contents were examined through forced regurgitation using a 30 cm catheter (1.5 cm diameter) and 300 cm³ syringe. The total number of ingested fingerlings was counted. The length of whole fingerlings was measured to the nearest mm, and the length of partially digested fingerlings was approximated. No attempt was made to estimate the length of heavily digested prey. I tested the null hypothesis that predators selected fingerlings based on length in proportion to their availability. For each training raceway separately, the null hypothesis was rejected if the 95% confidence interval calculated for the mean difference in length between fingerlings found in the experimental raceways and the stomachs of predators did not include zero (Zar 1996).

Approximately equal number of control and predator-trained fingerlings were stocked in ten lakes and reservoirs from April 30 through May 26, 2003 (Figure 5). Study waters were selected based on three criteria: 1) intermediate size to allow high probability of recapture, 2) intermediate density of predator populations that presumably would exert some predation pressure while allowing sufficient survival to evaluate relative performance, and 3) low chance of dewatering. Reservoirs were located in IDFG regions 2, 3, 4, and 5 (Table 7). Over the past several years, five of the study waters have been stocked only with catchable-size rainbow trout due to the presence of predator populations. However, the other five study waters, including Mann and Winchester lakes as well as Horsethief, Little Wood, and Succor Cr. reservoirs, have received annual plants of fingerling rainbow trout for at least the last three years.

Spring and fall sampling was conducted to evaluate the relative performance of the two test groups. In spring, in a subset of the study waters ($n = 4$), I conducted electrofishing surveys for 1-3 days immediately after stocking to assess short-term mortality of the test groups caused by predation. From May 2 through June 2, 2003, I sampled Glendale, Little Payette, Mann, and Winchester lakes. Potential predators, including large trout, northern pikeminnow *Ptychocheilus oregonensis*, and bass *Micropterus* sp., were captured and measured. Stomach contents of each predator were examined through forced regurgitation. Ingested fish were identified to species and measured, and all ingested rainbow trout fingerlings were examined for fin clips. Mean values and 95% confidence intervals were calculated by group.

During fall, approximately 5-6 months after stocking, experimental gill nets, trap nets, and electrofishing were used to assess the relative survival of the two test groups. I sampled seven of the ten study waters from September 25 through October 29, 2003. No fall sampling was conducted at Glendale or Condie reservoirs due to dewatering and lack of boat access, respectively. Additionally, no sampling was conducted at Dierkes Lake. Length in mm and weight in grams of all fin clipped rainbow trout were measured. Mean values and 95% confidence intervals were calculated by group.

