



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

By

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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 16 high mountain lakes during 2001. During 2004, 779 fish were sampled, including 99 test fish. Of these 99 test fish, 56 diploids and 29 triploids were identified, yielding a grit mark retention rate of 86%. This equaled a mean catch rate of 1.8 triploid (± 2.1) and 3.5 diploid fish per lake (± 2.6). Based on overlapping confidence intervals, there was no statistical difference in length or weight between the test groups, possibly due to very small sample sizes; however, there was a tendency for the diploids to be about 10 mm longer and 75 g heavier.

Methods for producing triploid rainbow trout, walleye *Sander vitreus*, and westslope cutthroat trout *O. clarkii lewisi* were investigated during 2004. A direct comparison of pressure and heat was tested on spring and fall rainbow trout eggs at Hayspur fish hatchery. No measurable difference was seen between the performances of heat or pressure-treated spring eggs. For the fall eggs, a pressure treatment of 9,500 psi applied at 33 minutes after fertilization (MAF) and five-minute duration yielded 100% induction rates across five replicates and survival rates that were 95% relative to control eggs. For walleye, no efficient treatment was identified for producing triploids. For westslope cutthroat trout, pressure outperformed heat. The highest survival (96% relative to controls with a 99% induction rate) was provided by a pressure treatment of 9,500 psi applied at 26.3 MAF and five minute duration, whereas the highest induction rates (100%) were provided by a 9,500 psi applied at 17.5 MAF and five-minute duration (34% survival). Although the optimal treatment was between these two levels of MAF, the 26.3 MAF treatment is adequate for production purposes without further testing.

A study was initiated to determine the relative performance and longevity of diploid and triploid kokanee in lowland reservoirs. A total of 725 female kokanee and 470,455 eggs were used for creation of the triploid group. Eye-up rate was 38.5%, somewhat less than expected from a previous experiment. Results from a calcein marking pilot study indicated that this technique would be efficient for marking test groups due to high survival and good short-term retention and quality.

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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 and often contradictory. For example, 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 Alaska lakes and South Dakota ponds, respectively. 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. Teuscher et al. (2003) found that triploid rainbow trout survived at higher rates than diploid fish and grew at the same rate as diploid rainbow trout in two productive Idaho reservoirs. 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 putative sterile fish in systems where reproduction between wild and hatchery fish was possible (IDFG 2001). The establishment of this policy was based on research that indicated sterile rainbow trout are able to perform well in a wide range of stream habitats (Dillon et al. 2000) and in productive reservoirs (Teuscher et al. 2003). The implementation of this policy has also resulted in the widespread stocking of sterile rainbow trout in hundreds of Idaho high mountain lakes. However, no research was conducted on the performance of sterile rainbow trout in high mountain lakes prior to the implementation of the above-noted policy. 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.

Development of techniques to induce sterility in other hatchery-produced fish including westslope cutthroat trout *O. clarki lewisi*, walleye *Sander vitreus*, and kokanee could further reduce impacts of stocking on Idaho's native and wild fish populations or improve fishing. However, sterilization techniques for these species have yet to be developed and implemented in IDFG hatchery programs. The development of sterile westslope cutthroat trout would give managers another alternative for stocking high mountain lakes and would further reduce the potential for intraspecific hybridization throughout central and northern Idaho. Although walleye are only stocked in a few waters in southern Idaho, concern exists that illegal transfers from

these waters could lead to establishment of self-sustaining populations in other water bodies, as has happened in neighboring states such as Wyoming and Montana (Bennett and McMahon 1996; Rahel 2004; Mike Ruggles, Montana Department of Fish, Wildlife and Parks, personal communication). The stocking of sterile walleye could greatly reduce the possibility that transferred fish could develop self-sustaining populations.

Another potential benefit of sterility is increased longevity through elimination of normal gonadal development and associated spawning mortality (Ihssen et al. 1990). This could be beneficial in put-grow-and-take kokanee fisheries, as triploid kokanee have been shown to live longer (Johnston et al. 1993). Increased longevity could provide additional harvest opportunity in subsequent years, possibly for larger fish, when diploids would have already perished. This would seem especially true considering that kokanee vulnerability to angling appears to be quite strongly and positively associated with fish size (Rieman and Maiolie 1995).

In this progress report, I compare survival and growth of triploid and diploid rainbow trout that were stocked as part of a large-scale study in 16 central Idaho high mountain lakes during 2001. I also summarize efforts to refine techniques for producing triploid rainbow trout and westslope cutthroat trout, as well as walleye. Finally, I document initial efforts to examine the performance of diploid and triploid kokanee in lowland lakes and reservoirs.

RESEARCH GOAL

1. To enhance hatchery-supported resident fisheries through the use of sterile fish.
2. To reduce the genetic risks of IDFG's resident trout stocking program on indigenous rainbow and cutthroat trout populations.

OBJECTIVES

1. To determine, during 2003-04, the relative survival and growth of diploid and triploid rainbow trout in 16 high mountain lakes across a wide geographical area of Idaho and provide information on environmental characteristics that affect survival and growth.
2. To compare the accuracy of scales and otoliths for estimating age of trout from high mountain lakes.
3. To refine techniques for inducing triploidy in rainbow trout, westslope cutthroat trout, and walleye that provide high induction rates (95-100%) while maintaining adequate survival (not less than 75% of untreated fish).
4. To increase the longevity of kokanee through sterilization by at least one year, and thereby increase harvest rates by 25%.

METHODS

Performance of Sterile Trout in High Mountain Lakes

During 2001, IDFG regional fishery managers and U.S. Forest Service personnel provided high mountain lake information that facilitated study site selection. Only lakes scheduled for stocking in 2001 were considered for stocking with test fish. Test fish were not stocked in drainages where conflicts with native westslope cutthroat trout populations were possible or in lakes where brook trout populations were established. We preferentially selected lakes that were from five to 10 acres in surface area and had reasonable access from roads, yet were remote enough to keep harvest to a minimal level. Additionally, past surveys must have indicated that lakes were capable of supporting trout fisheries. Sixteen lakes were selected throughout central Idaho (Figure 1). All test lakes are managed under the general trout regulation of six fish per day with no length limit, except for Blackwell and Brush lakes, which are managed under the trophy regulation of two fish per day with none under 508 mm.

Mixed-sex rainbow trout eggs were produced from 1:1 pairings at Hayspur Fish Hatchery (FH). After fertilization, egg lots were split. Half of the eggs was reared normally and serve as the control in this study. The other half was placed in a 26°C water bath at 20 minutes after fertilization (MAF) and thermal-shocked for 20 minutes to induce triploidy (Teuscher et al. 1998). Eggs were incubated, reared in 1 m tanks, and transferred to raceways. Both groups were adipose clipped to indicate inclusion in this study when sampled in the field, and the diploid and triploid groups were grit marked with green and red fluorescent dye, respectively (Evenson and Ewing 1985; Nielson 1990). From August 30 through September 15, 2001, each lake was stocked with 300 mixed-sex diploid and 300 triploid rainbow trout from a fixed-wing airplane (Kozfkay and Megargele 2001).

Lakes were surveyed with floating gillnets and angling from July 16 to August 24, 2004. Experimental gillnets had 19, 25, 30, 33, 38, and 48 mm bar mesh panels (7.7 m each) and were 46 m long by 1.5 m deep. Typically, four to seven gillnets were set in the early afternoon and pulled the following morning. While the nets fished, the two- or three-person crew used spin- and fly-fishing gear to collect additional samples.

All captured fish, both test and non-test fish, were identified to species, measured 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 an industrial-strength black plastic garbage bag. Scale and otolith samples were collected from most test fish. Scales were stored in coin envelopes, whereas otolith samples were stored in alcohol filled microcentrifuge tubes (DeVries and Frie 1996). In the laboratory, scales were embedded in acetate with a scale press set at 10,000 psi for 30 seconds, whereas otolith samples were viewed whole (Boxrucker 1986). Each structure was examined with a dissecting microscope on 2-4 power by three viewers independently. If a consensus was not reached, the structures were re-examined by a pair of experienced readers to determine the final age estimate. Age estimates were compared to known age-1 and age-3 fish to determine precision and accuracy for the two structures.

Similar to IDFG standard high lake monitoring protocols, abiotic measurements were collected at each of the 16 lakes. A series of three transects were placed at equal distances perpendicular to the long axis of the lake with the aid of a laser rangefinder. Three sampling points were equally spaced along each transect. Depth measurements (m) were collected with a

handheld sonar unit at each of the nine sampling points to estimate average depth. Maximum depth was also determined with this unit. At each of the middle sampling points pH, conductivity $\mu\text{S}/\text{cm}$, surface water temperature ($^{\circ}\text{C}$), and Secchi depth measurements were collected. Additionally, one Hobo recording thermometer was placed in the lake approximately 0.6 m below the water's surface and 2-3 meters from shore. Elevation (m) and location were determined with the use of a handheld Global Positioning System (GPS) unit. Lake area was determined with Geographic Information Systems (GIS). In addition, amphibian surveys were conducted by slowly walking the entire perimeter of each lake along the water shore interface and looking near and under woody debris.

To assess whether abiotic and biotic characteristics may have affected test and non-test fish populations, I used correlation procedures to look at the association of explanatory variables, and secondly used stepwise multiple regression selection techniques to determine which models best fit the data. Four stepwise analyses were conducted to assess the affect of explanatory variables on: 1) the length of test fish at age-3, 2) the total number of fish (test and non-test) caught per lake, 3) the number of triploid fish caught, and 4) the number of test fish (diploid and triploid) caught combined. Analyses 3 and 4 were logistical regression due to the large proportion of zeros in the response variables. All analyses were completed in SAS (Version 8.2, SAS Institute Inc, Cary, NC).