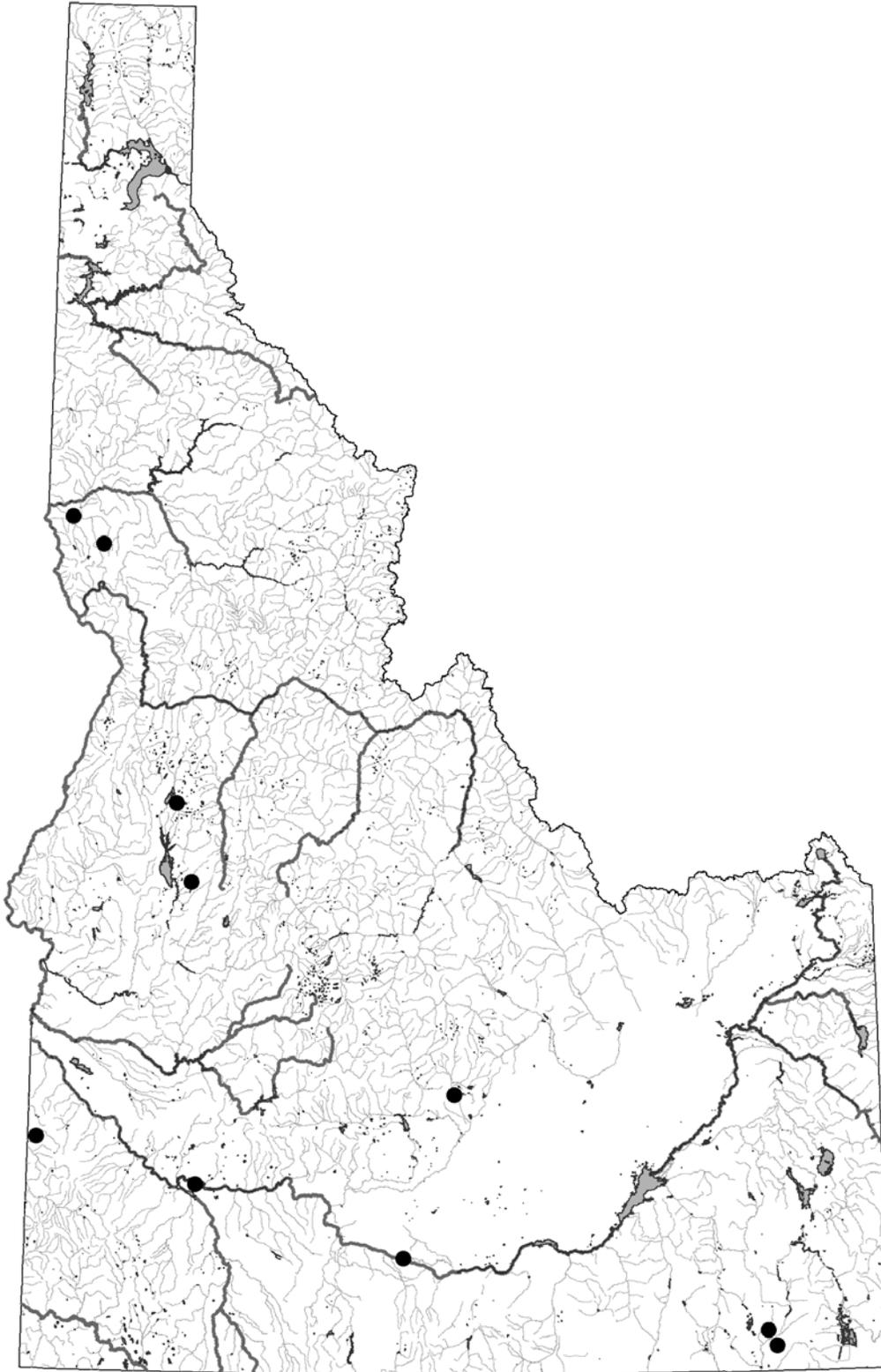


Figure 5. Location of the ten study waters in Idaho that were stocked with predator-trained and control fingerlings during 2003.

Table 7. Location and stocking information for ten study waters in Idaho stocked with predator-trained and control fingerlings during 2003.

Water Body	Region	Stocking Date	Hatchery Source	# of controls	# of trained
Condie Res.	5	5/15/2003	Nampa	6,945	6,945
Glendale Res.	5	5/12/2003	Nampa	4,984	4,984
Winchester Res.	2	5/1/2003	Hagerman	7,979	7,979
Mann Lake	2	5/1/2003	Hagerman	9,988	9,988
Little Payette Lake	3M	5/26/2003	Nampa	10,145	10,145
Succor Cr. Res.	3B	5/15/2003	Nampa	2,530	2,530
Little Wood Res.	4	5/13/2003	Nampa	9,935	9,935
Crane Falls Res.	3B	4/30/2003	Hagerman	5,012	5,012
Dierkes Lake	4	4/30/2003	Hagerman	2,005	2,005
Horsethief Res.	3M	5/21/2003	Nampa	5,330	5,330

RESULTS

At the end of the training period on April 30, 2003, the stomach contents of the five predators (one predator died during the training period) from Raceway 4 at Hagerman FH contained a total of 26 fingerlings with a mean length of 72 mm (95% CI \pm 15 mm; Figure 6). Over the 12 days since introduction, the predators had increased in weight by an average of 47 g (\pm 55 g). Mean length and weight of the fingerlings in the experimental raceway was 80 mm (\pm 3 mm) and 10 g (\pm 1 g), respectively. Based on overlapping confidence intervals, predators did not select smaller fingerlings from the raceways. Similarly, at the time of stocking, there was no statistical difference in length and weight between fingerlings from the control group (84 ± 3 mm, 12 ± 1 g) and the experimental group.

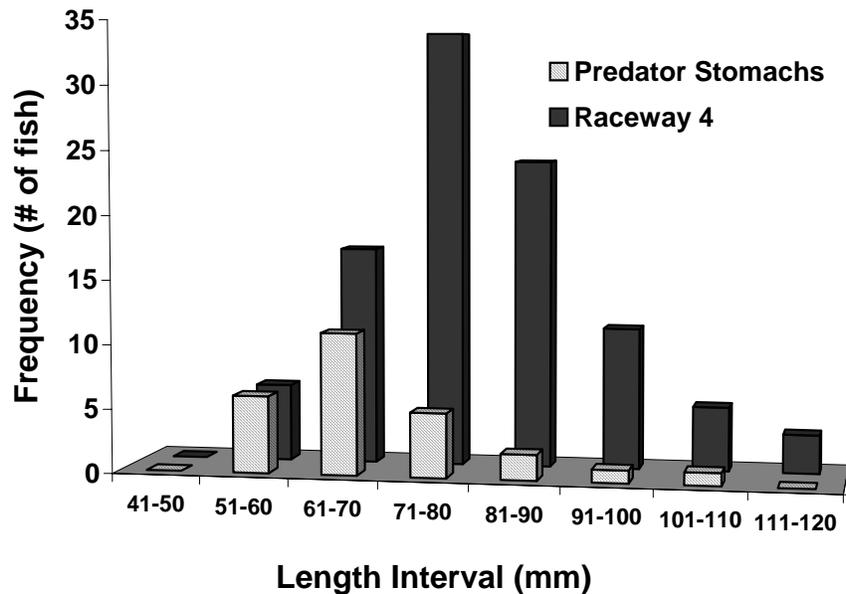


Figure 6. Length frequencies of fingerling rainbow trout available to predators (n = 100) in raceway 4 (black bars) and of those found in the stomachs of three predators (n = 26; white/gray bars).

For the second pair of raceways, stomach contents from the eight predators from raceway 10 were not examined. Mean length and weight of the fingerlings in the experimental raceways was 89 mm (± 3 mm) and 14 g (± 1 g). At the time of stocking, there was no statistical difference in length or weight between fingerlings from the control group (90 ± 2 mm; 15 ± 1 g) and the experimental group.

For the third pair of raceways, stomach contents from the five predators from raceway 11 at Nampa FH contained nine fingerlings with a mean length of 80 mm (± 8 mm; Figure 7). Over the 12 day training period ending on May 30, 2003, the predators had increased in weight by an average of 100 g (± 73 g). Mean length and weight of the fingerlings in the experimental raceways was 87 mm (± 2 mm) and 13 g (± 1 g). Based on overlapping confidence intervals, predators did not select smaller fingerlings from the raceways. Similarly, at the time of stocking there was no statistical difference in length and weight of fingerlings between the control group (91 ± 2 mm; 15 ± 1 g) and the experimental group.