Treatment Development for Sterile Fish Production

Rainbow Trout

Pressure or heat shocks are the two most commonly used techniques for inducing sterility in salmonids. In order to compare their relative effectiveness, I conducted thermal and pressure shock experiments to induce triploidy in rainbow trout at Hayspur FH on April 22, 2004. Fertilized eggs from each replicate were produced by combining the gametes of two females with two males. 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 five ways (four treatments and one control). The heat treatment was 26°C at 20 MAF for 20 minutes. The three pressure treatments were 9,500 psi and five-minute duration at 200, 300, & 400 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 L 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 treatment was applied. The heat shocking unit consisted of an individual 51 L insulated cooler. The cooler was 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 Hayspur Fish Hatchery. Eggs were enumerated at the eyed, hatch, and swim up stages. 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.

At Hayspur Fish Hatchery, the bulk of rainbow trout egg production and the highest quality eggs are collected from fall spawning fish. Additionally, survival rates and higher and more consistent during this time period. To provide a more robust assessment of the relative effectiveness of heat and pressure for inducing triploidy in rainbow trout, a second experiment was conducted at Hayspur FH on November 4, 2004. Fertilized eggs from each of the five replicates were produced by combining the gametes of six females with six males. 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 26°C at 20 MAF for 20 minutes. The two pressure treatments used 9,500 psi and five-minute duration at 300 and 375 CMAF. After treatment, eggs and fry were handled and sampled in the same manner as described in the previous paragraph, except that the commercial size heat bath was used instead of an experimental cooler (see Kozfkay 2003 for full description).

Walleye

Experiments designed to induce triploidy in walleye were performed in cooperation with personnel from Montana Department of Fish Wildlife and Parks' (MT FWP) Fort Peck Office and Miles City FH. Brood fish were captured with trap nets by MT FWP personnel from the Dry Arm of Fort Peck Reservoir, Montana and held in net pens until ready to spawn. On April 21, 2004, fertilized eggs were produced by combining the gametes of four females with four males. After one minute to allow fertilization, a slurry of Fuller's earth and lake water was added to the spawning bowl to make the eggs nonadhesive. After three minutes in the slurry, eggs were split in two; one-half was pressure treated, while the other half was not treated to allow comparisons of survival between treated and untreated eggs. The process was completed three times with additional groups of males and females to allow testing of three pressure treatments. The treatments consisted of 8,000 psi for 30 min duration, 9,000 psi for 12 min duration, and 9,500 psi for 5 min durations. The eggs for all treatments were introduced to the pressure chamber at 4 MAF. Due to the difficulty in rearing small groups of walleye at Miles City FH, I was unable to run replicates for any of the treatment-control combinations. Eggs were hatched in upwelling jars and were transferred as sac fry to outside rearing ponds. When fish reached approximately 30 mm, 60 blood samples were collected from each treatment by replicate group, stored in Aalsever's solution, and shipped to North Carolina State University where ploidy levels were determined with flow cytometry by Dr. Jeff Hinshaw.

Westslope Cutthroat Trout

I conducted a heat and pressure shock experiment to induce triploidy in westslope cutthroat trout at Hayspur FH on April 22, 2004. Two females were fertilized with the milt from four to five males for each of three replicates. Approximately equal numbers of fertilized eggs were split four ways (three treatments and one control). Survival and induction rates were compared. The most efficient heat treatment from a previous experiment was compared to pressure treatments that have worked well on other species. The treatments included 28°C at 10 MAF for 10 min duration as well as 9,500 psi at 200 and 300 CMAF for 5 min duration. After treatment, eggs and fry were handled and sampled in the same manner as described in the rainbow trout section.

Performance of Sterile Kokanee in Lowland Lakes and Reservoirs

In order to test the relative performance of diploid and triploid kokanee in lakes and reservoirs, I heat treated large groups of kokanee eggs (~2,500–9,000 per production treatment) from August 23 through September 7, 2004. Eggs were collected at a weir on the Deadwood River. The weir was installed and operated by Nampa FH personnel. Ripe kokanee were anesthetized and in each of four spawning bowls, the eggs of 4-13 females were fertilized with the milt of 4-13 males. An equal number of males and females were spawned for each bowl. After fertilization was initiated with the introduction of freshwater, eggs were allowed to sit for one minute, pooled, and transported to a temporary shelter. The eggs were then placed in a heat bath at 27°C at 20 MAF for 20 minutes. This treatment has been shown to perform well in a previous experiment on kokanee (induction rates 98% and survival rates of 64% relative to controls; Kozfkay 2002). After treatment, eggs were shipped to Mackay FH with other production eggs.

To evaluate the performance of large numbers of diploid and triploid kokanee stocked as fry, I needed a quick and efficient method for applying two distinct batch marks. Calcein has been shown to be a persistent mark for Atlantic and Chinook salmon *Salmo salar* and *O. tshawytscha*, as well as steelhead *O. mykiss* (Mohler 1997; Mohler 2003a; Mohler 2003b). However, since my experience with this technique is limited, I conducted a pilot marking study by immersing rainbow trout fry in calcein in the IDFG Eagle Fish Health Laboratory Wet Lab. Forty-five fry (\bar{x} = 30 mm and 0.5 g) were split into three separate groups and reared separately. On June 18, 2004, all 45 fish were immersed in a 1.5% salt solution for 3.5 minutes, followed by a brief rinse in freshwater and then transfer to a 0.5% calcein solution for 3.5 minutes. On August 6, 2004, I applied the second mark in the same manner, except that I varied the duration of immersion in the calcein bath. The durations were 3.5, 5, or 7 minutes. On October 26, 2004, I collected scale and otolith sample from the remaining fry. Scales and otolith were analyzed for mark quality using fluorescent microscopy.

RESULTS

Performance of Sterile Trout in High Mountain Lakes

During 2004 surveys, I sampled 779 salmonids from 16 lakes for a mean of 48.7 fish per lake (\pm 10.4; Table 1). The majority of fish collected (673 or 86%) were non-test fish that originated from previous stocking events or natural recruitment. Non-test fish sampled included rainbow trout, westslope cutthroat trout, Yellowstone cutthroat trout *O. clarkii bouvieri*, and hybrids *O. mykiss* X *O. clarkii*, as well as lesser numbers of brook by bull trout hybrids *Salvelinus fontinalis* X *confluentus*, grayling *Thymallus arcticus*, kokanee, and golden trout *O. mykiss aguabonita*.

Of the 779 fish caught, 99 were test fish from the 2001 stocking event. Nearly twice as many Hayspur-strain mixed-sex diploid rainbow trout (56) were sampled as Hayspur-strain mixed-sex triploid rainbow trout (29; Table 1). This yielded a mean catch rate of 1.8 triploid (\pm 2.1) and 3.5 diploid fish per lake (\pm 2.6). The highest catch of test fish (43) was collected from Raft Lake and was comprised of 19 triploids and 24 diploids. Catch for diploids was eight or less in all other lakes and four or less for triploids. No test fish from either group were caught in Brush, Cache Cr. #2, Josephine #2, and Squaw lakes. Moreover, no triploid fish were caught from six other lakes. Fourteen adipose clipped fish were caught for which no grit mark could be

found; thus, these fish could not be certainly identified to test group. This yielded a grit dye mark retention rate of 86% for the three-year period since test groups were marked. Three of the study lakes (Blue, Blackwell, and Ingeborg) used during this study were also stocked during 2003 for another assessment of the performance of sterile fish in high mountain lakes (Kozfkay 2003). Incidentally, seven test fish were caught from Blackwell Lake as age-1 fish from the 2003 stocking event. These fish consisted of six Trout Lodge all-female triploid rainbow trout and one diploid Hayspur-strain rainbow trout.

During 2004, gill nets were fished for 41 to 162 h per lake for a combined total effort of 1091 h (Table 2). Catch per unit effort (CPUE) with gill nets for all fish combined ranged from a low of 0.17 fish per hour in Blue Jay Lake to a high of 1.06 fish per hour in Long Lake. Angling surveys were also conducted in 15 of the 16 lakes surveyed. Effort ranged from one to 12 hours per lake for a combined total effort of 72.3 h. In Brush, Josephine Lake #3, Raft, and Washington lakes, CPUE for angling equaled zero. The highest CPUE recorded for angling was 7.1 fish per hour in Queens River Lake # 5. When only test fish were considered, there appeared to be a disparity in the ratio of triploid : diploid fish caught between the two methods. The ratio for gill nets was 1 triploids : 1.8 diploids, whereas the ratio for angled samples was 1 triploid : 6 diploids.

Due to small sample sizes, length and weight for diploid and triploid fish could only be compared for four lakes: Blue Jay, Ingeborg, Long, and Raft. Due to wide confidence intervals at least partially caused by the low number of samples, there were no statistical differences between any of the paired comparisons, except that the weight of triploid fish was significantly less than the weight of diploids in Raft Lake (Figures 2 and 3). For Raft Lake, the only lakes with adequate numbers from both groups, mean length and weight of diploids was 19 mm longer and 87 g heavier than that of triploids.

There were 11 habitat variables measured for the 16 lakes sampled (Table 3). Positive correlations were found between maximum depth and average depth ($r = 0.89$), elevation and pH ($r = 0.76$), conductivity and pH ($r = 0.63$), and surface water temperature and Secchi depth ($r = 0.46$; Table 4). Negative correlations were found between stocking density and lake surface area ($r = -0.85$) as well as average depth and conductivity ($r = -0.46$).

For the length of test fish at age-3, stepwise regression analysis indicated that the CPUE for all fish (test and non-test) and stocking density had the most effect on length achieved; combined they explained 64% of the variation in total fish length among the 16 lakes sampled. For the total number of fish caught per lake, including test and non-test fish, no abiotic or biotic variables were associated, and thus, no model fit the data. Also for the logistic regression analysis to determine what factors were associated with lakes where triploid fish were sampled, no model fit the data. Even though no logistic model fit the data, plots of stocking density versus whether triploid fish were present or absent seemed to indicate a pattern for stocking density. Although there was obvious overlap, triploid fish were never present in lakes that were stocked at densities greater than 261 FPH (Figure 4). For the total number of test fish caught (diploid and triploid combined), no logistic model fit the data. Plots of abiotic and biotic habitat variables and whether test fish were present or absent indicated that there was strong overlap.