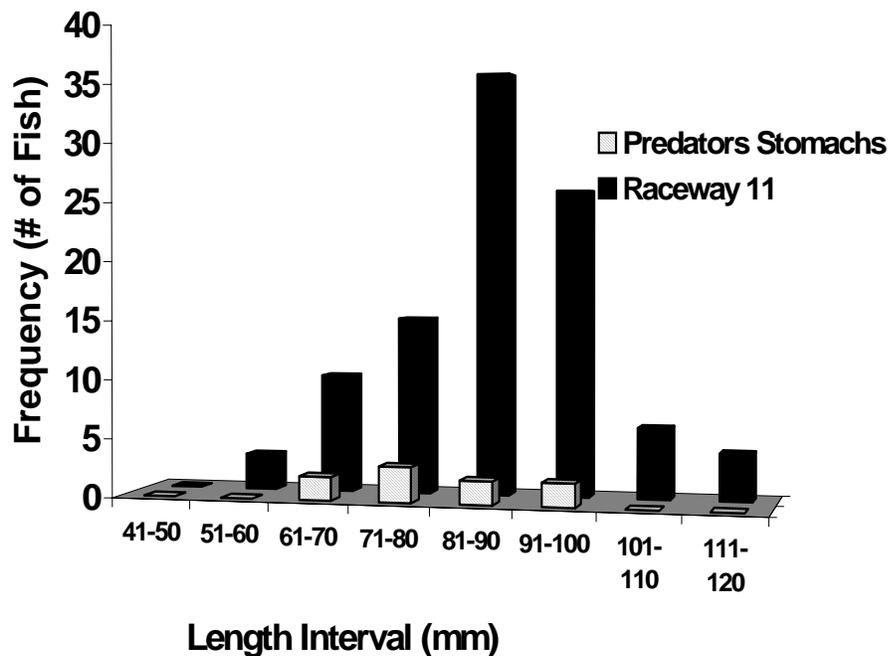


Figure 7. Length frequencies of fingerling rainbow trout available to predators (n = 100) in raceway 11 (black bars) and of those found in the stomachs of three predators (n = 9; white/gray bars).

In a subset of the study waters (n = 4), I assessed short-term, post-release mortality caused by predation. For the four reservoirs combined, 242 predators were caught and examined. These predators contained 327 prey fish. Fingerling rainbow trout were the primary prey item encountered (88%). Other prey items, including yellow perch *Perca flavescens*, sunfishes *Lepomis* sp., and crappie *Pomoxis* sp. were rarely consumed and comprised only 43 individuals or 12% of the total number. Of the 242 predators examined, 138, or 57%, had consumed at least one recently stocked test fish (Figure 8). Nearly all fingerlings were found in the stomachs of one predatory species, largemouth bass. The only exceptions were from Little

Payette Lake, where one smallmouth bass and one northern pikeminnow consumed fingerlings. Overall, 284 fingerling rainbow trout were recovered from stomachs that yielded an average 2.1 fingerlings per predator (excluding predators with empty stomachs) or 0.85 fingerlings per predator (including predators with empty stomachs).

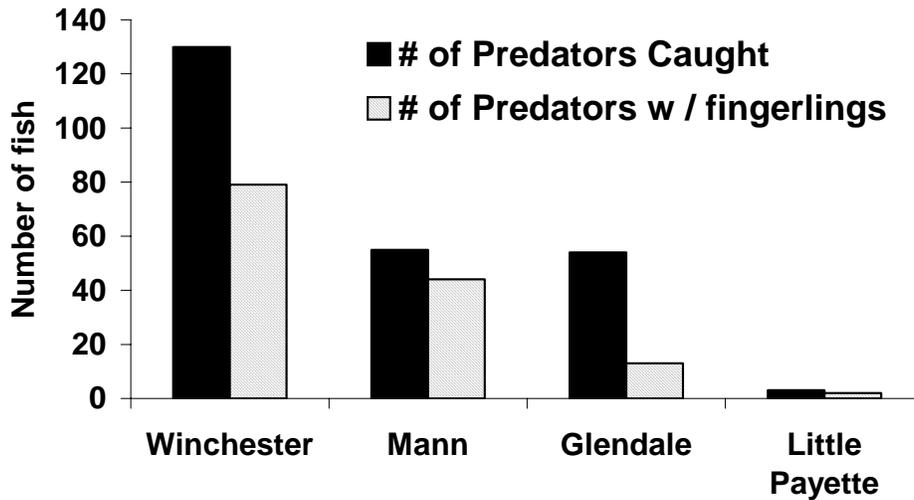


Figure 8. Number of potential and actual predators of fingerling rainbow trout caught during surveys conducted 1-3 days after stocking.

Fingerling consumption by predators did not differ between test groups. Due to partial digestion, only 99 of the 284 ingested rainbow trout fingerlings, or 35%, could be identified to test group. For the four reservoirs combined, 48 fingerlings were identified from the predator-trained group, whereas 51 fingerlings were identified from the control group. Relative consumption of the two test groups was equal in Little Payette Lake and was within one or two fingerlings in Winchester and Mann lakes (Figure 9). The largest difference in relative consumption occurred in Glendale Reservoir, where one predator-trained and four control fingerlings were recovered from stomachs. Based on overlapping confidence intervals, there was no statistical difference between the mean number of fingerlings ingested per reservoir for the control group, 12.8 (± 18) fingerlings/reservoir, and for the predator-trained group, 12 (± 19) fingerlings/reservoir.

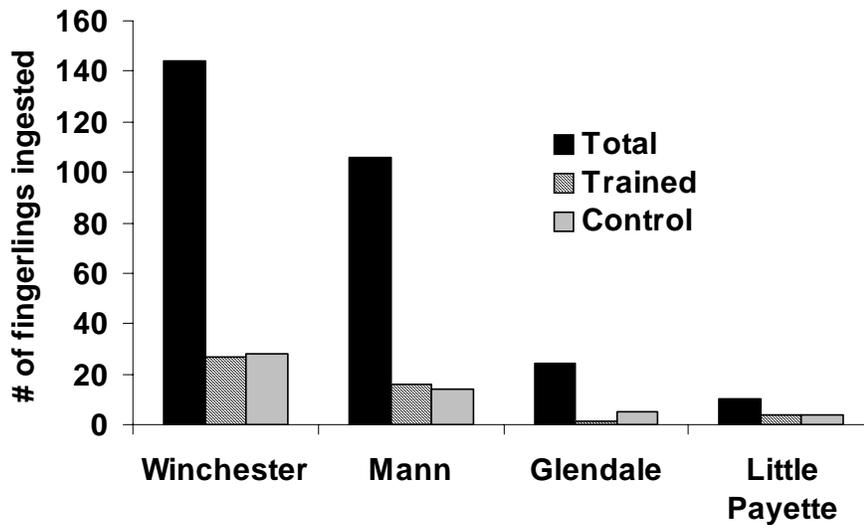


Figure 9. Relative number of predator-trained and control fingerlings sampled from predator stomachs during surveys conducted 1-3 days after stocking. Due to partial digestion of fin clips, not all individuals could be identified to group.

Overall survival of the test groups through the first fall after stocking was low. No test fish were caught from Crane Falls, Little Wood, or Succor Creek reservoirs. No electrofishing was conducted at these reservoirs due to low water and lack of boat access. From the other four study waters, 24 test fish were caught, primarily during night electrofishing surveys (Figure 10). No gill netting or trap netting was conducted at these reservoirs due to recent catchable plants or dense, near-shore macrophyte beds. By group, the catch consisted of 11 fish from the control group and 13 fish from the predator-trained group. The highest catch of test fish occurred at Horsethief Reservoir and was comprised of five control and six predator-trained fish. The catch per group for all other study waters was three test fish or less. No statistical comparisons were made due to the low sample sizes.