For known age-3 fish, consensus scale and otolith estimates were available from 63 fish. Mean ages for scale ($\bar{x} = 3.0 \pm 0.1$) and otolith samples ($\bar{x} = 2.9 \pm 0.1$) were not statistically different across the 63 fish. From scales, 50 out of 63 fish were correctly aged (79%; Figure 5). Of the remainder, seven were estimated as age-2 (11%), five as age-4 (8%), and one as age-5

(2%). From otolith samples, 56 out of 63 fish were correctly aged (89%). Of the remainder, six were estimated as age-2 (10%) and one as age-4 (1%).

For known age-1 fish, consensus scale and otolith estimates were available from six fish. From scales, five out of six fish were correctly aged (83%). Of the remainder, one was aged as age-2 (17%). From otolith samples, two out of six fish were correctly aged (33%). Of the remainder, four fish were estimated as age-2 (67%).

Consensus ages for both scale and otolith samples were reached for 313 fish, including known and unknown age fish. Mean ages for scale ($\bar{x} = 2.8 \pm 0.1$) and otolith samples ($\bar{x} = 2.9 \pm 0.1$) were not statistically different across the 313 fish. However, this alone is misleading. Scale samples tended to give higher estimates than otolith samples for fish aged as one and two (Figure 6). For fish aged as one from otolith samples ($n = 25$), average age from scales equaled 1.6 ± 0.2 . For fish aged as two from otolith samples ($n = 85$), average age from scales equaled 2.2 ± 0.1 . Estimates were equal for age three. For fish aged as three from otolith samples ($n = 134$), average age from scales equaled 3.0 ± 0.1 . Estimates from scales were much lower than from otolith samples for ages four through eight (Figure 6). For fish aged as four from otolith samples ($n = 43$), average age from scales equaled 3.3 ± 0.3 . For fish aged as five through eight from otolith samples ($n = 24$, $\bar{x} = 5.4 \pm 0.3$), average age from scales equaled 3.9 ± 0.4 .

Production of Sterile Trout

Rainbow Trout—Spring

Due to an error during enumeration of survival for the spring experiment, information from one replicate of the 26°C : 20 MAF : 20 duration treatment was lost. Therefore, only comparisons for the second and third replicates could be made across all treatments. For these two replicates, survival of treated eggs relative to controls ranged from 57-96% (mean control survival = 58%; Table 5). The highest survival of 56% for treated eggs was provided by the 26°C : 20 MAF : 20 duration treatment. Within the three pressure treatments tested, survival increased as CMAF increased.

Mean induction rates were high for three of the four treatments tested. For the 26°C : 20 MAF : 20 duration and the 9,500 psi : 300 CMAF : 5 min duration treatments, all samples ($n = 150$) tested were triploid. Additionally, for the 9,500 psi : 200 CMAF : 5 min duration treatment, 74 out of 75 samples analyzed were triploid, for a mean induction rate of 99%. Induction rates declined to 89% for pressure treatment at the highest interval after fertilization (400 CMAF).

Rainbow Trout—Fall

Overall survival of treated and untreated eggs for the fall groups was much higher than were those collected during the preceding spring. Survival of controls to eye-up was 95%, whereas that of treated eggs did not drop below 87% (Table 6). The highest survival of treated eggs was provided by the 26°C : 20 MAF : 20 duration heat treatment and equaled 91%; however, survival for the 9,500 psi pressure treatment at 33 MAF was only one percent less.

Nine of the 15 treatment by replicate combinations analyzed yielded 100% induction rates. All replicates ($n = 5$) for the 9,500 psi : 33 MAF : 5 min duration treatment equaled 100%.

Mean induction rate for the other pressure treatment (9,500 psi : 26.3 MAF : 5 min) equaled 98.6%, whereas the lowest mean induction rate was 96% for the best heat treatment identified in previous experiments (Kozfkay 2003).

Walleye

Survival of individual groups of treated and untreated walleye eggs was highly variable. For the 8,000 psi for 30 min duration treatment, survival for both groups was <1%, and no individuals survived to reach a size large enough for blood analysis. For the 9,000 psi : 12 min duration treatment, survival of treated eggs was 1%, whereas that of untreated was 17%, indicating that this treatment performed poorly. The highest survival was provided by the 9,500 psi : 5 min duration treatment for which survival of controls was 43% compared to 32% for treated eggs.

Induction rates were only analyzed for the 9,000 psi for 12 min duration and 9,500 psi for 5 min duration treatments. Induction rate for the 9,000 psi treatment was 93% (56 triploids out of 60 fish tested). Induction rate for the 9,500 psi treatment was 47% (28 triploids out of 60).

Westslope Cutthroat Trout

Survival of treated eggs to eye-up was 63-97% of untreated eggs. The highest survival of 52% was provided by pressure shocking eggs at 9,500 psi : 300 CMAF : for 5 min duration (Table 7). Survival decreased to 34% when pressure shocks were applied earlier at 200 CMAF. Survival of the best heat treatment identified previously (Kozfkay 2003) was intermediate at 41%.

Triploid induction rates were high for all treatments tested. Of the nine groups (3 treatment x 3 replicates) analyzed, seven groups were determined to be 100% triploid. For the 9,500 psi : 200 CMAF : 5 min duration treatment, mean induction rate was 100%. For the 9,500 psi : 300 CMAF : 5 min duration treatment, the mean induction rate was 99%. The lowest induction rate of 96% was provided by the 28°C : 10 MAF : 10 duration treatment.

Performance of Sterile Kokanee in Lowland Lakes and Reservoirs

A total of 725 female kokanee were used for creation of the triploid group. Average fecundity was approximately 649 eggs, yielding 470,455 green eggs. From these eggs, 180,946 eggs survived to eye-up for an eye-up rate of 38.5%. Survival to eye-up was highly variable across spawning days and ranged from 35-58%. Although diploid groups were not true controls, they do act as a good reference for comparison of survival between groups. Over the five days when both diploid and triploid eggs were collected, relative eye-up for triploids ranged from 47-102% to that of diploids spawned on the same day. Mean survival to eye-up for diploids and triploids collected on the same day were 57 and 39%, respectively.

For the calcein marking pilot study, survival of marked fish was high, as was mark retention and quality. Only two fish (4%) did not survive the marking procedure and the 82 day rearing period until scale and otolith samples were collected. All scale and otolith samples possessed distinct clear green marks that showed up as a single band in the otoliths and multiple bands in the scales.

DISCUSSION

Performance of Sterile Trout in High Mountain Lakes

Gill net and angling surveys for high mountain lakes stocked in 2001 indicated that the relative survival of Hayspur-strain diploid fish far exceeded that of Hayspur-strain triploid fish. The overall ratio was two diploids caught for every one triploid caught. This result very closely mimics the results from the pilot study, where over three years of sampling (2001-2003), the ratio of diploid: triploid fish caught was 2.3 : 1 (Kozfkay 2003). These results contradict previous work designed to determine the relative survival of triploid and diploid rainbow trout in Idaho streams (Dillon et al. 2000) and reservoirs (Teuscher et al. 2003). There are some key differences between these studies. For instance, both Dillon et al. (2000) and Teuscher et al. (2003) used different strains of rainbow trout, Mt. Lassen Mixed Sex and Trout Lodge All-Female rainbow trout, respectively. Secondly, these studies were conducted in different habitats. The reservoir study was conducted in two of the most productive systems in Idaho, whereas the stream study encompassed streams with varying levels of productivity during the summer months using catchable-sized fish. High mountain lakes are thought to be harsher environments than streams in summer and reservoirs and the poor survival of triploid fish was likely due to these differences. In other systems with questionable water quality, such as South Dakota ponds, triploid rainbow trout have also been shown to perform poorly (Simon et al. 1993).

High mountain lakes may present stressful environments for triploid fish. Several studies have indicated that triploid fish may not perform well under intense competition or chronically stressful conditions. For instance, when given the opportunity to feed on *Daphnia* sp. in direct competition, juvenile triploid saugeye *S. canadense* x *S. vitreus* were less efficient predators than diploid saugeye (Czesny 2000). Similarly, Galbreath et al. (1994) demonstrated that triploid Atlantic salmon grew at faster rates than diploid fish when reared separately, but when fish were combined into a common rearing tank diploid fish grew faster than triploids. In the present study, if competition with diploids was a key factor, then I might have artificially lowered the survival of triploid fish by stocking them with diploids.

Triploid fish have lower hemoglobin-oxygen ratios than diploid fish (Graham et al. 1985), which does not allow them to store enough oxygen during times of sustained high demand. This factor is thought to contribute to higher mortality and poorer growth of triploid fish during periods of high water temperatures (Ojolick et al. 1995). Currently, I do not know if high water temperatures were a factor in the poor survival of triploid fish in some lakes. Retrieval of thermographs and analysis of this data during 2005 should further elucidate this relationship. Other chronic environmental stressors may include low temperatures or low dissolved oxygen levels. Measurements from high mountain lakes indicate that lakes may become hypoxic during winter (Nelson 1988). Nelson (1988) and Fredericks et al. (1999) also indicated that winterkills were not uncommon. However, I am aware of no studies that look at the effect of these characteristics (low temperature or oxygen levels) on triploid survival, and I was unable to measure oxygen levels throughout the study period.

Stocking density appeared to be associated with the survival of triploid fish in our study lakes, although this conclusion is admittedly speculative. Lakes that were stocked at lower stocking densities tended to have better triploid fish survival. This suggests to some degree that

competition was affecting survival of triploids. Presumably at higher stocking densities (>261 fish/ha), competition for limited resources was intense enough to negatively influence the survival of triploid rainbow trout. No other variables predicted or explained the number of test fish caught during this study.

Based on overlapping confidence intervals, there was no statistical difference in the length and weight of diploid test fish among the four lakes where more than two test fish were caught per group. Small sample sizes contributed to wide confidence intervals, making this result fairly inconclusive. Based on mean values alone, there seemed to be a tendency for the diploid group to be about 20 mm longer and 75 grams heavier. It is doubtful that additional samples collected during 2005 will provide tighter confidence intervals. However, the larger numbers of fish stocked during 2003 should allow greater returns and a better assessment of this question when lakes are sampled in 2006. Relative growth of diploid and triploid fish may be age specific. Teuscher et al. (2003) noted that diploid fish grew faster through age-3, but afterwards as diploid fish started to put more energy into gonadal development, growth of triploid fish exceeded that of diploid fish through age-4. Brock et al. (1994) found that all triploid female rainbow trout were smaller than mixed-sex diploid rainbow trout produced from the same parents in each of six Alaska lakes. This trend continued through age-2. Unfortunately, no samples were collected from older fish to see if relative growth changed after sexual maturation.

Growth of test fish through age-3 was related to CPUE for all fish (test and non-test fish) and stocking density. Based on this information, growth in the high mountain lakes I examined seemed to be density dependent. CPUE effort for all fish was likely a good index of abundance (Schindler et al. 2001). In those lakes with the higher CPUE (abundances), growth rates were slower. Secondly, at higher stocking densities, growth was slower. For two out of three of the slowest growing lakes, stocking densities exceeded 425 fish/ha. In high mountain lakes of western Alberta, the variation in growth of rainbow trout was explained by three factors: total dissolved solids (42%), stocking density (30%), and to a lesser amount mean depth (3%; Donald and Anderson 1982). Additionally, they studied growth of brook trout in the same study area (Donald et al. 1980). Over the 23 lakes sampled, the variation in brook trout length at age-5 was explained by amphipod density (54%), maximum depth (11%), and specific conductance (7%).

Consensus age estimates from whole otoliths were more accurate than were those from scales for known age-3 fish. Estimates from scales correctly indicated three annuli 79% of the time, whereas estimates from otoliths correctly indicated three annuli 89% of the time. Since no preparation is required for aging whole otoliths, use of this structure is more efficient in terms of accuracy and time efficiency. During 2004, otoliths were stored in alcohol as recommended by DeVries and Frie (1996). This storage method led to clearing and crystallization to varying degrees in most otoliths, making some otoliths unreadable. I suspect that if the otoliths were stored dry, as done by other IDFG researchers, the disparity in accuracy would have been even greater. Assuming this pattern of accuracy between structures was consistent for older unknown age fish, the disparity seemed to increase markedly for fish aged as five or greater from otoliths. Scale estimates for these fish were on average 1.5 years younger. Age estimates from otoliths have been shown to be more accurate in other species, including white crappie *Pomoxis annularis* (Boxrucker 1986), walleye *Sander vitreus* (Isermann et al. 2003), and striped bass *Morone saxatilis* (Welch et al. 1993).

In terms of natural reproduction and whether stocked fish were a substantial part of existing fish populations, lakes were classified into one of three categories: 1) high percentage of fish from previous stocking events and limited or no natural reproduction, 2) intermediate

percentage of fish from previous stocking events and intermediate levels of natural reproduction, and 3) nearly all fish from natural production and limited or no contribution from previous stocking events. Raft and Blue Jay fell into category 1 (Table 8). Only two age classes of fish were present, age estimates coincided with past stocking events, and marked fish represented a high proportion of the catch: 98 and 65%, respectively. Blackwell, Blue, Long, and Shaw Twins most likely also fell into category 1. However, marked fish represented a smaller portion of the catch (6-15%). Since these lakes are stocked at frequent intervals (1-2 years), it was impossible to separate wild and hatchery fish based on age estimates (due to aging error). Josephine #2, Ingeborg, NF 20 mile #3 and #4, and Washington lakes fell into category 2. For these lakes, a combination of factors were present, such as a considerable number of hybrids (indicative of at least some successful spawning), an intermediate number of marked fish present in the catch, and fish populations that did not closely mimic past stocking records. Brush, Cache Cr. #1, Queens River #5, and Squaw lakes fell into category 3. Fish populations were characterized by one or a combination of factors, such as considerable number of hybrids, lack of marked fish present, or near total lack of fish present from previous stocking events. Cache Creek Lake #2 was not placed into any of the categories, because it lacks a stocking history and the lake is too shallow to hold substantial fish populations.

Treatment Development for Sterile Fish Production

A central question of sterile fish research is whether heat or pressure shock is more effective at producing triploid fish. For Hayspur rainbow trout, the spring 2004 experiment was inconclusive due to the loss of one replicate from the heat shock group. Induction rates were 100% for both the heat shock treatment and one of the pressure treatments (9,500 psi and 26.3 MAF). Based on the two comparable replicates, survival to eye-up was slightly higher for the heat shock treatment. Within the three pressure treatments, survival was highest when the shock was applied at 400 CMAF, but by applying a shock this late, induction rates declined. For the second comparison of heat and pressure on Hayspur rainbow trout during fall, overall survival was much higher due to better egg quality of fall fish. For this comparison, one pressure treatment provided 100% induction rates across all five replicates, while the mean induction rate for the heat treatment was 96%, exactly the same as has been seen for induction rate monitoring over the past several years. Survival to eye-up was 91% for heat and 90% for pressure treatment at 9,500 psi at 33 MAF, whereas survival of controls was 95%. Due to the small drop in survival and 100% induction rates, Hayspur rainbows should be treated with 9,500 psi at 33 MAF for 5 min duration instead of the 26°C at 20 MAF for 20 min duration treatment used during the last several years.

Survival and induction rates for pressure treated walleye were highly variable and did not indicate that a specific treatment performed well enough to be used for production purposes. The treatment that has been identified in the literature as being effective (Malison et al. 2001) provided survival rates of approximately 1%; however, survival of controls for this treatment were also about 1%. Therefore, this result was likely due to poor egg quality. Relatively high survival was provided by a 9,500 psi treatment but induction rates were poor. Conversely, the 9,000 psi treatment provided good induction rates but poor survival. Additional testing with modifications of these treatments will be needed to identify an effective treatment.

For westslope cutthroat trout, pressure treatment was more effective than heat. The highest survival was provided by 9,500 psi at 300 CMAF, whereas the highest induction rates were provided by 9,500 psi at 200 CMAF. Survival was much lower for the 200 CMAF treatment

than for the 300 CMAF treatment. Based on this information, the optimal treatment is probably between the two levels of CMAF tested.

Performance of Sterile Kokanee in Lowland Lakes and Reservoirs

Survival for mass produced sterile kokanee was highly variable. The variable survival may have been due in part to fluctuating river water temperatures. Higher or lower river water temperatures would submit fish to a more mild or intense heat shock than for fish held at a constant temperature as has been done in all of our previous (in-hatchery) sterilization experiments. Under these circumstances, pressure shock would probably be a much better alternative because water temperature remains fairly constant during treatment and, therefore, treatment across groups of eggs would be more consistent. Nonetheless, survival was sufficient to allow field evaluation of triploid and diploid kokanee provided that induction rates were high. Flow cytometric analysis will not be available for these groups until summer 2005.

The use of calcein during the pilot marking study appeared to be a viable option for use in the field evaluation. Very little mortality was seen in marked groups, and mark quality was good over the short term. However, we were unable to assess mark retention over the long term. Additionally, we were unable to assess whether a second marking period led to a second mark in scales due to the inability to hold these fish for a sufficient time between mark applications.

RECOMMENDATIONS

1. Re-evaluate current stocking strategies for sterile Hayspur rainbow trout in high mountain lakes. From the pilot study and the present evaluation, it is evident that triploid Hayspur rainbow trout survive at about half the rate of diploids when stocked together. A simple solution to this problem would seem to be to double our current stocking rates. However, there did seem to be an effect of stocking density, so increasing stocking density might not increase the number of stocked fish available to anglers. Alternatively, other strains of rainbow trout may perform better. Results from an additional stocking in 2003 will become available during 2006-2007 and will provide information as to whether Troutlodge all-female rainbow trout provide better performance in high lakes.
2. Refocus managerial high mountain lake surveys to determine whether lakes actually need stocked fish to maintain quality fisheries. Standard high mountain lake surveys should include an assessment of natural recruitment and contribution of stocked fish to existing populations using batch marked fish. During this study, the use of visual estimation of spawning habitat was inefficient in predicting whether there was sufficient reproduction to maintain populations without further stocking. For instance, the subset of lakes used in this study, at least four to as many as nine of the 16 lakes studied, probably would sustain fishable populations without further stocking.
3. Stock Hayspur triploids at densities of 250 fish/ha or less in high mountain lakes.
4. Use a pressure treatment of 9,500 psi at 33 MAF for 5-minute duration to induce triploidy in rainbow trout at Hayspur Fish Hatchery.

5. Use a pressure treatment of 9,500 psi at 26.3 MAF for 5-minute duration to induce triploidy in westslope cutthroat trout at Hayspur FH.
6. Redesign and retest pressure treatment to induce triploidy in walleye.
7. Determine whether pressure treatment will provide higher eye-up rates for sterile kokanee.
8. Proceed with the use of calcein as a batch marking technique for differentially marking two groups of kokanee and evaluate long-term retention in the wild using a secondary mark.