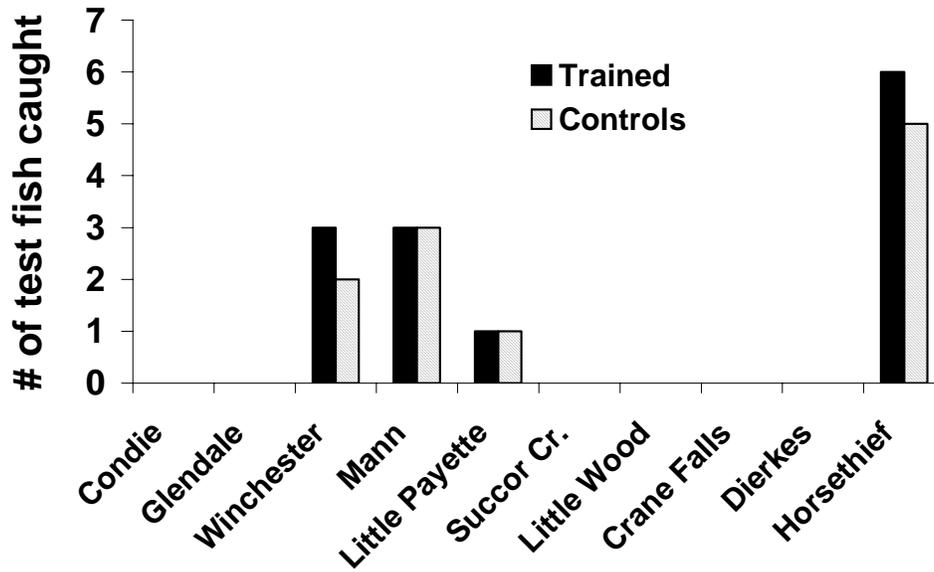


Figure 10. Number of predator-trained and control fingerlings sampled during fall surveys approximately five months after stocking. No test fish were caught from Succor Cr., Little Wood, or Crane Falls reservoirs. No sampling was conducted at Glendale and Condie reservoirs as well as Dierkes Lake.

Growth of test fish during the first summer after stocking was rapid. During fall sampling, mean length and weight for all test fish captured was 267 mm (± 10 mm) and 240 g (± 44 g). There was no statistical difference in size between the groups. The mean length ($\bar{x} = 269 \pm 17$ mm; $n = 11$) and weight ($\bar{x} = 238 \pm 73$ g) of fish from the control group was similar to that of the predator-trained group ($\bar{x} = 265 \pm 13$ mm; 242 ± 62 g; $n = 13$; Figure 11). For the four study waters combined where test fish were caught during the fall, mean length and weight had increased by 182 mm and 222 grams, respectively, over a 148 d period from the mean stock date on May 12 to the mean sampling date on October 7, 2003. Due to small sample sizes ($n \leq 6$), no comparisons of water-specific growth rates by test group were made.