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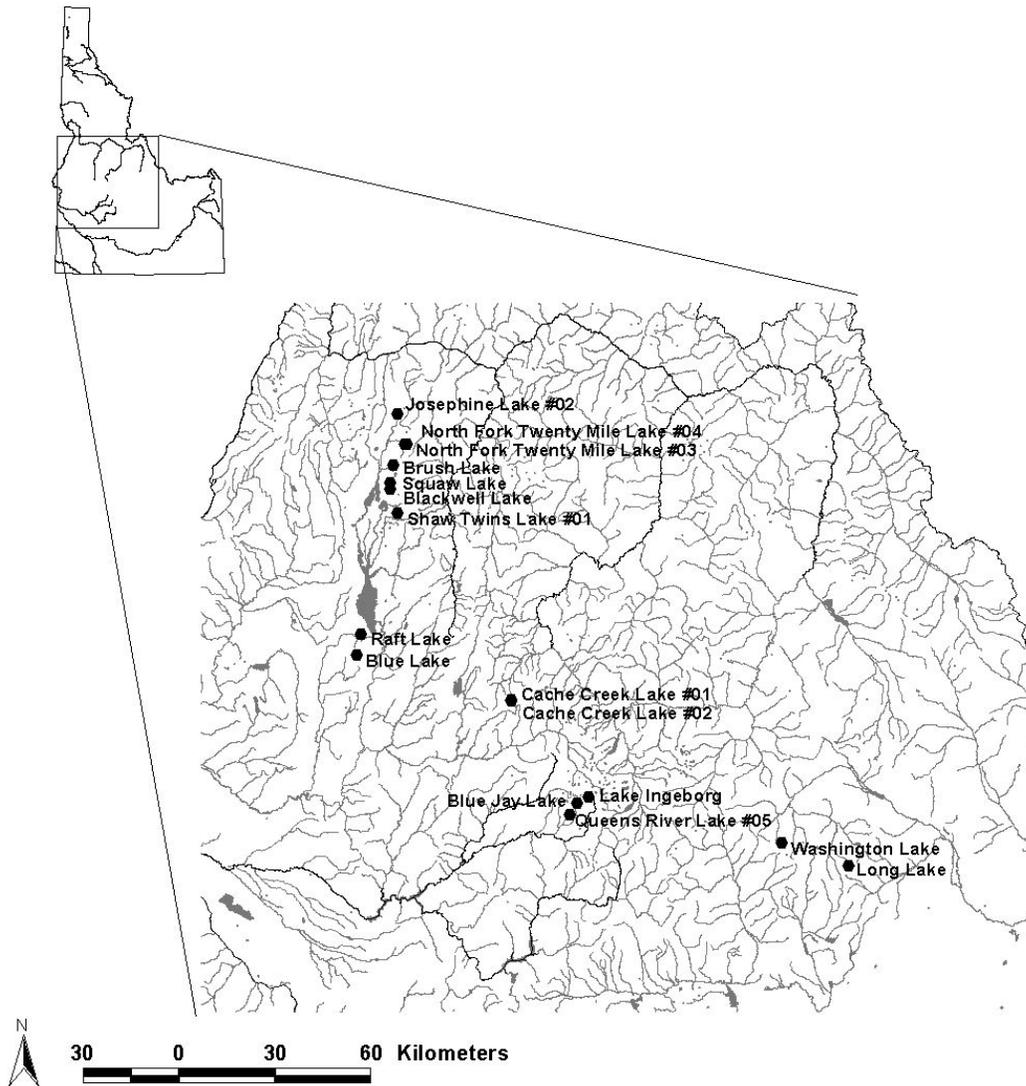


Figure 1. Locations of 16 mountain lakes in central Idaho used to compare the relative performance of mixed sex diploid and mixed sex triploid rainbow trout. Lakes were stocked with 300 fish of each group during 2001 and sampled during 2004.

Table 1. Total catch of non-test fish, diploid, and triploid rainbow trout caught during surveys conducted on 16 high mountain lakes during 2004. Test fish were stocked as fry during summer 2001. Additionally, as three of these lakes were used for another evaluation of sterile fish performance in high mountain lakes and stocked again 2003, seven test fish were caught early and incidentally to target test fish.

Lake Name	Total fish caught	Non test fish	2001 Stocking Event			2003 Stocking Event	
			Hayspur Triploid	Hayspur Diploid	No mark w/ad clip	Trout Lodge All Female	Hayspur Diploids
Blackwell	53	45	—	1	—	6	1
Blue Jay	20	7	4	8	1	—	—
Blue	82	77	1	1	3	—	—
Brush	24	24	—	—	—	—	—
Cache Creek #01	74	73	—	1	—	—	—
Cache Creek #02	23	23	—	—	—	—	—
Josephine #02	47	47	—	—	—	—	—
Ingeborg	35	27	2	6	—	—	—
Long Lake	64	56	2	6	—	—	—
NF 20 Mile #03	53	51	1	1	—	—	—
NF 20 Mile #04	80	74	—	3	3	—	—
Queens R. #05	90	89	—	1	—	—	—
Raft	50	1	19	24	6	—	—
Shaw Twins #01	26	23	—	2	1	—	—
Squaw	21	21	—	—	—	—	—
Washington	37	35	—	2	—	—	—
Totals	779	673	29	56	14	6	1

Table 2. Catch per unit effort (CPUE) for non-test fish, as well as diploid and triploid rainbow trout surveys conducted during 2004 on 16 high mountain lakes. Test fish were stocked as fry during summer 2001.

Lake Name	Angling CPUE				Gill Net CPUE			
	Total	Non test fish	Hayspur Triploid	Hayspur Diploids	Total	Non test fish	Hayspur Triploid	Hayspur Diploids
Blackwell	0.67	0.67	0.00	0.00	0.86	0.72	0.00	0.02
Blue Jay	5.60	2.40	0.80	2.40	0.17	0.07	0.04	0.07
Blue	1.45	1.45	0.00	0.00	1.32	1.29	0.02	0.02
Brush	0.00	0.00	0.00	0.00	0.59	0.59	0.00	0.00
Cache Creek #01	0.00	0.00	0.00	0.00	1.37	1.35	0.00	0.02
Cache Creek #02	0.33	0.33	0.00	0.00	0.33	0.33	0.00	0.00
Josephine #02	0.00	0.00	0.00	0.00	0.88	0.88	0.00	0.00
Ingeborg	1.09	1.09	0.00	0.00	0.34	0.25	0.02	0.07
Long Lake	0.77	0.46	0.00	0.31	1.06	0.95	0.04	0.07
NF 20 Mile #03	2.67	2.67	0.00	0.00	0.76	0.72	0.02	0.02
NF 20 Mile #04	0.59	0.47	0.00	0.12	0.93	0.91	0.00	0.02
Queens R. #05	7.14	7.14	0.00	0.00	0.80	0.79	0.00	0.01
Raft	0.00	0.00	0.00	0.00	0.31	0.04	0.12	0.15
Shaw Twins #01	0.67	0.67	0.00	0.00	0.38	0.34	0.00	0.03
Squaw	0.67	0.67	0.00	0.00	0.41	0.41	0.00	0.00
Washington	0.00	0.00	0.00	0.00	0.67	0.64	0.00	0.04

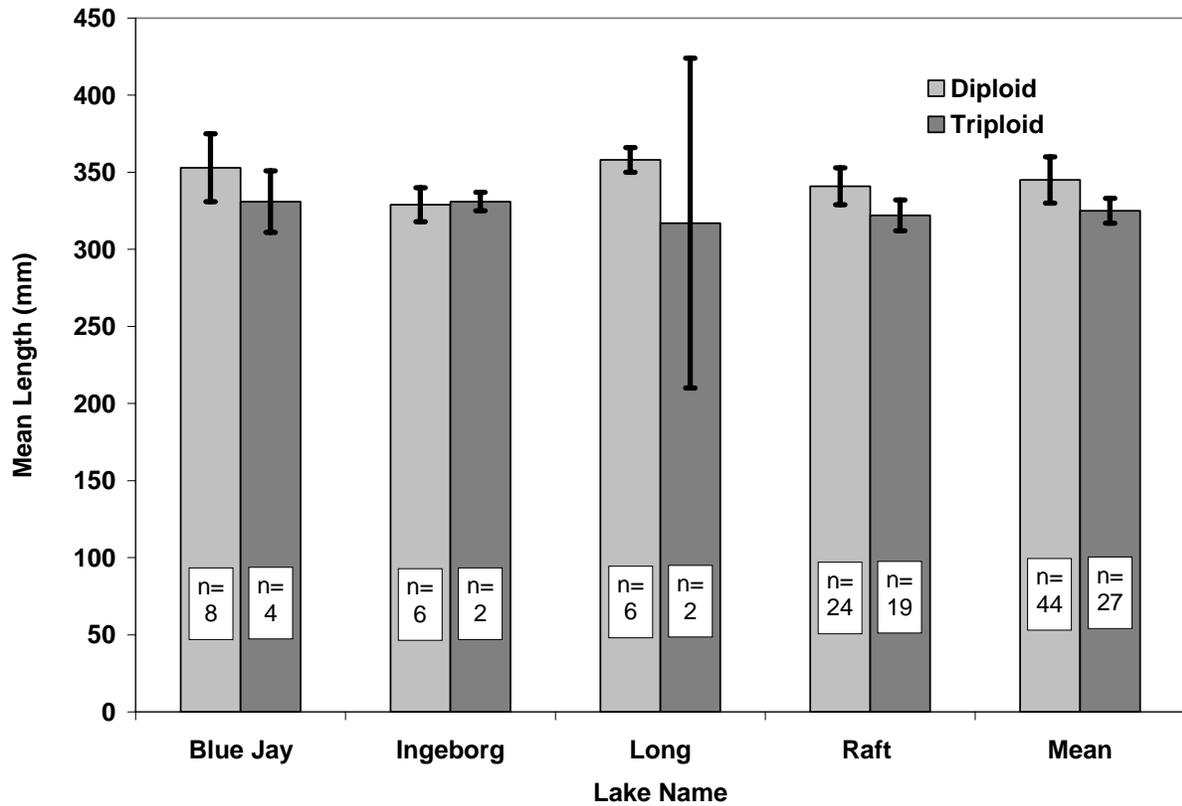


Figure 2. Mean length of triploid and diploid rainbow trout for lakes where two or more test fish were caught from each group. Lakes were stocked during 2001 and sampled during July 2004. Errors bars indicate 90% confidence intervals.