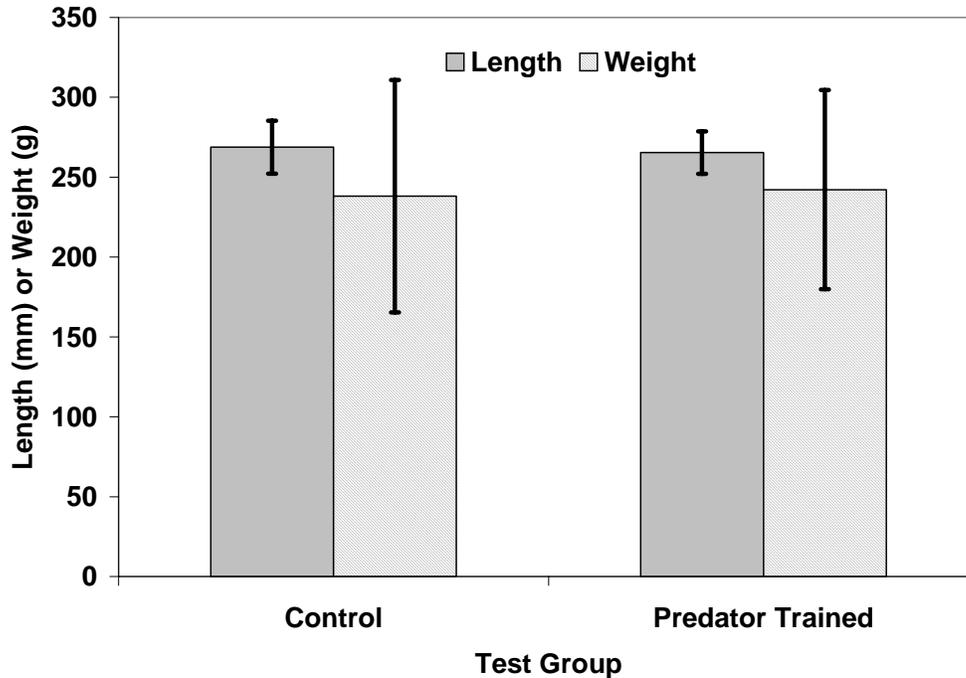


Figure 11. Mean length and weight of all control and predator-trained rainbow trout that were stocked during April and May and sampled during September and October 2003. Error bars indicate upper and lower 95% confidence limits.

DISCUSSION

In aquaria, juvenile salmonids have been trained to recognize predators and initiate avoidance behaviors. However, very few studies have attempted to train production-size groups of cultured fish for release into the wild. I sought to use training techniques that would increase the post-release survival of fingerling rainbow trout on a production scale without substantially interfering with normal hatchery operations. Large rainbow trout reared at the hatchery where training occurred offered the best alternative for this study, in that the potential for disease transfer was low and these fish were readily available at resident hatcheries. Also, large rainbow trout have been shown to be an effective predator of fingerlings in raceways (Kozfkay 2002). Unlike in the previous predator training study, there was no statistical difference in the length of ingested fingerlings and for those found in the training raceway (i.e., no size selection by predators). However, this conclusion is based on small sample sizes from predator stomachs that yielded wide confidence intervals, especially for raceway 11. In both raceways where stomachs were examined, mean lengths of fingerlings ingested were 8 and 7 mm shorter than the mean length of fingerlings in the experimental raceways. Visual interpretation of the graphical data clearly indicates that the length distribution of ingested fingerlings is skewed to the left towards smaller fingerlings, especially for raceway 4 where sample size from stomachs was relatively large. This leads me to believe that predators were selecting smaller individual prey based on size, but small sample sizes from stomachs and the ingestion of a few longer fingerlings created wide confidence intervals and prevented statistical differences.

Cultured fish may be most vulnerable to predation shortly after stocking. Fish reared in production raceways do not encounter predators until after stocking and do not initiate avoidance behaviors similar to wild fish or predator-trained hatchery fish (Berejikian 1995). I assumed that the potential for increasing survival through predator training would be greatest during the period shortly after stocking when fingerlings are presumably most vulnerable. However, I was unable to document a difference in the ability of control and predator-trained fingerlings to avoid being ingested by predators during the three day period after stocking. The numbers of control and predator-trained fingerlings ingested were nearly equal within study waters and summed across all study waters, although sample sizes were relatively small compared to the number of fingerlings stocked (<2%). This indicated that in this pilot study predator training did not improve short-term survival.

There are several possible explanations for the lack of an increase in short-term survival. The most obvious explanation is that training did not produce the desired effect. Fraser (1974) concluded that the use of an electrified loon model was unable to increase returns as the learned response, lateral movement, was not valuable in the wild. It is unlikely that this occurred in the present study, as large rainbow trout were observed to chase, strike at, and ingest fingerlings on a regular basis. Conversely, visual observations indicated that fingerling rainbow trout avoided proximity to predators and fled at their approach. Berejikian et al. (1999) also concluded that predator training did not increase survival of trained fish over untrained fish. They speculated that trained and control fish intermingled after stocking and that trained fish taught control fish to avoid predators through social communication. Since during the present study, predator-trained and control fingerlings were stocked from the same transport tanks, it is likely that fingerlings formed mixed-group aggregations after stocking. Predator avoidance behaviors may have been passed from trained to untrained fish. This could have caused an increase in the overall survival for test groups combined, but differences between groups would not be detectable with this study design. Lastly, stocking stress may have negated the benefits of predator training for the short time period studied. Stressed fingerling coho salmon were more susceptible to predators than unstressed fish for up to 90 minutes (Olla and Davis 1989). Other research has indicated that stress levels in salmonids may remain elevated for much longer time periods, up to 24 hours (Barton et al. 1986; Barton and Schreck 1988; Schreck 1981, cited in Olla and Davis 1989). If stocking stress influenced predator avoidance in the present study, the benefits of training, if any, would not be noticeable until after fish have recovered from transport stress.

The ultimate goal of this study was to increase overall survival of fingerling rainbow trout sufficiently to increase the return to creel rate. Due to very low catches of test fish in the fall, I was unable to address whether more predator-trained fingerlings survived to a catchable size than control fingerlings. The influence of several years of drought and low water levels in many of the study waters likely contributed to low survival rates. For instance, Glendale Reservoir was nearly dewatered, whereas Condie, Succor Cr., Little Wood, Mann, and Little Payette lakes/reservoirs were substantially drawn down throughout summer and fall. This likely created conditions unfavorable for fingerling trout survival such as high water temperatures or increased competition. Another possible explanation for low numbers of test fish in the fall was heavy predation. Winchester Lake maintained relatively stable water levels throughout this study, yet the catch of rainbow trout stocked in spring was still low. Cochnauer et al. (1999) also reported poor survival for stocked fingerlings in Winchester and indicated that during 1999 no fingerling rainbow trout contributed to the creel. They concluded that competition with illegally introduced yellow perch and crappie was the probable cause. My data suggest that predation by largemouth may also be contributing to the poor return to creel rates of rainbow trout stocked as fingerlings. Shortly after stocking, over 60% of the largemouth bass caught in Winchester Lake

had consumed at least one and up to as many as four fingerlings. Similar patterns of predation were evident, although less pronounced, in the other waters that had abundant population of largemouth bass.

Predator-trained and control fingerlings were equal in size at the time of stocking. After stocking, predator-trained fish should have spent more time avoiding predators, spent less time feeding, and took fewer risks to feed than untrained fish. Wild juvenile steelhead were less likely to feed under threat of predation than a domesticated strain (Johnsson and Abrahams 1991). Under threat of predation, zebra danio select feeding areas with less abundant prey and feed less often (Jakobsen and Johnsen 1989). Although these studies do not directly compare growth in trained and untrained fish, they do indicate that fish with more developed predator avoidance behaviors reduce feeding in the presence of predators and are less willing to take risks than hatchery fish. In the present study, this should have led to slower growth rates for predator-trained fish than control fish. This was not the case. Predator-trained and control fingerlings were not different in length or weight when recaptured during fall. Either the training program was insufficient to decrease foraging under threat of predation, or reduced foraging only occurred for a short time period after stocking.

RECOMMENDATIONS

1. Redesign/reattempt study to address the research question of whether the survival of fingerling rainbow trout stocked in large reservoirs may be improved sufficiently through predator training to increase return to creel rates. The two pilot studies have been heavily influenced by disease outbreaks (2002) and drought conditions (2003). These factors have reduced survival, introduced bias, and not allowed a sufficient assessment of the original research question.
2. Based on short-term sampling, predation of recently stocked fingerlings is intense, especially in reservoirs that possess abundant largemouth bass populations. The efficiency of fingerling plants in terms of contribution to the creel should be reevaluated on a case-by-case basis. Those systems where predation seemed excessively intense, such as Winchester Lake, may not be good candidates for future fingerlings plants.

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