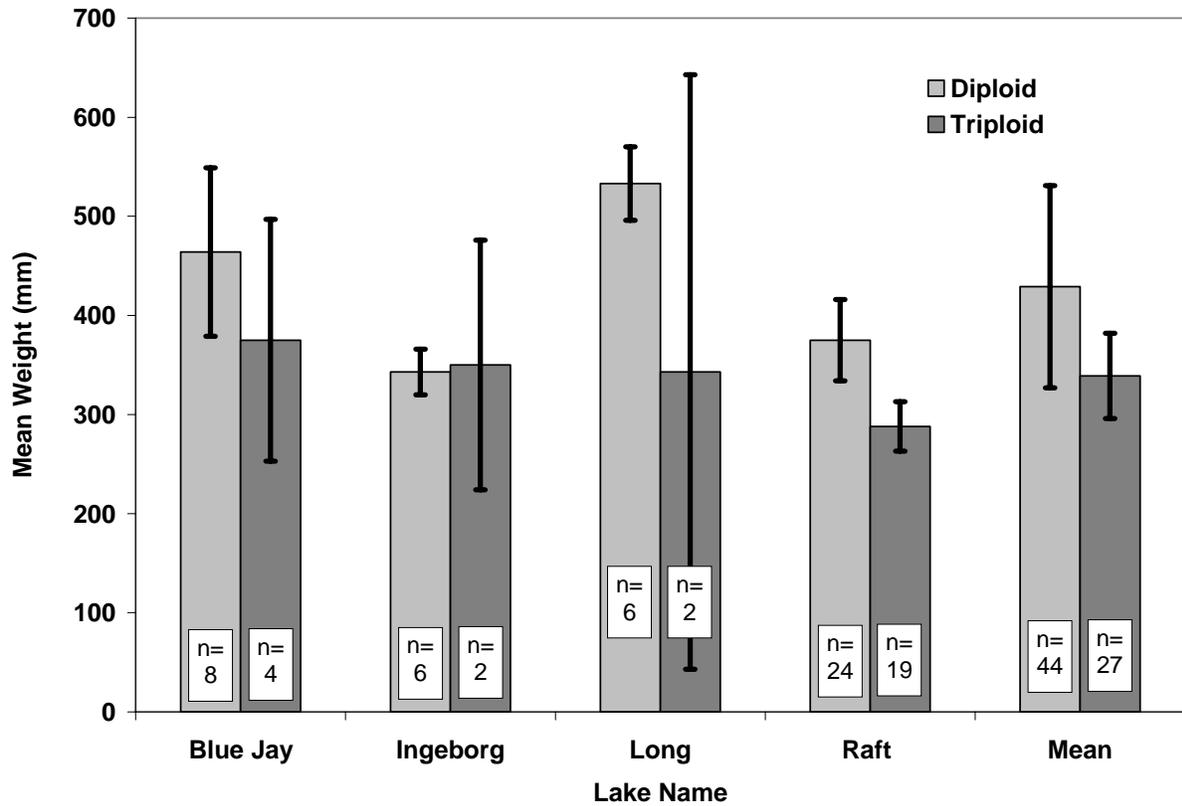


Figure 3. Mean weight of triploid and diploid rainbow trout for lakes where two or more test fish were caught from each group. Lakes were stocked during 2001 and sampled during July 2004. Errors bars indicate 90% confidence intervals.

Table 3. Description of study waters in Idaho stocked with diploid and triploid rainbow trout fry in 2001.

Lake Name	Stocking Density	Avg Depth (Meters)	Max Depth (Meters)	Size (ha)	Elevation (Meters)	Avg pH	Avg Conductivity	Avg Temp	Secchi
Blackwell Lake	113	7.3	10.8	5.3	2151	7.4	14.3	20.3	2.8
Blue Jay Lake	261	4.3	5.2	2.3	2614	7.8	5	18.4	6
Blue Lake	120	9.4	17.5	5	2250	7.5	9	14.2	4.2
Brush Lake	80	4.3	8.7	7.5	2179	7.3	4	15	5.5
Cache Creek Lake #01	429	4.5	8.3	1.4	2370	7.7	17	8.3	2.1
Cache Creek Lake #02	333	1.6	7.1	1.8	2334	7.6	20	12	3.1
Josephine Lake #02	120	7.5	17.3	5	2263	7.6	8	17.2	4.5
Lake Ingeborg	63	8.7	14.2	9.5	2723	7.6	1.7	16.8	6
Long Lake	107	1.6	2.8	5.6	2907	8.7	41.3	15.1	5.8
NF Twenty Mile Lake #03	167	4.5	7.8	3.6	2403	7.3	18.3	12.3	3.3
NF Twenty Mile Lake #04	92	6.1	7.8	6.5	2388	7.2	10.3	13.4	5
Queens River Lake #05	194	6.6	14.4	3.1	2561	7.8	3	18.2	4.2
Raft Lake	240	5.2	8.6	2.5	2043	6.9	8.3	14.8	2.7
Shaw Twins Lake #01	214	8.0	10.3	2.8	2213	7	6.7	19.7	7
Squaw Lake	286	4.5	6.6	2.1	2150	7.2	6	22.2	4.5
Washington Lake	500	12.5	20.2	1.2	3157	7.9	14	10	3.9

Table 4. Parameter estimates, confidence intervals, and correlation coefficients for habitat measurements collected during 2004 high mountain lake sampling. All units are metric. Statistically significant correlations are bolded. Correlations were deemed significant at $P = 0.10$.

	Correlation Matrix										
	Mean Value	90% CI	Stocking Density	Average Depth	Max Depth	Size	Elevation	pH	Conductivity	Temperature	Secchi
Stocking Density	207	56.2	1								
Average Depth	6	1.2	0.12	1							
Max Depth	10	2.1	0.09	0.89	1						
Size	4	1.0	-0.85	0.04	0.07	1					
Elevation	2419	133.1	0.28	0.25	0.20	0.00	1				
pH	8	0.2	0.08	-0.16	-0.05	0.05	0.76	1			
Conductivity	12	4.2	0.06	-0.46	-0.42	-0.05	0.35	0.63	1		
Temperature	15	1.7	-0.42	0.01	-0.10	0.16	-0.33	-0.22	-0.38	1	
Secchi	4	0.6	-0.37	0.08	-0.09	0.33	0.25	0.13	-0.18	0.46	1

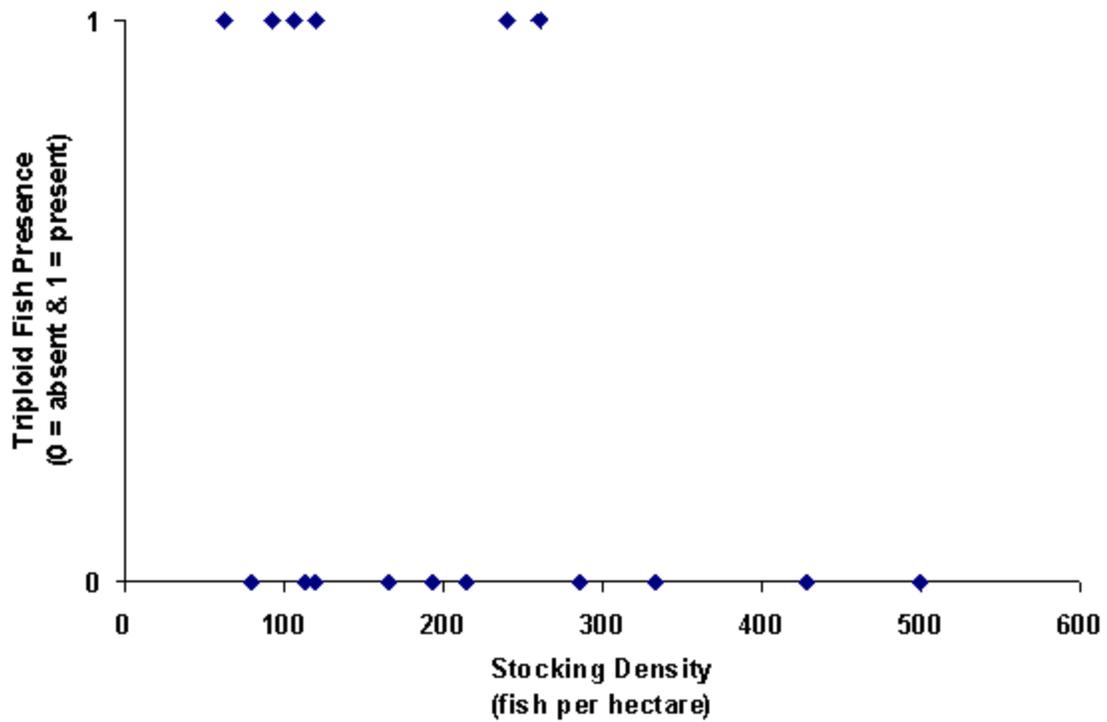


Figure 4. Relationship between stocking density and presence/absence of triploid Hayspur-strain rainbow trout in 16 high mountain lakes stocked during 2001 and sampled during 2004.

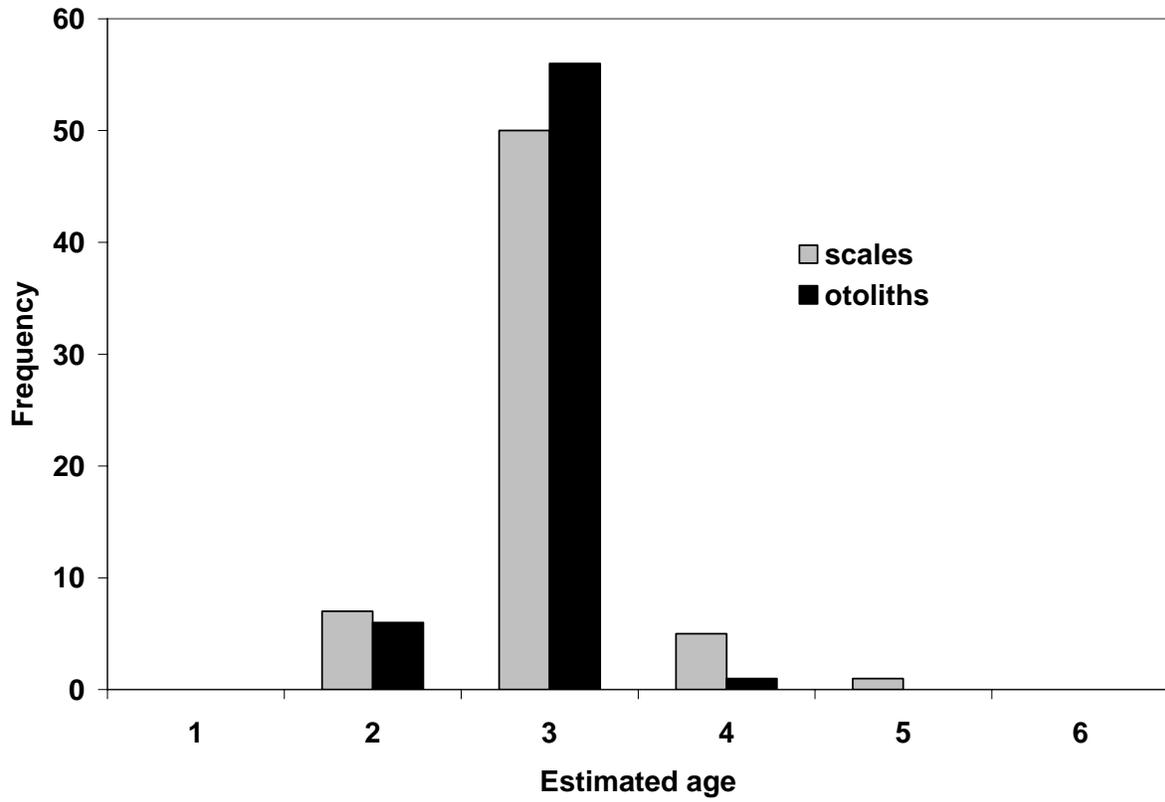


Figure 5. Frequency of age estimates from scale and otolith samples for known age-3 fish sampled from high mountain lakes during 2004.

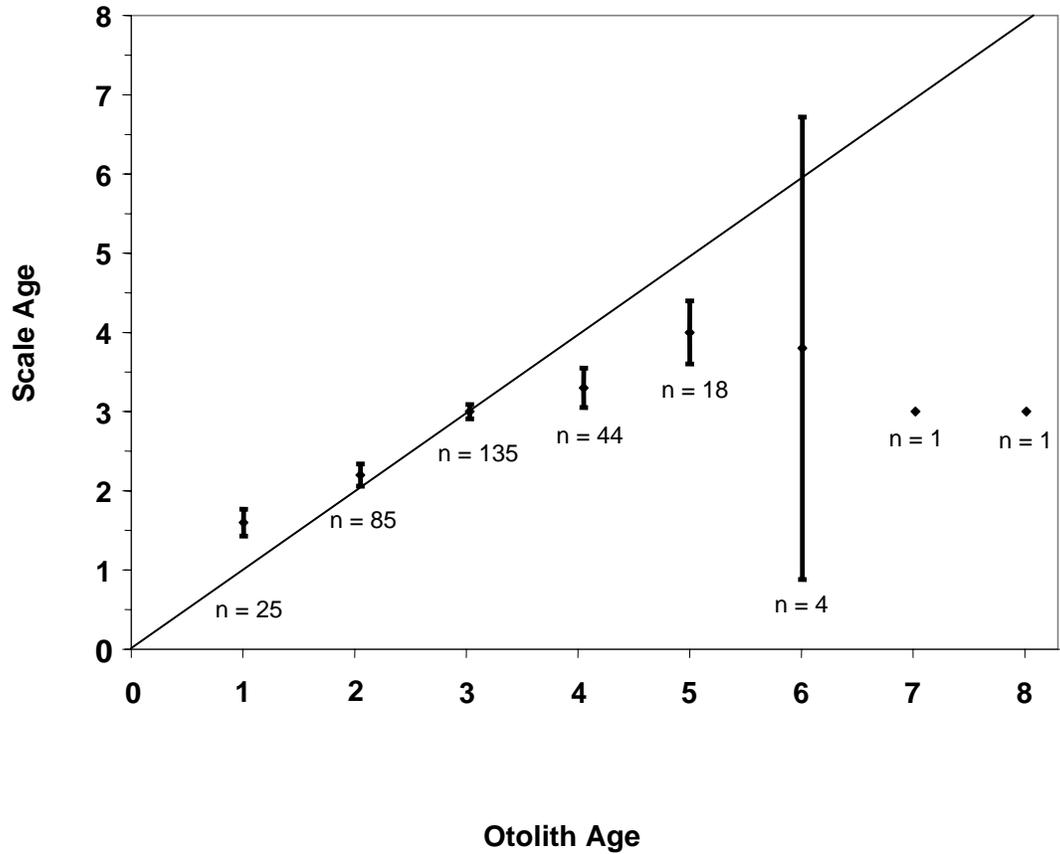


Figure 6. Comparison of consensus scale and otolith ages for known- and unknown- age trout sampled from high mountain lakes during 2004. Otolith samples (x-axis) were used as a reference to compare corresponding consensus scale sample age estimates across the range of ages sampled. Errors bars indicate 90% confidence intervals.

Table 5. Survival and triploid induction rates from an experiment designed to directly compare the efficiency of heat and pressure treatments for inducing triploidy in Hayspur-strain rainbow trout. Experiments were conducted at Hayspur Fish Hatchery during spring 2004. Minutes after fertilization and eyed eggs are abbreviated as MAF and EE, respectively.

Temperature / Pressure	MAF	Duration	Replicate	Survival to EE (%)	Mean Survival to EE (%)	Mean Survival to EE (%), excluding 1st rep.	Induction Rate (%)	Mean Induction Rate
Control	20	20	1	85	67	58	0	0
			2	30			0	
			3	86			0	
26	20	20	1	—	56	56	—	100
			2	31			100	
			3	81			100	
9500	200	5	1	71	46	33	96	99
			2	13			100	
			3	53			100	
9500	300	5	1	79	56	44	100	100
			2	24			100	
			3	64			100	
9500	400	5	1	80	63	55	100	89
			2	27			100	
			3	83			68	

Table 6. Survival and triploid induction rates from an experiment designed to directly compare the efficiency of heat and pressure treatments for inducing triploidy in Hayspur-strain rainbow trout. Experiments were conducted at Hayspur FH during fall 2004. Minutes after fertilization, Celsius minutes after fertilization, and eyed eggs are abbreviated as CMAF, MAF, and EE, respectively.

Temperature / Pressure	MAF/CMAF	Duration	Replicate	Survival to EE (%)	Mean Survival to EE (%)	SE	Induction Rate (%)	Mean Induction Rate	SE
26	20/230	20	1	92.8	91	2.3	96.7	96	1.6
			2	94.2			100.0		
			3	91.3			96.7		
			4	82.3			90.0		
			5	94.4			96.7		
9500	26.3/300	20	1	93.7	87	2.3	96.7	99	0.8
			2	90.2			96.7		
			3	82.5			100		
			4	83.0			100		
			5	83.1			100		
9500	33/370	5	1	93.7	90	2.1	100	100	0
			2	93.5			100		
			3	92.4			100		
			4	84.4			100		
			5	85.4			100		
	control		1	95.8	95	0.9	3.3	3	0
			2	95.5					
			3	96.6					
			4	91.2					
			5	94.5					

Table 7. Survival and triploid induction rates from an experiment designed to directly compare the efficiency of heat and pressure treatments for inducing triploidy in westslope cutthroat trout. Experiments were conducted at Hayspur FH during spring 2004. Eyed eggs and feeding fry developmental stages are abbreviated as EE and FF, respectively.

Temperature / Pressure	MAF	Duration	Replicate	Survival to Eye-up (%)	Mean Survival to EE (%)	Survival to FF (%)	Mean Survival to FF (%)	Induction Rate (%)	Mean Induction Rate (%)
Control	10	10	1	32		9		0	
			2	65	54	14	21	0	2
			3	64		39		6	
9500	200	5	1	36		16		100	
			2	35	34	9	10	100	100
			3	32		7		100	
9500	300	5	1	31		7		100	
			2	56	52	26	22	96	99
			3	70		34		100	
28	10	10	1	21		9		88	
			2	52	41	15	13	100	96
			3	50		14		100	

Table 8. Sample composition, stocking history, and importance of stocking in 16 high mountain study lakes. Lakes were qualitatively placed into three fish assemblage categories: 1) High percentage of fish from previous stocking events and limited or no natural reproduction, 2) Intermediate percentage of fish from previous stocking events and intermediate levels of natural reproduction, and 3) nearly all fish from natural production and limited or no contribution from previous stocking events. Westslope cutthroat, golden, rainbow, and hybrid trout were abbreviated as CUT, GNT, RBT, and RXC, respectively.

Lake Name	Sample content		Percent species composition				Rotation (yrs)	Recently stocked species	Preceding stocking to 2001 (species, yr)	Evidence of natural reproduction	Is stocking contributing to the fishery?	Category	Comments
	Non test fish (%)	2001 test fish (%)	CUT (%)	GNT (%)	RBT (%)	RXC (%)							
Blackwell	85	15	0	0	98	0	1	RBT	RBT,2000	Yes	Yes	1	Some recruitment
Blue Jay	35	65	0	0	100	0	3	RBT	RBT,1998	No	Yes	1	No recruitment
Blue	94	6	0	0	100	0	2	RBT	RBT,1999	No	Yes	1	
Brush	100	0	0	0	100	0	1-3	CUT, RBT, RXC, GRY	RXC,1998	Yes	No	3	Reproducing RBT pop.
Cache Cr. #1	99	1	0	0	100	0	3	CUT	WST,1999	Yes	No	3	Reproducing RBT pop.
Cache Cr. #2	100	0	0	0	78	0	NA	NA	NA	Yes	No	NA	Shallow, minimal pop.
Josephine #2	100	0	0	32	68	0	1	RBT, GNT	RBT,2000	Unknown	Yes	2	
Ingeborg	77	23	74	0	26	0	2	RBT, CUT	RBT,1999	Yes	Yes	2	Some wild CUT
Long	88	13	0	0	88	13	3	RBT	RBT,1998	Yes	Yes	1	No recruitment
NF 20 Mile #3	96	4	51	0	42	8	2-3	RBT	RBT,1999	Yes	Yes	2	Reproducing CUT pop.
NF 20 Mile #4	93	8	3	0	76	16	1-3	RBT, GRY	RBT, 2000	Yes	Yes	2	Wild fish present
Queens R. #5	99	1	33	8	40	18	2-3	RBT	RBT,1999	Yes	No	3	Reproducing hybrid pop.
Raft	2	98	0	0	100	0	1-5	RBT	RBT,1996	No	Yes	1	No recruitment
Shaw Twins #1	88	12	15	0	73	12	1	RBT, GRY	RBT,2000	Yes	Yes	1	Some recruitment
Squaw	100	0	90	0	0	10	3	RBT	RBT,1998	Yes	No	3	Reproducing CUT pop.
Washington	95	5	3	0	95	3	3	RBT	RBT,2000	Yes	Yes	2	Some recruitment

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

State of: Idaho

Grant No.: F-73-R-27, Fishery Research

Project No.: 4

Title: Hatchery Trout Evaluations

Subproject #3: Predator Training

Contract Period: July 1, 2004 to June 30, 2005

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 evaluation, 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, Hagerman, and Grace fish hatcheries 15 day periods prior to release of fingerlings during spring 2004. Equal numbers of predator trained and control fingerlings were stocked into Lucky Peak, Lake Walcott, and CJ Strike reservoirs, as well as Hayden Lake. Due to inaccessibility (Lake Walcott), lack of sampling due to slow growth rates (Hayden Lake), and no catch of test fish (CJ Strike and Lucky Peak reservoirs), I was unable to address whether more predator-trained fingerlings survived than control fingerlings. In order to fully answer this question, additional sampling in spring and fall 2005 is needed.

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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 (Dickson and MacCrimmon 1982; Berejikian et al. 1996). Hatchery trout are often more aggressive (Fenderson et al. 1968; Mesa 1991) 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 (Miller 1951; Miller 1953; Fraser 1981).

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* as 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 *O. mykiss* 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 laboratory-scale 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 study that within two years assesses 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 (FH), one production raceway at Hagerman Fish Hatchery on Tucker Springs water, and one pair of small raceways at Grace Fish Hatchery. 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. For the single raceway used at Hagerman, the upper half was used to rear control fish, while the bottom half was used for experimental purposes. 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 grit marking. Predator-trained fingerlings were marked with red dye, whereas control fingerlings were marked with green dye.

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 (~300 mm). These fish were reared at the hatchery where training occurred to reduce the possibility of introducing or transferring 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 March 22, 2004 at Nampa FH, seven predators were introduced into raceway 2 (\bar{x} = 308 ± 6 mm, 293 ± 15 g) and 11 predators were introduced into raceway 3 (\bar{x} = 304 ± 7 mm, 286 ± 18 g). The fingerlings from these raceways were stocked into CJ Strike Reservoir. On

April 27, 2004 at Hagerman FH, five predators were introduced into the bottom of raceway 6, and fingerlings from this raceway were stocked into Lake Walcott. On April 29, 2004, 10 predators were introduced into raceway 9 ($\bar{X} = 297 \pm 6$ mm, 320 ± 19 g) and raceway 12 ($\bar{X} = 304 \pm 11$ mm, 354 ± 35 g) at Nampa FH. Fingerlings from these raceways were stocked into Lucky Peak Reservoir. On May 4, 2004 at Grace FH, 13 predators were introduced into raceway S4 ($\bar{X} = 313 \pm 14$ mm, 326 ± 30 g), and fingerlings from these raceways were stocked into Hayden Lake. 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.

Approximately equal numbers of control and predator-trained fingerlings were stocked in four lakes and reservoirs from April 8 through May 20, 2004 (Table 9). Study waters were selected based on three criteria: 1) indicative of water normally stocked with fingerling rainbow trout by IDFG, 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 1, 3, and 4 (Table 9).

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. Floating gill nets measured 46 m long by 2 m deep and were comprised of six panels of 19, 25, and 32 mm stretch mesh monofilament. Trap nets had a 15.2 m lead that was 0.9 m deep with two 0.9 x 1.8 m frames and four 0.9 m hops with a 10 cm diameter throat; all mesh was 25 mm bar knotless nylon. I collected fish by electrofishing at night in the littoral zone using a Smith Root electrofishing boat. Pulsed direct current was produced by a 5,000 watt generator. Frequency was set at 60 or 120 pulses per second and an output of 4-5 amps. I sampled two of the four study waters from September 20 through October 25, 2004. No fall sampling was conducted at Hayden Lake or Lake Walcott due to projected slow growth rates and lack of boat access, respectively.

RESULTS

For Hayden Lake, marking success on May 17, 2004, 14-15 days after impregnation of grit dye, equaled 94% for the predator trained group and 91% for the control group. Mean length of fingerlings was equal based on overlapping confidence intervals. Mean length of fingerlings in the predator training raceway equaled 85 ± 4 mm, whereas the length of the control fingerlings was 89 ± 3 mm.

For CJ Strike Reservoir, marking success on April 5, 2004, 14-21 days after impregnation of grit dye, equaled 94% for the predator trained group and 92% for the control group. Mean length of fingerlings were equal based on overlapping confidence intervals. Mean length of the predator trained fingerling was 70 ± 2 mm, whereas the length of the control fingerlings was 73 ± 2 mm.

For Lake Walcott, marking success on May 4, 2004, 15-16 days after impregnation of grit dye, equaled 89-90% for the two groups used in this study. Mean length of fingerlings was equal based on overlapping confidence intervals. Mean length of the predator trained fingerling was 105 ± 4 mm, whereas the length of the control fingerlings was 112 ± 3 mm.

For Lucky Peak Reservoir, marking success on May 12, 2004, 15 days after impregnation of grit dye, equaled 91-92% for the control and experimental raceways (pooled). Mean length of fingerlings in the predator raceways (9 and 12; $\bar{X} = 73 \pm 2$ mm) was equal to that of fingerlings in the control raceways (10 and 11; $\bar{X} = 73 \pm 2$ mm).

The four study waters were stocked with relatively equal numbers of predator trained and control fingerlings from April 8 through May 20, 2004. The number of predator trained fingerlings ranged from 86-114% of the number of control fingerlings stocked in three of the four study waters (Table 9). The only exception was Lucky Peak Reservoir, where the number of predator trained fingerlings dropped to 62% of the number of controls due to poor survival during rearing and marking.

In Lucky Peak Reservoir, sampling consisted of 109 h of gill net, 174 h of trap net, and 190 minutes of boat electrofishing effort. A total of 755 fish were sampled including 105 rainbow trout. Rainbow trout averaged 333 ± 7 mm and 387 ± 23 g. Control and predator trained fingerlings from this study should have grown to between 200 and 250 mm by fall when sampling was conducted. However, very few trout of this size were caught ($n = 3$), and none were marked.

In CJ Strike Reservoir, sampling consisted of 103 h of gill net and 288 minutes of boat electrofishing effort. A total of 181 fish were sampled including 12 rainbow trout. Rainbow trout averaged 335 ± 21 mm and 437 ± 87 g. Control and predator trained fingerlings from this study should have grown to between 200 and 250 mm by fall when sampling was conducted. However, no trout of this size were caught, and the smallest individual caught was 290 mm.

No sampling was conducted at Hayden Lake and Lake Walcott during fall 2004 due to projected slow growth rates and lack of boat access due to low water levels, respectively.

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).

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 inaccessibility (Lake Walcott), lack of sampling due to slow growth rates (Hayden Lake), and no catch of test fish (CJ Strike and Lucky Peak reservoirs), I was unable to address whether more predator-trained fingerlings survived than control fingerlings. In order to fully answer this question, additional sampling in

spring and fall 2005 is needed. Unfortunately, during fall 2004 Lucky Peak Reservoir was drained to less than 1% of total storage volume. The effect that this drawdown had on test groups is unknown at this time.

RECOMMENDATIONS

1. Complete additional surveys on Lake Walcott and Hayden Lake as well as CJ Strike and Lucky Peak reservoirs. It seemed that during fall 2004 surveys, spring-planted fingerlings had not yet fully recruited to the gears or behaved differently than larger rainbow trout that were caught readily. An additional 6-12 months of growth should allow better catches of test fish.

LITERATURE CITED

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Table 9. Study water name, date stocked, rearing facility, and the number of fingerlings stocked during 2004 to compare the performance of predator trained and control fingerlings.

Study Water	Hatchery	Stocking Date	Number of control fingerlings	Number of predator trained fingerlings
CJ Strike Res.	Nampa	5/19/2004	143,359	88,950
Hayden Lake	Grace	5/13/2004	56,387	64,457
Lake Walcott	Hagerman	5/20/2004	103,885	90,300
Lucky Peak Res.	Nampa	4/8/2004	135,776	138,925

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